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Aims

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Improving pregnancy outcomes and women's health in general

This issue of the journal contains fourteen articles covering various aspects of Medicine - both clinical and basic sciences of which five focus on women issues. The first is a review of gynaecological cancer care which highlighted the impact of poverty, lack of access to health care, inadequate or absence of basic infrastructure as the main hinderances to optimal care while delay in presentation contributed to the overall poor prognosis. The second article advocates the adoption of home-based post-natal care by trained health practitioners between the period of birth and forty-two days for improving maternal and family health. According to the authors, this method has been adopted by many countries for lowering maternal mortality and providing holistic care for women and their newborn babies.

Hypertension is referred to as a silent killer because of the damage it causes while the individual is still asymptomatic. It is the most important risk factor for chronic cardiovascular diseases – damaging the heart, brain, kidneys, eyes and blood vessels in general. When it affects women, the consequences are grave. The third article reported that about 7% of pregnant women studied had hypertension. Pre-eclampsia and eclampsia predominated followed by gestational hypertension. Women aged 31 years and over as well as those with previous history of hypertension constitute the group that should be closely monitored in pregnancy to avoid preventable complications. They should indeed be regarded as "high risk pregnancies". The fourth paper was based on a study of iodine levels in pregnant women in their first trimester. Low iodine levels (measured in the urine) and thyroid hormone levels were found in less than 10% of the participants showing that iodine deficiency was uncommon. Nutritional supplementation of iodine was adequate, and the finding would justify why cretinism is relatively uncommon. The fifth article was based on observations in laboratory animals and the finding can be extrapolated to human beings. Watermelon (*Citrilus lanatus*) was shown to have anti-oxidative properties and that its consumption can reduce craving for sugar as well as result in healthy placental development. This may have a place in managing gestational diabetes.

Although the topics covered are varied, the overall conclusion is that maternal health can be improved upon by using the findings from these studies. The other nine articles in the issue are no less interesting and challenging. Collaboration between basic and clinical sciences is essential for making significant contributions in Medicine. The time has indeed come to emphasize the importance of translational research so that research findings can bring about the much-needed improvement in health with better indices for international comparisons.

A. Ogunniyi
Editor-in-Chief

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Impact of educational interventions on undergraduate students' HIV/AIDS knowledge, attitudes and sexual behaviour in Ibadan, Nigeria

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Abstract
Background: Human Immunodeficiency Virus (HIV) and Acquired Immune-deficiency Syndrome (AIDS) interventions in the recent past have targeted young people but most of these interventions have not been properly evaluated.
Objective: To assess reported changes in HIV/AIDS knowledge, attitudes and sexual behaviours among undergraduate students of the University of Ibadan following exposure to HIV/AIDS prevention interventions.
Methodology: A descriptive and cross-sectional design was adopted to measure the impact of HIV/AIDS interventions program in the University in the last 5 years preceding the study. A stratified proportionate sampling technique was used to administer 676 standardized questionnaires on respondents who were randomly selected from the Ten undergraduate halls of residence in the University.
Result: Respondents' mean age was 22.9 ±3.27 years. Majority (64.6%) reported changes in behaviour directly attributed to the HIV/AIDS education they had received on campus. These changes included: reduction in number of sexual partners (45.0%), use of condoms (42.3%), and taking HIV test (50.0%). The overall mean attitudinal score of the respondents before exposure to educational programme was 7.98±2.61 and their overall mean score after exposure was 8.57±2.48 ($p<0.05$) and that of behaviour was (5.18±2.82 vs 5.90±2.58) ($p<0.05$)
Conclusion: Behavioural change interventions need to be intensified to reach larger proportion of students.

Keywords: *HIV/AIDS, sexual behaviour, undergraduate students*

Résumé
Contexte: Les interventions sur le Virus de l'Immunodéficience Humaine (VIH) et du Syndrome d'Immunodéficience Acquise (SIDA) ont récemment ciblé les jeunes, mais la plupart de ces interventions n'ont pas été correctement évaluées.
Objectif: Pour évaluer les changements signalés dans les connaissances, les attitudes et les comportements sexuels chez les étudiants en licence de l'Université d'Ibadan, suivant l'exposition aux interventions de prévention du VIH / SIDA.
Méthodologie: Un modèle descriptif et transversal a été adopté pour mesurer l'impact du programme d'intervention sur le VIH / SIDA à l'Université au cours des 5 dernières années précédant l'étude. Une technique d'échantillonnage proportionnelle stratifiée a été utilisée pour administrer 676 questionnaires standardisés aux répondants choisis au hasard parmi les dix résidences des étudiants en licence à l'Université.
Résultat: L'âge moyen des répondants était de 22,9 ± 3,27 ans. La majorité (64,6%) a signalé des changements de comportement directement attribués à l'éducation sur le VIH / SIDA qu'ils avaient reçue sur le campus. Ces changements comprenaient: la réduction du nombre de partenaires sexuels (45,0%), l'utilisation de préservatifs (42,3%) et la prise du test VIH (50,0%). Le score total moyen des attitudes des répondants avant l'exposition au programme éducatif était de 7,98 ± 2,61 et leur score total moyen après l'exposition était de 8,57 ± 2,48 ($p <0,05$) et celui du comportement était (5,18 ± 2,82 vs 5,90 ± 2,58) ($p < 0,05$)
Conclusion: Les interventions focus le changement de comportement doivent être intensifiées pour atteindre une plus grande proportion des étudiants.

Mots-clés: *VIH / SIDA, comportement sexuel, étudiants en licence*

Introduction

AIDS is a global crisis. Three decades after the discovery of HIV/AIDS, the disease has infected more than 40 million people worldwide [1]. Sub-Saharan Africa with only 10% of the world's population has two thirds of the people living with HIV worldwide [1]. Young people aged between15-24 years represent 45% of all new HIV infections; with almost 3,500 infected with HIV each day [2, 3]. It is one of the leading causes of death among people aged 25 to 44 years [2].Since the first case of

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AIDS was identified in 1986 in Nigeria, HIV prevalence has been on the increase but it is currently stabilized at 3.1% sero-prevalence rate as at 2015 [2]. Nigeria's STD/HIV Control Programme estimates that over 60% of new HIV infections are in the 15-24 year old age group [2] and the country currently had the second largest number of people living with HIV in the world as at 2015 [3]. Response to the HIV/AIDS pandemic among adolescents is crucial if progress is to be made in the prevention, impact mitigation and the provision of care and support for PLWHA and People Affected by HIV/AIDS (PABA).

Young persons should be a priority for HIV/AIDS intervention activities because they are at the centre of the HIV epidemic in terms of transmission, vulnerability, impact and potential for change [4]. The University campus offers an excellent opportunity for reaching a large number of young persons with HIV prevention interventions because students represent a captive audience. However, an evaluation carried out by International Institute of Educational Planning (IIEP) in 2007 [5] to examine the response of higher education institutions to HIV and AIDS in three East African countries found that universities and teacher training institutions are inadequately addressing HIV and AIDS because of a culture of denial and concealment. The dissemination of this finding led to the launch of different programmes across the globe targeting students in the campuses across different African countries [6-9]

As with most African countries, in Nigeria there have been several initiatives targeting university students [10]. Students from the University of Ibadan (UI), Nigeria, have been targets of several HIV prevention interventions including awareness campaigns during World AIDS Day, bill board messages like the 'Zip Up' programme, posters and handbill messages implemented by non-governmental organizations. One of the sustainable AIDS programme on UI campus is the McArthur Peer Education initiative organized by the Centre for HIV/AIDS Intervention in Nigeria (CEHAIN), a university-based organization. CEHAIN implemented the project using Peer education (PE), a strategy that afforded students the opportunity to make inputs in planning and implementation of HIV prevention intervention. Over a four-year period, the project trained 1,157 volunteers as peer educators who in turn reached 23,930 fellow students with appropriate messages on HIV and AIDS, distributed 14, 609 educational materials and 16,644 male condoms [11]. We conducted an evaluation of these interventions on students' knowledge, attitude towards persons living with HIV and sexual behaviour. The results of the evaluation are presented in this article.

Materials and methods

The setting and study population

The UI was established in 1948 and it is located on the northern edge of the city of Ibadan (7 20'N, 30 50'E) occupying about 10.4 square kilometers landscape. As at 2007, when the study was conducted, U.I. had overall enrollment of 17,461 students, out of which 6,558 (37.5%) were for postgraduate programme. The University has 13 Faculties and 12 Halls of residence. Two of these Halls of residence are exclusively for post-graduate students; one is a mixture of both the undergraduate and post-graduate students while others are for the undergraduate students. The study population consisted of the undergraduate students' resident in the halls in the university. This is because the Mac Arthur Peer Education Initiative programme organized by CEHAIN was carried out in the halls of residence.

Study procedures

Data were collected using 60-item pretested copies of questionnaires. The questionnaire was designed to collect both the Pre and Post intervention information since there was no Pre-intervention data before the intervention was conducted. The questionnaires elicited information on demographic profile, respondent's exposure to HIV/AIDS education programmes in the campus, knowledge of HIV, sexual behaviour, attitudes towards persons living with HIV, utilization and HCT. The trained Peer Educators were expected to reach fellow students in the halls of residence. The study was planned to assess the effects of the CEHAIN-intervention on the knowledge, perception and attitude of students to HIV/AIDS. Students were requested to confirm whether or not they had received any HIV prevention intervention messages on campus and if so to report changes they had made in response to HIV/AIDS education received. Those who indicated that they have not been reached by the Peer Educators were excluded from this study. The questionnaires were assessed for internal consistency and its Cronbach's alpha coefficient analysis was 0.6. The 700 respondents recruited for the study were proportionately selected from the halls. The stratified sampling technique was employed in selecting the respondents. The block in the halls served as the basis for stratification. One

block was randomly selected to represent each Hall making 10 blocks in all. For each selected block, a list of all rooms was compiled. All students found in the rooms during the day of the visit were invited to participate in the study since the intervention was carried out in the halls. The main criterion for inclusion in the study was that a respondent is an undergraduate student of the University and resident in the hall. Four trained Research Assistants administered the questionnaires and a total of 700 questionnaires were administered and 676 (96.5%) of these questionnaires were retrieved and analysed.

Data analysis

The completed questionnaires were checked for completeness and open-ended questions were coded and analyzed with SPSS software package, version 15.0. There were 11 knowledge items in the questionnaire; each item attracts a score of 2 points making a total of 22. The attitudinal questions were categorized into two (negative and positive). The maximum attitude score was 12 score. Attitude score between 0-7 was regarded as negative and score between 8-12 was regarded as positive. Also behavioural questions were categorized into two (negative and positive). The maximum behaviour score was 10 score. Behaviour score between 0-5 was regarded as negative and score between 6-10 are regarded as positive. These questions were systematically asked to ascertain both their behaviour and attitude towards a PLWHA before and after their exposure to HIV intervention programme on the campus. Questions on attitude, behaviour and use of HCT were described in proportions and percentages. The Chi-square, independent and paired t-tests were used to compare attitudes and behaviour score while the f-test was used to examine differences within faculty, level and age-group both before and after the interventions.

Results

Socio-demographic characteristics

The demographic profile of the respondents is shown in table 1. Majority of respondents (62.2%) were between 20-24 years age group with the mean age of 22.9 ±3.27 years and a range of 16 to 40 years old; 98.2% were single. More (59.5%) were males; 24.1% were in 400 level of their course of study and 27.5% are in the Faculty of Arts and more (21.0%) were also recruited from Awolowo hall.

Knowledge of HIV/AIDS

Virtually all (99%) of respondents had heard about HIV/AIDS and the main source of information for this was the television (50.3%). Many respondents (62%) reported ever receiving educational/ information on HIV/AIDS from a fellow UI student. Of these, 32% received these educational/ information in 2004/2005 session while 23.2% received it in 2005/2006 session. Only 28.1% had ever participated in HIV/AIDS programme on campus and these included, 22.0% lecture/ symposium, 3.7% attended a rally and 1% HIV test programme. The overall mean score of students' level of knowledge of HIV and AIDS using a 22 point score scale was 19.4±2.8. Almost all the respondents (98%) knew that there is a relationship between HIV and AIDS. Knowledge of modes of transmission was high (table 2).

Table 1: Socio-demographic characteristics of respondents

Variables	Number (n)	Percentage %
Age (in years)		
15-19	76	11.2
20-24	420	62.2
25-29	153	22.6
≥30	27	4
Marital status		
Married	12	1.8
Single	664	98.2
Levels		
600	29	4.3
500	28	4.1
400	163	24.1
300	136	20.1
200	159	23.5
100	161	23.9
Faculty affiliation		
Humanities	186	27.5
Social Sciences	116	17.2
Sciences	367	54.3
Gender		
Male	402	59%
Female	274	41%
Total	676	100

HIV sexual risk related behaviour

Majority (65.0%) agreed that they had changed their behaviours as a result of the educational programmes they received about HIV/AIDS prevention. These changes were: Avoidance of blood transfusion (78%); Avoidance of sharing sharp objects (82.4%); Abstinence from sex (76.5%); Reduction in number of sexual partners (45%); Use of condoms (42.3%); Taking HIV test (50%).

Table 2: Respondents level of Knowledge of HIV/AIDS

S/N	Statement	Percent (%) answering correctly
1	There is a relationship between HIV and AIDS	98.0
2	HIV can be transmitted through seminal fluid	87.0
3	HIV can be transmitted through Vaginal fluid	91.0
4	HIV can be transmitted through blood and blood products	99.0
5	HIV can be transmitted through placenta	75.0
6	HIV can be transmitted through breast milk	74.0
7	An HIV infected person can be known by mere looking at the person	92.0
8	HIV/AIDS has no cure	79.1
9	Anybody can be infected by HIV if he/she is involved in risky Practices	92.0
10	Someone can get HIV by having unprotected sexual intercourse just once?	95.0
	Mean numbers of item correctly answered	19.4±2.8.
	Possible range of scale	0-22

Table 3: Reported positive changes in attitude and behaviour before and after exposure to intervention (N=658) *

S/N	Statement	Before intervention (%)	After intervention (%)
1	Anyone can get HIV/AIDS; so persons with the disease should not be discriminated against.	513 (75.9)	594 (87.9)
2	The U.I. authority should make HIV testing compulsory for all students	343 (50.7)	416 (61.5)
3	HIV is a serious issue, so it is my view that students should be tested Periodically.	423 (62.6)	503 (74.4)
4	I do not think any student has HIV/AIDS because they are very healthy and Productive	143 (21.2)	95 (14.1)
5	If your lecturer has HIV, should he or she be allowed to continue in your department?	500 (74.0)	572 (84.6)
6	If a student has HIV but is not sick should he or she be allowed to continue in your department	514 (76.0)	567 (83.8)
7	Can you sleep in the same room with someone who has HIV?	428 (63.3)	509 (75.3)
8	I am willing to eat from the same plate with a colleague I know Has HIV	326 (48.2)	421 (62.3)
9	Issues about HIV/AIDS should not be discussed openly in the Department.	166 (24.6)	170 (25.1)

** Those that give the correct answer*

Changes in attitude towards persons living with HIV/AIDS after exposure to intervention

Several questions were asked to know the impact of the interventions on behaviour and attitudes of students to PLWHA in this study. Most respondents (88.0%) agreed that PLWHA should not be discriminated against (Table 3).

Use of HCT Services after exposure to intervention

There was an increase in the number of students who took HIV test from 195 (29.0%) before the intervention programme to 50.0% after exposure to the programme on the campus. An increase from 24.0% to 35.0% was also observed in the number of respondents who claimed that they would be willing to disclose their status to their colleague/departments if they test positive to HIV/AIDS after exposure to intervention programme (Table 4).

Respondents' attitudes and behaviour to PLWHA before and after exposure to HIV educational programme

There was an increase in the overall mean score of the respondents. The overall mean attitudinal score

Table 4: Reported positive changes in the use of HCT services before and after exposure to intervention (N=658) *

S/N	Statement	Before intervention (%)	After intervention (%)
1	I will let my colleagues/department know if I test positive for HIV.	161 (23.8)	234 (34.6)
2	I will disclose my status if there is provision for treatment in the campus.	331 (49.0)	413 (61.1)
3	Do you know of any student who has lost his studentship due to his HIV positive status?	26 (3.8)	27 (4.0)
4	I have found out my HIV status	195 (28.8)	224 (33.1)
5	I know that the university have counseling and testing unit	156 (23.1)	203 (30.0)
6	I am willing to encourage others to have HIV testing	486 (71.9)	534 (79.0)

** Those that give the correct answer*

Table 5: Attitude and behaviour score before and after intervention

	Overall attitude and behaviour score before and after intervention			
	Before intervention	After intervention	t-test	*p-value*
Attitude	7.98±2.61	8.57±2.48	5.243	0.000
Behaviour	5.18±2.82	5.90±2.58	7.124	0.000
	Attitude and behaviour score before and after intervention by Gender			
Sex (Attitude)				
Male	8.04±2.56	8.50±2.49	-3.07	0.000
Female	7.88±2.66	8.65±2.45	-4.60	0.000
Sex (Behaviour)				
Male	5.25±2.76	5.82±2.52	-4.30	0.000
Female	5.05±2.89	6.00±2.66	-5.97	0.000
	Attitude and behaviour score before and after intervention by Faculty			
Faculty	Humanities (n=186)	Social sciences (n=116)	Sciences (n=374)	F-test	*p-value*
Before Intervention					
Attitude	7.81±2.56	7.89±2.60	7.9±2.60	0.729	0.483
Behaviour	4.86±2.80	5.46±2.80	5.24±2.81	1.605	0.150
After Intervention					
Attitude	8.29±2.41	8.62±2.56	8.68±2.48	1.904	0.202
Behaviour	5.84±2.55	5.96±2.70	5.89±2.55	0.072	0.930

of the respondents before intervention was 7.98±2.61 and their overall mean score after the intervention was 8.57±2.48 ($p<0.05$) (Table 5). There was also an increase in the overall mean behavioural score of respondent after intervention (5.18±2.82 vs 5.90±2.58) ($p<0.05$) (Table 5).

A significant relationship was noticed when respondents sex was compared with attitude and behaviour both before and after intervention ($p<0.05$) (Table 5). The result also showed an increase in the mean score of respondents on both attitude and behaviour (before and after intervention) when compared with respondents' faculties but the noticed differences were not significant. The noticed increase in score of respondents in both attitude and behaviour (before and after intervention) when compared with respondents' age was also not significant.

Discussion

The high proportion of respondents who had heard about HIV/AIDS is an indication that HIV/AIDS has remained top in the agenda in public discussions in the media in Nigeria. This finding agreed with previous studies on higher knowledge about HIV/AIDS among university students in Nigeria [12-17]. Television and Radio were the major sources of

information on HIV/AIDS and this was in line with the previous study conducted among students in Nigeria on sources of information on HIV/AIDS [18, 12, 13]. This has implications for planning and dissemination of HIV/AIDS information by policy makers and service providers.

A large proportion reported they received information on HIV/AIDS from a fellow student in the campus. This may be due to the fact that the PE approach is a popular educational strategy on UI campus and this further affirmed the assertion in previous studies that suggest that Peer Education can bring about short-term positive changes in attitude [18], self-efficacy and intention to use condom [18], attitude towards abstinence [18], self-efficacy to refuse sex [19], more conservative sexual norms [20], delay in the initiation of sexual behaviours [19], reduction in the frequency of intercourse [20] and the use of condoms among sexually active teens [18] is not a ruse. The authorities and stakeholders concerned could use this strategy in reaching students in future programme since there are willing volunteers among the students. Lecture/symposium was claimed by some to be the nature of the program on HIV/AIDS they attended and 33.0% claimed to have heard of Macarthur Peer Education Programme through friends, Peer Educators in the halls and during the orientation programme for new students. This suggests that it will be easy to integrate HIV/AIDS programme in the school curriculum as suggested by Oladepo and Brieger and Ogbuji [12, 14].

Despite high levels of awareness of HIV/AIDS, misconceptions persist. The implication of this may be that students who believe AIDS is curable may not take adequate precaution to prevent infection. Concerted efforts are therefore needed to clear these gray areas on the mode of transmission. Though respondents first heard of HIV/AIDS through different means, it is important to note that employing multi-purpose approach in disseminating information among youth in tackling the scourge will yield more result than expected. The increase in knowledge that is expected to lead to decrease in risky behaviour was found to be true in this study. Majority of the respondents were ready to avoid any behaviour that will expose them to infection. Peer Educators should be equipped and armed with newsletters that would spell out and simplify all about HIV and AIDS and the means of transmission. This will help to clarify the misconceptions about HIV/AIDS among the students and it will in turn affect positively the way they will relate with PLWHA. The social taboo the society places on issues of sex and the female gender might also contribute to the way that the female respondents were mostly uncomfortable in providing answers to questions during the study. It is important to break these cultural and social barriers that often stand, as hindrance to sexual and reproductive health education among young females if resources committed to such efforts would yield the desired result.

Majority of the students held positive attitude towards PLWHA after exposure to intervention as indicated by the fact that many would be willing to sleep in the same room and eat from the same plate with PLWHA. This positive change may be a recent development as previous studies showed that the attitudes of students towards students living with HIV/AIDS (SLWHA) are that of discrimination, rejection and stigmatization [11, 16]. This needs to be reinforced and promoted as this will further give self-confidence and encourage SLWHA to make known their status without fear of being rejected and stigmatized which has been the experience in the past.

The study also showed that more male respondents claimed to use condom with partners than their female counterparts. It is possible that the males were more truthful than the female in reporting their sexual behaviour. Another factor that might be responsible for this is the social taboo the society places on discussing sexual issues as it relates to female. This finding corroborate with previous studies that stated that consistent use of condom remains low among young females [13, 25-27]. More efforts are needed to be channeled towards encouraging the use of condom among students of both sexes since condom is still one of the safest means of preventing HIV infection.

The result of this study showed that a large number of the respondents have reduced their risky sexual behaviour. This finding is in line with other studies, which reported that younger students when compared with their older colleagues took more precautions and steps to reduce their risk of HIV/AIDS [14, 19]. This suggests that if this age group is recruited as peer educators it would be much easier and effective to reach to larger portion of the students with preventive messages.

The majority of the students reported changes in behaviour directly attributed to the HIV/AIDS education they had received on campus. This is contrary to previous findings, which revealed that respondents were knowledgeable about transmission and symptomology yet; this did not prevent them from engaging in unprotected coitus and other risky behavior [14, 15, 21, 22]. This finding is encouraging and it will further boost effort to reach out more

aggressively to adolescents who have been tagged as the AIDS generation.

The various positive changes noticed in attitude and behaviour of respondents towards HIV/AIDS and PLWHA is due to the fact that there is consistency in the promotion of HCT services through the media and PE and these services are free at the University Health Centre (Jaja Clinic). Also, HCT services are now more readily available than in the past [23]. This finding corroborates the previous study which asserted that youths when given the opportunity are likely to take advantage of the voluntary, confidential counselling and testing programmes especially if results are released at the same time of visit and services are free [23-25]. It is imperative that more efforts and support should be given to VCT among the students with adequate provision of support to those that will test positive. Assurance also must be given for confidentiality of result of test.

The study showed that youths are beginning to appreciate the fact that HIV/AIDS is not punishment for sins on PLWHA. Most of the respondents agreed that PLWHA should be encouraged rather than rejected. They claimed they can eat, sleep and work with them in the campus. The study also shows that having a right knowledge can affect perception and behaviour.

Limitations of the study

The study focused on sexual behaviour, which is personal and sensitive, and as such some respondents are not willing to give all information required by the researcher because of cultural norms, which forbid premarital sexual activity. Efforts were made to reduce this problem by assuring them of confidentiality of all information provided. The questionnaires were designed to be unanimous and self-completed. At the same time this data may be an underestimation of actual levels of sexual activities because there was no baseline study to refer to so respondents have to fall back to recollect what they were doing before that they are no more doing. Ascertaining the authenticity of responses provided by the study participants is a challenge in survey research. This study however is no exception. It would be assumed that since participation was voluntary then all the responses provided which form the basis of the findings of this study would be honestly made.

Future directions

The present paper calls for future research to be conducted to know what impact will the use of peer education strategy have in encouraging students who are living outside the university campus and are at risk to alter their behaviour and adopt safer sexual practices since a good number of students of the university of Ibadan now live outside the university campus due to shortage of accommodation in the campus.

Conclusion

The use of peer educators can be an effective tool in promoting positive sexual behaviour among youth, reduce the incidence of discrimination against PLWHA and make HCT services clients friendly in the campus as long as confidentiality is guaranteed. Although, not every student agreed to have met with a peer educator before but if the present trend is maintained and further encouraged more can still be achieved in the quest to tame the scourge among the youth in the nearest future.

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Neuropharmacologic effects of whole plant extract of *Digitaria horizontalis* in mice

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Abstract
*Background***:** *Digitaria horizontalis* Willd (Poaceae) is used in traditional African medicine in the treatment of various ailment including neurological disorders. In this study, an attempt was made to investigate the neuropharmacological activities of the hydroethanolic whole plant extract of *Digitaria horizontalis* (DH) in mice.
*Methodology***:** DH (12.5, 25, 50 or 100 mg/kg, p.o.) was administered 1 h before the behavioural assays. The formalin, open field, picrotoxin/pentylenetetrazol, elevated plus maze (EPM), hexobarbitone-induced hypnosis and tail suspension tests (TST) were used to assay for antinociceptive, spontaneous locomotor, anticonvulsant, anxiolytic, hypnotic and antidepressant activities of DH in mice, respectively.
*Results***:** Intraplantar injection of formalin (1% in saline, 20 µl) into the right hind paw induced biphasic nociceptive behaviours which was prevented by DH pretreatment in both the early and late phases. DH-induced antinociception was reversed by naloxone (opioid receptor antagonist). No significant change in spontaneous locomotor activity in the open field test [OFT]. DH prevented the occurrence and reduced the duration of seizure in picrotoxin and pentylenetetrazol models. Conversely, DH failed to increase the time spent by mice in open arms of the EPM. DH reduced latency to sleep but not the duration of sleep in hypnotic assay. Interestingly, DH dose dependently and significantly reduced the immobility time in TST in mice. DH showed potent antioxidant capacity due to its richness in phenolic, flavonoid, and tannins. DH possesses wide margin of safety, the extract up to 4000 mg/kg, *p.o*. did not induce mortality, however, a median lethal dose (LD50) of 1259 mg/kg was obtained following intraperitoneal injection.
Conclusion: Findings from this study showed that *D. horizontalis* possesses antinociceptive, sedative and antidepressant effects. The anticonvulsant activity could involve enhancement of GABAergic neurotransmission. Thus could be a potential phytotherapeutic agent in the treatment of neurological disorders.

Keywords: *Antinociceptive; anticonvulsant; antidepressant; antioxidant capacity; GABA*

Résumé
*Contexte***:** *Digitaria horizontalis* Willd (Poaceae) est utilisé en médecine traditionnelle africaine dans le traitement de maladies diverses, y compris les troubles neurologiques. Dans cette étude, une tentative a été faite pour étudier les activités neuropharmacologiques de l'extrait de plante entière hydro-éthanoïque de *Digitaria horizontalis* (DH) chez les souris.
Méthodologie **:** DH (12,5 ; 25 ; 50 ou 100 mg/kg, po) a été administré 1h avant les dosages comportementaux.Le formol, champ ouvert, picrotoxine/pentylenetetrazol, élevé plus labyrinthe (EPM), hypnose induite par hexobarbitone et test de suspension de queue (TST) ont été utilisés pour doser les activités anti-nociceptives spontanées locomotrices, anti-convulsivantes, anxiolytiques, hypnotiques et antidépressives de DH chez les souris, respectivement.
Résultats **:** L'injection intra-plantaire de formol (1% dans une solution saline, 20µl) dans la patte arrière droite induit des comportements nociceptifs bi-phasiques qui ont été prévenue par prétraitement avec DH à la fois dans les phases précoces et tardives. L'anti-nociception induit par DH a été inversée par naloxone (antagoniste des récepteurs opioïdes). Pas de changement significatif dans l'activité locomotrice spontanée dans le test en plein champ [OFT]. DH a prévenu l'apparition et réduit la durée de la saisie dans les modèles de picrotoxine et de pentylènetétrazole. Inversement, DH a échoué à augmenter le temps passé par les souris dans les bras ouverts de l'EPM. DH réduit la latence pour dormir, mais pas la durée du sommeil dans le test hypnotique. Fait intéressant, la dose DH a de manière dépendante et significative réduit le temps d'immobilité du TST chez les souris. DH a montré une capacité anti-oxydante puissante en raison de sa richesse en phénoliques, flavonoïdes et tanins. DH possède une large marge de sécurité, l'extrait jusqu'à 4000 mg /

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kg, *po* n'a pas induit de mortalité, cependant, une dose létale médiane (DL50) de 1259 mg / kg a été obtenue après injection intra-péritonéale.
Conclusion: Les résultats de cette étude ont montré que *D. horizontalis* possède des effets anti-nociceptifs, sédatifs et antidépresseurs. L'activité anticonvulsivant pourrait impliquer une amélioration de la neurotransmission GABAergique. Ainsi pourrait être un agent phyto-thérapeutique potentiel dans le traitement des troubles neurologiques.

Mots clés: *Antinociceptif ; anticonvulsivant; antidépresseur; capacité antioxydant; GABA*

Introduction

Natural products have contributed significantly towards the development of modern medicine. The use of traditional medicine in Nigeria is widespread, with as high as 80% of the population using herbal products either as medicament or food supplements. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant therapeutic activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects, and low cost [1]. Ethnopharmacological knowledge continues to contribute to the discovery of new drugs from plants [2]. *Digitaria horizontalis* Willd (Poaceae) also known as Jamaican crab grass are slender monocotyledonous annual and perennial lawn, pasture, and forage plants; some are often considered lawn pests. They are distinguished by the long, finger-like inflorescences they produce. The decoction of the plant is used in traditional African Medicine for gonorrhoea, cataracts, debility, induce emesis and nervous disorders [3]. Moreover, ethnomedicinal reports on *D. horizontalis* whole plant demonstrate its extensive application in different painful conditions and neurological disorders [3, 4]. However, there is lack of scientific study regarding the neuropharmacological activities of *D. horizontalis* whole plant. Therefore, we aimed to investigate the activity of *D. horizontalis* whole plant extract on the central nervous system behaviours in mice using standardized scientific models.

Materials and methods

Plant materials

Whole plant of *Digitaria horizontalis* was collected from a farmland in Ikotun, Lagos state, Nigeria. The plant material was identified and authenticated by Mr. T.K. Odewo (formerly Senior Superintendent, Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria), now in the Department of Botany and Microbiology, University of Lagos, Lagos, Nigeria, where voucher specimen (LUH 6157) was deposited for reference purpose.

Plant extraction

The whole plant of *D. horizontalis* (779 g) was soaked in 3.95 L of 70% ethanol and the mixture left to stand for 72 h and the procedure was repeated twice for exhaustive extraction. The combined extracts filtered and the filtrates was concentrated to dryness under reduced pressure in BUCHI Rotavapor® (Switzerland) and further dried in an oven at 40°C to give a brownish solid extract 5.79 g. The extract was preserved in the refrigerator and reconstituted in normal saline prior to administration.

Drugs and reagents

Picrotoxin, hexobarbitone, naloxone and pentylenetetrazol (Sigma–Aldrich, St. Loiuis, MO, USA), aspirin (Emzor Pharmaceutical, Nigeria), fluoxetine (Medibios Laboratories ltd, India), diazepam (Swiss Pharmaceutical Company, Switzerland), flumazenil (Hikma Farmaceutica, Portugal), morphine (Martindale Pharmaceuticals, UK), normal saline (Unique Pharmaceutical, Nigeria).

Quantitative phytochemical analysis

The quantitative estimation of total phenolic, flavonoid, tannins, and alkaloids contents in *D. horizontalis* whole plant extract were carried out using the methods of Harbone [5] or Sankhalkar and Vernekar [6].

Laboratory animals

Albino mice (18 -25 g) were obtained from the Laboratory Animal Centre, College of Medicine, University of Lagos. The animals were maintained under standard environmental conditions, and had free access to water and standard powdered diet (Livestock Feeds Plc, Lagos, Nigeria). All the animals were acclimatized for one week before the commencement of the investigation. The experimental procedures adopted in this study were in accordance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [7].

Acute toxicity study

The possible acute toxic effect of the extract was determined using the limit test and fixed dose protocol of the Organization of Economic Co-operation and Development [8] guidelines for testing of chemicals for oral and intraperitoneal

administration, respectively. In limit test for oral acute toxicity [8]. Five female mice were given DH (4000 mg/kg, *p.o*.). However, in fixed dose test; 11 mice were given DH (50 mg/kg, *i.p*., n =1; 200 mg/kg, *i.p*., n =5; and 2000 mg/kg, *i.p*., n = 5). Behavioural signs of toxicity and mortality were observed following extract administration; during the first 30 minutes, then the second, fourth, sixth hour and once daily for 14 days for delayed toxicity or mortality.

Pharmacological activities

Formalin-induced nociception

The formalin test was carried out according to the method of Hunskaar and Hole [9]. Thirty mice were randomly divided into six groups (n =5). Group I, vehicle (10 ml/kg, p.o., normal saline; control), Group II, aspirin (100 mg/kg, *p.o.,* standard reference), Group III, IV, V and VI, DH (12.5, 25, 50 or 100 mg/kg, p.o., respectively). One hour after extract or vehicle administration and 30 min after subcutaneous injection of morphine, 20µl of 1% formalin in saline was injected into the right hind paw of mice. The duration of paw licking or biting (an index of painful response) was recorded at 0-5 min (early phase, neurogenic pain) and 15-30 min (late phase, inflammatory pain) after formalin injection.

To investigate the involvement of opioidergic system in DH-induced antinociceptive effect, mice were pretreated with naloxone (5 mg/kg, s.c., opioid receptor antagonist), fifteen minutes later, DH (100 mg/kg, p.o.) was given. One hour post-treatment formalin was administered intraplantarly and the procedure was repeated.

Open field test

The changes in locomotor activity may lead to false negative/positive results in the tests. To assess the possible effects of *D. horizontalis* on spontaneous motor activity, mice were evaluated in the open-field paradigm as previously described by Ishola *et al* [2014]. Twenty-five mice were randomly divided into five groups (n =5). Group I, vehicle (10 ml/kg, p.o., normal saline; control), Group II, III, IV and V, DH (12.5, 25, 50 or 100 mg/kg, p.o., respectively). One hour post-treatment, mice were individually placed in a box (60 × 60 × 50 cm) with the floor divided into 16 squares (15 × 15 cm). The number of lines crossed, rearing and duration of grooming were recorded over a period of 5 min after 1 min of acclimatization. The box was cleaned with 10% ethanol to prevent auditory cue.

Picrotoxin-induced seizures

The method of White [10] was used to assess the anticonvulsant effect of extract. Mice were kept individually in transparent mice cages (25×15×15 cm) for 60 min to acclimatize to their new environment before the commencement of the experiment. Thirty mice were randomly divided into six groups (n =5). Group I, vehicle (10 ml/kg, p.o., normal saline; control), Group II, diazepam (5 mg/kg, p.o., standard drug), Group III, IV, V, and VI, DH (12.5, 25, 50 or 100 mg/kg, p.o., respectively). One hour later, seizure was induced by intraperitoneal injection of picrotoxin (10 mg/kg). Animals were observed for convulsion for a period of 30 min. Onset and duration of convulsions in mice were noted and recorded as well as percentage protection.

Pentylenetetrazol (PTZ)-induced seizures

The method of White [10] was employed to induce convulsion in mice. Thirty mice were randomly divided into six groups (n =5). Group I, vehicle (10 ml/kg, p.o., normal saline; control), Group II, diazepam (5 mg/kg, p.o., standard drug), Group III, IV, V, and VI, DH (12.5, 25, 50 or 100 mg/kg, p.o., respectively). One hour later, seizure was induced by intraperitoneal injection of PTZ (90 mg/kg). Mice were observed over a period of 30 min. The onset of tonic convulsion and the number of animals convulsing or not convulsing within the observation period were noted.

Elevated plus maze test

The elevated plus maze test was carried out as described by Ishola *et al.* [11]. The apparatus consisted of two opposite open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 40 cm) extended from a common central platform (10 × 10 cm) based on a design validated by Lister (1987). Forty-eight mice were randomly divided into six groups (n =8). Group I, vehicle (10 ml/kg, p.o., normal saline; control), Group II, diazepam (1 mg/kg, p.o., standard drug), Group III, IV, V, and VI, DH (12.5, 25, 50 or 100 mg/kg, p.o., respectively). One hour later, mice were placed individually onto the centre of the apparatus facing an open arm, and the time spent in each arm were noted for a period of 5 min. The maze was carefully cleaned up with 10% ethanol solution and dried after each trial. An arm entry was recorded when all four paws of the mouse were in the arm.

Hexobarbitone-induced sleeping time

Male Swiss albino mice (n = 5) were pre-treated with; Group I, vehicle (10 ml/kg, p.o., normal saline; control), Group II, diazepam (5 mg/kg, p.o., standard drug), Group III, IV, V, and VI, DH (12.5, 25, 50 or

100 mg/kg, p.o., respectively). One hour later, hexobarbitone (100 mg/kg, *i.p.*) was administered to each mouse in turn. The mice were placed on their backs in separate chambers, latency and duration of sleep depicted as loss of righting reflex after the administration of hexobarbitone, until they regained their righting reflexes were recorded. When there was any doubt, the animal was placed gently on its back again and if it righted itself within 1 min, this was regarded as the end point [12].

Tail suspension test

This test is based on the observation that a mouse suspended by the tail alternates periods of immobility and agitation [13]. Forty-eight mice were randomly divided into six groups (n =8). Group I, vehicle (10 ml/kg, p.o., normal saline; control), Group II, fluoxetine (20 mg/kg, p.o., standard drug) [14], Group III, IV, V, and VI, DH (12.5, 25, 50 or 100 mg/kg, p.o., respectively). One hour after test drugs or vehicle administration, mouse was hung on the hook by an adhesive tape placed 1 cm from the tip of the tail, 35 cm above the table top. Animals were suspended for 6 min and the duration of immobility were recorded for 5 min after 1 min of habituation. The duration of immobility was measured by a trained observer who remained unaware of the drug treatments. Mice were considered immobile only when they hung passively and completely motionless.

Results

Quantitative phytochemical analysis

The results depicted in table 1, revealed the gallic acid equivalents of total phenolic, rutin equivalent of flavonoids, the tannic acid equivalents of total tannins and the total alkaloid content of DH. Also, the total antioxidant capacity of DH expressed as the number of equivalents ascorbic acid.

Table 1: Quantitative estimation of phytochemical constituents

Constituents	*D. horizontalis*
Total Tannin Content (mg TAE/ 100g of DH)	3.01±0.06
Total Flavonoid Content (mg RE/ 100g of DH)	76.73±1.50
Total Phenolic Content (GAE/ 100g of DH)	21.27±0.69
Total Alkaloid Content (mg/100g of DH)	11.69±0.08
Total Antioxidant Capacity	12.69±0.50

Values are expressed as mean±SEM (n=3); RE- rutin equivalent; GAE- gallic acid equivalent; TAE- tannic acid equivalent.

Acute toxicity test

DH possesses wide margin of safety, the extract up to 4000 mg/kg, *p.o.* did not induce mortality nor behavioural toxic effect. However, the intraperitoneally administered mice recorded a median lethal dose (LD_{50}) of 1259 mg/kg.

Effect of DH on formalin-induced nociception in mice

As shown in fig. 1A-B, Intraplantar injection of formalin 1% in saline induced flinching and biting behaviour in the early (0-5min) and late phase (15-30 min) post formalin injection. Two way ANOVA revealed significant treatment effect of DH [$F(5,30)=115.20, P<0.0001$] at 0-5 min and [$F(5,30)=71.28, P<0.0001$] in the late phase (15-30 min). Post hoc analysis indicated significant effect of DH (12.5, 25, 50 and 100 mg/kg) with peak antinociceptive effect at 50 mg/kg (51.05% inhibition of neurogenic pain) (Fig. 1A). Furthermore, significant dose dependent inhibition of inflammatory pain was also recorded with peak inhibitory effect 82.44% at 100 mg/kg (Fig. 1B).

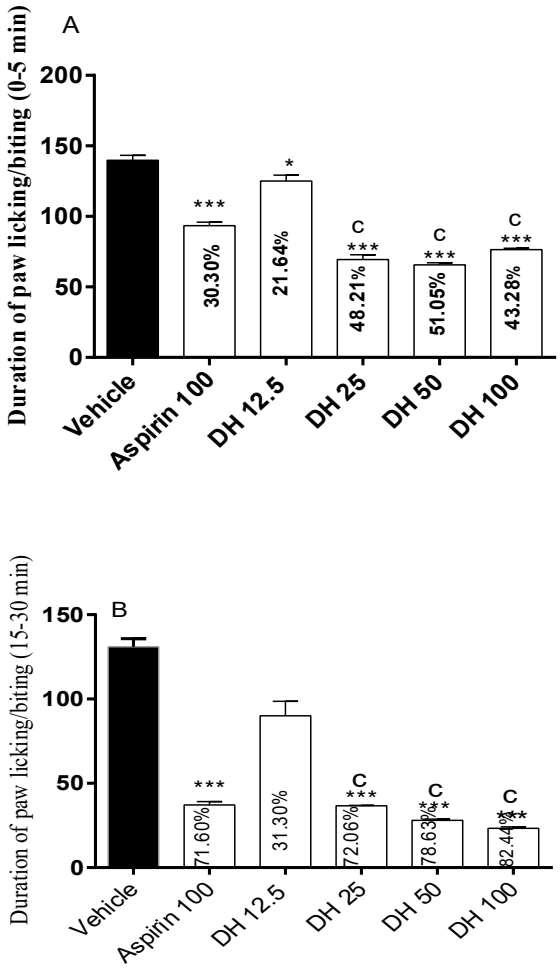


Fig. 1A-B: Effect of DH (12.5, 25, 50 and 100 mg/kg) on formalin-induced nociception in mice. Values are expressed as mean±SEM (n = 6). $^{*}P<0.05$; $^{***}P<0.001$ when compared with vehicle-treated control; $^{c}P<0.001$ versus DH (12.5 mg/kg) treated. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison tests.

Similarly, oral acute administration of aspirin (100 mg/kg) significantly inhibited early and late phases by 30.30 and 71.60%, respectively. Moreover, the antinociceptive effect of DH and aspirin were comparatively similar. However, the antinociceptive effect elicited by DH in mice was blocked by subcutaneous injection of naloxone (non-selective opioid receptor antagonist). Two way ANOVA revealed significant differences of treatment [F (2, 71) =62.73, P<0.0001] in early and late phases of nociception. Tukey's post hoc analysis showed that the pretreatment of mice with naloxone blocked DH (100 mg/kg)-induced antinociceptive effect in formalin test. (Fig. 2).

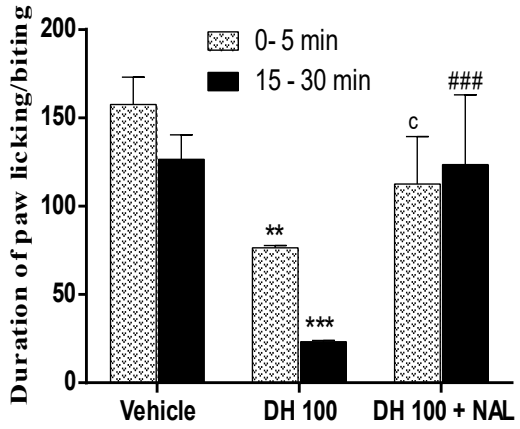


Fig. 2: Effect of pretreatment of mice with naloxone on DH (100 mg/kg)-induced antinociceptive effect in formalin test. Values are expressed as mean±SEM (n = 6). $^{**}P<0.05$; $^{***}P<0.001$ when compared with vehicle-treated control; $^{c}P<0.001$ versus DH (100 mg/kg) treated at 0-5 min; $^{###}P<0.001$ versus DH (100 mg/kg) treated at 15- 30 min. Statistical level of significance analysis by two way ANOVA followed by Tukey *post hoc* multiple comparison tests.

Effect of DH on locomotor activity in OFT

Oral acute administration of DH did not affect the animal spontaneous locomotor activity in open field test, as shown in Fig. 3. One way ANOVA revealed no significant effect of DH treatment [F (4, 24) =0.4712, P=0.7564].

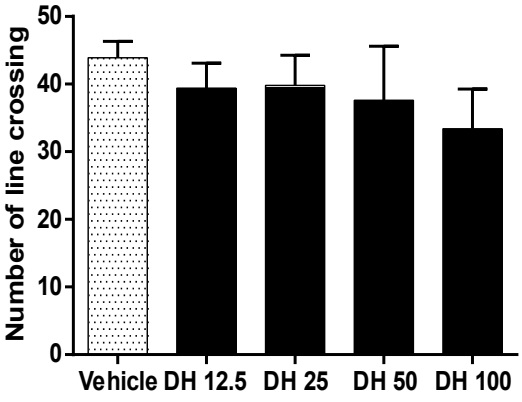


Fig. 3: Effect of DH (12.5- 100 mg/kg, p.o.) on spontaneous locomotor activity in open field test. Values are expressed as mean±SEM (n = 6). Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison tests.

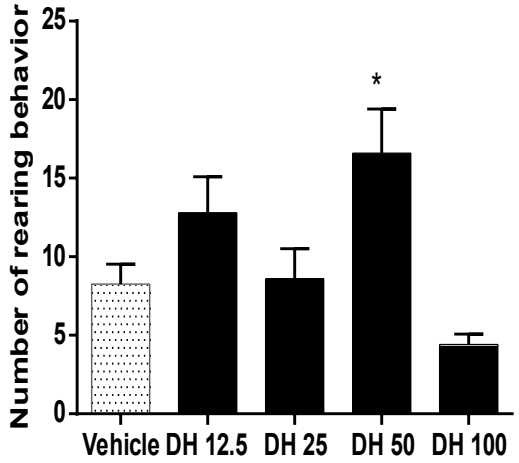


Fig. 4: Effect of DH (12.5- 100 mg/kg, p.o.) on exploratory behaviour in open field test. Values are expressed as mean±SEM (n = 6). P<0.05 when compared with vehicle-treated control. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison tests

Effect of DH on rearing behaviour in OFT

As shown in Fig. 4, acute administration of DH did not affect the number of rearing (exploratory behaviour) of animal except at 50 mg/kg treatment. Moreover, one way ANOVA showed significant main effect of DH (50 mg/kg) [F (4, 24) =5.69, P=0.0028]. Post hoc analysis indicated significant increase in number of rearing in DH (50 mg/kg) treated animals compared with vehicle-treated control.

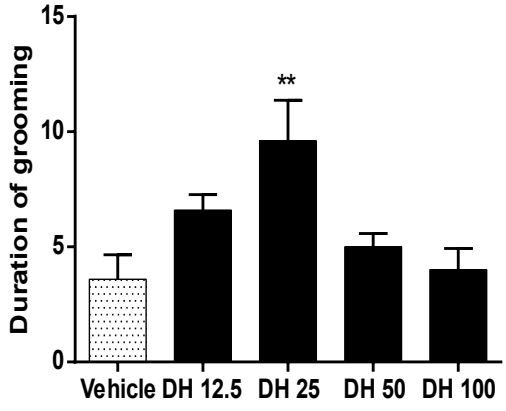


Fig. 5: Effect of DH (12.5- 100 mg/kg, p.o.) on grooming behaviour in open field test. Values are expressed as mean±SEM (n = 6). $^{**}P<0.05$ when compared with vehicle-treated control. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison tests.

Effect of DH on grooming behaviour in OFT

Acute administration of DH produced no significant effect on grooming behaviour except in 25 mg/kg treatment group. One way ANOVA revealed main effect of DH (25 mg/kg) [F (4, 25) =5.081, P=0.0039]. Post hoc analysis indicated significant increase in duration of grooming in DH (25 mg/kg) treated mice (Fig. 5).

Effect of DH on picrotoxin-induced seizure in mice

Intraperitoneal injection of picrotoxin induced clonic-tonic seizure in vehicle treated control mice. However, pretreatment of mice with diazepam before picrotoxin injection prolonged the occurrence of clonic seizure and prevents tonic seizure and death in 60% of the population. Similarly, acute oral administration of DH (25, 50 and 100 mg/kg) significantly [F (5, 29) =14.43, P<0.001] increase latency to clonic seizure induced by picrotoxin (Fig. 6A) and tonic seizure [F (4, 24) =7.92, P=0.0006] (Fig. 6B).

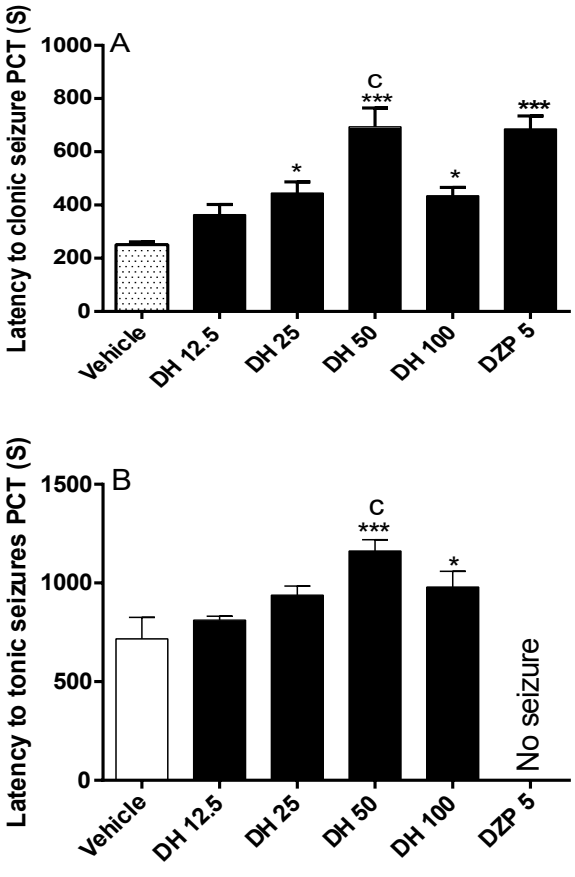


Fig. 6A-B: Effect of DH (12.5, 25, 50 and 100 mg/kg) on picrotoxin induced (A) clonic and (B) tonic seizures in mice. Values are expressed as mean±SEM (n = 6). *P<0.05; ***P<0.001 when compared with vehicle-treated control; cP<0.001 versus DH (12.5 mg/kg) treated. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison tests.

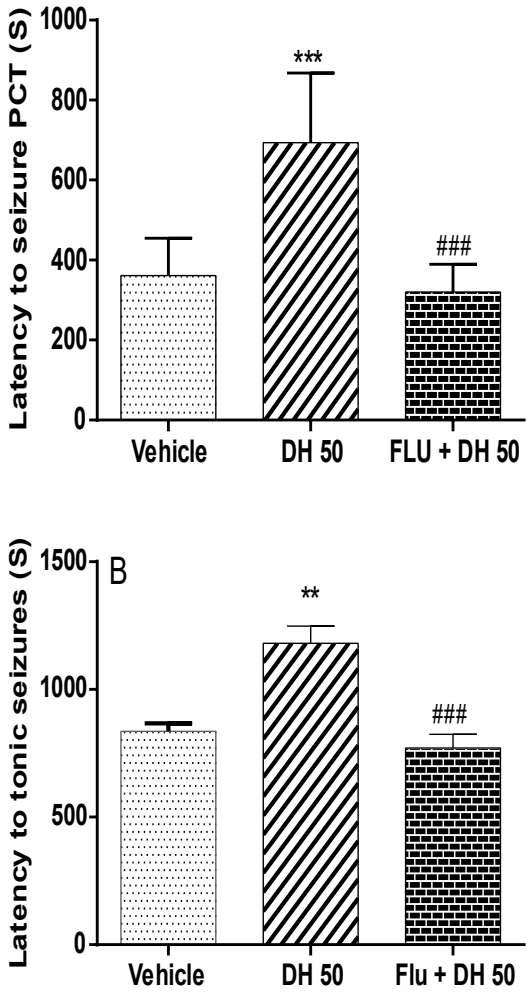


Fig. 7A-B: Effect of pretreatment of mice with flumazenil (3 mg/kg) on anti-epileptic effect of DH (50 mg/kg) in picrotoxin-induced (A) clonic and (B) tonic seizures. Values are expressed as mean±SEM (n = 6). ***P<0.001 when compared with vehicle-treated control; ###P<0.001 versus DH (50 mg/kg) treated. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison tests.

In addition, the increase in latencies to clonic and tonic seizures elicited by DH (50 mg/kg) was reversed by flumazenil [F(2,15)=17.14, P=0.0001] and [F(2,15)=17.58,P=0.0003], respectively (Fig. 7A and B). Tukey post hoc analysis revealed that DH (25, 50 and 100 mg/kg) treatment significantly (P<0.05) increase latency to seizure with peak effect at 50 mg/kg treated group. The effect of DH (50 mg/kg) was significantly prevented by pretreatment of mice with flumazenil.

Effect of DH on Pentylenetetrazol-induced seizure in mice

Intraperitoneal injection of PTZ (90 mg/kg) induced clonic and tonic seizure in vehicle-treated control mice with 100% mortality. Diazepam (benzodiazepine receptor agonist) completely

prevented the clonic and tonic seizures with 100% protection. Similarly, DH produced U-dose response effect, inhibited PTZ induced clonic and tonic seizures. One way ANOVA revealed significant increase in latencies to clonic [F(4,24)=22.87,P<0.001] (Fig. 8A) and tonic [F(4,24)=17.88,P<0.001] seizures (Fig. 8B). Post hoc analysis indicated significant effect of DH on PTZ-induced clonic and tonic seizures with effect at 12.5 mg/kg.

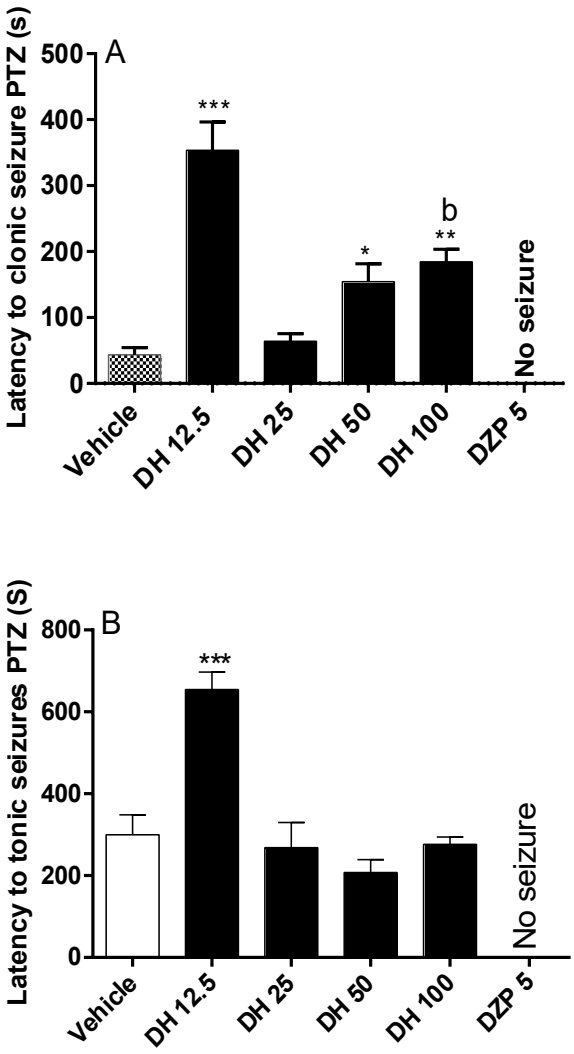


Fig. 8A-B: Effect of DH (12.5, 25, 50 and 100 mg/kg) on pentylenetetrazol-induced (A) clonic and (B) tonic seizures in mice. Values are expressed as mean±SEM (n = 6). *P<0.05; ***P<0.001 when compared with vehicle-treated control; ^{b}P<0.001 versus DH (25 mg/kg) treated. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison tests.

Effect of DH on elevated plus maze test

As shown in fig. 9, one way ANOVA revealed no significant treatment effect of DH (12.5, 25 and 50). However, main effect of diazepam was observed [F(4,20)=11.25, P=0.002]. Post hoc analysis revealed that DH treatment did not affect exploration of open arms by the animal. As expected, diazepam significant increased open arm exploration by the animal.

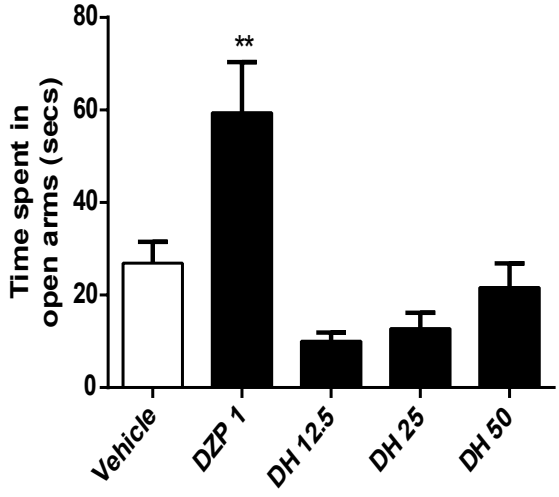
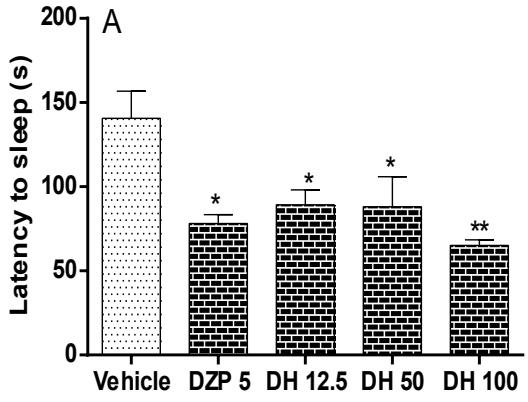


Fig. 9: Effect of DH (12.5, 25 and 50 mg/kg) on elevated plus maze test in mice. Values are expressed as mean±SEM (n = 6). **P<0.01; ***P<0.001 when compared with vehicle-treated control. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison tests

Effect of DH on hexobarbitone-induced hypnosis in mice

Intraperitoneal injection of hexobarbitone (90 mg/kg) induced hypnosis in mice. However, oral administration of diazepam (5 mg/kg) or DH (12.5, 25, 50 and 100 mg/kg) significantly reduced time to sleep. One way ANOVA indicate significant treatment effect [F(4,25)=7.99,P=0.003] (Fig 10A). Furthermore, diazepam treatment also increased the duration of sleep (P<0.05) but not DH treatment. One way ANOVA showed main effect of diazepam treatment [F(4,25)=5.45,P<0.01] (Fig. 10B). Post hoc analysis revealed that diazepam significantly prolonged the duration of sleep in mice.



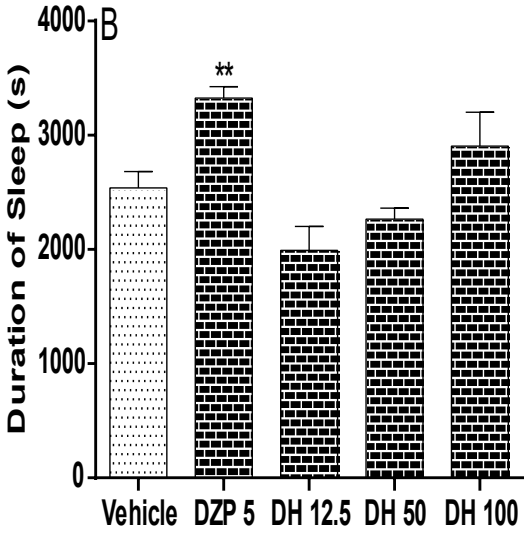


Fig. 10A-B: Effect of DH (12.5, 25 and 50 mg/kg) on hexobarbitone-induced hypnosis in mice. Values are expressed as mean±SEM (n = 6). *P<0.05; **P<0.01 when compared with vehicle-treated control. Statistical level of significance analysis by one way ANOVA followed by Tukey *post hoc* multiple comparison tests

Effect of DH on TST

Acute oral treatment with DH, dose dependently and significantly reduced immobility time in the TST in mice, at the dose of 50 and 100 mg/kg. One way ANOVA revealed significant effect of treatment with DH or fluoxetine (20 mg/kg) [F(5,24)=9.89,P<0.001] (Fig. 11).

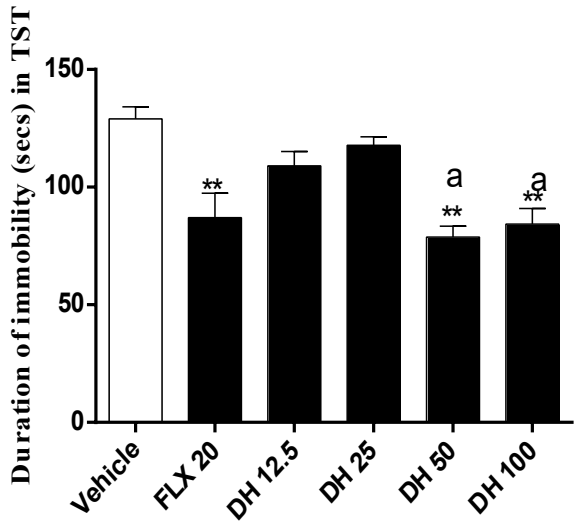


Fig. 11: Effect of DH on immobility time in TST in mice. Values are expressed as mean±SEM (n=6). P<0.01 versus vehicle-treated, control group; p<0.05 versus DH 25 mg/kg treated. Statistical level of significance analysis by one way ANOVA followed by Tukey post hoc multiple comparison tests.

Discussion

The results obtained from this study showed that the hydroethanolic whole plant extract of *D. horizontalis* possesses antinociceptive, anticonvulsant, hypnotic and antidepressant activities without affecting the spontaneous locomotor activity thus ruling out psychostimulant effect. Moreover, the extract is very rich in flavonoid, phenolics, tannins and alkaloid, with potential antioxidant capacity. More importantly wide margin of safety evidenced in the acute toxicity study.

The formalin test is a well-established model of persistent pain characterized by a transient, biphasic pattern of pain behaviour. The early phase is characterized by acute activation of C and Aδ fibres. The late phase involves an inflammatory reaction in peripheral tissue [15], the development of CNS sensitization [16] and additionally involves activation of primary afferent nociceptors [17]. In this study, pretreatment of mice with *D. horizontalis* increases the pain threshold both in the early and late phases of formalin-induced nociception suggesting possible inhibition of centrally and peripherally mediated forms of pain. DH-induced antinociception was reversed by pretreatment of mice with naloxone (non-selective opioid receptor antagonist) indicating involvement of opioidergic mechanism.

D. horizontalis protected about 40% of mice population against picrotoxin-induced seizure with peak effect observed at 50 mg/kg. Similarly, the standard AED, diazepam protected 60% of mice with complete prevention of tonic seizure. Picrotoxin exerts its convulsants effect by blocking the $GABA_A$ receptor-linked chloride ion channel which normally opens to allow increased chloride ion conductance (The $GABA_A$ receptors are ligand-gated chloride-selective ion channels that are activated by GABA). As expected, diazepam (a benzodiazepine) prevented picrotoxin-induced seizures in mice. Benzodiazepines are positive allosteric modulators of the GABA type A receptors ($GABA_A$). Binding of benzodiazepines to this receptor complex promotes binding of GABA, which in turn increases the total conduction of chloride ions across the neuronal cell membrane [18]. This increased chloride ion influx hyperpolarizes the neuron's membrane potential. Hence, the involvement of GABAergic system in the anticonvulsant effect of the extract was investigated, through pretreatment of mice with flumazenil (benzodiazepine receptor antagonist). Interestingly, flumazenil prevented *D. horizontalis*-induced anticonvulsant activity. Thus, showing possible involvement of GABAergic

neurotransmission in the activity of *D. horizontalis*. To corroborate this finding, the pretreatment of mice with pentylenetetrazol delay the occurrence of seizure but did not affect the incidence of seizure. Pentylenetetrazol exerts its convulsant effect by inhibiting the activity of GABA which is implicated in epilepsy [19]. This further supports the hypothesis that *D. horizontalis* may be affecting GABAergic mechanism to produce its anticonvulsant action.

For evaluation of anti-anxiety activity, EPM was used since it provides spontaneous or natural aversive stimuli such as height, unprotected opening and novelty which generate fear and made naïve mice to spend more time in closed arms; therefore any agent that increase open arm exploration are considered anxiolytic. The present study showed that that extract failed to increase the time spent by the animals in open arms of the EPM despite the fact the extract did not affect the locomotor activity in open field test. Interestingly, diazepam exhibited the typical anxiolytic effect in the EPM test [20].

However, DH produced a U-dose response effect on rearing and grooming behaviours, with peak effect at 50 and 25 mg/kg, respectively. DH (100 mg/kg) on the other hand produced significant decrease in rearing and grooming behaviours corroborating its sedative effect. The sedative activity of the extract is evident from the decrease in latency to sleep and increase in the duration of sleep. We also evaluated the antidepressant-like effect of the extract using the tail suspension test. The tail suspension test has become one of the most widely used models for assessing antidepressant-like activity in mice. The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. Various antidepressant medications reverse the immobility and promote the occurrence of escape-related behaviour [21]. In the present study, administration of fluoxetine (standard antidepressant drug) or *D. horizontalis* showed significant decrease in the duration of immobility suggesting possible antidepressant-like effect. The antidepressant-like effect is devoid of any psychostimulant effect of the extract evidence in no significant change in locomotor activity in OFT. Further studies are needed to determine the responsible chemical constituents and possible mechanisms of action.

In conclusion, the findings obtained from this study showed that the hydroethanolic whole plant extract of *D. horizontalis* possesses neuropharmacologic effects such as; antinociceptive (via opioidergic system) anxiolytic, and anticonvulsant (through enhancement of GABAergic neurotransmission) as well as antidepressant activities.

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Awareness, knowledge and participation of National Health Insurance Scheme (NHIS) among nurses in a tertiary healthcare institution in Southwest Nigeria

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Abstract

Background: The National Health Insurance Scheme (NHIS) was implemented as part of health reform programmes aimed at providing effective health care to all Nigerian citizens. Despite the noble objectives and the enormous benefits of the scheme, little is known about how involved the health workers are.

Aim: This study assessed the awareness, knowledge and participation of NHIS among nurses in a tertiary healthcare setting.

Methods: The study population was nurses in the Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu, Southwest Nigeria. Data was collected using a validated, semi-structured self-administered questionnaire. Variables assessed included; sociodemographic data, awareness, knowledge and participation regarding NHIS. Data were analysed using Statistical package for Social Sciences (SPSS) software version 16 then presented as descriptive analysis and cross tabulation of variables.

Results: The mean age of the respondents was 38.3±5.4 years, 98.0% were females and 85.6% were married. There was high level of awareness of the existence of NHIS as evidenced by 57.9% of the respondents having information on NHIS from seminars. Majority (76.5%) had good knowledge of the NHIS (scores of 4-5/5 correct responses) with the remaining 23.3% having fair knowledge (scores of 2-3/5 correct responses). However, only 3.3% of the nurses were currently registered and participating in the scheme. The age of respondents had statistically significant relationship with the level of knowledge ($X^2 = 9.40$; $p<0.05$).

Conclusion: There was high level of awareness on National Health Insurance Scheme among nurses in Olabisi Onabanjo University Teaching Hospital, which did not translate to participation in the scheme.

Keywords: *Awareness, knowledge, National Health Insurance Scheme, Nurses, Nigeria.*

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Résumé

Contexte : Le Projet National d'Assurance Santé (NHIS) a été mis en œuvre dans le cadre des programmes de réforme de la santé visant à fournir des soins de santé efficaces à tous les citoyens nigérians. Malgré les nobles objectifs et les énormes avantages du programme, on sait peu sur comment les agents de santé y sont impliqué.

But : Cette étude a évalué la sensibilisation, les connaissances et la participation du NHIS parmi les infirmiers dans un établissement de soins tertiaires.

Méthodes : La population étudiée était les infirmiers dans l'Hôpital d'Enseignement Universitaire Olabisi Onabanjo (OOUTH), Sagamu, Sud-Ouest du Nigeria. Les données ont été recueillies à l'aide d'un semi-structuré questionnaire validé, auto-administré. Les variables évaluées incluses; les données sociodémographiques, de sensibilisation, de connaissance et de participation concernant NHIS. Les données ont été analysées en utilisant le logiciel Statistiques pour les Sciences Sociales (SPSS), version 16, présenté ensuite comme une analyse descriptive et un tableau croisé des variables.

Résultats : L'âge moyen des répondants était de 38,3 ± 5,4 ans, 98,0% étaient des femmes et 85,6% étaient mariés. Il y avait un grand niveau de sensibilité sur l'existence de l'NHIS comme évident que 57,9% des personnes interrogées avaient des informations sur l'NHIS au cours des séminaires. La majorité (76,5%) avait une bonne connaissance de l'NHIS (scores de 4-5 / 5 réponses correctes), avec les 23,3% restants ayant une connaissance adéquate (scores de 2-3 / 5 réponses correctes). Cependant, seulement 3,3% des infirmières étaient actuellement inscrites et participaient au programme. L'âge des répondants avait une relation statistiquement significative avec le niveau de connaissance ($X^2 = 9,40$; $p <0,05$).

Conclusion : Il y avait un grand niveau de sensibilité sur le Projet National d'Assurance Santé parmi les infirmiers de l'Hôpital d'Enseignement Universitaire Olabisi Onabanjo, qui ne s'est pas traduit à une participation au programme.

Mots - clés: *Sensibilisation, Connaissances, Projet National d'Assurance Santé (NHIS), Infirmiers, Nigeria.*

Introduction

The National Health Insurance Scheme (NHIS) was introduced to ensure that every Nigerian citizen has access to quality healthcare. The scheme was basically designed to facilitate fair financing of healthcare costs through the pooling of resources together within the population [1]. This is against the backdrop that in most developing countries, there is a lack of universal coverage of health care and little equity [2]. In Nigeria, due to numerous factors, access to healthcare is severely limited. The unaffordability of services has been one of the major limitations to healthcare delivery and utilization in the country [3]. Unfortunately, healthcare needs among the population are increasing due to the rising socioeconomic burden [4]. Generally, government expenditure on health in sub-Saharan Africa has been described as "inadequate, insufficient, inequitable and unsustainable" [5]. In Nigeria, despite the significant improvement in government expenditure on health care from 2.8% of the total Gross Domestic Product (GDP) in 1995 to 3.9%in 2013; this still accounts for just 27.6% of total healthcare expenditure [6].The burden of paying for health care has thus become a performance indicator for assessment of national health systems. In addition, the Nigerian health care delivery system is characterized by poor access to health care services for vulnerable members of the society, especially women and children [7].

The Nigerian NHIS was established under Decree No 35 of May 1999 [8]. The scheme was officially launched on the 6th June, 2005, while commencement of services to enrollees started in September 2005. NHIS was designed to provide minimum economic security for employees with regard to unfavourable losses resulting from accidental injury, sickness, old age, unemployment and premature death of family wage earner. In this scheme, the healthcare of the employee is paid for with funds created by pooling together the contributions of employees (5.0% of basic salary) and employers (10.0% of employer's basic salary)[9]. This 15.0% contribution covers health care benefit package for the employee, a spouse and four biological children below the ages of 18 years. As at 2013, only 3.5% of the target population had registered on the scheme [10].

The national health scheme was launched with the major aim of improving accessibility and equity in health care delivery. The scheme suffered a long lag between conception and implementation partly due to the opposition by health care professionals and administrators, owing to misconception and inadequate awareness of the principle of health insurance. The key element in assessing the level of implementation of the scheme in Nigeria is a regular assessment of the awareness and attitude of the health care professionals.

Low awareness has been reported as the major reason for the poor utilization of NHIS [11]. Surprisingly, health care providers who may have the perception that they can take care of themselves medically when ill, do not seem to be exempted from this trend [12]. These healthcare providers (nurses inclusive) influence the quality of care rendered. Nurses form an essential cache of health workers in the delivery of health services. It is therefore imperative that nurses are sufficiently aware and participate actively in this scheme. It is against this background that this study sought to assess the awareness, knowledge and participation of nurses in a tertiary level health care centre of the National Health Insurance Scheme.

Methods

Study design

This study was a descriptive cross-sectional type.

Setting

The study was carried out in Olabisi Onabanjo University Teaching Hospital (OOUTH) situated in Sagamu, Ogun State, South West Nigeria. Ogun state is one of the 36 states in Nigeria. It has three senatorial districts and fifteen local government areas. There are two public (one federal and one state) and one private tertiary health facilities in the state. Olabisi Onabanjo University Teaching Hospital is located in the East senatorial district of Sagamu local government area. The hospital was founded on the 1stof January, 1986. It is the only state tertiary health facility and has a workforce of about 1000 staff, including specialists in various fields. The study was carried out between October and December, 2015. Data was collected using validated, semi-structured self-administered questionnaire which was in line with the objectives of the study. In order to ensure that the tool was valid and appropriate for the data collection, it was pre-tested among twenty nurses (>10.0% of the sample size) at Dideolu Specialist Hospital in Ikenne, another local government area in the state and necessary corrections made.

Participants

This comprised of all the 153 nurses from the twelve different departments in Olabisi Onabanjo University Teaching Hospital (OOUTH) Sagamu. The eligibility

criterion used was that the nurse must be currently employed in OOUTH. All the 153 nurses in the teaching hospital including those on leave, participated in the study. Data was cleaned for inconsistencies in the responses.

Variables
Demographic variables were analysed with the awareness, knowledge scores and practice.

Data sources/measurement
Information were gathered from the nurses who were the respondents.

Bias
The tool was validated before it was used and data was exclusively collected by the investigator.

Statistical methods
The Statistical Package for Social Sciences (SPSS) software version 16 was used for data analysis. The questionnaires were analysed using descriptive statistics to compute percentages and averages.

Sample size determination and sampling technique
The sample size for this study was determined using the formula for estimation of population prevalence [1] and was based on a 95% confidence level.

$$n = \frac{z^2pq}{d^2}$$

where n = calculated sample size; z = confidence limit (z = 1.96 at 95% confidence interval); p = the response rate in a similar study (which was 88.9% [15]); q = 1-p; d = degree of accuracy (= 0.05). Calculated minimum sample size was 151. This was then corrected for population <10,000 to obtain a corrected minimum sample size of 131.

Departmental distribution of respondents
The distribution of nurses in OOUTH according to department is as follows: Medicine (27.0%), Surgery (25.0%), Paediatrics (21.0%), Obstetrics and Gynaecology (20.0%), Community Medicine and Primary Care (4.0%) and Central Sterile Supply (3.0%).

Determination and classification of knowledge scores
A total of five questions were asked which were graded and scored as follows; a total score of 4-5 correct responses was used to indicate good knowledge, 2-3 as fair knowledge and 0-1 as poor knowledge.

Ethical considerations
Ethical approval was obtained from the Ogun State Ministry of Health Scientific Committee. Written informed consent was obtained from the respondents, and confidentiality was maintained.

Results

Demographic characteristics
The demographic characteristic of the respondents is shown in table 1. Majority of the nurses in Olabisi Onabanjo University Teaching Hospital were between 40-49 years of age (45.1%) while the minority were between 60 years and above (9.8%) and a very large percentage of them are female (98.0%). It was found that most of them were married (85.6%) while single and widowed nurses constituted 13.7% and 0.7% respectively. In addition, it was also observed that majority of the nurses have 1-2 children (36.6%) and about 24.2% of the nurses have no children (Table 1).

Table 1: Demographic characteristics of the respondents

	Frequency	Percentage
Age (years)		
20-29	21.0	13.7
30-39	48.0	31.4
40-49	69.0	45.1
50 and above	15.0	9.8
Sex Distribution		
Male	3.0	2.0
Female	150.0	98.0
Marital Status		
Single	21.0	13.7
Married	131.0	85.6
Widowed	1.0	0.7
Religion		
Christianity	77.0	50.0
Islam	73.0	47.4
Others	4.0	2.6
Number of Children		
None	37.0	24.2
1-2	56.0	36.6
3-4	54.0	35.3
> 4	6.0	3.9

Awareness of National Health Insurance Scheme (NHIS)
Nearly all the nurses in Olabisi Onabanjo University Teaching Hospital have heard about NHIS (96.0%) (Figure 1) and 55.6% of them heard about it through Mass/Electronic Media. It was found that 81.0% of the Nurses had correct awareness on at least one of the objectives of NHIS and 65.5% had correct awareness

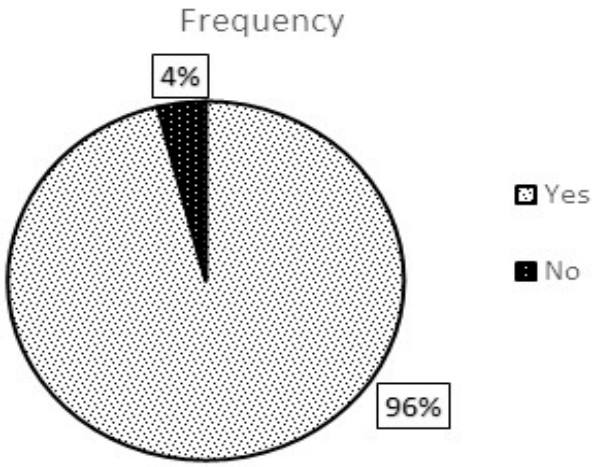

Fig. 1: Have you ever heard about NHIS

on the contribution of NHIS while 26.1% had no awareness on the contribution of NHIS (Table 2).

Table 2: Sources of information and awareness on NHIS

Means of acquisition of awareness about NHIS	Frequency	Percentage
Mass/electronic media	85.0	55.6
Workshop, seminar or conference	56.0	36.6
Workshop and media	12.0	7.8

Majority of the respondents (82.0%) were aware of the benefit package of NHIS, while 18.0% of the nurses did not have any idea of this benefit package.

Knowledge scores of respondents

Majority (76.5%) had good knowledge of the NHIS (scores of 4-5/5 correct responses) with the remaining 23.3% having fair knowledge (scores of 2-3/5 correct responses) (Fig. 2).

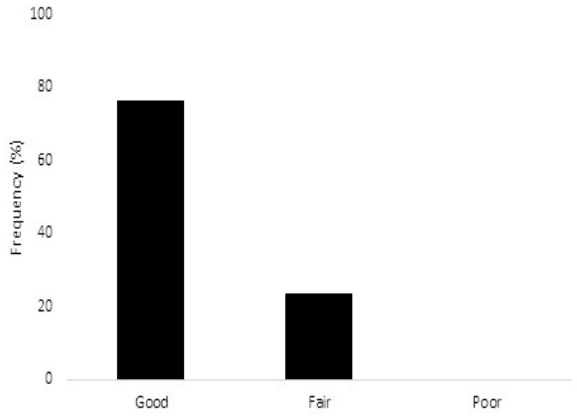

Fig.2: Knowledge scores of respondents

Participation in NHIS

Only 3.3% of the nurses were currently registered and participating in the scheme (Table 3).

Table 3: Participation in NHIS

	Frequency	Percentage
Yes	5.0	3.3
No	148.0	96.7
Total	153.0	100.0

Relationship between age and knowledge

The age of respondents had statistically significant relationship with the level of knowledge ($X^2 = 9.40$; $p<0.05$) (Table 4).

Table 4: Relationship between Age of respondents and knowledge

	knowledge		
Age	Good	Fair	Total
20-29	15.0(12.8%)	6.0(16.7%)	21.0
30-39	40.0(34.2%)	8.0(22.2%)	48.0
40-49	55.0(47.0%)	14.0(38.9%)	69.0
≥50	7.0(6.0%)	8.0(22.2%)	15.0
Total	117.0	36.0	153.0

$X^2 = 9.40$; dl = 3; $p<0.05$

Discussion

Majority of the respondents in this study were married females. This showed similar pattern with a previous study [1] which had 85.0% females and

75.0% of them married. There was also similarity in the modal age group (40-49 years).

The results of this research showed that there was generally a high level of awareness of the existence of NHIS among nurses in OOUTH which is similar to other Nigerian studies on medical health workers that found out that majority of their respondents were aware of the scheme [1,13,14].

Most of the respondents indicated that they got the information on NHIS from seminars. This finding tends to underscore the importance of educational sessions in hospitals where the target audiences are health care professionals. Majority of the respondents had good knowledge of NHIS. They knew at least one objective of the scheme and its benefits which is in line with previous studies [10,15]. A larger percentage of respondents in this study knew the appropriate contribution to be made by employers and employees.

The results showed that majority of the married respondents were not currently on the scheme as their participation was very low. This is contrary to findings from the Jos study [1] where a high level of awareness translated to a high level of participation. This was unexpected as it would be assumed that this group would appreciate the opportunity to ease their health-related financial burden by participating in the scheme.

Limitation of the study

The study was a descriptive type which could only generate and test hypothesis. Further studies are required to broaden the knowledge established in this study.

Conclusion

This study showed that there was a high level of awareness of the National Health Insurance Scheme among the nurses in Olabisi Onabanjo University Teaching Hospital which did not translate to participation in the scheme. More enlightenment and re-sensitization would improve on the gaps observed in this study.

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Urinary iodine levels and thyroid hormones in first trimester pregnant women in Nigeria

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Abstract

*Background/Aim***:** Iodine is an essential component of thyroid hormones required for the normal growth, development and functioning of the body. Its deficiency causes intellectual impairment, reproductive risks such as overt hypothyroidism, infertility, gestational hypertension, increased first trimester abortions and still births. Paradoxically, iodine deficiency disorders (IDD) are among the easiest and cheapest preventable disorders. This study therefore evaluated dietary iodine status and some thyroid parameters in first trimester (10th–12th week) pregnant women

Methods: Forty-two healthy pregnant women, mean age and gestational age of 30±5.22 years and 11.43±0.83 weeks respectively were recruited in consecutive manner for this study after obtaining their informed consents. Urinary iodine levels were analysed in casual urine samples using the ammonium persulphate method as described by Dunn *et al* while thyroid stimulating hormone (TSH) and free thyroxine (fT_4) were measured in serum using Enzyme Immunoassay technique.

Results: 40.5% of the participants had adequate dietary iodine, 47.6% had more than adequate, 9.5% had mild iodine deficiency, while 2.4% had excess dietary iodine. 92.9% of the participants had normal TSH values, 4.8% and 2.4% fell in the hypothyroid and hyperthyroid ranges respectively. 91.4% of the participants had normal values for fT_4, 8.6% had below normal while none had above. Qualitative salt analysis shows iodization of salt in all the salt samples tested.

Conclusion: This study reveals adequate iodine nutrition in the first trimester sub-population, however, screening for overt and subclinical hypothyroidism should still be strongly considered.

Keywords: *Iodine, iodine deficiency disorders, thyroid hormones, first trimester pregnant women*

Résumé

Contexte / But **:** L'iode est un composant essentiel des hormones thyroïdiennes nécessaires à la croissance, au développement et au fonctionnement normaux du corps. Sa carence entraîne une déficience intellectuelle, des risques de reproduction tels que l'hypothyroïdie manifeste, l'infertilité, l'hypertension gestationnelle, l'augmentation des avortements au premier trimestre et des d'enfants mort-nés. Paradoxalement, les troubles dus à la carence en iode (IDD) sont parmi les troubles évitables les plus faciles et moins chers. Cette étude a donc évalué l'état de l'iode alimentaire et certains paramètres thyroïdiens chez les femmes enceintes du premier trimestre (10^{eme} - 12^{eme} semaine).

Méthodes : Quarante-deux femmes enceintes en bonne santé, âge moyen et âge gestationnel de 30 ± 5,22 ans et 11,43 ± 0,83 semaines respectivement ont été recrutés de façon consécutive pour cette étude après avoir obtenu leur consentement informé. Les niveaux d'iode urinaire analysés dans des échantillons d'urine occasionnels en utilisant la méthode du persulfate d'ammonium décrite par Dunn *et al* tandis que la thyréostimuline (TSH) et la thyroxine libre (fT_4) ont été mesurées dans le sérum en utilisant la technique d'essai d'immune enzymatique.

Résultats : 40,5% des participants avaient un apport alimentaire adéquat en iode, 47,6% avaient plus qu'adéquat, 9,5 % avaient une légère carence en iode et 2,4% avaient un excès d'iode alimentaire. 92,9% des participants avaient des valeurs de TSH normales, 4,8% et 2,4% étaient à portée avec l'hypothyroïdie et l'hyperthyroïdie respectivement. 91,4% des participants avaient des valeurs normales pour l'fT_4, 8,6% avaient un taux inférieur à la normale alors qu'aucun n'avait au-delà. L'analyse qualitative des sels a montrée l'iodation du sel dans tous les échantillons de sel testés.

Conclusion: Cette étude révèle une nutrition adéquate en iode dans la sous-population du premier trimestre; cependant, le dépistage de l'hypothyroïdie manifeste et sub-clinique devrait être fortement envisagé.

Mots clés: *Iode, troubles de la carence en iode, hormones thyroïdiennes, femmes enceintes au premier trimestre*

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Introduction

Iodine is required for the synthesis of thyroid hormones, which are important for the development of foetus especially for the maturation of the central nervous system [1]. Adequate dietary iodine intake during pregnancy is essential to prevent adverse maternal and neonatal outcomes. In fact, mild to moderate degrees of iodine deficiency during foetal development have been linked to reduced intellectual function [2, 3]. Insufficient iodine intake as well as its associated thyroid disorders negatively and irreversibly affects the psychoneurotic- intellectual development of the foetus, especially when the deficiency occurs during the first trimester [4]. Furthermore, there is also evidence for an increased risk of adverse obstetrical outcomes such as pre-eclampsia, placental abruption, and negative effects on the offspring which include preterm birth, low-birth weight or even foetal death [5].

Pregnancy is associated with changes in maternal thyroid physiological function which can be viewed globally as a balance between hormone requirements and the availability of iodine [6, 7]. During pregnancy, synthesis of thyroid hormones is increased by up to 50% with an increase in the requirement of thyroxin (T_4) in order to maintain a normal global metabolism in the mother [8-10]. This increase in hormone demands is due to several factors that concur to exert stimulatory effects on the thyroid machinery. These factors include the adjustment of the thyroidal economy during the first trimester to the marked increase in the circulating levels of thyroxin-binding globulin (TBG) in response to increased oestrogen production caused by human chorionic gonadotropin (hCG) [9].

The second factor is thyrotropic action of hCG, also occurring in the first trimester, which tends to transiently elevate free thyroxin (fT_4) levels and decrease serum thyroid stimulating hormone (TSH). A third factor which intervenes later in gestation is related to modification in the peripheral metabolism of the thyroid hormones, particularly at the placenta level [9]. Other factors include the transportation of iodide and T_4 from maternal circulation to the foetus. The foetal thyroid hormone production increases during the second half of gestation [11] and after delivery; iodide is also transported into the breast milk [11].

Another factor is the increased loss of iodide through the kidney [6, 11]. The majority of dietary iodine (90%) is excreted in the urine. Urinary iodine excretion is largely a passive process dependent on glomerular filtration rate (GFR) [12]. Pregnancy results in increased loss of renal iodine. In circumstances of borderline or overt iodine deficiency, pregnancy-related increases in GFR could deplete total body iodine reserves without the capacity for replenishment if dietary intake remains low [13]. This is due to GFR increases within the first month after conception peaking by the end of the first trimester at approximately 40-50% above pre-pregnancy levels in normal pregnancy [14].

A consensus statement from the American Thyroid Association (ATA) highlights the importance of higher dietary iodine intake in pregnancy and higher pregnancy-specific urinary iodine concentration (UIC), to allow for physiological changes during pregnancy [15]. The recommendation stipulates that a median UIC of 150-249μg/L is expected in iodine sufficient pregnancy [15].Thyroid hormone synthesis is impaired when nutritional iodine intake is low. The body reacts to low iodine intake by increasing thyroidal uptake efficiency (up to 4 times) in order to maintain the T_4 output. This favours the preferential production of the more potent triiodothyronine (T_3) over T_4 for efficient utilization of the available iodine. Clinically, euthyroidism is thus maintained but biochemically, the pattern of low T_4, normal or moderately elevated TSH and normal or elevated T_3 is often observed [16, 17].

Previous reports have shown that TSH levels maybe decreased in some women with otherwise healthy thyroid glands in the first trimester. Approximately 10% of women have TSH levels below normal and up to 10% of women have suppressed levels of TSH [18]. However, the lower TSH level in the first trimester mirrors the highest level of hCG and a significant negative correlation between these levels has been reported [19]. The hCG and TSH molecules share similarities as do the hCG and TSH receptors. Consequently, hCG weakly stimulates the thyroid gland. The TSH receptor stimulation depends on the amplitude and duration of the hCG peak which may induce gestational hyperthyroidism that occurs in 2-3% of pregnancies [9]. During pregnancy, TSH level increases and reach the highest value in the third trimester, irrespective of iodine supply [20 - 23]. In mild iodine deficiency, lower levels of fT_4 and free triiodothyronine (fT_3) and higher levels of TSH, thyroglobulin and TBG were observed in the second and third trimester of pregnancy compared with the first trimester [19].Total thyroxin slightly increased in the first trimester and decreased by approximately 30% to low normal values in the second and third trimester [24].

Although Ojule and Osotimehin [25, 26] and Akanji *et al.* [27] have reported maternal iodine and thyroid status in Ibadan, none addressed specifically, the status of first trimester pregnant sub-population. More so, the finding by Pop *et al.*[28] that neurological impairment of the foetus is most critical at 12weeks gestation and that treatment may be ineffective if given after this period, it becomes imperative to understand the interplay between UIC and thyroid hormones status during the first trimester pregnancy

Materials and methods

The study group consists of 42 consecutive apparently healthy pregnant women aged between 20-40years with gestational age of 10-12weeks. They were recruited in a consecutive manner at St-Gregory Specialist Ultrasound Clinic, Ibadan. Pregnant women with previous history of thyroid diseases or any IDD and those on any medication that could affect iodine and thyroid hormones homeostasis were excluded from the study. Short structured questionnaire was administered on each participant to obtain information on education, socio economic background, medication use, dietary intake and awareness about universal salt iodization. Informed consent was obtained from each participant and study approved by the University of Ibadan/University College Hospital Joint Ethics Review Committee.

Three millilitres of venous blood samples were collected from each participant into bottles without anticoagulant. The samples were allowed to clot and retract then centrifuged at 3000rpm for 5min to obtain the serum. Similarly, 10ml of spot urine samples were collected from each participant into clean universal bottles and cooking salt samples being used at home were collected for salt iodine analysis from the participants. The sera and urine samples were stored frozen at 20ºC until the day of analysis.

Determination of Urinary Iodine Concentration

Urinary iodine concentration was determined using ammonium persulphate method as described by Dunn *et al.*[29]. After an initial heating of samples at 100ºC for 60 minutes, iodide was measured by its catalytic action on the reduction of the ceric ion (Ce^{4+}) to the cerrous ion (Ce^{3+}) coupled to the oxidation of arsenite (As^{3+}) to (As^{5+}). The absorbance was measured at a wavelength of 420 nm using a spectrophotometer.

Determination of serum TSH

The Ultra-TSH EIAgen assay was based on the one-step immune-enzymatic sandwich principle, in conjunction with the Biotin-Streptavidin technology using enzyme immunoassay test kit obtained from Adaltis Italia S.P.A., Italy and following the manufacturer's instruction.. In this method, two monoclonal anti-TSHs of high affinity and specificities were used: one was labelled with horseradish peroxidase (HRP) and the other with biotin while the microplate wells were coated with streptavidin. Samples, calibrators and controls were dispensed into the wells, followed by the mixture of the two labelled anti-TSH. During the incubation, the two monoclonal antibodies bind the TSH molecule to two different and specific sites and at the same time, the streptavidin immobilizes the forming immunological sandwich to the wells through the binding to the biotin moiety of the biotinilated antibody. After washing to eliminate the un-reacted species, the mixture of chromogen/substrate was added. The reaction was then blocked by adding the stop solution and the developed colour was measured photometrically at wavelength of 450 nm. The intensity of the colour was directly proportional, within the working range of the assay, to the concentration of TSH in the sample. The concentration of TSH in a participant's sample or control was then determined by the interpolation on the calibration curve.

Determination of serum fT4

The fT_4 was measured using enzyme immunoassay test kit obtained from Adaltis Italia S.P.A., Italy and following the manufacturer's instruction. The method is based on a solid phase competitive enzyme immunoassay. Serum samples, standards and thyroxin-enzyme conjugate working reagent were added to wells coated with monoclonal T_4 antibody, fT_4 in the specimen and the T_4 labelled conjugate compete for available binding sites on the antibody. After washing to remove unbound T_4 conjugate, the substrate solution was added resulting in a colour development. The colour development was stopped with the addition of the stop solution and absorbance read spectrophotometrically at 450 nm. The intensity of the colour formed was proportional to the amount of enzyme present and was inversely related to the amount of un-labelled fT_4 in the sample. The concentrations of fT_4 in unknown samples were quantified by reference to a series of fT_4 standards assayed in the same way.

Determination of salt iodine levels

The rapid test kit is a stabilized starch-based solution from UNICEF for the qualitative determination of iodine in salt. In this method, iodine (if present in

salt) react with starch in the solution to give a blue/purple colouration.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences, version 20.0. Values were reported as mean ± standard deviation (SD), median and range. Pearson's correlation was used to establish the relationship between the variables and Chi-square test was used to estimate the statistical difference in the level of awareness of Universal Salt Iodisation (USI) and the levels of education.

Results

The mean, standard deviation (SD), median and range of age, gestational age, serum TSH, fT4 and urinary iodine (UI) are shown in table (1). Table 2 shows the relationship between the level of education and knowledge of universal salt iodization. In this table, 32 women had tertiary education while 9 women and 1 woman had secondary and primary education respectively. Despite the increasing awareness about USI, only 12 women (28.57%) were aware of USI regardless of the educational background. In table [3], the mean, percentage and frequency distribution of the study group sub-classification according to the reference values for the measured parameters are depicted. Using WHO

Table 1: Characteristics of the studied pregnant women

Characteristics	Mean (SD)	Median	Range
Age (years)	30.00 (5.22)	30.00	20.00 – 40.00
Gestational age (weeks)	11.43 (0.83)	12.00	10.00 – 12.00
TSH (mIU/L)	1.83 (1.55)	1.40	0.20 – 8.00
fT4 (pmol/L)	11.35 (0.24)	10.32	6.45 – 20.64
UI excretion (µg/dL)	191.20 (63.00)	197.50	80.00 – 300.00

Table 2: Educational Status and Knowledge about Universal Salt Iodisation (USI)

Level of Education	Knowledge of USI n (%)	No knowledge of USI n (%)	Total n (%)
Primary	0 (0.00)	1 (2.38)	1 (2.38)
Secondary	1 (2.38)	8 (19.05)	9 (21.43)
Tertiary	11 (26.19)	21 (50.00)	32 (76.19)
Total	12 (28.57)	30 (71.43)	42 (100)

X^2 = 2.273; p-value = 0.321 and df = 2; n = number; % = percentage

Table 3: Sub-classification of the pregnant women based on reference interval

	Classification	Reference Interval	n (%)	mean (SD)
UI (µg/L)	Mild deficiency	50 – 99	4 (9.5)	88.75 (6.30)
	Adequate*	100 – 199	17 (40.5)	148.75 (29.50)
	More than adequate	200 – 299	20 (47.6)	242.20 (24.90)
	Excess	≥300	1 (2.4)	300.00 (0.00)
TSH (mIU/L)	Hyperthyroidism	<0.3	1 (2.4)	0.20 (0.00)
	Euthyroidism**	0.3 – 4.5	39 (92.9)	1.60 (0.98)
	Hypothyroidism	>4.5	2 (4.8)	7.10 (0.27)
fT_4 (pmol/L)	Low	<9.0	3 (8.6)	6.84 (0.06)
	Normal**	9.0 – 25.7	32 (91.4)	11.87 (0.23)
	High	>25.7	0 (0.0)	0.00 (0.00)

**Reference interval for the general population [30] **Reference interval for first trimester pregnancy [31]*

conventional reference interval, the sub-classification of the women revealed that none of the women had severe iodine deficiency. Thirty-nine women (92.9%) had euthyroidism while 2 women (4.8%) and 1 woman (2.4%) had hypothyroidism and hyperthyroidism respectively. An inverse relationship was observed when fT4 was correlated with TSH and UI. The correlation between fT4 and TSH was significant (Table 4).

Table 4: Pearson Correlation between fT4, TSH and UI

	FT_4 (ng/dL) r-value	p-value
TSH (mIU/L)	0.42*	0.012
UI (µg/L)	0.18	0.300

*significant at $p<0.05$

Discussion

The validity of UIC as an indicator of dietary intake is dependent on the relationship between dietary iodine intake and urinary excretion [13]. Using the conventional World Health Organization (WHO) reference criteria, our study showed that as much as 40.5% of the participants had adequate iodine intake between 100 - 199 µg/L [30]. This could be a reflection of the aggressive campaign on salt iodization by various Health Institutions in Nigeria. Likewise, it could be a pseudo-UIC since the conventional WHO reference criteria did not account for the commonly observed increased ioduria which occurs during early pregnancy as a result of increased GFR which could hitherto overestimate iodine nutrition. Similar concern was raised by Stilwell *et al.* [13].

Similarly, our findings showed that 47.6% of the participants had more than adequate iodine nutrition while 2.4% had excess iodine intake. Thus, implying that about 50% of the studied participants had iodine nutritional value greater than 200µg/L. This observation requires attention in order to prevent iodine induced hyperthyroidism as experienced in Tasmania [32], Zimbabwe [33] and Zaire [34].Therefore, imminent solutions of regular assessment of nutritional iodine and effective quality control of salt iodization are required.

Furthermore, the observed median urinary iodine value for the entire study population is in line with the WHO criteria for median urinary iodine concentration which should be greater than 100 µg/L in 'iodine sufficient' population [30]. Although, National Health and Nutrition Studies in the United States between 1971 and 2002 put forth inconsistent reports on UIC median levels. Their reports showed that median urinary iodine excretion increased in pregnancy when compared with non-pregnant women of reproductive age[35-37].Therefore, the absence of clearly defined reference intervals for iodine excretion in pregnancy underscores the urgent need for appropriate and specific UIC ranges in pregnancy.

Surprisingly, educational status did not affect the UIC. The result shows that 50% of the studied women with adequate UI Chad no knowledge of USI despite having attended a tertiary institution. This is a good success for USI campaign which has been in place in Nigeria and strongly supported by legislation since 1995. The result implies that majority of Nigerians consume table salt without even considering whether they are fortified or not. Thus signifying that eradication of IDDs is achievable if 100% of USI can be implemented. This observation further shows that government does not need to fund awareness campaign any longer but instead, divert the funds to USI implementation which, as shown in this study, could be more cost effective.

Equally of interest, in this study, is the finding that 92.9% and 91.4% of the studied women had normal levels of TSH and fT_4 respectively showing that thyroid homeostasis was maintained despite the various physiological changes in pregnancy. This could be due to adequate dietary iodine intake found in the pregnant women as revealed by the UIC. Surprisingly, one woman had normal value of fT_4 (1.1ng/dL) with a mildly depressed TSH (0.2mIU/L) in the presence of normal UIC (160µg/L). This might be due to transient fall in serum TSH near the end of the first trimester in normal pregnancy in association with elevated circulating hCG [38]. The hCG weakly stimulates the thyroid gland thus inducing gestational hyperthyroidism which occurs in 2-3% of the pregnancies [9]. The overt hypothyroidism observed in 2 women despite adequate urinary iodine excretion, could be due to underlying factors such as antibodies to thyroid tissues and goitrogenic factors which were not measured in this study. The significant negative correlation observed between fT_4 and TSH is in accordance with previous reports [39, 40]. This is due to the usual negative feedback mechanism.

Conclusion

Iodine deficiency poses reproductive risks such as hypothyroidism, infertility, gestational hypertension, increased first trimester abortions and still births. Although the studied pregnant women in Ibadan had adequate dietary iodine intake, screening for overt

as well as subclinical hypothyroidism during pregnancy should still, be strongly considered.

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Cytotoxic, membrane stabilizing and anti-arthritic effects of methanol extract of *Ocimum gratissimum* Linn. leaf.

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Abstract

*Background***:** *Ocimum gratissimum* Linn. leaves have been shown to be useful in arthritis as well as related inflammatory oxidative conditions. The study investigated the cytotoxicity, membrane stabilizing and anti-arthritic effects of sequential methanol extract of the leaves.

Methods: Powdered leaves were sequentially extracted with n-hexane, chloroform and methanol to obtain a resultant methanol extract (MEO*g*). The extract was tested for cytotoxicity using Chinese harmster ovary cell (CHO-k1) and murine macrophages (RAW 264.7) cell lines. Membrane stabilizing effect was tested by heat-induced erythrocytes haemolysis assay. Antiarthritic properties were tested by egg-albumin, formalin-induced inflammation and carrageenan/kaolin induced monoarthritis models in rats. Thereafter, biomarkers of oxidative stress were evaluated using standard biochemical assays.

*Results***:** The extract did not reduce viability of CHO-k1 and RAW 264.7 cells. MEOg showed significant (p <0.05) membrane stabilizing properties. MEOg at 100, 200, and 400 mg/kg dose-dependently reduced egg albumin-induced paw oedema by 24.0, 32.2, and 37.8%, respectively. Similarly, MEOg significantly (p < 0.05) decreased paw thickness compared to control in formalin-induced arthritis in rats. Furthermore, MEOg dose-dependently showed anti-inflammatory activity which was evident with decrease in paw and knee swelling, and decreased inflammatory pain in carrageenan/kaolin-induced monoarthritis in rats. The treatment decreased plasma TBARS and NO, and increased GSH and SOD levels.

Conclusion: These findings support the ethno-pharmacological use of *Ocimum gratissimum* leaves for ameliorating inflammation and pain of arthritis.

Keywords: *Cytotoxicity, Anti-arthritic, Ocimum gratissimum, carrageenan, kaolin*

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Résumé

Contexte **:** Il a été montré que les feuilles d'*ocimum gratissimum* Linn. sont utiles dans l'arthrite ainsi que dans les conditions oxydatives inflammatoires associées. L'étude a étudié la propriété cytotoxique, la stabilisation membranaire et les effets antiarthritiques de l'extrait séquentiel de méthanol des feuilles.

Méthodes: Les feuilles en poudre ont été extraites séquentiellement avec du n-hexane, du chloroforme et du méthanol pour obtenir un extrait méthanoïque résultant (MEO*g*). L'extrait a été testé pour sa propriété cytotoxique en utilisant des lignées cellulaires d'ovaire harmonique chinois (CHO-k1) et de macrophages murins (RAW 264.7). L'effet de stabilisation de la membrane a été testé par un test d'hémolyse des érythrocytes induit par la chaleur. Les propriétés antiarthritiques ont été testées par des modèles d'albumine d'œuf, de l'inflammation induite par le formol et de mono-arthrite induite par la carraghénane/kaolin chez les rats. Par la suite, les bio-marqueurs du stress oxydatif ont été évalués en utilisant des dosages biochimiques standards.

Résultats **:** L'extrait n'a pas réduit la viabilité des cellules CHO-k1 et RAW 264.7. MEOg a montré des propriétés de stabilisation membranaires significatives (p <0,05). La MEOg à 100, 200 et 400 mg/kg réduit de façon dose-dépendante de la patte d'œdème induit par l'albumine de l'œuf de 24,0, 32,2 et 37,8%, respectivement. De même, MEOg significativement (p < 0,05) réduit l'épaisseur de la patte par rapport au contrôle dans l'arthrite induite par le formol chez les rats. En outre, MEOg a dose-dépendamment montré une activité anti-inflammatoire qui était évidente avec la diminution du gonflement des pattes et des genoux, et une diminution de la douleur inflammatoire dans la mono-arthrite induite par la carraghénane /kaolin chez les rats. Le traitement a diminué les concentrations plasmatiques de TBARS et de NO et a augmenté les niveaux de GSH et de SOD.

Conclusion: Ces résultats soutiennent l'utilisation ethno-pharmacologique des feuilles d'*ocimum gratissimum* pour améliorer l'inflammation et la douleur de l'arthrite.

Mot-clé s: *Propriété cytotoxique, Antiarthritique, Ocimum gratissimum, carraghénane, kaolin*

Introduction

Age-related diseases of bone, joint, and muscle are on the increase globally, affecting the health of millions of people across the world. Osteoarthritis, the most common form of arthritis, also known as degenerative joint disease is a major cause of pain and disability in the elderly [1]. The incidence and magnitude of osteoarthritis and associated risk are on the increase in Nigeria [2, 3]. It is believed that the growing burden of arthritic, rheumatic and musculoskeletal diseases will place a greater socioeconomic burden on health systems [1]. The existing treatments for arthritis usually focus on reducing inflammation and accelerating the repair process with an ultimate goal of preventing structural joint damage and loss of function [4]. Drugs like the non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are used to suppress the symptoms while disease-modifying anti-rheumatic drugs (DMARDs) and the newer biological response modifiers are used to halt the underlying immune process. Chronic diseases are multigenic and thus multitargeted approach is needed. The existing drugs have not been entirely successful in curing chronic inflammatory disorders. Most of these drugs are enormously expensive and are associated with serious side effects and morbidity [5, 6]. Thus the need to seek for alternatives from medicinal plants.

Ocimum gratissimum Linn. has been reported in several ethnopharmacological surveys as a plant widely used and readily accessible to the communities. It is a shrub commonly found around village huts and gardens. The plant is often employed for different ailments and menus in Africa and Asia [7]. *O. gratissimum* is commonly referred to as the wild basil, African basil, tree basil, East Indian basil and clove basil. In Nigeria, It is known as Efinrin nla in Yoruba, Ebavbokho in Bini, Dadoya in Hausa, and Nchanwu by Igbo speaking people of Nigeria [8].

The flowers and the leaves of *O. gratissimum* are rich in essential oils thus useful in the preparation of teas and infusion [9]. The leaves are prepared in several ways as infusion, decoction, maceration in water or palm wine or in other alcohol beverages. Sometimes the leaves can also be rubbed on body parts or used as incense to bring relief to inflammatory pain such as in rheumatoid arthritis [10]. Previous report had shown that the aqueous alcohol extract from the leaves caused significant reduction in carrageenan-induced rat paw oedema [11]. Also sequential extraction of *O. gratissimum* leaves with hexane, chloroform and methanol showed that the methanol extract is rich in phenolic content. Extracts rich in polyphenols are known to be safe with multipronged properties. As part of our continuing investigation of *O. gratissimum* leaf, this paper reports the cytotoxic, membrane stabilizing and antiarthritic effects in non – immunologically-induced arthritis models.

Methods

Chemicals and reagents

Indomethacin, doxorubicin, caffeic acid, Ellman's reagent, thiobarbituric acid, and carrageenan, were products of Sigma-Aldrich Co. (Germany). Formaldehyde, n-hexane, chloroform, methanol were analytical grade reagents.

Experimental animals

Female Wistar rats (150 – 180g) were obtained from the College of Medicine Central Animal House, University of Ibadan. The animals were, acclimatized in the laboratory for one week at room temperature of 28 ± 2°C, relative humidity of 60 - 70%, and 12:12 light: dark cycle. They were allowed free access to water and fed with standard commercial rat chow pellets (Ladokun Feeds Ltd, Ibadan, Nigeria). All experiments followed approved protocols with strict compliance to the "Principle of Laboratory Animal Care" (NIH Publication No. 85-23) [12] and ethical guidelines for investigation of experimental pain in conscious animals by Zimmermann [13]. Ethical approval was obtained from the University of Ibadan Animal Ethics Committee (UI-ACUREC/app/2015/026).

Plant material collection and extraction

The plant was identified and authenticated by a curator at the herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan by comparison with existing herbarium specimen. The voucher specimen of collected plant sample was deposited at FRIN herbarium and given specimen number F.H.I 110191. A sequential extraction of dried powdered leaves (800 g) of *O. gratissimum* in solvents of increasing polarity, n-Hexane, Chloroform and 80% Methanol, was carried out for 48 hours in each case. The extracts were filtered and concentrated in a Rotary evaporator (BuchiRotavapor R-124) under reduced pressure. The methanol extract (MEO*g* yield= 5.42%) was stored in a glass chamber desiccator containing activated silica gel (BDH, England). The extract was freshly prepared daily by dissolving in 1% tween 80.

In vitro cytotoxicity testing of MEOg

Murine macrophage cell line RAW 264.7 (ATCC TIB-71) and Chinese harmster ovary (CHO-k1) were

obtained from the Cell Bank of Rio de Janeiro, Brazil. Cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL), and maintained at 37°C in a humidified incubator of 5% CO_2.

Cell viability was measured with Alamar blue [14] according to manufacturer's instruction. Briefly, RAW 264.7 cells or CHO-k1 cells (2 x 10^4 cells/well in 96-well culture plate) were incubated at 37°C (5% CO_2) overnight. The cells were treated with/without MEO*g* (3.125 -200 µg/mL) and Doxorubucin (10 µM) as positive control. After incubation for 24h, the treatments were removed from the plates and then 200 µL of 10% Alamar blue solution was added. Twenty hours after, the absorbance was read at 540 nm (oxidized state) and 620 nm (reduced state) with Mulitscan® EX ELISA plate reader (Thermo Scientific, USA). Cell viability was expressed as a percentage of untreated control cells and the median inhibitory concentration (IC_{50}) was analyzed with a non-linear regression coefficient.

Heat- induced rat erythrocytes haemolysis

The effect of MEO*g* on haemolysis of rat red blood cell (RBC) induced by heat was evaluated according to the method of Brown *et al.* [15] as modified by Anosike *et al*. [16]. The MEO*g* and caffeic acid were dissolved in isotonic phosphate buffer solution (154 mMNaCl in 10 mM Sodium phosphate buffer, pH 7.4). The assay mixture consisted of 5mL graded concentration of extract (125, 250 and 500 µg/mL); and 0.5 ml of 10% RBC suspension. The control was prepared as above without the extract, while erythrocytes were omitted from the extracts control tubes. Each sample was prepared and arranged in quadruplicate sets (4 sets per concentration). A pair of each mixture was incubated at 54°C for 20 min in a regulated water bath. The other pair was maintained at 10°C in a refrigerator for 20 min. Afterwards, the mixture were centrifuged at 4000 rpm for 3 min and the haemoglobin contents of the supernatant were estimated at 540 nm using a UV/VIS Spectrophotometer (INESA). The percentage inhibition of haemolysis by the extract was calculated as follows:

$$\% \text{ Inhibition of Haemolysis} = 100 - \frac{OD1 - OD2}{OD3 - OD4} \times 100$$

Where: OD1 = absorbance of test sample heated; OD2 = absorbance of test sample refrigerated; OD3 = absorbance of control sample heated; OD4 = absorbance of control sample refrigerated.

Egg albumin –induced acute inflammation in rat paw

The Phlogistic agent employed in this model to induce acute inflammation was fresh egg albumin [17]. Rats were divided into five groups (n = 5) and pretreated with vehicle (1% tween 80), MEO*g* (100, 200 and 400 mg/kg) and indomethacin (10mg/kg). All treatments were administered orally 1 h before a sub plantar injection of 0.1 ml of raw egg albumin to the left hind paw. Oedema formation was taken as increase in paw circumference measured by wrapping a white cotton thread around the injected paw. Paw circumference was measured every 30 min for a total duration of 120 min after egg albumin injection.

The area under the curve for increase in paw circumference against time was computed from 0 to120 min using GraphPad. The percentage of inhibition of total oedema formation was calculated with the formula below:

% inhibition = [(AUC of control – AUC of treatment)/AUC of control] x 100%.

Formalin -induced arthritis in rats

In this experiment, female Wistar rats were divided into five groups of five rats each. Group 1 received vehicle (1% tween 80; 10 ml/kg); groups 2-4 received MEO*g* (100, 200 and 400 mg/kg); group 5 received indomethacin (2 mg/kg). All treatments were administered orally for seven consecutive days. On day 1 and 3, animals were injected with 0.02 ml of 3.75% formalin (BDH England) under a subaponeurotic of the left hind paw (Plantapedis). The thickness of the left hind paws were measured using an electronic digital caliper (Mytutoyo, Japan) and recorded on day 0, 3 and 7. The ability of the extract to suppress inflammation was expressed as percentage inhibition of paw oedema which was calculated as follows:

Percentage of inhibition (%) = (C – T)/C X 100, where C= increase in paw thickness of control group of rats and, T = increase in paw thickness of treated group of rats.

The rats were sacrificed after the last measurement on day 7; the digits were removed, cleaned, weighed and homogenized in Tris-KCl buffer at 4 °C. The homogenate was used to assay formalondialdehyde as a measure of lipid peroxidation in the paw tissues.

Carrageenan/kaolin – induced monoarthritis

Monoarthritis of the knee was induced by injection of mixture of 3% carrageenan (Sigma, type IV) and 3% kaolin into the right knee joint cavity according to Sluka and Westlund, [18] with slight modification.

Rats were divided into five groups and pretreated with vehicle (1% tween 80; 10 ml/kg), MEO*g* (100, 200, and 400 mg/kg) and indomethacin (5 mg/kg) for consecutive three days. One hour after the last treatment, rats were lightly anaesthetized with ether, then 0.1 ml of a mix of 3% carrageenan and 3% kaolin were injected into the right knee joint cavity and the leg was flexed and extended for about 2 min. The following parameters were then assessed:

Paw volume and knee diameter

Inflammation and swelling of the paw and knee were measured at 1, 3 and 5th hour after induction of inflammation. Baseline paw volume was measured with the Ugo Basileplethysmometer (7140 model, Italy) before intra-articular injection of CK and after at 1, 3, and 5th hour. The swelling caused by intra-articular CK injection was also assessed by measuring the knee circumference with the aid of electronic digital vernier caliper (Mytutoyo, Japan).

Mechanical paw withdrawal threshold.

Pain latencies were measured using Randall-Selitto Analgesymeter (Ugobasile model 7200). Mechanical hyperalgesia withdrawal threshold was measured in rats at the fifth hour after CK injection. The injected and/or non-injected rat paw was placed on a plinth under a cone-shaped pusher of the instrument and increased pressure applied to the middle dorsum of the paw. Mechanical paw withdrawal thresholds were calculated by subtracting the mean right from the left paw threshold.

Locomotory activity

Reduction in locomotory activity as an index of chronic pain was determined in CK injected rats [19]. Rats induced with monoarthritis following injection of 3% carrageenan/kaolin mixture were placed individually in the UgoBasile activity meter cage. The apparatus consists of an animal cage (with a transparent cover) and an electronic unit. The horizontal activity detector relies on horizontal sensors, designed for the assessment of the ambulatory activities. Each rat was placed in the centre of the cage; the movements it makes inside the cage can interrupt one or more infrared beam/s. The beam interruptions were counted and recorded by the electronic unit. The locomotory activity was quantified for 5 min.

Biochemical assessment

Twenty four hours after the injection of Carrageenan/kaolin into the knee joint cavity, all animals were subjected to deep ether anaesthesia. Blood was collected by cardiac puncture into heparinized bottles. The plasma was separated by centrifugation at 3000 rpm below 30^{o}C for 15 min and used for the biochemical assessment. Non-enzymic antioxidant marker, reduced gluthathione (GSH) levels in plasma was determined using the Ellman's reagent as described by Sin *et al.,*[20]. The index of lipid peroxidation measured as thethiobarbituric reacting substance (TBARS) using a method described by Nagababu [21]. Enzymatic activity of superoxide dismutase in plasma was assayed according to the method described by Misra and Fridovich [22], and total protein content by the biuret method [23].

Histopathological analysis of ankle joints

The rats were euthanized and the ankle joints were separated from the hind paw, weighed and fixed in 10% buffered formalin for 24 h. After decalcification in 5% formic acid, the tissues were dissected longitudinally, embedded in paraffin, and then cut into 4µm section. Tissue sections were stained with hematoxylin and eosin (H & E) and examined with light microscope.

Statistical analysis

Data were expressed as Mean ± SEM (standard error of the mean), and statistical significance was set at $P< 0.05$. Data were analyzed using one-way analysis of variance (ANOVA), significant main effects were further analyzed by Bonferroni's *post hoc* test for multiple comparison of treatment groups. Graphs and statistical analysis were done using Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism® software version 5.01 (GraphPad Software, Inc. La Jolla, CA 92037 USA).

Results

In vitro cytotoxicity in RAW 264.7 and CHO-K1 cells

The cytotoxic effect of MEO*g* in RAW 264.7 cells and CHO-k1 cells were evaluated by the Alamar blue assay at 24 h of exposure. The results showed that MEOg (3.125 – 200 µg/mL) did not reduce cell viability in RAW 264.7 (Fig 1A) and CHO-k1 (Fig 1B) cells during 24 h exposure. The cell viability produced by MEOg at all concentrations was similar to that of negative control ($p > 0.05$) but significantly higher ($p < 0.05$) than that of Doxorubicin (10 µM). The MEOg showed IC_{50} values > 200 µg/mL.

Membrane stabilizing effect of MEOg in heat-induced haemolysis of rat erythrocytes

MEO*g* (125, 250 and 500 µg/mL) demonstrated *in vitro*-anti-inflammatory activity by its ability to inhibit heat-induced rat erythrocytes haemolysis

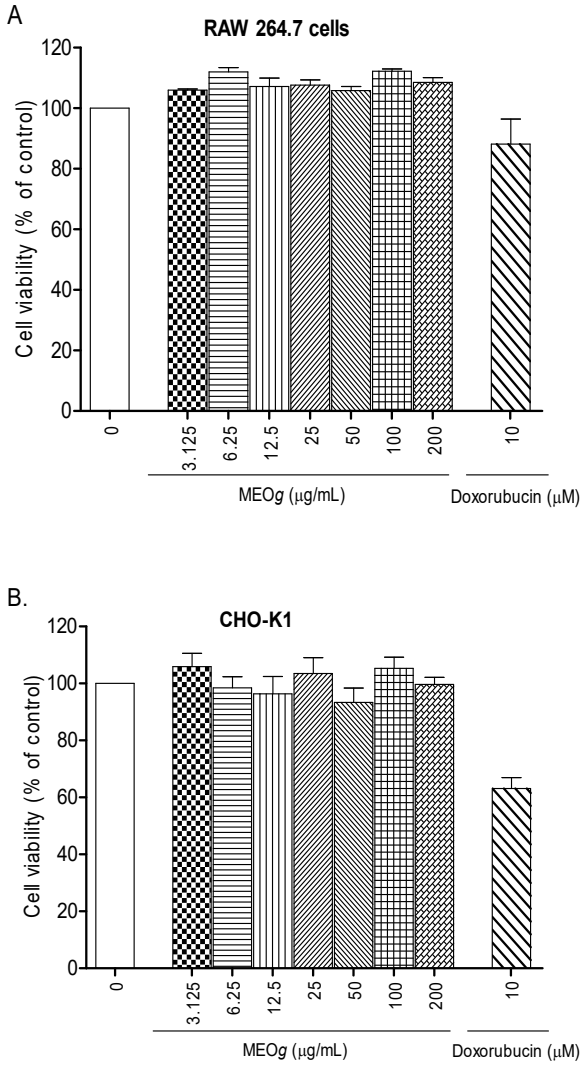


Fig 1: Effects of MEO*g* on cell viability in (A) RAW 264.7 cells and (B) CHO-K1

µg/mL) also offered protection against heat-induced heamolysis at 13.9, 14.5 and 64.8 % respectively.

In vivo anti-inflammatory activity of MEOg in egg albumin-induced paw oedema in rats.

The results obtained from this experiment are shown in fig 2. MEOg suppressed rat paw oedema formation when compared with the untreated control group (Fig 2A). Pretreatment with MEOg (100, 200 and 400 mg/kg) produced statistically significant (p<0.05) and dose dependent inhibition of oedema response as calculated from area under the curve (AUC) (Fig 2B)

Anti-inflammatory effects of MEOg in formalin-induced chronic inflammation in rats.

The results in table [2] showed that treatment of rats induced with chronic inflammation using formalin with MEO*g* (100, 200 and 400 mg/kg) significantly (p<0.05) reduced paw swelling when compared to the control animals. Indomethacin (2 mg/kg) produced a higher inhibition of paw oedema (25.7%, 41.3%) as compared to the extract at 100 mg/kg (6.78%, 15.07%); 200 mg/kg (11.38%, 17.39%) and 400 mg/kg (19.37%, 18.84%) on days 3 and 7 respectively. The suppressive effect of indomethacin was more pronounced on the seventh day than on the third day, while the extract (400 mg/kg) remained reduced.

Anti-lipoperoxidation effects in formalin-induced paw tissues.

Treatment with MEOg significantly (p <0.001) reduced formalin-induced lipid peroxidation in rat

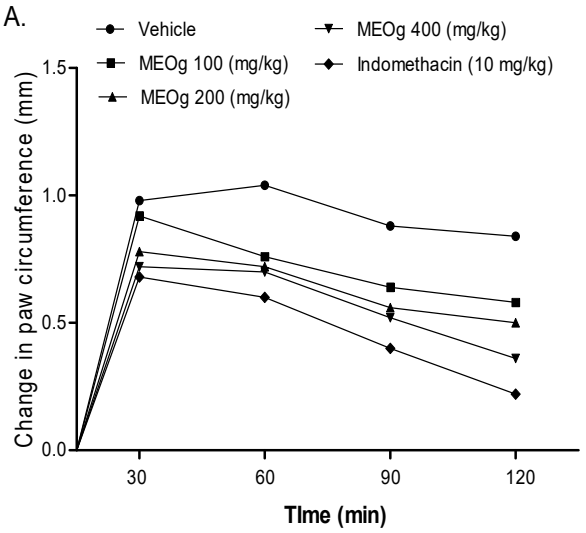
Table 1: Effect of ME*Og* on heat induced haemolysis of Red Blood Cell

Treatment	Conc. (µg/mL)	Mean absorbance ± SEM Heated Solution	Unheated solution	Percentage inhibition of haemolysis
Control	-	0.853 ± 0.005	0.016 ± 0.002	-
MEOg	125	0.601 ± 0.004*	0.153 ± 0.003	46.5
MEOg	250	0.578 ± 0.008*	0.122 ± 0.003	45.5
MEOg	500	0.344 ± 0.014*	0.089 ± 0.002	69.5
Caffeic acid	125	0.742 ± 0.006*	0.021 ± 0.005	13.9
Caffeic acid	250	0.784 ± 0.005*	0.068 ± 0.003	14.5
Caffeic acid	500	0.347 ± 0.006*	0.044 ± 0.001	64.8

* Each value is statistically significant at p<0.05, compared with control using the one –way ANOVA

(Table 1). The percentage inhibition of heat-induced haemolysis (46.5, 45.5 and 69.5%) obtained for MEO*g* (125, 250 and 500 µg/mL) was statistically significant (p< 0.05). Caffeic acid (125, 250 and 500

paw tissues in a dose-dependent manner (Fig 3). The mean values of TBARS are 32.4 ± 5.4(vehicle), 11.9 ± 2.3 (100 mg/kg), 7.0 ± 2.6 (200 mg/kg), 5.9 ± 1.2 (400 mg/kg) and 11.3 ± 2.7(indomethacin 2 mg/kg).



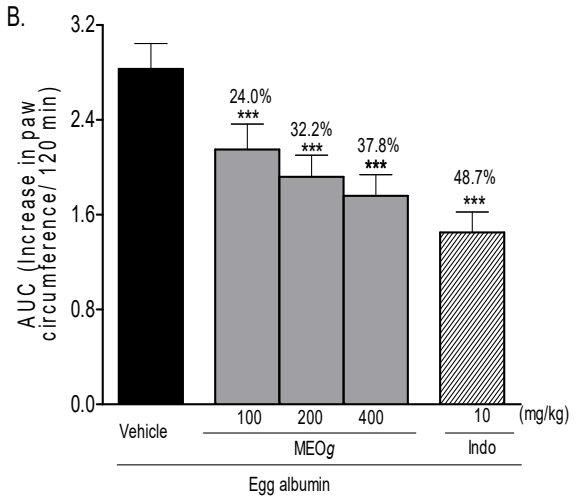


Fig 2: Effect of MEO*g* on egg albumin-induced paw oedema in rats (A) Change in paw circumference, (B) AUC. Data represent Mean ± SEM of five rats. *** $p < 0.001$ by 1-way ANOVA followed by Bonferroni's post hoc test compared to control group.

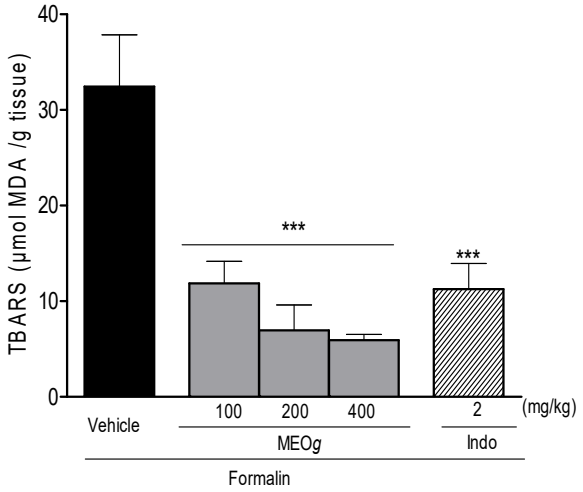


Fig 3: Anti-lipoperoxidative Effect of MEO*g* on formalin-induced chronic inflammation in rat paw. Data represent Mean ± SEM of five rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by 1-way ANOVA followed by Bonferroni's post hoc test compared to vehicle group. TBARS- Thiobarbituric Acid Reacting Substances

Effect of MEOg on carrageenan/kaolin induced monoarthritis in rat

MEOg reduces paw volume and joint diameter in CK-induced monoarthritic rats

Intra-articular injection of rats with CK caused swelling in both the paw and the knees in a time dependent manner. There was significant ($p < 0.05$) inhibition of the swelling in rats treated with MEO*g* (100, 200 and 400 mg/kg) and indomethacin (5 mg/kg) compared to rats that received vehicle alone (1% tween 80). The effects were dose-dependent and as well as time dependent. MEO*g* (400 mg/kg) gave comparable reduction of 81.30 % when compared o indomethacin of 89.2% (Table 3).

Table 2: Effect of *O gratissimum* extract on paw thickness in formalin –induced paw chronic inflammation in rats.

Treatment	Change in paw thicknesses (mm) Day 3	Day 7
Control (1% Tween 80)	2.65 ± 0.05	2.07 ± 0.04
ME (100 mg/kg)	2.47 ± 0.06(6.79)	1.74 ± 0.06(15.94)*
ME (200 mg/kg)	2.35 ± 0.06(11.32)*	1.71 ± 0.10(17.39)*
ME (400 mg/kg)	2.14 ± 0.05(19.25)*	1.68 ± 0.03(18.84)*
Indomethacin (2 mg/kg)	1.95 ± 0.07(26.41)*	1.22 ± 0.06(41.06)*

Each value is the mean SEM of five rats (n = 5)
Values in parenthesis represents percentage inhibition of edema
* p< 0.05 by one way ANOVA followed by post hoc Bonferroni's test compared to control group (pretreated orally with 1% tween 80)

Table 3: Effect of MEO*g* on paw oedema formation at different time points in the CK rats.

Experimental groups	Change in paw volume (ml) 1hr	3hr	5hr
Control (1% Tween 80)	0.345 ± 0.016	0.790 ± 0.019	0.722 ± 0.028
MEO*g* (100 mg/kg)	0.290 ± 0.065(15.94)	0.525 ± 0.033 (33.54.99)*	0.375 ± 0.005 (48.06)*
MEO*g* (200 mg/kg)	0.282 ± 0.053(18.26)	0.420 ± 0.045 (46.84)*	0.335 ±0.059 (53.60)*
MEO*g* (400 mg/kg)	0.220 ± 0.043(36.23)	0.200 ± 0.042(74.64)*	0.135 ± 0.033(81.30)*
Indomethacin (5 mg/kg)	0.248 ± 0.017(28.11)	0.105 ± 0.034 (86.70)*	0.078 ± 0.034 (89.20)*

Each value is the mean ± SEM of five rats (n = 5),
Values in parenthesis represents percentage inhibition of oedema
* $p < 0.05$ relative to control (1% Tween 80).

The result of measurement of the knee diameter as presented in table 4 showed an increase in knee circumference at different time intervals. There was a significant ($p < 0.05$) dose dependent reduction in joint diameter of rats that received MEO*g* (100, 200 and 400 mg/kg) and indomethacin (5 mg/kg) as compared to vehicle treated control.

MEOg increases mechanical withdrawal threshold

Mechanical paw withdrawal threshold at the fifth hour post CK injection was lower in vehicle group as compared to groups pretreated with MEO*g* (100, 200 and 400 mg/kg) and indomethacin (5 mg/kg). Pretreatment with MeO*g* (100, 200 and 400 mg/kg) protected against carrageenan –induced hyperalgesia in a significant ($p < 0.001$) and dose-dependent manner (Fig 4).

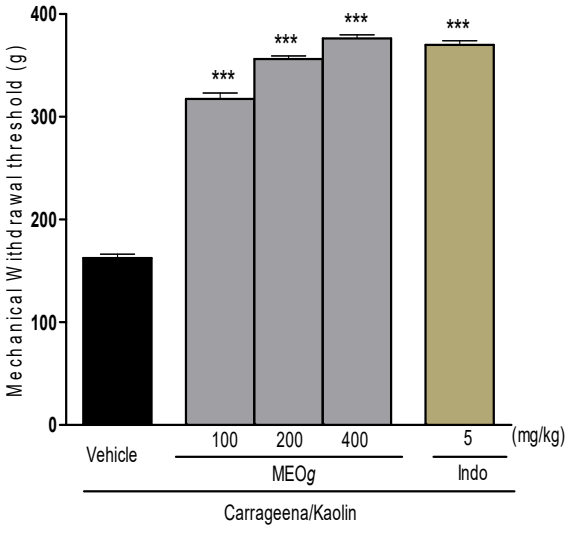


Fig 4: Antihyperalgesic effect of MEO*g* (100, 200, and 400 mg/kg, orally) and Indomethacin (5 mg/kg) in carrageenan/ kaolin –induced arthritic rats. Each bar represent Mean ± SEM, n = 5; one-way ANOVA followed by Bonferroni's post hoc test for multiple comparison, ***$p < 0.01$, vs vehicle.

MEOg ameliorates CK-induced impairment of locomotory activity

The result of exploratory activity determined twenty -four hours after intraarticular injection of CK in rats are presented in fig 5. The exploratory activity (total horizontal beam breaks in five minutes was lower in rats that received vehicle. Exploratory activity in rats pretreated with MEO*g* (200 mg/kg) was similar to that of indomethacin (5 mg/kg)-pretreated rats ($p>0.05$) but significantly ($p <0.001$) higher than in rats that received vehicle. The dose response function for MEO*g* reveals an inverted U-function indicating that MEO*g* (100 and 400 mg/kg), did not show any significant difference from the control.

Effects of MEOg on Plasma TBARS, Nitrite, GSH and SOD in CK rats

Table 4 shows the effect of MEO*g* (100, 200 and 400 mg/kg) on the plasma TBARS, nitrite, GSH and SOD. Treatments with MEO*g* significantly ($p <0.05$) reduced index of TBARS in a dose dependent manner compared to control, while plasma nitrite level was reduced in both extract and Indomethacin treated CK rats, it was however not statistically significant. GSH and SOD levels in rats treated with MEO*g* (100 - 400 mg/kg) and Indomethacin (5 mg/kg) were significantly ($p <0.05$) elevated in comparison to control rats.

Effects of MEOg on histology of joints

Histopathological analysis of the CK-injected ankle joints in vehicle-treated rats showed oedema and infiltration with inflammatory cells (mainly neutrophils and some macrophages). There is marked diffuse presence of inflammatory cells which are almost entirely neutrophils (intact and degenerate), suggestive of purulent (acute) arthritis (Fig 6A). MEO*g* (100 – 400 mg/kg) showed multiple foci of moderate aggregates of inflammatory cells which are

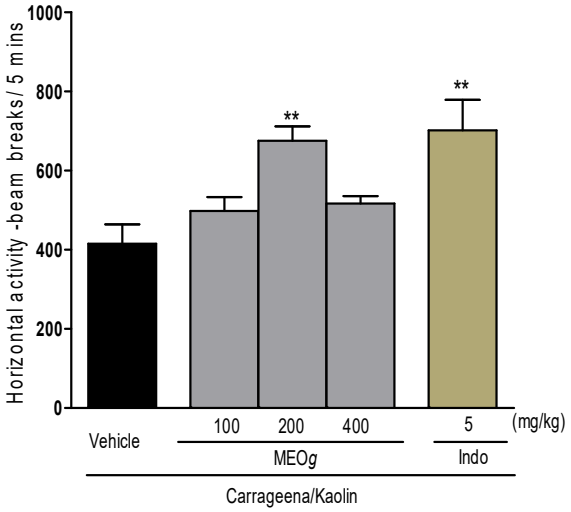


Fig 5: Effect of MEO*g* (100, 200, and 400 mg/kg, orally) and Indomethacin (5 mg/kg) locomotory activity in carrageenan/ kaolin –induced arthritic rats. Each bar represent Mean ± SEM, n = 5; one-way ANOVA followed by Bonferroni's post hoc test for multiple comparison, $^{**}p < 0.01$, vs vehicle.

macrophages and lymphocytes as well as a few plasma cells (fig 6E).

Discussion

O. gratissimum leaves are generally regarded as safe due to their longstanding use in traditional medicine to treat various forms of diseases. However, in view of the different extraction method and keeping in mind that sequential extraction may abrogate the safety ascribed to traditional use based mainly on aqueous extract, we tested the cytotoxicity effect of the phenolic-enriched extract.

The cytotoxicity testing of sequential methanol extract in RAW 264.7 and CHO-k1 cells did not show reduction in the viability of the cells. An increase in cell viability is an indication of cellular proliferation, while a decrease might be as a result of cell death either due to toxic effects of test extracts or sub optimal culture conditions. In categorizing plant extract safety, IC_{50} values of 20 µg/mL and below are considered to be toxic [24],

Table 4: Effect of MEO*g* on knee swelling at different time points in the CK rats.

Experimental groups	Increase in Knee Circumference (mm)		
	1hr	3hr	5hr
Control (1% Tween 80)	1.045 ± 0.114	2.150 ± 0.360	2.507 ± 0.143
MEO*g* (100 mg/kg)	0.433 ± 0.178(58.6)*	0.865 ± 0.247(59.8)*	0.760 ± 0.240(69.7)*
MEO*g* (200 mg/kg)	0.398 ± 0.115(61.9)*	0.755 ± 0.061(64.9)*	0.655 ± 0.141(73.9)*
MEO*g* (400 mg/kg)	0.303 ± 0.039(71.0)*	0.688 ± 0.174(68.0)*	0.435 ± 0.125(82.6)*
Indomethacin (5 mg/kg)	0.265 ± 0.084(74.6)*	0.490 ± 0.103(77.2)*	0.380 ± 0.073(84.8)*

Each value is the mean ± SEM of five rats (n = 5),
Values in parenthesis represents percentage inhibition of joint swelling
* $p < 0.05$ relative to control (1% Tween 80).

Table 5: Effect of MEO*g* on TBARS, Nitrites, GSH and SOD in carrageenan/kaolin –induced monoarthritis in rats

Treatment	TBARS (ηM MDA/ mg protein)	Nitrites (µM)	GSH(µM GSH/ mg protein)	SOD (mIU/ mg protein)
Control (1% Tween 80)	26.15 ± 1.19	50.25 ± 0.48	0.198 ± 0.003	3.25 ± 0.16
MEO*g* (100 mg/kg)	12.76 ± 0.96*	43.75 ± 2.32	0.237 ± 0.009*	4.93 ± 0.28*
MEO*g* (200 mg/kg)	14.10 ± 0.46*	42.50 ± 1.19	0.237 ± 0.007*	5.11 ± 0.46*
MEO*g* (400 mg/kg)	17.10 ± 1.68*	42.00 ± 3.34	0.230 ± 0.008*	4.97 ± 0.34*
Indo (5 mg/kg)	12.80 ± 0.62*	46.75 ± 2.39	0.210 ± 0.004*	5.75 ± 0.25*

* $p < 0.05$ by one way ANOVA followed by Bonferroni's post hoc test compared to control group (pretreated orally with 1% tween 80).

mostly neutrophils and a few macrophages (Fig 6B-D). Indomethacin treatment showed moderate aggregates of inflammatory cells which are mostly

therefore MEO*g* with IC_{50} greater than 200 µg/mL showed no preliminary indication of toxicity. This is in agreement with findings from Kpadonou

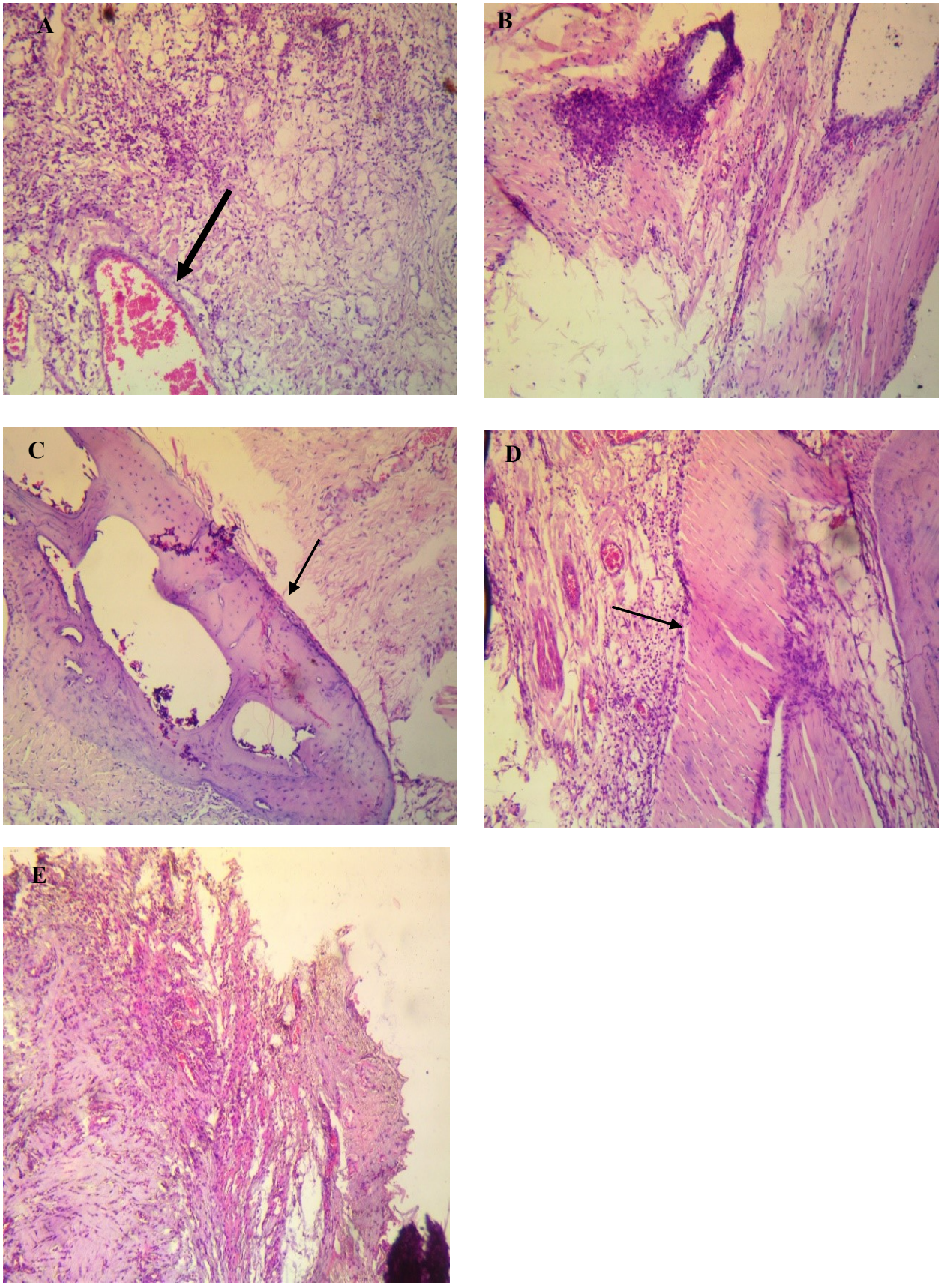


Fig 6: Histological evaluation of hind paw joints of formalin-fixed decalcified tissue from affected joints stained with haematoxylin and eosin. Photomicrographs are at magnification of 100X. Neutrophil infiltration around the synovial lining and congestion of blood vessels in the capsule (black arrow). (A) Negative control (B) MEO*g* (100 mg/kg); (C) MEO*g* (200 mg/kg); (D) MEO*g* (400 mg/kg); (E) Indomethacin (5 mg/kg).

Kpoviessi *et al.*, [25] which reported an IC_{50} value of 287.46 ± 3.76 for crude ethanol extract of *O. gratissimum* leaves tested in CHO-k1 cells. This suggests that sequential methanol extract of *O. gratissimum* leaf is safe for use if the plasma concentration is below the cytotoxic level.

Erythrocytes haemolysis assay which measures the release of haemoglobin from disrupted erythrocytes is fast and less laborious screening methods to assess at cytotoxicity [26]. MEOg showed concentration dependent protective effect in the heat-induced haemolysis of rat erythrocytes. The ability to protect the erythrocytes from stress-induced lytic effect of heat also demonstrate the *in-vitro* anti-inflammatory properties of the sequential methanol extract. Brown [15] suggested the membrane stabilization assay as a technique for the rapid screening of potential anti-inflammatory compounds based on their ability to inhibit heat-induced haemolysis. During inflammation, there are lyses of lysosomes which release their component enzymes that produce a variety of disorders. The lysosomal enzymes are implicated in the pathogenesis of articular tissue degradation in several rheumatic diseases. MEO*g* by stabilizing the membrane may prevent the rupture of the lysosomes and inhibit the release of lysosomal enzymes as do by anti-inflammatory drugs [27].

The ability of MEO*g* (100-400 mg/kg) to considerably suppress the inflammation induced by egg-albumin in a dose-dependent manner is suggestive that it has strong anti-oedema properties in acute inflammation. Egg albumin induces a robust but short lived oedema in rat paw, as it is known to release the early mediators like histamine and serotonin to the site of inflammation and the fluid accumulation through the mast cell degradation [17, 28].

Formalin-induced paw oedema has been widely used classic model to screen compounds with anti-inflammatory properties in chronic inflammatory state such as arthritis in human [29, 30]. Formalin injury induces a cascade of cellular reactions in two phases. Cytokines such as TNF-α and IL-1β are released during the cascade and,in turn induce the release of pain mediators such as bradykinin and calcitonin gene-related peptide [31, 32]. In this study, the anti-inflammatory effects of MEOg were evaluated in formalin-induced chronic inflammation in rat. The results indicate that MEOg suppressed the chronic inflammatory response induced by formalin, and ameliorates formalin-induced lipid peroxidation in rat paw tissues. Although the observed anti-inflammatory efficacy was modest compared to indomethacin, it may offer an alternative in the treatment of chronic inflammatory diseases.

In the carrageenan/kaolin induced monoarthritis in rats experiment, MEO*g* suppressed the swelling of the paw and knee in the CK injected rats. Carrageenan –induced oedema formation has been associated with release of early inflammatory mediators like histamine, bradykinin, platelet activating factor, nitric oxide and prostaglandins [33-35]. Involvement of cyclooxygenase (COX-2) in the production of prostaglandins that promotes hyperemia and oedema in the carrageenan-induced rat knee has been previously described [36]. While COX-2 is said to be important in development of hyperemia and oedema, maintenance of acute inflammatory hyperemia is associated with nitric oxide [36].

In addition to swelling of joint and hind paw in rats, carrageenan injection may cause activation of neurons in the dorsal horn of the spinal cord leading to central sensitization. The CK model has been a useful model for assessing hyperalgesia in rodents. A paw withdrawal latency measure can be used to infer pain and hyperalgesia in inflamed rat limbs. The hyperalgesia in the CK model starts to develop at 4^{th} hour and is maintained for 24 hours [18, 37]. MEOg significantly suppressed hyperalgesia in a dose dependent manner. This is consistent with similar findings on the anti-arthritic activity of *O. gratissimum* extract [38, 39].

Furthermore, MEO*g* pre treatment alleviates the suppression of locomotory exploratory function in rats injected with CK. Assessment of exploratory activity as a measure of spontaneous pain has been used to elucidate the mechanisms of arthritis and evaluate the effects of anti-arthritic treatments [19,40]. Reduction of locomotory activity is considered an expression of pain. Previous studies have shown similar pattern of increase in locomotory activity with some non-steroidal anti-inflammatory drugs like indomethacin, acetaminophen, celecoxib while drugs like aspirin has been ineffective [19, 41-43].

Considerable evidence had shown that the production of reactive oxygen species (ROS) at site of inflammation contributes to tissue damage. The late phase of carrageenan-induced inflammation contributes to the release of Nitric oxide through the activation of inducible nitric oxide synthase (iNOS) [44]. ROS/RNS promotes lipid peroxidation, which is a critical mechanism of the injury that occurs during rheumatoid arthritis. Carrageenan stimulated production and release of nitric oxide contributes to

tissue injury, inflammation-induced oedema and hyperalgesia [44]. The protective effect of MEO*g* was demonstrated by the reduction of NO released into plasma. The GSH and SOD levels is usually a reflection of the endogenous defense against damage caused by ROS, and they are important non-enzymic and enzymic endogenous antioxidant defense system involved in oxidation-reduction process [45]. Depletion of GSH in plasma and reduced SOD activity in CK arthritic group might be due to the increased oxidative stress in the animals. Interestingly, pretreatment with MEOg significantly re-established the depleted levels of GSH and SOD, probably by competing for scavenging of free radicals.

In conclusion, significant reductions in inflammation and pain in rats treated with MEOg might be due to the effects of polyphenols acting via multiple mechanisms such as antioxidant, membrane stabilizing and cytoprotective activities.

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Laparoscopic treatment of symptomatic renal cysts in overweight patients at Ibadan: an initial experience

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Abstract

Background: Symptomatic renal cysts are eminently suitable for treatment through laparoscopic approach. We report our initial experience with the laparoscopic treatment of symptomatic renal cysts in overweight patients at Ibadan.

*Results***:** Five patients were treated between June 2015 and April 2016 comprising of 3 males and 2 females. The mean age was 61 (age range, 40-75) years. The mean body mass index was 29.9 (range 25.5-31.1) kg/m^2. Four of the five patients presented with loin pain. Three patients were hypertensive; one had peptic ulcer disease and another diabetes with prostate cancer. Abdominal ultrasound and CT scan showed thin-walled cortical cysts, with sizes varying from 35cc to 380cc. Four cysts were unilateral, located in the left kidney while a patient had bilateral cysts. The mean operating time was 222 minutes (range 180-310 minutes). The estimated blood loss was 20-100mls. The five patients had laparoscopic transperitoneal deroofing and excision of the renal cortical cysts. The postoperative period was uneventful and all were discharged on the second day after surgery. The median period of follow up was 14 months (range 8-18 months) and these patients have remained asymptomatic with no radiological evidence of persistence of cysts.

Conclusion: Laparoscopic deroofing and excision of simple renal cyst in overweight patients is feasible and safe in our environment.

Keywords*: symptomatic renal cyst, overweight, laparoscopic deroofing and excision, initial experience, Ibadan, Nigeria*.

Résumé

Contexte: Les kystes rénaux symptomatiques sont éminemment convenables au traitement par la technique de laparoscopie. Nous rapportons notre expérience initiale avec le traitement par laparoscopie des kystes rénaux symptomatiques chez les patients en surpoids à Ibadan.

Résultats **:** Cinq patients ont été traités entre juin 2015 et avril 2016 comprenant 3 hommes et 2 femmes. L'âge moyen était de 61 ans (tranche d'âge, 40-75 ans). L'indice de masse corporelle moyen était de 29,9 (intervalle 25,5-31,1) kg/m^2. Quatre des cinq patients présentaient avec une douleur lombaire. Trois patients étaient hypertendus; un avait un ulcère gastroduodénal et un autre diabétique avec un cancer de la prostate. L'échographie abdominale et la scanographie ont montré des kystes corticaux à parois minces, avec des tailles variant de 35cc à 380cc. Quatre kystes étaient unilatéraux, situés dans le rein gauche alors qu'un patient avait des kystes bilatéraux. La durée moyenne de fonctionnement était de 222 minutes (intervalle 180-310 minutes). La perte de sang estimée était de 20 à 100 ml. Les cinq patients ont été traités par la découverture de laparoscopie transpéritonéale et l'excision des kystes corticaux rénaux. La période postopératoire s'est déroulée sans incident et tous ont été libérés le deuxième jour après la chirurgie. La période médiane de suivi était de 14 mois (intervalle 8-18 mois) et ces patients sont restés asymptomatiques sans preuve radiologique de persistance des kystes.

Conclusion : La découverture de laparoscopie et l'excision du kyste rénal simple chez les patients en surpoids sont faisables et sécuritaires dans notre environnement.

Mots - clés *: kyste rénal symptomatique, surpoids, découverture de laparoscopie et excision, expérience initiale, Ibadan, Nigeria*.

Introduction

The availability of diagnostic imaging facilities such as abdominal ultrasonography and computed tomography (CT) scan have increased the detection rate of asymptomatic renal cysts [1]. Renal cysts are commonly seen in individuals above the age of 50 years and in those with background hypertension and significant history of cigarette smoking. Renal cysts are classified as congenital or hereditary. They may be solitary, multiple, unilateral in three-quarter or bilateral in one-third of cases [1,2].

In 1986, Bosniak classified renal cysts based on the thickness of the wall as thin or thick, the presence of septa, echogenicity or contrast enhancement of the content of the cysts. These

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characteristics are seen on CT scan and by extension on abdominal ultrasound. These cysts are classified into four main categories with a further subdivision of category II into II and IIF, where IIF has a 5% chance of underlying malignancy [3,4]. The asymptomatic renal cysts do not require surgical intervention because they grow at a rate of 3.2% and increase in cyst size of 1.6mm per year [1]. Hence, they are usually observed with serial imaging studies [1,4]. However, the large size renal cysts, may present with flank pain in addition they may rupture spontaneously or from external trauma, may be infected or may present with hypertension [1,2]. Thus, necessitating intervention by either Ultrasound or CT-guided aspiration with or without injection of sclerosing agents [5,6,7,8] Alternatively, the symptomatic or complicated simple renal cysts can be treated by surgical deroofing, unroofing, decortication or excision through open surgical approach or laparoscopy [2,5,9,10].

We present our initial experience with the laparoscopic treatment of symptomatic simple renal cysts at a major University Teaching Hospital in Nigeria.

Materials and methods

This is a prospective collection of data of 5patients with symptomatic renal cysts between June 2015 and April 2016.

The demographic data which include sex, body mass index and the clinical features of these patients were collected. The abdominal ultrasound and computerized tomographic features were also reviewed. The intraoperative findings, estimated blood loss, the duration of surgery and the subjective outcome of surgery based on relief of symptoms were analyzed. All the patients had transperitoneal laparoscopic deroofing and excision of renal cysts. The aspirated cystic fluids and excised cystic wall were sent for cytology and histological assessment. All the patients were discharge on the second day after laparoscopic surgery.

Operative Technique

The laparoscopic access was obtained by using the "Direct trocar technique". The 10mm trocar was inserted through the periumbilical opening and subsequently the carbon dioxide insufflator was attached with the pneumoperitoneum increased to 15-18mmHg at a flow rate of 500-1000ml/second. The peritoneum was thereafter inspected for intraperitoneal injury. Other secondary ports were inserted under direct visualization. Through these ports, the ligament of Toldt was identified, incised, and the descending colon was mobilized medially. The renal cyst was then identified, grasped dissected with Maryland grasper and laparoscopic suction nozzle. The cyst was incised, the content aspirated and aspirate sent for cytology. The cyst wall was then excised with cautery and sent for histological analysis. This was the procedure performed for all the unilateral cyst as well as the bilateral cysts.

Results

There were 3 males and 2 females. The mean age was 61 (age range 40-75) years. All the patients were over-weight with an average body mass index (BMI) 29.9 (range 25.5 – 31.1) kg/m^2 (Table 1). The complete blood count, serum electrolyte, urea and creatinine were all within normal limits.Four patients presented with recurrent or intermittent left loin pain and one had cough, chest pain and fever (table 2) The co-morbid conditions in the patients were hypertension in 3 patients, peptic ulcer disease and both diabetes mellitus and prostate cancer in 1 in patient each. The abdominal ultrasound and CT scan (figure 1)showed thin-walled cortical cysts with sizes varying from 35cc to 293.7cc. Four patients had unilateral renal cyst while one had multiple bilateral renal cysts.

Tables 1: Demography of patients with symptomatic renal cysts in Ibadan

Serial No.	age (years)	sex	BMI (kg/m^2)
1	75	female (F)	30.2
2	55	female (F)	27.4
3	40	male (M)	31.1
4	75	male (M)	30.9
5	60	male (M)	25.5
	61 (40-75)	2F/3M	29.9 (25.5-31.1)

BMI – body mass index

The mean operating time was 222 minutes (range 180-310). The estimated blood loss was 20-100mls. All five patients had transperitoneal laparoscopic deroofing and excision of the renal cortical cysts (figures 2 and 3). There were no cytologic and histologic evidence of malignancy in any of the aspirate or excised cyst. The postoperative period was uneventful and all were discharged on the second day after surgery. They were asymptomatic and ultrasound revealed absent cysts at median period of 14 (8-18) months postoperatively.

Tables 2: The clinical and radiological features of patients with symptomatic renal cysts in Ibadan

Serial No.	presentation	comorbidity	location of cyst	Bosniak Classification (CT scan/USS)
1.	left loin pain	hypertension	LURP	I
2.	left loin pain	hypertension	LMRP	I
3.	Rec. left loin pain	peptic ulcer disease	LMRP	I
4.	fever-1/12, cough-2/52, left lower chest pain	hypertension	LURP	II
5.	bilateral loin pain	diabetes / prostate cancer	BU-LRP	I

LURP – left upper renal pole, LMRP – left middle renal portion, BU-LRP – both upper and lower renal poles, USS – ultrasound, CT – computerized tomography

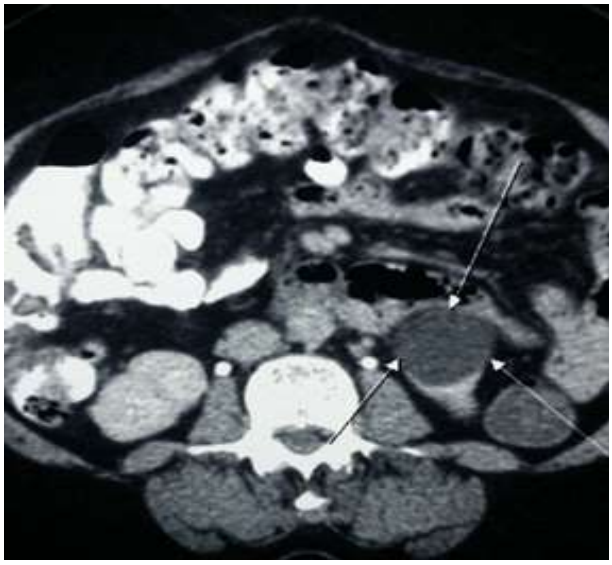

Fig. 1: Transverse CT section showing left upper pole renal cyst surrounded by white arrows.

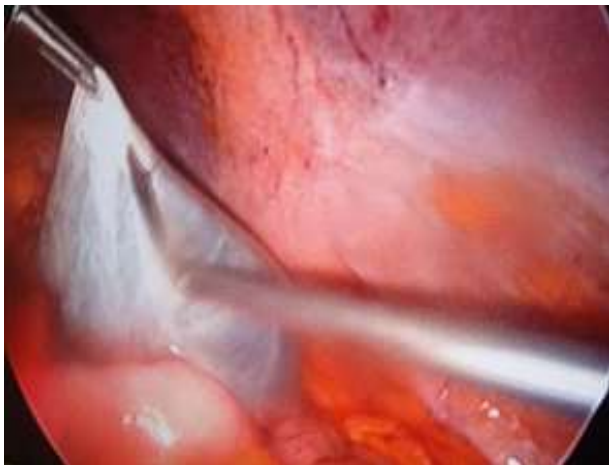

Fig. 2: Operative deroofing and aspiration of symptomatic left renal cyst

One of the female had organized haematoma at the primary trocar site that resolved 4 weeks after surgery. The other four patients resumed work a week after discharge.

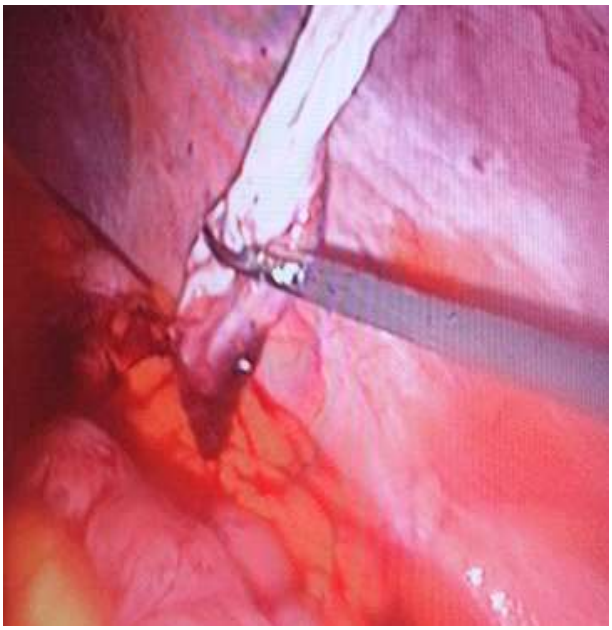

Fig. 3: Laparoscopic excision of renal cyst wall

Discussion

Renal cysts are commonly seen with increasing age after 50 years of age as seen in majority of patients in this series [2]. Majority of patients with renal cysts have flank pain and hypertension, as seen in 60% of patients in this study. [2] Similarly 80% of patients often present with unilateral and solitary renal cyst as observed in this series [2].

Preoperative abdominal ultrasound and CT scans were very instrumental in characterizing the symptomatic renal cysts [1-3,6]. In this series, 4 patients had Bosniak type I and one was type II. Laparoscopic renal surgery is safe in obese patient though associated with prolong operation time with minimal complication rate and minimal perioperative blood loss [11].

The first line treatment for small symptomatic renal cyst is either ultrasound guided or CT/MRI guided aspiration with or without sclerotherapy

Table 3: Characteristics of laparoscopic surgery

Serial No.	Cyst-shape, wall/size (cc)	EBL (mLs)	Operation time(min)	Procedure
1¥	round, thin-walled, (33.9)	80	200	LDE
2¥	round, thin-walled, (293.7)	80	180	LDE
3¥	round, thin-walled, (68.7)	50	225	LDE
4¥	round, enhanced wall, (380)	20	195	LDE
5¥	multiple round, thin-wall(30-31.9)	100	310	LDE
Mean		66(20-100)	222(180-310)	

EBL – estimated blood loss, LDE – laparoscopic deroofing/excision, ¥ - discharged 2nd day after surgery

[2,6,7]. Ali *et al* [8], reported symptomatic success in 93.4% of patients and 2% had failed ultrasound-guided percutaneous sclerotherapy for symptomatic cyst because the cysts were greater than 10cm. Skolarikos *et al* [6], in their comprehensive review, found that only 2-4% of renal cysts become symptomatic due to haemorrhage, infection or rupture of cysts. Patients treated with sclerosing agents such as ethanol may have pain, fever and alcohol intoxication leading to shock.

Bas *et al*. [12], reported that laparoscopic decortication for symptomatic renal cyst had a high success rate, low recurrence rate and minimal morbidity when compared to percutaneous aspiration with a minimally high recurrence rate of 22.8% after a mean follow up period of 34.9 months. They also found 2% conversion rate from laparoscopic decortication due to bleeding or adhesions [12]. All patients in this study had their procedures completed laparoscopically. Farhan [13], reported a 90.9% symptomatic and radiographic success rate in their series after a median follow up of 12 (range 6-18) months and mean operation time of 100 (80-120) minutes. Though our follow up period was comparable to Farhan, our operation time was double. This may be explained by our early learning curve for laparoscopy and we do hope to report shorter periods in subsequent update.

Patients with symptomatic renal cyst often present with pain, hypertension and pressure effect in this series as cough and chest pain due to the large size of the renal cyst. In our series, all the patients had renal cysts successfully deroofed and excised through laparoscopic transperitoneal approach. In patients with infected cysts, retroperitoneal approach is preferred for large symptomatic renal cysts because it ensures complete excision of the cysts. [9-14] In this series, all the patients had 100% symptomatic relief and 80% radiographic success. One of 5 patients (20%) developed a new cyst at 6 months postoperative that is consistent with Bas *et al* [12], findings of development of new renal cysts in 5 of 149 patients who had Laparoscopic decortication of symptomatic renal cyst.

In conclusion, transperitoneal laparoscopic deroofing and excision of symptomatic renal cyst is feasible, safe and effective in our environment. The advantages are reduction in duration of admission, early return to work. One of the challenges was the long operation time which can be adduced to learning curve and which should reduce with more experience.

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Home-based postnatal care: An indicator for improved maternal and neonatal health outcome in Nigerian rural communities

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Abstract
Introduction: Maternal and neonatal mortality is a major reproductive health concern in Nigeria. At present, the country has the highest maternal mortality rate in Africa and the second globally after India. Most of these maternal and neonatal deaths have been reported to occur at the postnatal period, complicated by a large number of home deliveries and poor uptake of postnatal care.
Approach: In 2009, WHO and UNICEF recommended community-based approach to postnatal care. Providing home visits during the six-week period following childbirth by skilled health care provider is the key strategy. With respect to this, HBPNC has been piloted and adopted by many countries such as Bangladesh, Kenya, Madagascar, Egypt, Ethiopia, Indonesia, India, Iran and Parkistan (to mention but a few) and found to be effective, acceptable, affordable and cost-effective.
Findings: In the past years, diverse interventions to combat maternal and neonatal mortality in Nigeria have been employed. Most of these interventions targeted the antenatal and intranatal period. Although, this has yielded remarkable success, HBPNC will immensely assist in reaching the unreached and meet the unmet need of mothers and neonates at this crucial period especially in the rural communities.
Conclusion: Since the SDG 3 is targeted at reducing maternal and neonatal mortalities by 2030, a successful implementation of the Integrated Maternal, Newborn and Child Health Strategy and indeed HBPNC will contribute to the realization of this goal.

Keywords: *Home-based, postnatal care, maternal health outcomes, rural communities*.

Résumé
Introduction: La mortalité maternelle et néonatale est un problème majeur de santé reproductive au Nigeria. Actuellement, le pays a le taux de mortalité maternelle le plus élevé en Afrique et le deuxième dans le monde après l'Inde. La plupart de ces décès maternels et néonatals ont été signalés à la période postnatale, compliquée par un grand nombre d'accouchements à domicile et une faible prise en charge des soins postnataux.
Approche **:** En 2009, l'OMS et l'UNICEF ont recommandé une approche communautaire des soins postnataux. Fournir des visites à domicile au cours de la période de six semaines suivant l'accouchement par un fournisseur de soins de santé qualifié est la stratégie clé. À cet égard, HBPNC a été testé et adopté par de nombreux pays tels que le Bangladesh, le Kenya, Madagascar, l'Égypte, l'Éthiopie, l'Indonésie, l'Inde, l'Iran et le Pakistan (pour ne citer que quelques exemples) et trouvé effective, acceptable et rentable.
Résultats: Au cours des dernières années, diverses interventions ont été menées pour lutter contre la mortalité maternelle et néonatale au Nigéria. La plupart de ces interventions ciblaient la période prénatale et intra-natale. Bien que cela ait connu un succès remarquable, HBPNC aidera immensément à atteindre les personnes non atteintes et à répondre aux besoins non satisfaits des mères et des nouveau-nés à cette période cruciale, en particulier dans les communautés rurales.
Conclusion: Étant donné que SDG 3 vise à réduire la mortalité maternelle et néonatale d'ici 2030, une mise en œuvre réussie de la Stratégie intégrée pour la santé maternelle, néonatale et infantile et certainement du HBPNC contribuera à la réalisation de cet objectif.

Mots clés: *à domicile, soins postnataux, résultats de santé maternelle, communautés rurales*.

Introduction

Nigeria loses about 145 women of childbearing age and 2,300 under-five year olds daily [1]. Globally, 303 000 maternal deaths occurred in 2015; highest in Africa [2]. Nigeria had about 58,000 maternal deaths making the country the second largest contributor in the world [2], most occurring during postnatal period. Similarly, 2.9 million newborn deaths occurred in 2012; about half occurred within first 24 hours after birth and three-quarter in the first week of life [2, 3]. Victims were often babies born too early, too small, with infections, or asphyxiated

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around the time of delivery [1]. Most of these deaths are preventable with quality post natal care (PNC). Meanwhile, the uptake of PNC services is very poor and inadequate (thirty-six percent) in Nigeria [4, 5].

Although there is global decline in maternal mortality, this remains high in many low-resource settings including Nigeria. Maternal complications (psychological & mental health problems) and neonatal morbidity are also prevalent [6,7]. The strategy to improve this in Nigeria has traditionally focused on the antenatal and intranatal period with little focus on postnatal period. Despite various interventions, Nigeria was far from meeting the Millennium Development Goal (MDG) 4 & 5. With the Sustainable Development Goal (SDG 3) now in focus for 2030, concerns are increasing on how to overcome the challenges militating against this goal. HBPNC in Nigeria will go a long way to address these challenges.

This paper hopes to review some of the specific challenges to PNC in Nigeria and how they can be addressed through HBPNC.

Emergence of HBPNC

Maternity care traditionally focuses on the postnatal clinic where women are seen six weeks after delivery. The uterus is checked for complete involution among other things and further counselling on any relevant matter is completed. This visit is concluded by referring the mother to family planning clinic while the baby is referred to infant welfare clinic for continuation of care [15-17].

This traditional model was criticized for neglecting the first four weeks following childbirth crucial to the survival of the newborn and mother. As a result, in 2009, UNICEF and WHO recommended home visits especially during the first week of newborn life [8, 9]. Further review in 2013 focused low resource settings/ low and middle income countries with the goal of improving maternal and newborn survival. Target was to reduce the global maternal mortality ratio to less than 70 per 100 000 live births and end preventable deaths of newborns and children under five years of age by year 2030 (SDG3.1 and 3.2). HBPNC has been piloted and adopted by many countries among which were Kenya, Madagascar, Egypt, Ethiopia, Indonesia, India ,Iran and Pakistan (to mention but a few) and found to be effective, acceptable, affordable and cost-effective [8,10-12]. A global review of level of implementation of HBPNC was recently conducted in five countries including Bangladesh, Malawi, Nepal, Nigeria and Rwanda of which only Nigeria is yet to adopt PNC home visits as a national policy or strategy. However, Nigeria has developed an Integrated Maternal, Newborn and Child Health Strategy (IMNCH) [13,14] which holds that each state in Nigeria develop her own approach to improving early PNC contacts including home visits based on local system and barriers to access. About twenty four states out of thirty-six and the Federal Capital Territory have begun implementing the IMNCH activities. Although progress with implementation is highly varied between and within the states, it was gathered that three northern states supported by USAID, ACCESS and MCHIP projects reported that HBPNC is effective and efficient.

HBPNC is a community-based intervention given by trained health practitioners to women and their babies between the period of birth and forty-two days in their homes. HBPNC employs the principles of woman-centered care to enable women participate in informed decision making regarding their own care with preference to the first week following child birth to increase survival chances of mother and neonate. It provides a holistic individualized care to mothers, infant, family and indeed the community while assessing the family environment, strengths and challenges with aim of providing interventions to improve maternal and family health [18,19].

Objective of HBPNC

The major objective of HBPNC is to decrease maternal and neonatal mortality and morbidity through:

- The provision of essential maternal and neonatal care during postnatal period and prevention of complications
- Early detection and special care of preterm and low birth weight newborns
- Early identification of illness in the mother and newborn and provision of appropriate care and referral
- Support of the family for adoption of healthy practices and build confidence and skills of the mother to safeguard her health and that of the newborn.

Rationale for HBPNC

Table 1 illustrates reasons adduced for HBPNC being needful for reducing maternal and neonatal mortality.

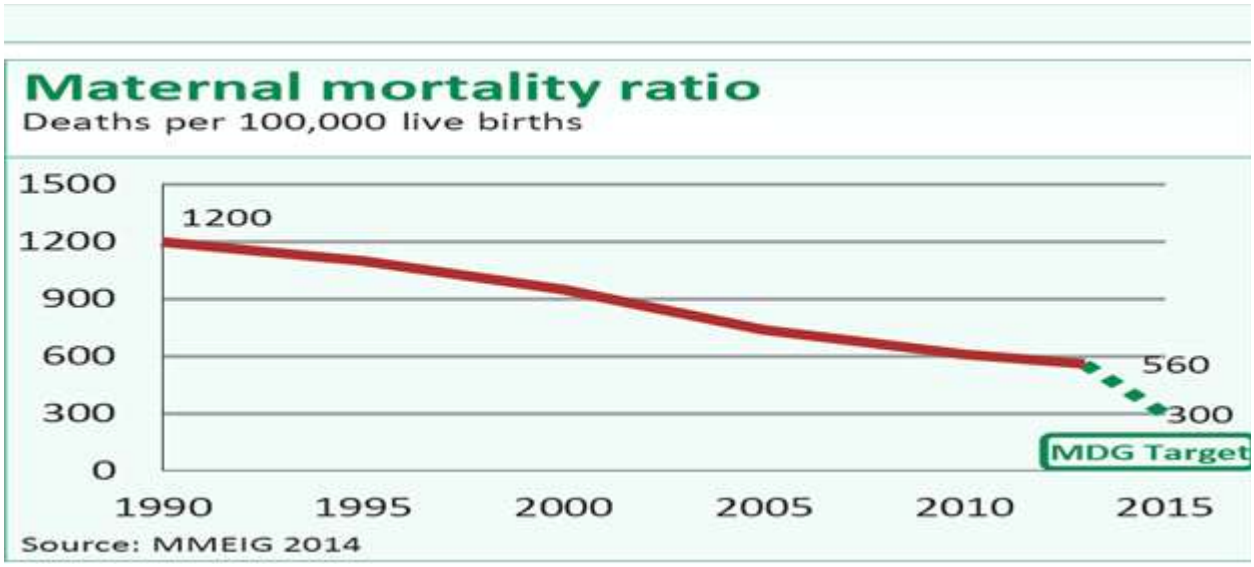
Current PNC programs in Nigeria

The HBPNC is not a new initiative in Nigeria although the level of implementation has not been evaluated. Some programs targeted at improving

Table 1: **Reasons for advocating adoption of HBPNC**

i.	To reduce the number of maternal and neonatal deaths [8] occurring which is still very high as shown in Figures 1 and 3 below. Most occur in developing countries during postnatal period in rural areas [4, 20, 21]. Figure 2 showed that mothers die from preventable causes.
ii	To reach mothers who had homebirths reportedly high in rural areas (77%) [4,8]. Even where deliveries take place in a health facility, mother and baby are discharged within few hours and may have no contact with the healthcare practitioner until six weeks after delivery.
iii.	To identify subgroups of women (in rural areas) who are underserved thus, reducing the wide and persistent disparities in the coverage of interventions between and within countries [4].
iv.	Nigeria needs to adopt PNC home visit as a national policy [3].A review on the current status of implementation of PNC home visit carried out by USAID, MCHIP and Save the Children in 2012 revealed that out of the two Asian countries and three African countries pilot tested, only Nigeria is defaulting.
v.	To enhance follow up of mothers and their babies since many do not keep follow up appointments [15]
vi.	HBPNC assists families in early detection of maternal and neonatal problems and in dealing with constraints to seeking appropriate help [13].
vii.	The facility based PNC has led to high congestion in the various health facilities consequently, there is overstretching of human and material resources available
viii.	To identify cultural beliefs and newborn care practices that do not conform to recommended standards [22]. Hence, it will inform behaviour change messages for newborn care practices targeting mothers, grandmothers, other relatives and traditional birth attendants.
ix.	HBPNC will be a good forum to continually assess, monitor manage the mother and newborn during postnatal period.

Fig.1



Source: WHO Fulfilling the health agenda for women and children, 2014

neonatal and maternal survival are: Midwife Service Scheme, Community-based Newborn Care, Integrated Management on Childhood Illness, Maternal Perinatal Death Surveillance and Response, Infant and Young Child feeding and Maternal Newborn and Child Health Week. However, they lack effective implementation, due to very low level of government spending on health care. Most of these programs were initiated by non-governmental organizations working with funding from international donors.

Home-based PNC delivery models

According to Koblinski (2005), the three main models are:

- Home visits by professional healthcare providers:

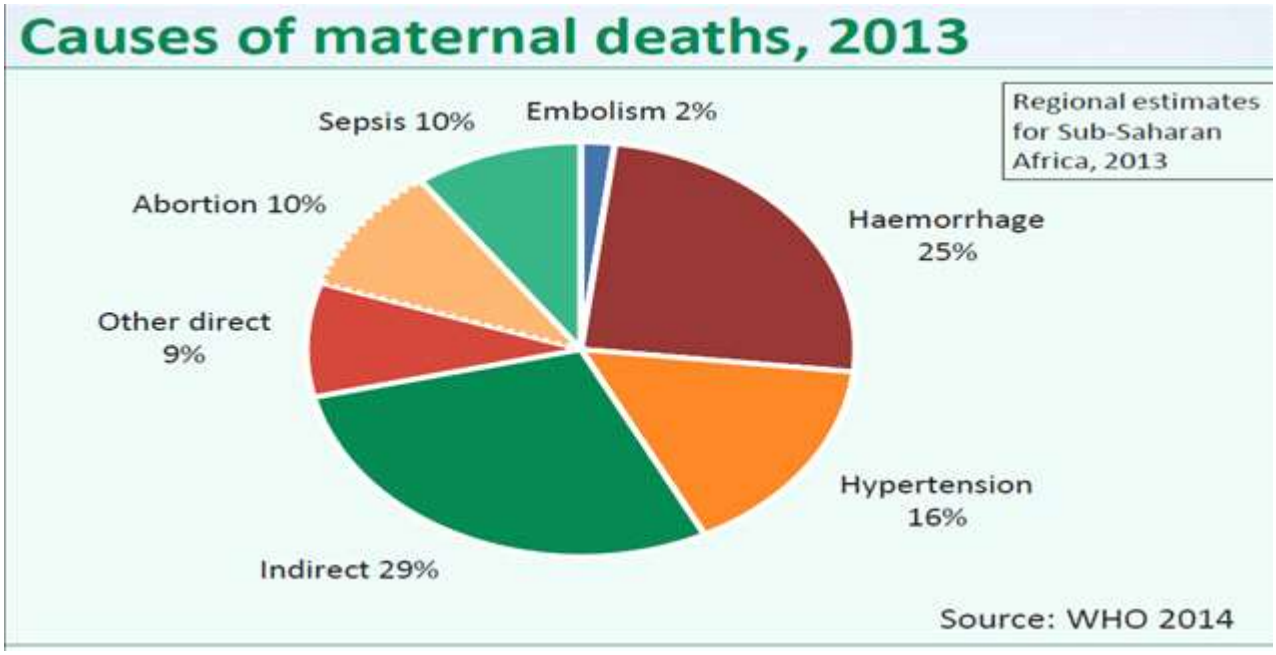


Fig. 2
Source: WHO Fulfilling the health agenda for women and children , 2014

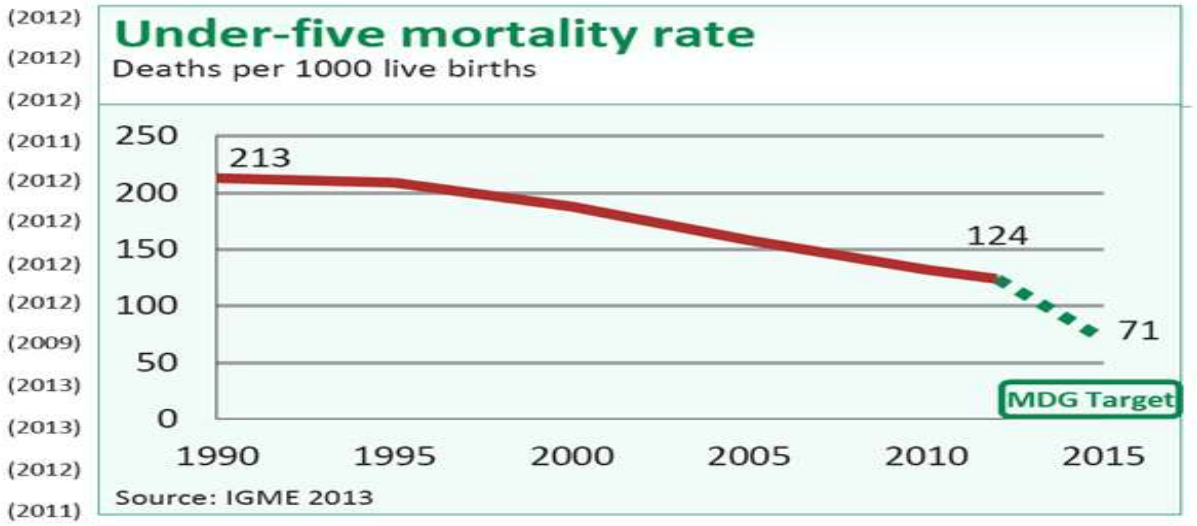


Fig.3
Source: WHO, UNICEF, UNFPA, World Bank, UNDP Trends in maternal mortality 1990-2015

- Home visits by non-professional community workers
- Home visits by community workers with referral or health facility support [23].

Home visits by professional health care providers
Focuses early identification, referral, and prompt management of complications. It also promotes healthy behaviours in the crucial postnatal period when women are trying new behaviours. Although, this model may be an appropriate long-term approach, it may be challenging to adopt in most developing countries, where the expense and demands related to regular home visits by professional health care providers may be too great. Furthermore, the logistics to reach women becomes the hurdle since HBPNC depends on locating the just delivered woman, the availability of transport to reach her, and the health provider's motivation to pay the visit. Some countries such as: Bangladesh, Indonesia, Zimbabwe, Egypt, Nepal and India have adopted this model. Literatures added that this model

Table 2 below captures key activities.

	Key activities of HBPNC
i.	Mobilization of all pregnant mothers to ensure that they receive the full antenatal package.
ii.	Undertaking birth planning and birth preparedness with the mother and family to ensure access to safe delivery.
iii.	Provision of maternal and newborn care through series of home visits depending on the need by a trained health worker in the first six weeks of life using the standard PNC checklist for first visit.
iv.	Examination of every newborn for prematurity and low birth weight and management.
v.	Extra home visits for preterm and low birth weight babies by the midwives and prompt referral when necessary.
vi.	Early identification of illness in the newborn and mother and provision of appropriate care as required.
vii.	Follow up for sick mothers and newborns after discharge from facilities.
viii.	Counselling the mother on postpartum care, recognition of post-partum complications and enabling referral to ensure better health outcomes.
ix.	Counselling the mother for the adoption of an appropriate family planning method.
x.	Skillful and competent PNC for babies and mothers who delivered outside the health facility.

of HBPNC could manage maternal and newborn complications and illnesses, but there is dearth of data to support this.

Home visit by non-professional community workers
Holds that non-professional community workers' pay household visits to provide home care or facilitate women's groups. This is commonly used in newborn care home visit programs. Studies from countries using this approach e.g. India reported success in the reduction of neonatal and perinatal mortality, timely initiation of exclusive breastfeeding, diarrhoea reduction and infant growth. Although, there is little or no provision for referral in this approach, community workers can play an important role in facilitating effective participatory group processes.

Home visits by community workers with referral or health facility support
It is a community-based outreach system with emergency referral support to a hospital for management of emergency obstetric complications, or outreach by family planning clinics to ensure counselling support, referral for broader contraceptive choice, and clinical management.

Studies from countries using this approach such as Honduras and Chile show improvement in the use of skilled birth attendants, birth planning, family planning continuation rates, and initiation and continuation of full breastfeeding. Utilizing the referral links may be challenging in very remote places for this approach however, locating referral centres closer to women may facilitate their use. Also, having birthing centres placed in remote, difficult to access localities and staffed by a skilled providers, will improve successful response.

Provisions of HBPNC
Barriers to optimal postnatal care in Nigeria
Improving the maternal and neonatal health outcome especially PNC in rural communities is faced with lots of challenges. However, most of these challenges can be surmounted using the HBPNC approach. Some of the challenges are outlined below:

Most deliveries take place outside health facilities
In Nigeria, although vast number of women attend antenatal care services, 65% of deliveries take place outside health facilities [4] mostly at home in rural communities [25]. This is not peculiar to Nigeria only; other African countries also experience same [24]. About 18 million women in Africa currently do not give birth in a health facility and this poses challenges for planning and implementing PNC for women and their newborns [24]. The major reasons reported for home, as opposed to facility-based birth include: lack of money, distance to the health facility, fear of caesarean section, lack of privacy or a dedicated labour room at the health facility and possibility of complications [25].

Cultural factors
The cultural perspective on the use of maternal health services suggests that medical need is determined not only by the presence of physical disease but also by cultural perception of illness [26]. In most African rural communities, maternal health services co-exist with indigenous health care services; therefore, women must choose between the options [26]. Also, many societies in sub-Saharan Africa acknowledge that grandmothers play an influential role in supporting the young women (daughters and daughters-in-law) during pregnancy, childbirth, and

throughout the care of the newborn but may endorse harmful practices. HBPNC will be a good strategy to identify and correct such harmful care practices through counselling [29].

Delay in seeking PNC

Delay in seeking care during the postnatal period could be due to: inability to detect illness early, tradition of keeping the baby indoors, need to wait for the 'decision maker' (husband) to give permission and pay for visiting a health facility or viewing the postnatal period with less concern [27,28]. HBPNC is crucial for shortening delays in seeking care after birth [10, 28].

Attitude of Health care workers

Unfriendly attitude of health care workers (verbal abuse and condescension) especially with poor patients reduces morale of mothers [27]. HBPNC provides an environment to treat mothers and their babies with love and empathy.

Misconception / Ignorance on the part of mothers

Nearly all mothers attend the PNC clinic for their children's immunization or weighing or growth monitoring. Onasoga, 2013 reported that although mothers have heard maternal health service, they do not have an in-depth knowledge of services rendered during the PNC [30] thus, minority (35.1%) return for PNC [31].

Funding

Lately, Nigerian government has taken pragmatic steps in reducing maternal and neonatal mortality. Notable among these efforts is the Midwife Service Scheme and CBNC which commenced in 2012. However, it is worthy of note that if the SDG-3 must be met, government needs to scale up funding in this respect.

Level of maternal education

Level of education is a significant predictor to utilization of maternal health care services. Wong *et al*. (2004) reported that the higher the educational level and experience, the more likely the utilization of health cares. In other words educated women are more likely to use maternal health care services than women with no formal education [4, 26, 32].

Age of mother

Studies have shown that younger mothers are more likely to deliver in health facilities than their older counterparts [33]. A study conducted in a rural community in Sokoto State, Nigeria revealed that socio-demographic characteristics such parity, age, educational level of mother and even the husband influence the uptake of PNC [34].

Parity

There is a significant negative association between parity and utilization of maternal health care services. Kebebe *et al*. (2012) reported their study that women in a larger household are less likely to deliver at health facilities [30]. HBPNC will reach such women.

Poor accessibility to health facility

Ugboja *et al*, posited that although most mothers concur about the importance of PNC and encourage each other to attend the clinic, long distances and inaccessibility to a health facility (especially during the rainy season), negligence and unplanned pregnancy were important barriers to use of PNC. Mothers often find the traditional birth attendants more accessible [10,26,27,29,30].

Low level manpower in health

Attendance by a skilled health provider is one of the health indicators of a nation but grossly inadequate in rural health facilities. WHO and UNPF reported that 73 countries in Africa, Asia and Latin America account for 96% of the world's maternal deaths, 91% of stillbirths and 93% of newborn deaths [2, 35]. Yet these countries only have 42 percent of the world's doctors, midwives and nurses [35]. For every 10,000 people in Africa, there are two doctors and 11 nurses or midwives, compared with 19 doctors and 49 nurses/midwives per 10,000 in America, and 32 doctors and 78 nurses/midwives per 10,000 in Europe [35]. Only one in five countries has enough adequately educated midwives to meet the basic needs of women and newborns [35].

HBPNC in centuries

HBPNC has potential to improve the quality of maternal and neonatal healthcare in Nigeria. It has been estimated that if routine PNC and curative care in the postnatal period reached 90 percent of babies and their mothers, 10 to 27 percent of newborn deaths could be averted [24]. Thus, high PNC coverage could save up to 310,000 newborn lives a year in Africa [24]. To achieve this goal, the Nigerian government must make a commitment to provide adequate resources, trained providers, up-to-date equipment and, most importantly, sufficient funding to end the many needless deaths associated with childbearing among Nigerian women. Focus should include:

i. Planning HBPNC for all home deliveries especially in the rural communities and follow up visits for hospital deliveries
ii. Conducting proper analyses of current policies and practices related to PNC to bridge identified gaps.
iii. Encouraging public private partnership. Entrepreneurs, non-governmental organizations and the government may partner together to implement HBPNC.
iv. Continuous recruitment, training and supervision of midwives/health workers should be done.
v. Continued effort is required to make quality antenatal and postnatal care accessible and affordable to the majority of the women in the rural community. The frequency, timing, duration and intensity of visits should be based upon local needs [36, 37].

Conclusion

Many African women and their newborns do not have access to health care during the early postnatal period, putting them at an increased risk of illness and death. HBPNC has been proven to be effective, efficient, culturally acceptable, cost effective in achieving a remarkable reduction in the maternal and neonatal mortality. There is an incredible opportunity to adapt HBPNC to rural settings to reach women and their newborns, especially for the numerous population of women who give birth at home. Nigeria being the Giant of Africa should consider reaching the unreached mothers in rural communities through adoption of HBPNC as a national policy in order to address the high maternal and neonatal mortality rate being reported in rural areas.

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Antioxidant effect of *Citrullus lanatus* ameliorates fructose-induced placental aberrations

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Abstract
Background: Fructose consumption during pregnancy has been associated with exacerbation of placental oxidative stress. The hypoglycemic and anti-oxidant properties of *Citrullus lanatus* juice (CLJ) previously reported may provide remedy to the oxidative stress.
Objective: The study investigated the effects of *C. lanatus* juice on fructose-induced placental changes in Wistar rats.
Methods: Twenty pregnant rats were assigned into four groups (n=5) and treated from Gestation Day (GD) 1-21 with water (control), 10% Fructose (w/v), 50% CLJ (v/v) and Fructose + CLJ. All treatments were given *ad libitum*. Caesarean section was performed on GD 21 during which the pups and placentas were harvested and weighed. Blood glucose level, progesterone concentration, placental morphometric indices (weight, circumference and thickness), oxidative status (using spectrophotometer) and histology were assessed. Data were analyzed using ANOVA and $P<0.05$ was considered statistically significant.
Results: The weight and circumference of placentas of fructose group were lower ($p < 0.05$) than that of control. Placental thickness was higher ($p < 0.05$) in fructose group compared with control. Placental malondialdehyde was higher in fructose group ($p < 0.05$) and lower in fructose + *C. lanatus* group ($p < 0.05$) compared with control and fructose groups respectively. Placental histology showed severe and mild infarction of chorionic villi in the fructose and fructose + *C. lanatus* groups, respectively.
Conclusion: C. lanatus juice ameliorated fructose-induced changes in placental oxidative status and morphology. Thus, intake of *C. lanatus* juice may be beneficial for optimal and healthy development of placenta and fetus of mothers who experience sugar cravings during pregnancy.

Keywords: *Placenta, Fructose, Citrullus lanatus, Oxidative stress, Rats.*

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Résumé
Contexte: La consommation de fructose pendant la grossesse a été associée à une exacerbation du stress oxydatif placentaire. Les propriétés hypo glycémiques et anti-oxydantes du jus de *Citrullus lanatus* (CLJ) précédemment rapporté peut apporter un remède au stress oxydatif.
Objectif: L'étude a étudié les effets du jus de *C. lanatus* sur les changements placentaires induits par le fructose chez les rats Wistar.
Méthodes: Vingt rates gravides ont été réparties en quatre groupes (n = 5) et traitées à partir du jour de gestation (JG) 1-21 avec de l'eau (témoin), 10% de fructose (w / v), 50% de CLJ (v / v) et Fructose + CLJ. Tous les traitements ont été donnés *ad libitum*. Une césarienne a été effectuée le JG 21 au cours de laquelle les souriceaux et les placentas ont été arrachés et pesés. La glycémie, la concentration de progestérone, les indices de morphométries placentaires (poids, circonférence et épaisseur), le statut oxydatif (à l'aide d'un spectrophotomètre) et l'histologie ont été évalués. Les données ont été analysées en utilisant ANOVA et $P <0,05$ a été considéré comme statistiquement significatif.
Résultats: Le poids et la circonférence des placentas du groupe fructose étaient plus faibles ($p <0,05$) que ceux du groupe témoin. L'épaisseur placentaire était plus élevée ($p <0,05$) dans le groupe fructose par rapport au témoin. Le malondialdéhyde placentaire était plus élevé dans le groupe fructose ($p <0,05$) et plus faible dans le groupe fructose + *C. lanatus* ($p <0,05$) comparativement aux groupes témoin et fructose respectivement. L'histologie placentaire a montré un infarctus sévère et bénin des villosités choriales dans les groupes fructose et fructose + *C. lanatus*, respectivement.
Conclusion: Le jus de *C. lanatus a* amélioré les changements induits par le fructose dans l'état oxydatif et la morphologie du placenta. Ainsi, la consommation de jus de *C. lanatus* peut être bénéfique pour le développement optimal et sain du placenta et du fœtus des mères qui éprouvent l'appétit excessif de sucrerie pendant la grossesse.

Mots-clés: *Placenta, Fructose, Citrullus lanatus, Stress oxydatif, Rats.*

Introduction

Women normally experience cravings for certain foods during pregnancy [1] and fructose-containing foods are the most commonly craved food items [2]. There have been reports on the negative effects of maternal consumption of fructose during gestation

on maternal metabolic status [3], placental growth, fetal development and future health of offspring [4].

Fructose is a simple sugar found naturally in honey, fruits, and vegetables [5]. It is commonly used in the food industry to prepare chocolate, drinks, candy, ice-cream and fast foods [6]. Fructose intake has been shown to contribute to the increased prevalence of obesity [7- 8], diabetes [9] and metabolic disorders [10] which are all associated with an increase in systemic oxidative stress [11].

Oxidative stress in pregnancy is caused by increased metabolic activity of placental mitochondria [12]. This oxidative stress plays physiologic roles in the development of the placenta [13], embryo [14], and fetus [15]. However, aggravated placental oxidative stress alters placental morphometric indices and may result in pregnancy complications [16]. The physiological adaptations made by the placenta in response to maternal perturbations are generally believed to be responsible for the altered pattern of fetal development and the resultant predisposition of offspring to disease in the future [17]. Also, the contribution of oxidative stress in fetal origins of adult disease is supported by epidemiological evidence of placental oxidant indices in association with type 2 diabetes [18] and preeclampsia [19]. Thus, placental oxidative stress and altered placental morphometric indices are proposed links between intrauterine insults and disease pattern in adult life.

Citrullus lanatus (Watermelon) is a natural product originally from a vine of South Africa [20]. It consists of about 93% water; hence the name "Watermelon'' [21]. The potent antioxidant and antidiabetic properties of *C. lanatus* juice have been reported [22, 23]. It was hypothesized in this study that concomitant consumption of *C. lanatus* juice along with fructose rich foods during pregnancy may prevent additional oxidative stress and its associated adverse placental and fetal effects. Thus, the aim of this study was to investigate the effects of *C. lanatus* juice on fructose-induced placental morphometric derangements and oxidative stress in Wistar rats.

Materials and methods

Experimental Animals

Twenty virgin female Wistar rats (120-150 g) and ten proven male breeders (250-300 g) were obtained from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. They were acclimatized for two weeks and had access to pelletized feed and water *ad libitum* throughout the study. The female rats were paired with the proven male breeders at a ratio 2:1 (female: male). Mating was confirmed by the presence of sperm cells in the vaginal smear and the day on which sperm cells were observed was designated as Gestation Day (GD) 1 for each rat.

Preparation of C. lanatus juice and fructose solution

C. lanatus fruits were obtained from a farm in Egbeda, Oyo state, Nigeria. Identification was done at Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo state where a voucher specimen with FHI number 110505 was deposited. Each *C. lanatus* fruit was washed and cut into small pieces. The thick epicarp layer and the seeds were removed. The fleshy red-coloured endocarp was blended using an electric blender and passed through a sieve to obtain the juice on a daily basis. A 50% concentration was prepared daily by diluting *C. lanatus* juice with drinking water at ratio of 1:1 v/v [24]. A 10% fructose (Qualikem Fine Chemical P. LTD, India) solution was freshly prepared daily by dissolving 10 g of fructose in 100 ml of drinking water [4].

Treatments

The pregnant rats were assigned into four groups (n=5) on GD 1. They were treated from GD 1-21 by replacing their drinking water with fructose solution and/or watermelon juice as follows:

Group 1 - Control (water)
Group 2 - Fructose solution (10% w/v)

Group 3 - *C. lanatus* juice (50% v/v)
Group 4 - Fructose solution (50% v/v) + *C. lanatus* juice (10% w/v)

Measurement of body weight

Maternal body weight was measured every week using a weighing balance.

Blood glucose level measurement

On GD 21, blood was collected from rat tails by nipping with a pair of fine scissors. Blood sugar was estimated from a drop of the blood with a glucometer (Accu-Check Active, Germany).

Measurement of placental morphometric indices

Caesarian section was performed under thiopentone anaesthesia (50mg/kg i.p) on GD 21 [25]. The pups and placentas were harvested and weighed on an electric balance (Lisay, China). Placental volume was measured by water displacement method [26]. Placental circumference and thickness were measured using a digital Vernier calliper (Dial, India).

Homogenization of placenta

The placentas were washed in ice cold 1.15% KCl solution, blotted with filter paper and weighed. These were then chopped into bits and homogenized into volumes of the homogenizing buffer (pH 7.4) using a Teflon homogenizer. The resulting homogenates were centrifuged at 10,000 revolutions for 15 minutes in a cold centrifuge (4^0C), to obtain the post mitochondrial fraction (PMF). The supernatant were collected and used for biochemical analyses.

Determination of protein concentration

The protein concentration of the supernatant was determined by means of the Biuret method described by Gornal *et al*. [27] using bovine serum albumin (BSA) as the standard.

Assessment of lipid peroxidation

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation as described by Rice-Evans *et al*. [28]. This method is based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde, an end product of lipid peroxidation. Briefly, an aliquot of 0.4ml of the supernatant was mixed with 1.6ml of Tris- KCL buffer to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in water bath for 45minutes at 80^0C. This was then cooled in ice and centrifuged for 15minutes at 3000rpm. The resulted clear pink solution was measured at an absorbance of 532nm against a reference blank of distilled water. The MDA level was calculated according to the method of Adam-Vizi and Sergi (1982). Lipid peroxidation in units /mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5 M^{-1}Cm^{-1}$.

Estimation of superoxide dismutase (SOD) activities

The level of SOD activity was determined by the method of Mistra and Fridovich [29]. The ability of SOD to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for SOD. Briefly, 1ml of the supernatant was diluted in 9ml of distilled water to make 1 in 10 dilutions. An aliquot of 0.2ml of the diluted supernatant was added to 2.5ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was started by addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. The absorbance at 480nm was monitored every 30 seconds for 150 seconds.

Estimation of catalase activities

Catalase activity was determined according to the method of Sinha [30]. This method is based on the fact that dichromate in acetic acid is reduced to chronic acetate when heated in the presence of hydrogen peroxide with the formation of perchromic acid as an unstable intermediate. Briefly, 1ml of the supernatant was mixed with 49ml of distilled water to give a 1 in 50 dilution of the sample. The assay mixture contained 4ml of H_2O_2 solution (800 µmoles) and 5ml of Phosphate buffer in a 10ml flat bottom flask. One milliliter of properly diluted enzyme preparation was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1ml portion of the reaction mixture was blown into 2ml of dichromate acetic acid reagent at 60s intervals. The chromic acetate produced is measured calorimetrically at 570 nm for 3 min at 60s intervals after heating the reaction mixture in a boiling water bath for 10 min. Catalase activity expressed as µmol H_2O_2 consumed/ min/mg protein.

Estimation of reduced glutathione (GSH) level

The method of Beutler *et al*. [31] was followed in estimating the level of reduced glutathione (GSH). This method is therefore based upon the development of a relatively stable (yellow) colour when 5', 5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) is added to sulfhydryl compounds present in reduced GSH. Briefly, 0.1ml of test sample (placenta homogenates) was diluted with 0.9ml of distilled water to give 1 in 10 dilutions. Then, 3ml of 4% sulphosalicyclic acid solution (precipitating solution) was added to the diluted test sample to deproteinize it. The mixture was centrifuged at 3,000g for 10 minutes. Thereafter, 0.5ml of the supernatant was added to 4ml of 0.1M phosphate buffer and finally, 4.5ml of Ellman's reagent was added. A blank was prepared with reaction mixture of 4ml of 0.1M phosphate buffer, 0.5ml of the diluted precipitating solution (addition of 3ml of precipitating solution and 2ml of distilled water) and 4.5ml of Ellman's reagent. All readings were taken within 5 minutes at 412nm, as colour produced is not stable following addition of Ellman's reagent. Reduced GSH, is proportional to the absorbance at 412nm.

Measurement of progesterone level

The progesterone ELISA kit (Calbiotech) was used for the quantitative measurement of progesterone level in the dam. The supernatant and progesterone enzyme conjugate were added to wells coated with anti-progesterone monoclonal antibody.

Progesterone in the sample competes with a progesterone enzyme conjugate for binding sites. Unbound progesterone and progesterone enzyme conjugate was washed off by washing buffer. Upon the addition of the substrate, the intensity of colour was inversely proportional to the concentration of progesterone in the samples. A standard curve was prepared relating colour intensity to the concentration of the progesterone.

Histology of placenta

A section of the placenta tissue fixed in 10% formalin was dewax in Xylene, then dehydrated in Absolute Alcohol, 95% and 70% Alcohol. Micro sections (about 4 μm) were prepared and stained with haematoxylin and eosin (H&E) dye according to Avwioro [32] and were examined under a light microscope by a Histopathologist who was ignorant of the treatment groups.

Statistical analysis

Data were presented as mean ± Standard Error of Mean (SEM). Means were compared using one-way ANOVA with Bonferroni post hoc tests. P<0.05 was considered statistically significant. All analyses were performed using Graph Pad Prism (version 5) software.

Results

There was no significant difference (p>0.05) in the body weight of dams among the groups (Fig.1). Serum progesterone level of dams showed no significant difference (p>0.05) among the groups (Fig. 2). Maternal blood glucose level at GD 21 was higher in the fructose group compared with the control group (p<0.05) and was similar in both the *C. lanatus* and fructose + *C. lanatus* groups when compared with the control group (p>0.05) (Figure 3). Placental weight and circumference were lower while placental thickness was higher in the fructose group in comparison with the control group (p<0.05) and this was similar in both the *C. lanatus* and fructose + *C. lanatus* groups when compared with the control group (p>0.05). The fetal weight on GD 21 was higher (p<0.05) in fructose group when compared with the control group and was similar in the *C. lanatus* and fructose + *C. lanatus* group in comparison with the control group (p>0.05). The feto-placental weight ratio was higher (p<0.05) in

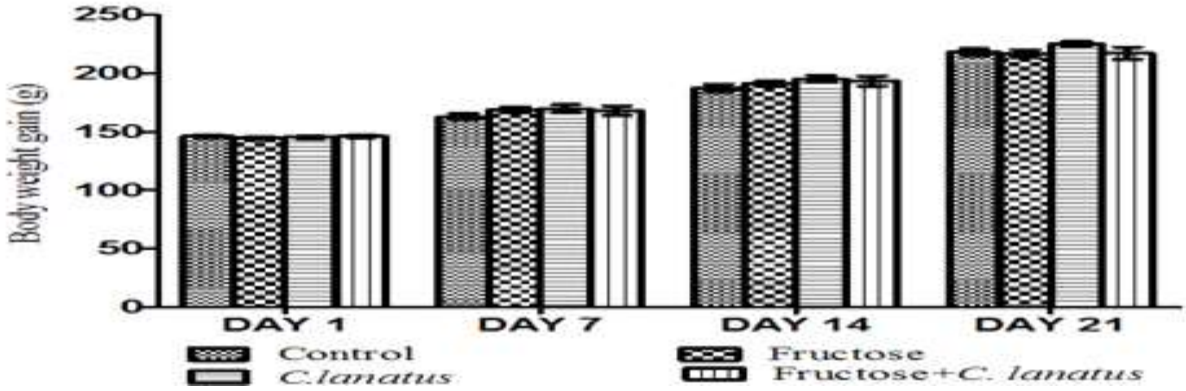

Fig. 1: Effect of fructose and *C. lanatus* juice on body weight gain of pregnant rats

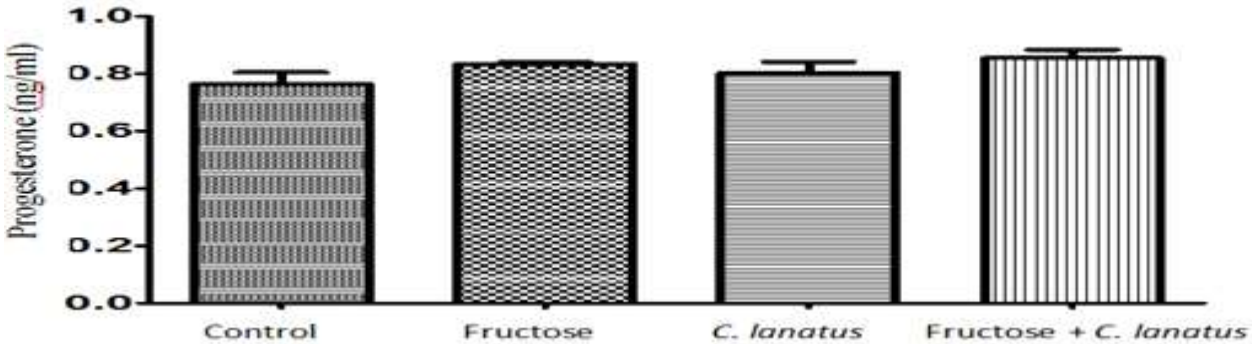

Fig. 2: Effect of fructose and *C. lanatus* juice on progesterone levels of pregnant rats

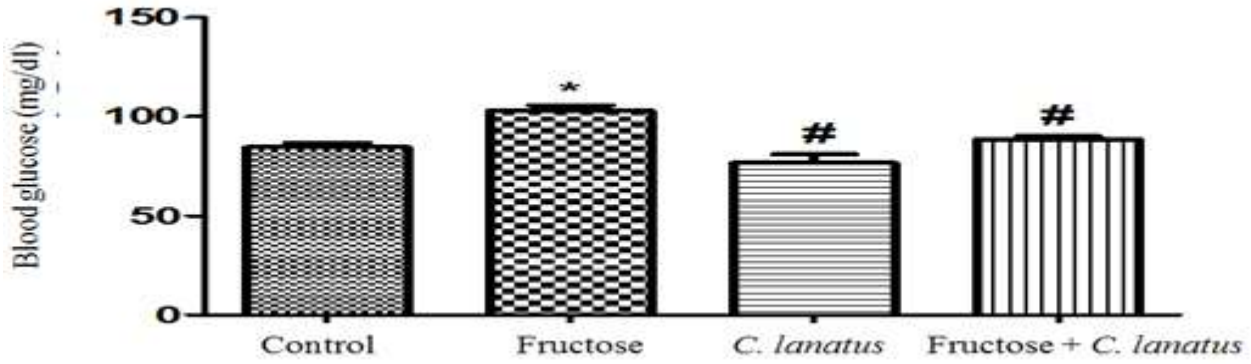

Fig. 3: Effect of fructose and *C. lanatus* juice on blood glucose level of pregnant rats
*P<0.05 when compared with control group. #P> 0.05 when compared with fructose group.

Table 1: Effect of fructose and *C. lanatus* juice on feto-placental morphometric indices.

Index	Control	Fructose	*C. lanatus*	Fructose + *C. lanatus* juice
Fetal weight (g)	3.5 ± 0.0	3.8 ± 0.0*	3.6 ± 0.1#	3.6 ± 0.0#
Placental Weight (g)	0.6 ± 0.0	0.5 ± 0.0*	0.7 ± 0.0#	0.6 ± 0.0
Circumference (mm)	4.8 ± 0.1	4.5 ± 0.0*	4.7 ± 0.1#	4.5 ± 0.0*
Thickness (mm)	2.5 ± 0.0	2.9 ± 0.1*	2.6 ± 0.1#	2.6 ± 0.0#
Volume (cm^3)	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0
Feto-Placental weight ratio	5.8 ± 0.1	7.0 ± 0.2*	5.5 ± 0.1#	6.3 ± 0.2#

n=5, *p<0.05 when compared with control group, #p>0.05 when compared with fructose group.

Table 2: Effects of fructose and *C. lanatus* juice on placental oxidative status

	Control	Fructose	C. *lanatus*	Fructose + C. *lanatus*
MDA(U/mg protein)	0.001±0.0001	0.002± 0.0002*	0.0003±0.00001*#	0.001±0.00003#
SOD(U/mg protein)	5.8 ± 0.4	2.2 ± 0.3*	10.3 ± 0.9*#	6.1 ± 0.4#
Catalase (IU/L)	0.4 ± 0.01	0.4 ± 0.004	0.5 ± 0.003*	0.4 ±0. 0002
GSH(U/mg protein)	38.8± 2.1	25.5 ± 7.7	78.0 ± 4.4*	39.1±3.4#
Protein conc. (mg)	1.6±0.01	1.7±0.01	0.8±0.02*#	1.5±0.02

n=5, *P<0.05 when compared with control group, # P>0.05 when compared with fructose group
MDA = Malondialdehyde; SOD = Superoxide dismutase; GSH = Reduced glutathione

the fructose group than in the control group and was similar (p>0.05) in the *C. lanatus* and fructose + *C. lanatus* groups when compared with the control group (Table 1). Placental superoxide dismutase and reduced glutathione are lower in the fructose group compared with control (p < 0.05) and was similar in the fructose + *C. lanatus* group compared with the control group (p > 0.05). Placental malondialdehyde was higher in the fructose group and lower in the fructose + *C. lanatus* group compared with the control and the fructose groups respectively (Table 2). Photomicrograph of the control and *C. lanatus* groups showed normal chorionic villi, while the fructose and fructose + *C. lanatus* groups showed severe and moderate infarction of the chorionic, respectively (Fig. 4).

Discussion

Maternal fructose consumption alters placental growth and pattern of fetal development [4]. Fructose consumption causes oxidative stress and metabolic derangements in the placenta [33-34], both of which

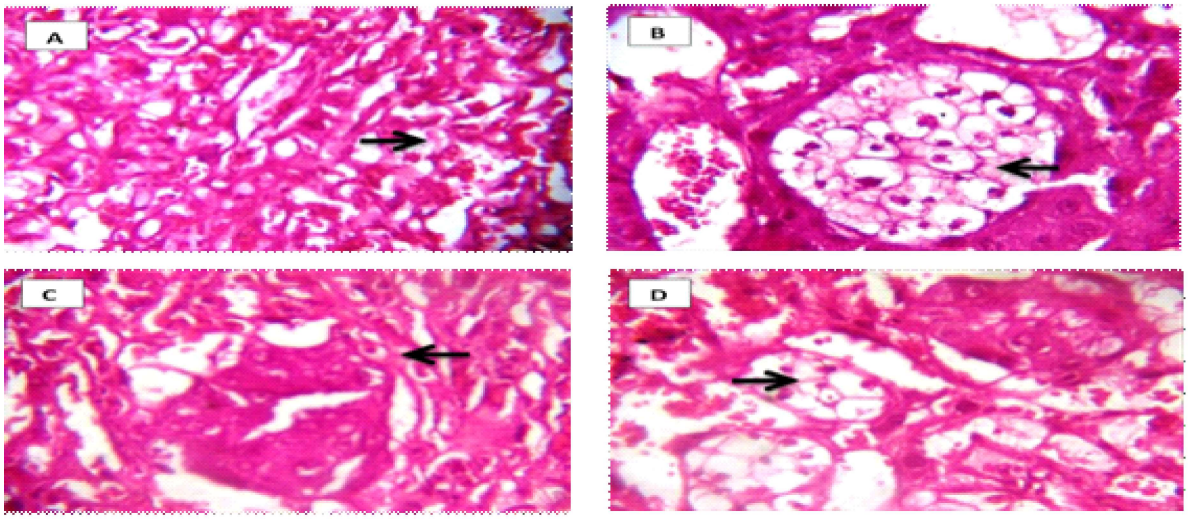

Fig. 4: Photomicrographs of placentas from pregnant rats given fructose solution and *C. lanatus* juice throughout gestation. H &E. Magnification X400. A = Control, B = Fructose, C = *C. lanatus,* D = Fructose + *C. lanatus.*

are detrimental to fetal development. There have been reports on the antioxidant and hypoglycemic effects of *C. lanatus* juice [23]. This study demonstrated the effects of *C. lanatus* juice on fructose-induced placental morphometric aberrations and oxidative stress in Wistar rats.

The higher maternal blood glucose level in the fructose group suggests that increased fructose consumption during pregnancy may cause hyperglycemia. This confirms results from previous studies that associated high consumption of fructose and fructose-rich foods with development of hyperglycemia and diabetes [3]. These conditions during pregnancy are known to affect fetal development with resultant susceptibility to health issues in adult life [3, 11, 35]. The rats which received both fructose and *C. lanatus* juice were normoglycemic suggesting that *C. lanatus* juice has the ability to prevent development of hyperglycemia in pregnant rats. Lycopene, a hypolipidemic agent present in *C. lanatus* may be attributed to this effect [36]. Maternal hyperglycemia throughout gestational period predisposes to an increase in glucose transport across the placenta [37]. Since maternal insulin does not cross the placenta [38], the pancreas of the fetus causes compensatory increase in insulin secretion thus stimulating increase in pup growth and adiposity which can result in the higher birth weight that was recorded in the fructose group. The birth weight of the group that had *C. lanatus* plus fructose was comparable with that of the control group. Again, the adiposity-countering effect of lycopene becomes evident.

Feto-placental ratio is often used as a proxy for placental efficiency and it is defined as the weight of fetus produced per weight of placenta [39]. In the present work, the observed increase in the fructose group feto-placental ratio might be due to low placental weight and high birth weight. Adequate fetal growth is dependent on nutrient transfer by the placenta [40] and evidence has shown that the placenta adapts anatomically and physiologically to changes in the environment in order to achieve optimal fetal growth [41]. These adaptations normally occur in response to maternal and/or fetal insults [41] and failure of such placental adaptations may result in high or low birth weight [42]. Thus, increased fetal weight might have resulted from the failure of placental adaptation. The failure of placental adaptation may also have contributed to the asymmetric increment in body weight. In addition, fructose consumption is reported to elevate uric acid production [43] which can lead to increased *de novo* lipogenesis [44] in the placenta and resulting in increased lipid traffic across the feto-placental unit causing high birth weight. Reduction in placental weight in dams fed with fructose has been reported [4] but why maternal fructose consumption causes reduced placental weight remains elusive. In the fructose + *C. lanatus* group, the placental weight, birth weight and feto-placental ratio were normal and comparable with that of control group. Again, the hypolipidemic effect of lycopene becomes evident.

Malondialdehyde (MDA) is an index of lipid peroxidation. The elevated malondialdehyde

levels observed in the placenta of fructose-fed dams in this study suggests the accumulation of superoxide radicals and hydrogen peroxide in the placenta thereby making it prone to oxidative stress [45]. As explained earlier, high fructose consumption during pregnancy may predispose to uremia with resultant oxidative stress. Oxidative stress is normal during pregnancy; however, exaggerated levels have been reported in pregnancies associated with complications. The low activity of superoxide dismutase in the fructose group placenta indicates that there was an accumulation of superoxide radicals in the placenta of fructose-fed dam. Superoxide dismutase (SOD) constitutes an important link in the biological defense mechanism through conversion of endogenous cytotoxic superoxide radicals to H_2O_2 which are harmful to polyunsaturated fatty acids and proteins [46- 47]. Catalase further detoxifies H_2O_2 into H_2O and O_2 [46].

Similarly, the reduction of catalase activity observed in placenta of fructose-fed dam clearly reflects the inability of the tissue to eliminate H_2O_2 produced by the inactivation of the enzymes probably due to the excess generation of ROS. Results suggest the reduction in the levels of these enzymes led to oxidative stress in the placenta of the fructose group. However, *C. lanatus* supplementation successfully preserved the levels of these enzymes. Reduced glutathione (GSH) is considered to be one of the most significant constituents of the antioxidant defense of living cells. The reduction of GSH may be one of the reasons for the increased vulnerability added to free-radical-induced damage.

Therefore, the decreased in GSH level observed in this study suggests that its toxic effects may expose the placenta to damage. *C. lanatus* supplementation successfully ameliorates the levels of these antioxidant compared to the fructose-fed group. The observed oxidative stress as demonstrated by increased MDA reduced SOD, catalase and GSH may be responsible for the severe infarction of chorionic villi seen within the frondosum layer as shown by the photomicographs of placenta.

However, supplementation with *C. lanatus* juice successfully ameliorated the fructose-induced placental oxidative stress and histological aberrations. This is in line with the studies of Oseni *et al.* [23] and Mohd *et al.* [24] which reported that watermelon juice decrease lipid peroxidation. This could be an indication that this medicinal fruit has the essential potentials to mitigate oxidative processes caused by maternal high fructose intake.

Conclusion

Citrullus lanatus juice modulated fructose-induced placental morphometric changes and oxidative stress in Wistar rats. Thus intake of *C. lanatus* juice may be beneficial for optimal and healthy development of placenta and fetus of mothers who experience sugar cravings during pregnancy.

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Comparison of post-operative pain control and stress response from rectal diclofenac and pre-incisional wound infiltration with bupivacaine in paediatric herniotomy

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Abstract
Background: Herniotomy is one of the most common procedures performed by the paediatric surgeon. Its most common complication is post-operative pain. Furthermore, is the known stress response associated with surgery, which may also have deleterious effects. Analgesics are effective in reducing both these effects. This study compares the effectiveness of two analgesic options- rectal diclofenac and wound-infiltrated bupivacaine, on post-operative pain and stress response.
Methods: This was a prospective, double blinded clinical study. Seventy two children undergoing herniotomy were randomized into group 1 (Bupivacaine group) and group 2 (Diclofenac group). The children were similar in all other aspects except for the treatment they received. Pain was evaluated by a blinded assessor on arrival of the patient in the recovery room using the FLACC scale. Blood samples were taken following induction of anaesthesia and at the end of surgery for cortisol and glucose estimations.
Results: The mean pain scores were similar in both groups post-operatively and it remained below a score of 4 throughout the study period. There was no significant difference between the pre- and post-operative serum cortisol and glucose levels in both groups ($p > 0.05$)
Conclusion: Rectal diclofenac provides a comparative effectiveness of analgesia to wound-infiltrated bupivacaine in children undergoing herniotomy.

Keywords: *Paediatric herniotomy, Rectal Diclofenac, stress response, pre-incisional wound infiltration, Bupivacaine, post-operative pain*.

Résumé
Contexte: L'herniotomie est l'une des procédures les plus courantes effectuées par les chirurgiens pédiatriques. Sa complication la plus fréquente est la douleur post-opératoire. En outre, est la réponse au stress connue associée à la chirurgie, qui peut également avoir des effets délétères. Les analgésiques sont efficaces pour réduire ces deux effets. Cette étude compare l'efficacité de deux options analgésiques - le diclofénac rectal et la bupivacaïne infiltrée par la plaie, sur la douleur post-opératoire et la réponse au stress.
Méthodes: Ceci était une étude clinique prospective, aveuglement doublée. Soixante-douze enfants subissant une herniotomie ont été randomisés dans le groupe 1 (groupe bupivacaïne) et groupe 2 (groupe diclofénac). Les enfants étaient dans tous les autres aspects sauf pour le traitement qu'ils ont reçu. La douleur a été évaluée par un évaluateur aveugle à l'arrivée du patient dans la salle de réveil en utilisant l'échelle FLACC. Des échantillons de sang ont été prélevés après l'induction de l'anesthésie et à la fin de la chirurgie pour les estimations de cortisol et de glucose.
Résultats: Les scores de douleur moyens étaient similaires dans les deux groupes après l'opération et ils sont restés sous le score de 4 tout au long de la période d'étude. Il n'y avait pas de différence significative entre les niveaux de cortisol et de glucose sériques pré- et post-opératoires dans les deux groupes ($p > 0,05$)
Conclusion: Le diclofénac rectal fournit une efficacité comparative de l'analgésie à la bupivacaïne infiltrée par la plaie chez les enfants subissant une herniotomie.

Mots clés: *Herniotomie pédiatrique, diclofénac rectal, réponse au stress, infiltration pré-incision, bupivacaïne, douleur postopératoire*.

Introduction

Herniotomy is one of the most common procedures performed by paediatric surgeons worldwide [1–3] and it is safely performed as a day-case procedure [4–7]. Post-operative pain has been reported as the commonest complication of this procedure and one of the commonest causes of unanticipated hospital admission after day-case procedures [5,8]. The treatment of pain is, therefore, an integral part of

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day-case herniotomy. Various modalities of analgesia exist and multimodal approach to preventing and treating pain is now being employed [9]. The multimodal approach involves combination of mild analgesics with regional and local analgesia to achieve effective pain relief and reduce opioids-related side effects [9–11]. Opioids, which had hitherto been the mainstay in the treatment of acute post-operative pain, are increasingly being avoided because of their documented side effects such as respiratory depression and delayed recovery from anaesthesia [12]. The use of non-opioid analgesics, such as the non-steroidal anti-inflammatory drugs (NSAIDs), is much favoured recently as they are effective and safe in children [12–14]. This practice is, however, not common in developing countries where options of medications may be limited. The use of bupivacaine for wound infiltration and oral paracetamol for post-operative pain are the generally employed methods of post-operative pain management in our centre. Oral paracetamol, however, has been shown to be more effective in children with mild to moderate pain [15].

Surgery is associated with stress response, which is increased in the presence of post-operative pain, due to secretion of cortisol and other stress response hormones [16–18]. This response can be attenuated by various analgesic modalities. Stress response hormone levels can, therefore, be used as an objective method of assessment of the analgesic efficacy of these modalities [16,19].

The aim of this study was to compare the effect of rectal diclofenac and wound-infiltrated bupivacaine on post-operative pain and cortisol levels in children undergoing herniotomy in our centre.

Materials and methods

This was a prospective, randomized, double-blinded clinical study of 72 children that underwent unilateral herniotomy at the University College Hospital (UCH), Ibadan, Nigeria between July 2013 and October 2014. Ethical approval was obtained from the Joint University of Ibadan and University College Hospital Ethics Review Committee and the Oyo State Research Ethical Review Committee. ASA class I or II patients (of consenting parents) with congenital inguinal hernias and hydroceles undergoing herniotomy in the Paediatric Surgery Unit, UCH were recruited for the study. While patients with bilateral, recurrent and complicated hernias, undescended testes, history of dyspepsia and known allergies to either test agents used were excluded from this study.

No premedication was given. General anaesthesia was induced and maintained using isoflurane and oxygen via facemask in younger children and laryngeal mask in older children, with spontaneous respiration. All patients were given a single dose of intravenous fentanyl (at a dose of 1 $\mu g\ Kg^{-1}$) as intra-operative analgesia (protocol followed in our centre) immediately after the induction of anaesthesia. Routine intra-operative monitoring was used.

Patients were randomly assigned to two groups. Patients in the Bupivacaine group (Group 1) had the operation site infiltrated with bupivacaine, 0.5 ml Kg^{-1} at a concentration of 2.5 mg ml^{-1}, immediately after induction of anaesthesia and two minutes before making the incision. Those in the diclofenac group (Group 2) had rectal diclofenac administered at a dose of 1-2 mg Kg^{-1} immediately after the induction of anaesthesia, and two minutes before incision was made, while no skin infiltration was done. Diclofenac suppository preparations of 12.5 mg were used and whole preparations were administered at all times, ensuring that the given dose fell within the recommended dose range. We avoided breaking the suppositories to avoid the uncertainty about the drug distribution within the suppository. Standard surgical procedure of herniotomy was performed on all the patients. The same surgical team performed all the surgeries. The patients' peri-operative pulse rate, respiratory rate and blood pressure were recorded. The length of incision, duration of procedure and estimated blood loss in each case were determined and recorded.

Pain measurement

In the recovery room, pain assessments were done at 15 minutes, one hour, two hours and four hours by independent anaesthetists, who were not involved in the procedures and were blinded to the group that the patients were allocated to. The University of Michigan Health System "FLACC" scale [20] was used for an objective assessment of pain (Table 1). Patients with pain score > 4 were treated with intravenous paracetamol or pentazocine as rescue analgesics. The time and frequency of giving rescue analgesia were also recorded.

Endocrine measurement

Venous blood samples were obtained from each patient for the estimation of cortisol and glucose 1-2 minutes after induction of anaesthesia and before administration of the test drug, and 1-2 minutes after the last stitch (before recovery from anaesthesia). Enzyme linked immunosorbent assay (ELISA) was

used to measure the serum cortisol levels (GenWay Biotech Inc. ELISA Cortisol Kit, San Diego, US). Serum glucose estimation was done using the Contour® Glucometer (Bayer Health Care LLC, Mishawaka, IN 46544, USA), in which a drop of blood was placed on a strip, which was then inserted into the reader to give an automatic estimation of the glucose level.

Statistical analysis

Data obtained were analysed using SPSS version 21 software. The sample size was determined with the formula for comparing means using the mean and standard deviations from a previous study and using a power of 80% [21,22]. Values were expressed as mean ± standard deviation. Logarithmic transformation of the serum cortisol levels was done and comparison of means between the study groups was achieved using the Independent t-test. Paired sample t test was used to analyse the differences between pre- and post-operative serum cortisol and glucose levels in all the participants. Categorical variables were analyzed using the Chi Square and the Fisher's Exact Test. A p value of < 0.05 was considered significant.

Results

A total of 72 patients (36 in each group) were recruited for this study. Their ages ranged from six months to 12 years, with a mean of 61.86 ± 43.86 months. There was no significant difference between the two groups with regards to the ages of patients, their weights and length of surgical incisions (Table 2). The two groups were also similar in terms of the length of pre-operative fast and duration of surgery. There was no significant difference between the mean FLACC pain score in both groups at the various times of assessment (Table 3). The mean pain score in both groups remained below 4 for the entire four hours of assessment. The mean pain score reduced from a maximum at the time of first assessment to a minimum by the fourth hour of assessment (Fig. 1).

Table 1. FLACC Pain Score

	0	**1**	**2**
Face	No particular expression or smile	Occasional grimace or frown, withdrawn, disinterested	Frequent to constant frown, clenched jaw, quivering chin
Legs	Normal position or relaxed	Uneasy, restless, tense	Kicking or legs drawn up
Activity	Lying quietly, normal position, moves easily	Squirming, shifting back and forth. tense	Arched, rigid, or jerking
Cry	No cry (awake or asleep)	Moans or whimpers, occasional complaints	Crying steadily, screams or sobs, frequent complaints
Consolability	Content, relaxed	Reassured by occasional touching. hugging or "talking to : distracable	Difficult to console or comfort

Table 2. Patients' characteristics, clinical data, duration of surgery, length of pre-operative fast, length of incision and rescue analgesia requirement.

	Bupivacaine group (Group 1, n = 36)	Diclofenac group (Group 2, n = 36)	95% confidence interval of the difference
Age (months)	66.61 ± 46.77	57.11 ± 40.85	-11.14 – 30.14
Sex (M/F)	31/5	34/2	
Weight (Kg)	20.56 ± 11.61	17.56 ± 8.22	-1.74 – 7.72
Heart rate			
Pre-operative	102.61 ± 21.27	104.67 ± 27.92	-13.72 – 9.61
Post-operative	111.00 ± 20.12	110.03 ± 23.76	-9.38 – 11.32
Duration of surgery (mins)	35.91 ± 12.78	36.77 ± 11.35	-6.62 – 4.91
Length of pre-operative fast (hours)	15.13 ± 9.53	13.48 ± 3.20	-1.70 – 4.99
Length of incision (cm)	3.27 ± 0.59	3.30 ± 0.69	-0.82 – 0.10
Rescue analgesia	4	3	

Values are expressed in mean ± SD

Table 3. FLACC pain scores

`	Bupivacaine group	Diclofenac group	95% confidence interval of the difference
FLACC at 15 mins	1.36 ± 2.80	0.89 ± 2.14	-0.70 – 1.64
FLACC at 1 hour	1.19 ± 2.42	0.50 ± 1.52	-0.26 – 1.65
FLACC at 2 hours	0.56 ± 1.23	0.28 ± 0.78	-0.21 – 0.76
FLACC at 4 hours	0.17 ± 0.45	0.14 ± 0.83	-0.29 – 0.34

Values are expressed in mean ± SD

Four patients in Group 1 had rescue analgesia (intravenous paracetamol) for FLACC pain score of > 4, while 3 in Group 2 required rescue analgesia. These patients had the rescue analgesics after the first assessment, except for 2 (1 in each group), who required it after the second assessment. None of them required a repeat dose of rescue analgesia. There was no significant difference between the two groups in terms of the patients' requirement for rescue analgesia.

There was no significant difference between the baseline serum cortisol levels of both groups (Table 4). The post-operative serum cortisol level was significantly higher than at baseline in the bupivacaine group but not in the rectal diclofenac group (p = 0.008 in Group 1 and p = 0.127 in Group 2). There was, however, no significant difference between the mean post-operative serum cortisol levels in the two groups. The serum glucose levels also followed a similar trend to that of serum cortisol.

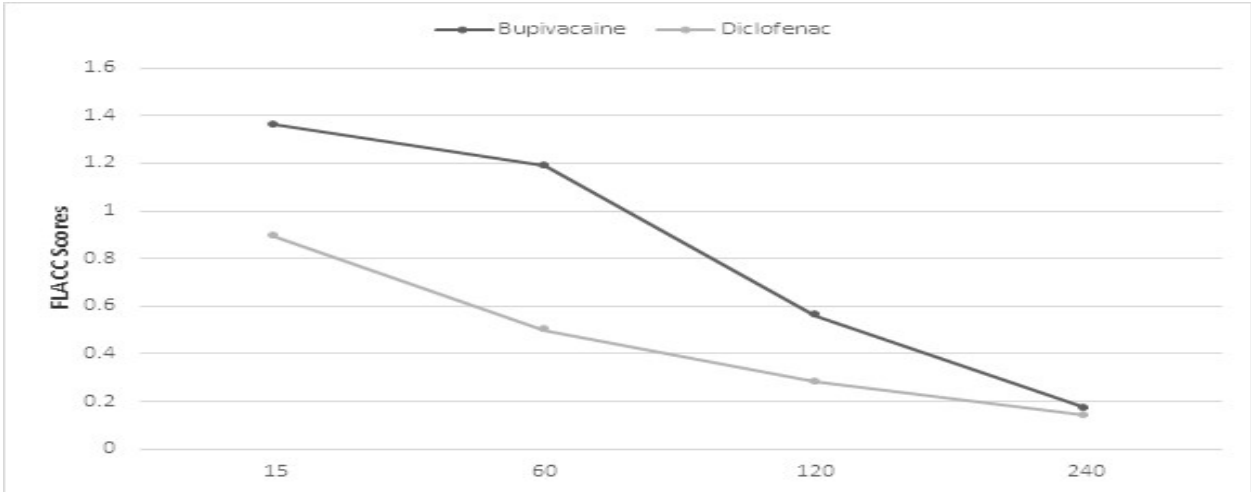

Table 4. Cortisol and Glucose values

Serum Cortisol (ng ml^{-1})	Bupivacaine group	Diclofenac group	95% confidence interval of the difference
Pre-operative	234.77 ± 191.69	284.92 ± 279.83	-179.45 – 79.17
Post-operative	430.30 ± 355.37	396.21 ± 316.16	-148.07 – 216.25
Serum Glucose (mg dl^{-1})			
Pre-operative	74.49 ± 10.28	81.27 ± 31.60	-17.95 – 4.40
Post-operative	102.14 ± 41.48	129.66 ± 93.09	-61.67 – 6.63

Values are expressed in mean ± SD

The pre-operative heart rates were also similar in both groups with a consistent and also similar increase post-operatively. There was no significant difference in the estimated blood loss between the two groups.

Discussion

Hernia repair in children is a day-case surgery. Hence, most of the post-operative care is carried out at home and by the parents. Pain is the most common complication and cause of unanticipated hospital admission following day-case procedures with much of this being underplayed and pain relief often inadequate [5,8,23].

Multimodal approach, which involves the combination of two or more modalities to prevent and treat pain, has been advocated [24]. Pre-incisional and post-incisional infiltration of the wound with levobupivacaine, caudal anaesthesia and the use of intra-muscular and suppository diclofenac have all been shown to be effective modalities. They reduce the requirement for opioids, increase the duration of post-operative analgesia and promote early recovery [8,16,25,26]. Bupivacaine, a long-acting anaesthetic agent, has been variously shown to be effective for post-operative pain but is also known to be associated with systemic toxicity when inadvertently administered into the vascular system [24,27,28]. This has stimulated interest in the administration of alternative agents.

The use of NSAIDs is not popular in our centre and most other centres across Nigeria. The protocol for the treatment of post-operative pain has been limited to the use of wound-infiltration with either lidocaine or bupivacaine at the end of surgery, in addition to oral paracetamol, which is given as the take-home medication.

This study provides evidence that either infiltrating the wound with bupivacaine or administering rectal diclofenac pre-operatively was effective for post-operative analgesia following herniotomy in children. In the present study, the mean pain scores remained low (< 4) throughout the post-operative study period in both groups. The mean pain score (FLACC scale) in both study groups gradually fell from a maximum in the immediate post-operative period to a minimum at 4 hours. Solanki *et al* in a comparison of bupivacaine infiltration and diclofenac suppository in 50 patients undergoing tonsillectomy found the latter to be a better option in terms of its convenience and duration of analgesia [29]. However, the pain experience of the patient and the effectiveness of analgesia of the test drugs may be different between tonsillectomy and herniotomy.

The mean pain score was relatively higher in the immediate post-operative period in both groups in the present study. This is similar to what was reported by Moores *et al,* who studied post-operative analgesia in 43 children, comparing rectal diclofenac to caudal bupivacaine [30]. They reported that rectal diclofenac was less potent in the immediate post-operative period but of comparable efficacy to caudal blockade after two hours. This delayed efficacy was ascribed to the possibility of some delay in the absorption of diclofenac from the rectal mucosa [30]. This report did not agree with our study in which, bupivacaine infiltration was not more effective but comparable to rectal diclofenac as the mean pain score at 15 minutes and 1 hour post-operation were comparable to the rectal diclofenac group.

The present study showed a comparable analgesic efficacy between rectal diclofenac and bupivacaine wound infiltration. Gupta *et al,* in a similar comparative study of the analgesic efficacy of rectal diclofenac and caudal block alone reported a mean duration of analgesia of 12.45 hours with rectal diclofenac administered at a dose of 1 mg Kg^{-1} as against 8.2 hours provided with caudal block [25]. Gupta *et al* in that study noted that rectal diclofenac alone or in combination with caudal block provided excellent analgesia and lasted for a longer time than caudal block alone in children undergoing infra-umbilical surgeries [25]. Gupta *et al*, however, also observed the delay in the onset of analgesia with diclofenac when compared to caudal block as reported by Moores *et al*. The mean pain scores only became comparable at 1 hour post-operation [25]. This delay was not observed in our study, similar to the findings of the study by Solanki *et al* [29]. This difference in the onset of analgesia was ascribed to the dose of rectal diclofenac used in the study by Solanki *et al*, which was 2mg Kg^{-1} as against 1mg Kg^{-1} used in the studies by Moores *et al* and Gupta *et al*. A dose of 1 to 2mg Kg^{-1} of rectal diclofenac was used in this study to avoid having to divide the suppositories. The 12.5mg formulation of rectal diclofenac was used for this study. Whole suppositories were, therefore, used ensuring that the dose given fell within the specified range. It is possible that difference in dosage in the various studies is responsible for the variation in the findings on the onset of analgesic efficacy of rectal diclofenac.

Three of the patients in the diclofenac group and four in the bupivacaine group required additional analgesics for pain scores greater than 4. This is comparable to the study by Moores *et al* who reported comparable requirement for additional analgesia between patients who had rectal diclofenac

and those who had caudal bupivacaine for day-case herniotomy [30].

The serum cortisol level is expected to rise as part of the usual stress response to surgery. The level of rise can, however, be reduced by attenuation of the stress response, which can be achieved by various analgesic modalities [16]. The mean baseline serum cortisol level in this study (259.39 ng ml^{-1}) falls within the range of 60 to 300ng ml^{-1} estimated for adult Nigerians at the University College Hospital, Ibadan [31] and the levels were comparable in both groups. There was comparable difference between pre- and post-op serum cortisol levels between both groups. This finding corroborates our finding of a comparable mean pain scores between the two study groups. There was a similar trend in the serum glucose response in this study, which mirrored that of the serum cortisol response.

Theoretically, NSAIDs can cause increased bleeding because of their known anti-platelets effect. None of the participants in this study, however, was observed to have excessive bleeding following surgery and estimated blood loss was comparable between the two groups. A similar finding was reported by Adarsh *et al* who found no significant bleeding in excess of usual in children who had pre-operative rectal diclofenac for cleft palate repair [12]. No incident of significant post-operative bleeding was also reported in the studies by Gupta *et al* and Moores *et al* [25,30]. This finding also agrees with the conclusion by the Cochrane review on the safety of the use of diclofenac in children and the review by Rømsing and Walther-Larsen [13,14]. These reviews on the use of NSAIDs noted no excessive bleeding in any child and found no difference in the rates of bleeding requiring surgical intervention in tonsillectomy (a high risk surgery for bleeding) between participants randomized to diclofenac and non-NSAIDs. We, however, excluded patients with either personal or family history of asthma or dyspepsia because of the known effects of NSAIDs on prostaglandin synthesis. No adverse drug reaction was recorded amongst any of the participants in this study. A major limitation of this study was the subjective nature of the FLACC scale that was used for the evaluation of pain control. In a way to reduce this subjectivity bias, we compared the vital signs, which were noted to change with experience of pain in children.

Conclusion

Rectal diclofenac provides a comparative effectiveness of analgesia to wound-infiltrated bupivacaine in children aged six months and older undergoing day-case herniotomies. It also has similar effects on stress response to surgery, measured with serum cortisol and blood glucose, in the early post-operative period to that produced by bupivacaine infiltration. It can be safely given to patients who do not have personal or family history of asthma, dyspepsia, bleeding diathesis and known allergy to NSAIDs. The findings of the current study suggest that rectal diclofenac is an effective alternative to bupivacaine infiltration for early post-operative analgesia in children.

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Comparative study of physico-chemical properties of saliva in caries free and caries active Nigerian children

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Abstract

*Background***:** Saliva is a body fluid with several functions involved in the maintenance of homeostasis and oral health. However, the physico-chemical properties of saliva may predispose an individual to caries.

Aim: To compare the saliva flow rate, pH, buffering capacity, total antioxidant capacity, calcium and phosphate levels and *Streptococcus mutans* counts in caries free and caries active children.

*Methods***:** The cross sectional study comprised of two groups of children aged 6-12-years-old, 44 caries free (dmft/DMFT < 1) and 44 caries active (dmft/DMFT > 1) from Ile-Ife. Unstimulated saliva was collected to evaluate the physico-chemical properties. The pH and buffering capacity were estimated using the digital pH meter, total antioxidant capacity was determined by phosphomolybdenum method and *Streptococcus mutans* count estimated using Mitis Salivarius Bacitracin (MSB) agar. The mean values of the two groups were compared using Student's t-tests. Statistically significant value was inferred at $p<0.05$.

Results: The mean saliva flow rate and buffering capacity were significantly higher in children without caries (0.55 ± 0.10 and 8.93 ± 0.53) compared to children with caries (0.50 ± 0.08 and 8.21 ± 0.43) respectively. Mean total antioxidant capacity was significantly increased in children without caries than caries active children (0.25 ± 0.20 vs 0.17 ± 0.11, $p=0.04$). Similarly, mean *Streptococcus mutans* counts was significantly higher in children with caries compared to those without caries ($1.10 \pm 0.35 \times 10^4$ vs $0.21 \pm 0.21 \times 10^4$, $p=0.01$).

Conclusion: The physico-chemical properties of saliva such as flow rate, buffering capacity, phosphate level and *Steptococcus mutans* counts play a role in caries process.

Keywords: *Saliva, flow rate, Streptococcus mutans, caries*

Résumé

Contexte **:** La salive est un fluide corporel avec plusieurs fonctions impliquées dans le maintien de l'homéostasie et la santé bucco-dentaire. Cependant, les propriétés physicochimiques de la salive peuvent prédisposer un individu à la carie.

Objectif: Pour comparer la vitesse d'écoulement de la salive, le pH, la capacité tampon, la capacité antioxydant totale, les taux de calcium et de phosphate et l'estimation de *Streptococcus mutans* parmi les enfants sans caries et avec caries actives.

Méthodes **:** L'étude transversale comprenait deux groupes d'enfants âgés de 6 à 12 ans, 44 sans caries (dmft / DMFT <1) et 44 avec caries actives (dmft / DMFT> 1) provenant d'Ile-Ife. La salive non stimulée a été recueillie pour évaluer les propriétés physicochimiques. Le pH et la capacité tampon ont été estimés en utilisant le pH-mètre numérique, la capacité antioxydant totale a été déterminée par la méthode au phospho-molybdène et le nombre de *Streptococcus mutans* estimé en utilisant Mitis Gélose salivaire à la bacitracine (MSB). Les valeurs moyennes des deux groupes ont été comparées en utilisant le Student t-test. Une valeur statistiquement significative a été déduite à $p <0,05$.

Résultats: Le débit salivaire moyen et la capacité tampon étaient significativement plus élevés chez les enfants sans caries ($0,55 \pm 0,10$ et $8,93 \pm 0,53$) que chez les enfants avec caries ($0,50 \pm 0,08$ et $8,21 \pm 0,43$) respectivement. La capacité antioxydant totale moyenne était significativement augmentée chez les enfants sans caries que chez les enfants avec caries actives ($0,25 \pm 0,20$ vs $0,17 \pm 0,11$, $p = 0,04$). De même, le nombre moyen de *Streptococcus mutans* était significativement plus élevé chez les enfants atteints de caries que chez ceux sans carie ($1,10 \pm 0,35 \times 10^4$ vs $0,21 \pm 0,21 \times 10^4$, $p = 0,01$).

Conclusion: Les propriétés physicochimiques de la salive telles que le débit, la capacité tampon, le taux de phosphate et le nombre de *Streptococcus mutans* joue un rôle dans le processus de caries.

Mots clés: *Salive, débit, Streptococcus mutans, caries*

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Introduction

Oral health is an essential part of the general health. Poor oral health is detrimental to children as it affects nutrition, growth and development. Dental caries is one of the most common childhood diseases and a major cause of tooth loss in the younger population [1]. If untreated, it could lead to pain, dental abscess, disturbance of mastication and growth, destruction of bone and septicaemia [2].

Dental caries is a multifactorial problem, caused by the action of bacteria on refined carbohydrates over a period of time, with resultant production of lactic acid [3]. The acid results in demineralization of the enamel [3]. However, saliva can reverse this process through increased salivary flow and fluoride effect [4]. Saliva is a body fluid with numerous functions that is involved in maintenance of homeostasis and oral health [5]. It has been identified to play an important role in caries initiation and prevention through its buffering, mechanical cleansing, antimicrobial, and remineralization activities [6, 7]. However, some researchers have suggested salivary characteristics and components as predisposing factors to early childhood caries [8-10].

Some studies found a difference in the mean salivary pH of children without caries compared with caries active children while others found no difference [9–12]. Some researchers demonstrated that children without caries had significantly higher mean saliva pH than those with caries [9, 10] Indicating that children with low saliva pH may be predisposed to caries. However, other studies found mean pH to be similar in children with or without caries [11, 12].

The ability of the salivary pH to be maintained at its normal level is dependent on its buffering capacity. The bicarbonate and phosphate ions constitute the principal buffering components of the saliva [5]. Zehetbauer *et al.*, [8] found buffering capacity to be significantly high in children without caries while other studies found no difference in the buffering capacity of saliva [10, 11, 13].

Different types of bacteria inhabit the oral cavity, amongst these is *Streptococcus mutans* (SM), which has been implicated in the initiation of dental caries and considered a risk factor for onset of caries [14]. Studies have demonstrated a correlation between the frequency of *Streptococcus mutans* and dental caries in children [15,16].

Saliva plays an important role in the onset and development of dental caries [17]. It can act as a good medium for individual caries risk assessment especially in children. It is easy and quick to collect and less infectious to health care provider. Therefore, this study aimed to compare the saliva flow rate, pH, buffering capacity, total antioxidant capacity (TAC), calcium and phosphate levels and *Streptococcus mutans* counts in children with and without caries.

Material and methods

The study was carried out in Ile- Ife, southwestern Nigeria. An ancient Yoruba city located in the tropical savanna climate and lies on longitude 4^0 69'E and latitude 70^0 50'N. It has two Local Government Areas (LGA), Ife East and Ife Central. This was a cross sectional study that compared the saliva flow rate, pH, buffering capacity, total antioxidant capacity, calcium and phosphate levels and *Streptococcus mutans* counts in children with and without caries. It comprised of a sample of 88 children aged 6-12 years randomly selected from three primary schools (1 private and 2 public) in Ife Central LGA. Ife Central LGA was selected out of the two local government areas by balloting. Three primary schools were randomly selected from a list of primary schools in the LGA (The number of public primary schools is twice the private primary schools). Within each school, children with and without caries were identified. The participants were randomly selected from the identified group using the class register until the sample size was attained.

The participants were given consent forms and questionnaires for their parents/guardians. Each parent/guardian had to give consent for the child to participate in the study and also fill the questionnaire regarding use of antibiotics by the child, the duration for which the antibiotics was taken and also if the child has any medical conditions. The children were divided into two groups; 44 children with caries (dmft/DMFT >1) and 44 children without caries (dmft/DMFT = 0). The children in the caries group had at least one decayed tooth. Caries status was assessed according to World Health Organization index [18]. The index comprises of the following components; d/D -decayed tooth, m/M - missing tooth due to caries and f/F- filled tooth due to caries. Children with chronic medical conditions, those on antibiotics 3 weeks before the study and those who had orthodontic appliances were excluded because these conditions could predispose the children to caries.

Ethical approval (ERC/2014/03/01) for the study was obtained from the Ethics and Research committee, Obafemi Awolowo University Teaching Hospitals Complex, Ile Ife. Approval was also obtained from the Local Education Authority. Informed consent was obtained from the parents or

legal guardians of the children and assents from children aged 8 years and above. Only children whose parents or legal guardian gave informed consent participated in the study.

Intra oral examination was carried out on each child using a sterile dental mirror and probe to assess for dental caries. The participants were examined sitted on a plastic chair in a suitable environment with good day light source. No radiographic investigation was carried out to determine presence of caries.

The participants were asked to rinse the mouth with water and swallow existing saliva. Unstimulated mid-morning whole saliva sample was collected for each child. The unstimulated saliva is the major determinant of salivary clearance [19]. Sample was taken by one of the investigators OAM at least one hour and thirty minutes after breakfast to allow for sufficient time. Each participant was asked to allow saliva to drool from the oral cavity into a sterile labelled disposable plastic container to determine the salivary flow rate (ml/min). A total of 5ml of saliva was collected from each pupil and the duration noted. Following collection, the saliva was transported in the sealed plastic container in a refrigerated medium to the laboratory for analysis.

The saliva sample collected for each pupil was aliquoted into five Eppendorf tubes. Four saliva-containing tubes were frozen immediately at -20^{0}C for analysis of pH, buffering capacity, calcium and phosphate levels and total antioxidant level. The fifth tube was used for *Streptococcus mutans* count analysis.

The pH of each saliva sample was measured using a digital pH meter (Microfield England) after calibration against a known buffer. The buffering capacity was determined by the method described by Ericsson, [20] and modified by Damle *et al*., [1]. A 0.5ml of saliva was titrated to 1.5mls of 5mmol/L hydrochloric acid. The mixture was vigorously shaken and centrifuged for 1 minute. The cover was removed to eliminate carbon dioxide and allowed to stand for ten minutes after which the pH was then measured using digital pH meter. Salivary calcium was estimated using the method described by Preethi *et al*. [21]. The saliva sample was treated with calcium reagent and the precipitate mixed with Ethylenediaminetetraacetic acid (EDTA). A spectrophotometer (Randox UK) at 570nm under room temperature was used to measure the calcium level. The level of salivary inorganic phosphate was measured using Fiske and Subarrow's method [1]. The colour complex and intensities were read at 675nm using spectrophotometer (Teco Diagnostic Canada) at room temperature. The total antioxidant capacity of the saliva was determined by a method based on reduction of Molybdenum (VI) to Molybdenum (V) by the saliva and subsequent formation of a green phosphate/Molybdenum (V) complex at an acidic pH. 0.1ml of the saliva containing reducing antioxidant specie was combined with 1ml of the reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in an Eppendorf tube. The tubes containing the reacting mixture were capped and incubated in a water bath at 95^{o}C for 90mins. The mixture was then allowed to stand and cool to room temperature and the absorbance measured at 695nm against a blank which consisted of the reacting mixture and distilled water in place of the saliva. The total antioxidant capacity of saliva was expressed as an ascorbic acid equivalent.

The saliva sample for *Streptococcus mutans* analysis was vortexed for 15 seconds, an inoculating loop was used to streak the saliva in mitis salivarius bacitracin agar (MSB) selective for *Streptococcus mutans*. This was incubated anaerobically for 24-48 hours at 37^{0}C. Colony counting was done with a magnifying glass and the count expressed as the number of colony forming unit per millimeter of saliva (cfu/ml).

Data were analyzed using SPSS (version 16). The mean saliva flow rate, pH, buffering capacity, total antioxidant level, calcium and phosphate levels and *Streptococcus mutans* counts for children with and without caries were calculated. The means for the two groups were compared using Student's t-test. Statistically significant value was inferred at $p < 0.05$.

Results

The mean saliva flow rate of children without caries (0.55 ± 0.10) ml/min was significantly higher than children with caries (0.50 ± 0.08) ml/min ($p = 0.01$). There was no significant difference in the mean pH of children with caries compared to those who were caries free (7.46 ± 0.31 vs 7.49 ± 0.37, $p = 0.77$). The mean buffering capacity of children without caries (8.93 ± 0.53) was significantly increased than those with caries (8.21 ± 0.43) ($p = 0.01$). The mean saliva phosphate level of children with caries was significantly increased than caries free children (9.55 ± 2.84 vs 7.83 ± 3.96, $p = 0.02$). Mean total antioxidant capacity of children without caries was significantly higher than those with caries (0.25 ± 0.20 vs 0.17 ± 0.11, $p=0.04$). The mean *Steptococcus mutans* count was significantly higher in children with caries ($1.10 \pm 0.35 \times 10^4$) cfu/ml compared to

those without caries (0.21 ± 0.21 x 10^4) cfu/ml (p=0.01). (Table 1).

assessed due to its predominance most of the daytime. Children without caries had significantly

Table 1: Mean salivary variables and *Streptococcus mutans* counts in caries free and caries active pupils

Saliva variables	Caries free (N = 44)	Caries active (N = 44)	p-value
Flow rate(ml/min)	0.55 ± 0.10	0.50 ± 0.08	0.01*
pH	7.49 ± 0.37	7.46 ± 0.31	0.77
Buffering capacity	8.93 ± 0.53	8.21 ± 0.43	0.01*
Calcium(mg/dl)	3.04 ± 2.83	3.32 ± 2.09	0.71
Phosphate(mg/dl)	7.83 ± 3.96	9.55 ± 2.84	0.02*
Total Antioxidant Capacity (mgAAE/ml)	0.25 ± 0.20	0.17 ± 0.11	0.04*
Streptococcus mutans count (10^4-10^5cfu/ml)	0.21 ± 0.21	1.10 ± 0.35	0.01*

* $p < 0.05$

Table 2: Correlation between physico-chemical saliva variables and dmft/DMFT

Saliva variables	Flow rate	pH	Buffering capacity	Calcium level	Phosphate level	TAC	*SM count	dmft/ DMFT
Flow rate	-	0.45	0.19	-0.14	-0.25	-0.18	-0.02	-0.06
p -value		0.01	0.21	0.36	0.10	0.25	0.90	0.69
pH	0.45	-	0.05	0.30	0.22	0.29	0.30	0.11
p -value	0.01		0.76	0.04	0.15	0.06	0.04	0.49
Buffering capacity	0.19	0.05	-	-0.11	-0.01	-0.25	-0.13	0.02
p -value	0.21	0.76		0.47	0.99	0.10	0.41	0.88
Calcium	0.14	0.30	-0.11	-	-0.24	0.12	-0.12	0.21
p -value	0.36	0.04	0.47		-0.11	0.41	0.44	0.16
Phosphate	0.25	-0.22	-0.01	-0.24	-	0.25	0.10	0.06
p -value	0.10	0.15	0.99	0.11		0.10	0.53	0.70
TAC	-0.18	-0.29	-0.25	0.12	0.25	-	-0.03	0.09
p -value	0.25	0.06	0.10	0.42	0.10		0.87	0.55
SM counts	-0.02	0.30	-0.13	-0.12	-0.10	-0.03	-	0.04
p -value	0.90	0.04	0.41	0.44	0.53	0.87		0.81
dmft/DMFT	-0.06	0.11	-0.02	-0.21	0.06	-0.09	0.04	-
p -value	0.69	0.49	0.88	0.70	0.70	0.55	0.81	

* SM- *Steptococcus mutans* count

Generally, there was a weak correlation between all the salivary variables assessed and dental caries. Saliva flow (-0.06), pH (0.11), buffering capacity (0.02) and *Steptococcus mutans* count (0.04). (Table2)

Discussion

Saliva cleanses the oral environment and its components may play a major role in caries formation. In this study, unstimulated saliva was higher mean saliva flow rate, buffering and total antioxidant capacity when compared to those with caries. Children with caries had significantly higher mean saliva inorganic phosphate level and *Streptococcus mutans* counts.

Saliva influences caries formation mainly by its flow rate [22]. This important preventive function is achieved through dilution of bacterial substrate (refined carbohydrate) and acid produced from bacteria action on substrate. The dilution and

elimination process is referred to as salivary clearance [23]. In the present study, children with caries had significantly reduced saliva flow rate compared to those without caries. Similarly, Lenarder-Lumikan and Loimaranta [17] observed hyposalivation in individuals with dental caries experience. Dogra *et al*. [24] reported rapid formation of caries in individuals and also on tooth surfaces that are usually immune to caries due to decreased salivary flow rate. Thus, it can be implied that the salivary clearance of caries active individuals is low due to reduced salivary flow rate. On the contrary, a study reported no relationship between salivary flow rate and caries activity [25].

There is no exact pH at which demineralization begins, the general range of 5.0 to 5.5 is considered critical for enamel demineralization [26]. In the present study, the mean pH of caries active children was 7.45. This is similar to the reports of other studies [21, 24, 27] where there is no association between pH value and caries activity. However, Shafer *et al.* [28] demonstrated a resting salivary pH of around 7.0 in children with low or no caries activity while those with extreme caries activity had a resting pH below critical pH of 5.5. The reason for the higher pH observed in caries active group in this study is not clear.

Caries activity is strongly dependent on the buffering capacity of the saliva. In the present study, caries active children had significantly lower buffering capacity compared to children without caries. Similarly, Ericson [20] reported low salivary buffering capacity in relation to caries incidence. Lagerlof *et al.* [23] demonstrated that low flow rate combined with low or moderate buffer effect indicates poor salivary resistance against microbial attack. The concentration of bicarbonate is dependent on saliva flow rate. Owing to this flow-dependent variation the pH of saliva is strongly dependent on its secretion rate [29]. However, Tunlunoglu [27] and Preethi *et al.* [21] proposed that factors such as microflora, diet and retention of food may dominate buffering capacity to initiate caries.

The role of saliva in remineralization of enamel is dependent on its saturation with calcium and phosphate [28]. The relationship of calcium and phosphate contents of saliva and caries is not clear. In this study, the mean calcium level was higher in children with caries. Shahrabi *et al*. [30] and Elizarora and Petrovich [31] reported high calcium level in children with severe caries. However, Preethi *et al*., [21] found a significantly low calcium level in caries active children. The high calcium observed in caries active children maybe due to loss of calcium from the demineralized enamel.

In this study, caries active children had significantly higher mean saliva phosphate level compared to their caries free counterparts. Similarly, Damle *et al*. [1] and Preethi *et al.* [21] found an increase inorganic phosphate in caries active pupils. However, Shahrabi *et al*. [30] reported low phosphate in pupils with high dental caries. The increase phosphate level in caries active children may be due to release of phosphate from the demineralized enamel.

An imbalance in the levels of free radicals, reactive oxygen and antioxidant in saliva play a major role in the initiation and progression of caries [21]. Free radicals are formed in caries process and their level depends on caries activity. This study assessed the total antioxidant level, a more representative form of antioxidants, as antioxidants do not act alone thus, measurement of a specific antioxidant will be inappropriate. The Total antioxidant capacity (TAC) of children without caries was significantly higher. Hedge *et al*. [32] reported a low antioxidant level in children with caries. However, Tulunoglu *et al.* [27] and Uberos *et al.* [33] reported a high total antioxidant level in children with caries. The reason for the high level in children without caries in this study is not clear although diet may have been contributory.

Streptococcus mutans are part of the normal oral flora, in certain situations they may become dominant to cause dental caries [34]. It has been reported to be strongly associated with the initiation of caries [14]. In this study, children with caries have significantly higher *Streptococcus mutans* counts compared to their caries free counterparts. Hedge *et al*. [35], in a study among 13-15-year-old Indian children found a positive relationship between *Streptococcus mutans* counts and caries experience. Similarly, Gabris *et al.* [36] found a positive relationship between *Streptococcus mutans* counts and caries in 14 – 16 –year-old Hungarian adolescents. . However, Giacaman *et al.* [14] and van-Palenstein *et al.* [37] reported no association between *Streptococcus mutans* counts and high caries experience. The colonisation of teeth by *Streptococcus mutans* in children without caries provides evidence that the aetiology of caries is multifactorial. The diet (refined carbohydrate) is needed for caries formation. The diet of the participants in this study was not considered. It is possible that the diet of the caries-active children differs from that of children without caries. Other

factors such as oral hygiene practices and use of fluoride that affect *Streptococcus mutans* counts were also not considered.

Saliva can act as good medium to measure and monitor caries risk assessment in children. It is easy, less painful and quick to collect. Therefore it can be used at the chair side for diagnostic screening and monitoring of caries activity in children. This will enable dental practitioners make specific recommendations tailored towards individual needs.

Conclusion

This study demonstrated that children with caries had significantly lower mean saliva flow rate, buffering capacity and total antioxidant capacity level compared to children without caries. Also caries active children had significantly higher *Streptococcus mutans* counts and phosphate level compared to their caries free counterparts. However, there was no significant difference in the mean saliva pH and calcium levels of both groups.

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Prevalence of hypertensive disorders in pregnant Nigerians and their related factors

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Abstract

Background: Pregnancies complicated with hypertensive disorders are regarded as high risk and contribute to increased maternal and perinatal morbidity and mortality.

Objective: To determine the prevalence of hypertensive disorders in pregnancy (HDP) and their related factors in defined areas of South West Nigeria.

Methods: The study is a prospective cohort study conducted in Antenatal Clinics of the departments of Obstetrics and Gynaecology of Ekiti State University Teaching Hospital, Ado-Ekiti, Federal Medical Centre, Ido-Ekiti, University College Hospital Ibadan and Adeoyo Maternity Hospital, Ibadan, Nigeria from June 2011 to October 2012. The data regarding demographic details, gestational age, obstetrics history, diagnosis, and blood pressure readings were obtained from each participant through a semi pretest questionnaire. Data entry and analysis was done using SPSS version 22 statistical package.

Result: A total of 521 pregnant women enrolled for the study and 34(7.2%) were hypertensive among whom 55.9% were diagnosed as preeclampsia-eclampsia (19), 35.3% as gestational hypertension (12), 5.9% as chronic hypertension (1) and 2.9% as preeclampsia superimposed on chronic hypertension. HDP was more prevalent among women aged ≥31years (64.7%), who had previous history of HDP (23.5%), the third trimester (70.6%) of pregnancy and in nulliparous women (67.6%).

Conclusion: Hypertensive disorders of pregnancy are among the most common medical complications worsening the outcome of pregnancy. Regular monitoring of the risk factors may help to mitigate the progression of the disorders.

Keywords: *Preeclampsia, pregnancy, hypertensive, prevalence, disorders*

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Résumé

Contexte: Les grossesses compliquées avec troubles d'hypertension sont considérées comme haute-risque et contribuent à l'augmentation de la morbidité et de la mortalité maternelle et périnatale.

Objectif: Pour déterminer la prévalence des troubles hypertensifs pendant la grossesse (HDP) et leurs facteurs associés dans des zones définies du Sud-Ouest du Nigeria.

Méthodes: L'étude est une étude de cohorte prospective menée dans les cliniques prénatales des départements d'obstétrique et de gynécologie de l'Hôpital d'Enseignement Universitaire de l'État d'Ekiti , Ado- Ekiti , Centre Médical Fédéral, Ido-Ekiti , Collège Hospitalier Universitaire Ibadan et l'Hôpital de Maternité Adeoyo, Ibadan, Nigeria de Juin 2011 à Octobre 2012. Les données concernant les détails démographiques, l'âge gestationnel, l'histoire obstétrique, diagnostic, et des lectures de pression artérielle ont été obtenues de chaque participant par le biais d'un semi pré-test questionnaire. La saisie et l'analyse des données ont été effectuées à l'aide du logiciel statistique SPSS version 22.

Résultat: Au total, 521 femmes enceintes ont participé à l'étude et 34 (7,2%) étaient hypertendues, parmi lesquelles 55,9% avaient reçu un diagnostic de pré-éclampsie - éclampsie (19), 35,3% d'hypertension gestationnelle (12), 5,9% d'hypertension chronique (2) et 2,9% comme pré-éclampsie super imposée à l'hypertension chronique. HDP était plus fréquente chez les femmes âgées de e"31 ans (64,7%), qui avaient des antécédents d'HDP (23,5%), le troisième trimestre (70,6%) de grossesse et chez les femmes nullipares (67,6%).

Conclusion : Les troubles hypertensifs de la grossesse sont parmi les complications médicales les plus fréquentes qui aggravent l'issue de la grossesse. Un suivi régulier des facteurs de risque peut aider à atténuer la progression des troubles.

Mots-clés: *Pré-éclampsie, grossesse, hypertension, prévalence, troubles*

Introduction

Pregnancy is a normal physiological event but in some circumstances pregnancy specific or other

medical conditions can cause maternal as well as foetal morbidities and even mortalities. Among pregnancy specific disorders, hypertensive disorders of pregnancy (HDP) are one of the major leading causes of maternal and fetal morbidity and mortality in different communities [1].

Hypertension in pregnancy can exist before pregnancy, be induced by the pregnancy, develop during delivery or all occurring and its clinical presentation is characterized by hypertension, proteinuria and edema [2, 3]. HDP can also trigger some severe forms of maternal complications, such as cardiovascular and cerebrovascular diseases, liver and kidney failure, placental abruption, disseminated intravascular coagulation (DIC) and HELLP syndrome. Under these circumstances, the placenta dysfunction may occur, leading to fetal growth restriction, fetal distress, preterm birth, intrauterine fetal demise, stillbirth and neonatal asphyxia [4, 5].

Hypertensive disorders during pregnancy are classified into 4 categories, as recommended by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy: 1) chronic hypertension, 2) preeclampsia-eclampsia, 3) preeclampsia superimposed on chronic hypertension, and 4) gestational hypertension [4].

Though HDP has been described as a maternal complication over the last several decades, its true aetiology and pathophysiology remain unknown; HDP-related complications are still threatening maternal and fetal life and health. The prognosis of HDP is associated with the severity of disease process and in general, the more severe the disease is, the poorer the prognosis [5, 6]. However, how pregnancy incites or aggravates hypertension remains unsolved despite decades of intensive research. Indeed, HDP remain among the most significant and intriguing unsolved medical problems complicating pregnancy (6, 7].

The incidence of hypertensive disorders of pregnancy varies widely ranging from 1-35% among different populations, probably due to variations in the definitions, classification and target population studied [3]. In a population-based study, Ye *et al.* [6] examined HDP in 112,386 pregnant women with prevalence of 5.22%. Another study conducted in Latur, Maharasthra, India on 1566 deliveries, the prevalence was found to be 6% [8]. Studies in Zambia and Pune also reported prevalence rates of 17.7% [9] and 7.8 % [4] respectively. The variations can be attributed to racial differences, ethnic background, socioeconomic status, age distribution and some other parameters like parity and gravidity [4. 6].

In Nigeria, it is estimated that 10% of pregnancies are complicated by HDP and it results in more admissions in the antenatal period than any other disorder [3, 10]. Salako *et al.* [11] reported a prevalence rate of hypertension at antenatal booking as 9.8% rising to 26.2% at delivery among pregnant patients at the University College Hospital, Ibadan, Nigeria while Singh *et al.* [3] reported a value as high as17% prevalence rate of HDP in a teaching hospital in Northern part of Nigeria. Kooffreh *et al.,* [12] also reported a prevalence rate of preeclampsia (1.2%), one of the types of HDP in University of Calabar Teaching Hospital, a South-South area in Nigeria. There is dearth of information on the prevalence of HDP in South West of Nigeria. This present study is therefore aimed at determining the prevalence of HDP and some related factors in South Western part of Nigeria.

Materials and methods

A total of 521 participants were enrolled into this prospective cohort study. The participants were pregnant women attending the clinics for antenatal care in four different tertiary health facilities, namely: Ekiti State University Teaching Hospital, Ado-Ekiti, Federal Medical Centre, Ido-Ekiti, University College Hospital and Adeoyo Maternity Hospital, Ibadan, Nigeria respectively. Participants were recruited from June 2011 to October 2012.

Inclusion criteria were women first seen at first or second trimester (< 20 weeks at booking) with systolic blood pressure below 140mm/Hg and diastolic blood pressure below 90mm/Hg and participants that gave consent. Exclusion criteria included pregnant women first seen at ≥20 weeks of pregnancy, women who were already hypertensive at entry into the study or had proteinuria by the dipstick measurement greater than 300mg/L (1+). The ethical approval for the study was obtained from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Committee Ibadan, Oyo State, Nigeria.

Classification into the different subtypes of hypertensive disorders in pregnancy (by the National High blood pressure Education Program) was as follows:

1. Preeclampsia-eclampsia: elevated blood pressure of ≥140mm/Hg systolic and ≥90mm/Hg (hypertension) appearing ≥20weeks of gestation, accompanied with proteinuria ≥300mg/24hr (or urinary dipstick proteinuria of ≥1+).
2. Gestational hypertension (Pregnancy Induced hypertension): elevated blood pressure of ≥140mm/Hg systolic and ≥90mm/Hg (hypertension) for the first time appearing

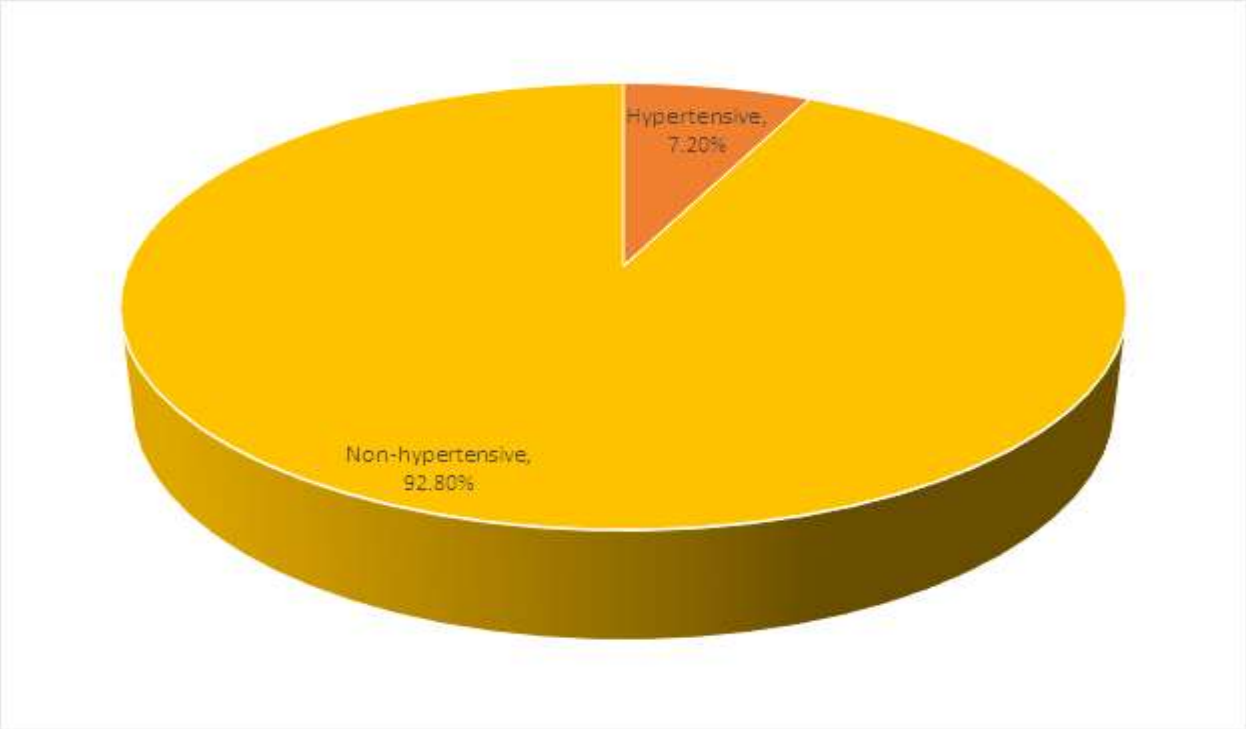

Fig.1: Prevalence of HDP in the study participants.

≥20weeks of gestation, no proteinuria, blood pressure returns to normal postpartum.

3. Chronic hypertension: elevated blood pressure of ≥140mm/Hg systolic and ≥90mm/Hg (hypertension) appearing >20weeks of gestation without a known cause (or known to exist prior to pregnancy), hypertension persistent for more than 12weeks after pregnancy.
4. Preeclampsia superimposed on chronic hypertension: elevated blood pressure of ≥140mm/Hg systolic and ≥90mm/Hg (hypertension) appearing ≥20weeks of gestation, accompanied with new onset of proteinuria or resistant hypertension

Age, educational status, parity, occupation, ethnic group, gestational age, maternal smoking, and previous history of hypertension were obtained from each participant through a semi pretest questionnaire. Urinalysis for protein and glucose was done at booking and at subsequent visits. The diastolic and systolic blood pressure readings were measured in a sitting position with sphygmomanometer after at least 10 minutes of rest at booking and at subsequent visits. All pregnant women were followed-up to delivery and information on the gestational age, mode of delivery and foetal outcome were recorded. Data collected were entered into a spread sheet and analysed using SPSS version 22 statistical package. Analysis of variance (ANOVA) and Student's t-test were used for comparison of continuous variables while Chi- square test was used for association between categorical variables. Survival analysis (time to event analysis) was employed using Cox proportional hazard regression model as the technique to measure the survival and hazard function. $P<.05$ is considered significant.

Results

Of the 521 pregnant women that were recruited at booking during the antenatal period and longitudinally followed-up till delivery, 50 were lost for follow-up. Out of the remaining 471 whose outcomes of pregnancy were known, 34 developed HDP giving a prevalence of 7.2% while 437 (92.8%) were normotensive. (Fig. 1).

Table 1 shows the different subtypes of HDP made up of 55.9% preeclampsia-eclampsia, 35.3% gestational hypertension, 5.9% chronic hypertension and 2.9% preeclampsia superimposed on chronic hypertension respectively. The calculated percentages for preeclampsia-eclampsia, gestational hypertension, chronic hypertension and preeclampsia superimposed on chronic hypertension in the study population were 4.0, 2.6, 0.4 and 0.2 respectively.

Table 2 shows the maternal age distribution of HDP and normotensive pregnant women. In both groups majority of the pregnant women were aged

Table 1: Distribution based on type of HDP developed in the study participants.

Type	Number (n)	Percentage (% within study group)	Percentage (% within HDP group: n=34)
Preeclampsia-Eclampsia	19	4.0	55.9
Gestational hypertension	12	2.6	35.3
Chronic hypertension	2	0.4	5.9
Preeclampsia superimposed on chronic hypertension	1	0.2	2.9
Normal	437	92.8	
Total	471	100	100
Those who developed in the second trimester only	10	2.1	29.4
Those who developed in the third trimester only	24	5.1	70.6

HDP= Hypertensive disorders in pregnancy, n= number of participants

Table 2: Comparison of the maternal age of normotensive women and HDP patients.

Variable	HDP	Normotensive	X^2	t-value	p-value
Age group (years)	n=34	n=483			
17-22	2 (5.9%)	15 (3.1%)	17.158		0.002*
23-28	4 (11.8%)	145 (30.0%)			
29-34	18 (52.9%)	231 (47.8%)			
35-40	7 (20.6%)	87 (18.0%)			
41-46	3 (8.8%)	5 (1.0%)			
Mean maternal age (yrs)	32.4±5.0	30.5±4.4		-2.392	0.017*
Gestational age group (weeks)	n=34	n=437			
<27	0	37 (8.5%)	41.280		0.000*
27-36	18 (52.9%)	54 (12.4%)			
37-42	16 (47.1%)	340 (77.8%)			
>42	0	6 (1.4%)			
Mean value	35.5±3.5	36.5±6.5		0.826	0.409

*HDP= Hypertensive disorders in pregnancy, n= number of participants, Values are in number of participants with percentage in each group in parenthesis, X^2 = Chi-Square, t= Student t-test, p = significant level, * =significant at p<0.05, Values are in mean ± standard deviation.*

between 20-40 years with 8.8% of the HDP group aged above 40 years while only 1.0% of the normotensive group were older than 40 years. More of the hypertensive women were aged between 29 and 46 years while the normotensive women were aged between 17 and 34 years. The mean maternal age for hypertensive women was 32.4 ± 5.0 years while that of normotensive was 30.5 ± 4.4 years (p<0.02).There is a significant difference in the mean gestational age between the HDP and normotensive women (p<0.001).

As indicated in table 3, 23.5% of HDP group had previous history of hypertension, while the respective value for the normotensive group was 1.8%. In the HDP group only 11.8% had term (47.1%) or preterm (52.9%) delivery by vaginal route, while the others had their babies by either EMCS (47.1%) or ELSCS (38.2%) respectively. On the other hand, as high as 59.7% of the normotensive group had term (78.3%), preterm (20.4%) or posterm (1.4%) delivery by vaginal route, while the others had their babies either by EMCS (24.9%) or ELSCS (10.8%) respectively (p<0.001). The incidence of HDP peaked in the nulliparous (67.6%) when compared with the normotensive women (60.4%) respectively.

In table 4, it was observed that none of the women developed HDP in the first trimester of pregnancy (<14weeks), only 10 (29.4%) had the disease in the second trimester (15-28weeks), more

Table 3: Obstetric characteristics of the participants.

Obstetric characteristics	HDP	Normotensive	Total	X^2	p-value
Gestation	n=34	n=437	n=471	19.242	0.000*
Term	16 (47.1%)	342 (78.3%)	358		
Preterm	18 (52.9%)	89 (20.4%)	107		
Posterm	0	6 (1.4%)	6		
Mode of delivery	n=34	n=437	n=471	37.600	0.000*
Vaginal	4 (11.8%)	261(59.7%)	265		
EMCS	16 (47.1%)	109 (24.9%)	125		
ELSCS	13 (38.2%)	47 (10.8%)	60		
Evacuation	1 (2.9%)	20 (4.6%)	21		
Outcome of pregnancy	n=34	n=437	n=471		
Live birth	30 (88.2%)	399 (91.3%)	429	4.276	0.233
Stillbirth	3 (8.8%)	15 (3.4%)	18		
Misabortion	1 (2.9%)	6 (1.4%)	7		
Miscarriage	0 (0.0%)	17 (3.9%)	17		
Previous history of HTX	n=34	n=487	n=521	46.666	0.000*
NO	26 (76.5%)	478 (98.2%)	504		
Yes	8 (23.5%)	9 (1.8%)	17		
Parity	n=34	n=487	n=521	2.497	0.476
Nulliparous	23 (67.6%)	294 (60.4%)	317		
Primiparous	4 (11.8%)	111 (22.8%)	115		
Multiparous (2-4)	7 (20.6%)	80 (16.4%)	84		
Grand multiparous (5-6)	0 (0%)	2 (0.4%)	2		

*HDP= Hypertensive disorders in pregnancy, n= number of participants, HTX= hypertension, Values are in number of participants with percentage in each group in parenthesis, X^2 = Chi-Square, p = significant level, * =significant at p<0.05*

than half of the women 24 (70.6%) had the disease in the third trimester of pregnancy.

Table 4: Gestation week of HDP women at the point of diagnosis of hypertension.

Gestational age (weeks)	Number (n=34)	Percentage (%)
17-28	10	29.4
29-36	18	52.9
37-39	6	17.6

HDP= Hypertensive disorders in pregnancy, n= number of participants

Table 5 shows adjusted Cox regression of maternal age, parity and previous history of hypertension in women with hypertensive disorders of pregnancy. After controlling or adjusting for maternal age, parity and previous history of hypertension, it was observed that pregnant women who are within the age of 23-28years were at lower risk of developing hypertension when compared with those within 17-22 years. The hazard was lower in pregnant women of age range 23-28 years compared to 17-22 years (HR= 0.10, 955CI = 0.01- 0.81). The data further showed that primiparous pregnant women had lower risk of developing hypertension when compared with nulliparous pregnant women. The hazard was less pronounced in primiparous women than nulliparous (HR = 0.16, 95% CI = 0.03 -0.82).

On the other hand, development of hypertension in pregnancy was 9.2 times higher in women who had previous history of hypertension. The hazard was more pronounced in pregnant women with previous history of hypertension (HR = 9.20, 95% CI = 3.78-22.44).

Table 6 shows un-adjusted Cox regression of maternal age, parity and previous history of hypertension in women with hypertensive disorders of pregnancy. After individual analysis, significant differences were observed in maternal age and previous history of hypertension (p= 0.02 and 0.000). An increase in maternal age of 1year will be associated with 1.1 fold increase in risk of development of hypertension in pregnancy (HR =1.10, 95% CI = 1.01-1.19). Previous history of hypertension was associated with greater risk of development of HDP and therefore shorter survival (HR =10.44, 95% CI= 4.72-23.08).

Table 5: Adjusted Cox Regression of Maternal age, Parity and Previous History of Hypertension in Women with Hypertensive Disorders of Pregnancy

Index	B	Exp (β)	95% CI Lower	For Exp (β) Upper	p- value
Maternal age					
17-22					
23-28	-2.262	0.104	0.013	0.814	0.031*
29-34	-2.322	0.098	0.006	1.526	0.097
35-40	-3.054	0.047	0.001	2.649	0.137
41-46	-2.314	0.099	0.001	14.305	0.362
Parity group					
Nulliparous					
Primiparous	-1.864	0.155	0.029	0.817	0.028*
Multiparous (2-4)	-2.413	0.090	0.004	2.248	0.142
Grand multiparous (5-6)	-15.425	0.000	0.000		0.971
Previous history of HTX	2.220	9.204	3.776	22.437	0.000*
Maternal age	0.170	1.185	0.933	1.505	0.165
Parity	0.640	1.896	0.595	6.045	0.279

95% CI = 95 percentage confidence interval

Table 6: Un-adjusted Cox regression of maternal age, parity and previous history of hypertension in women with hypertensive disorders of pregnancy

Index	B	Exp (β)	95% CI Lower	For Exp (β) Upper	p- value
Maternal age	0.091	1.096	1.014	1.185	0.022*
Parity	-0.012	.988	.695	1.405	0.947
Previous history of HTX	2.346	10.440	4.723	23.077	0.000*
Maternal age					
17-22					
23-28	-1.483	0.227	0.042	1.239	0.087
29-34	-0.629	0.533	0.124	2.298	0.399
35-40	-0.543	0.581	0.121	2.799	0.499
41-46	1.324	3.760	0.625	22.613	0.148
Parity group					
Nulliparous					
Primiparous	-0.827	0.437	0.151	1.265	0.127
Multiparous (2-4)	0.054	1.056	0.453	2.461	0.900
Grand multiparous (5-6)	-10.175	0.000	0.000		0.980

95% CI = 95 percentage confidence interval

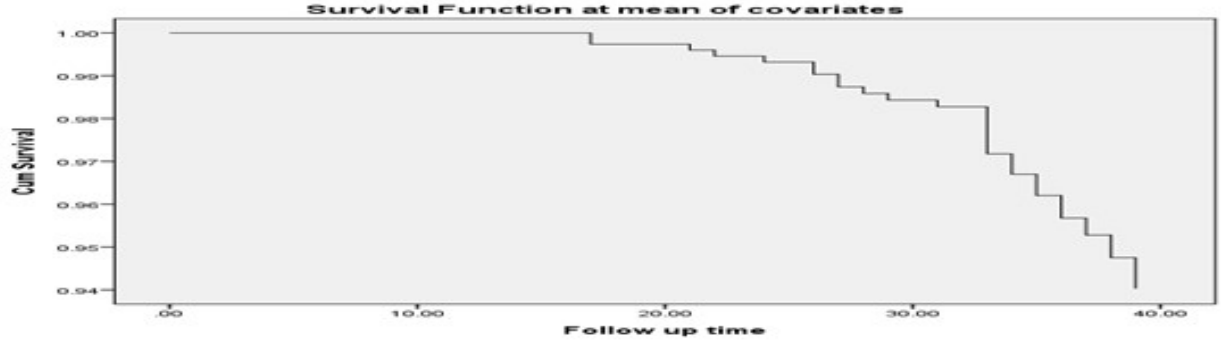

Fig. 2: Survival function for adjusted maternal age, parity and previous history of hypertension

Figures 2-4 show the survival function for the adjusted and un-adjusted maternal age, parity and previous history of hypertension in women with hypertensive disorders of pregnancy. In figures 2-4, the women survived up to around 15th week of gestation. The hazard began around the 17th week of gestation (second trimester) and continued till around 38th week (third trimester).

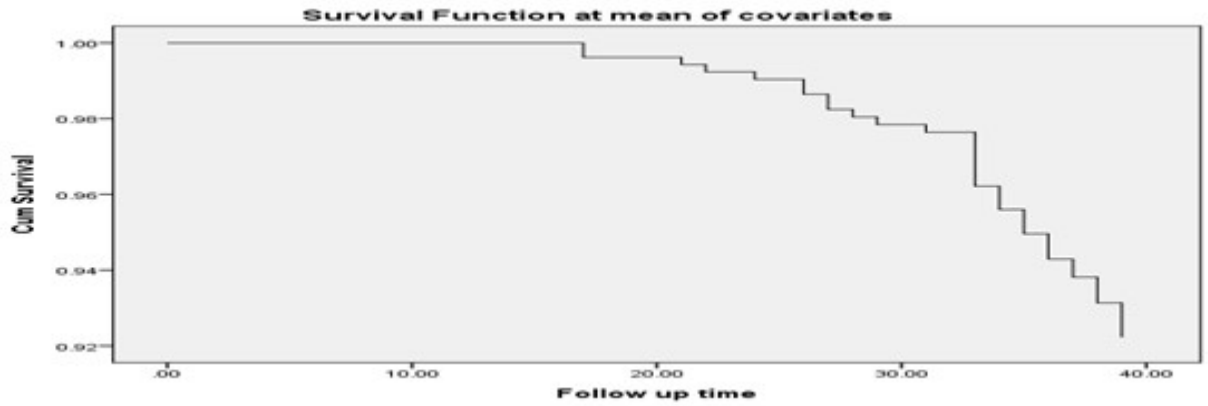

Fig.3: Survival function for un-adjusted maternal age

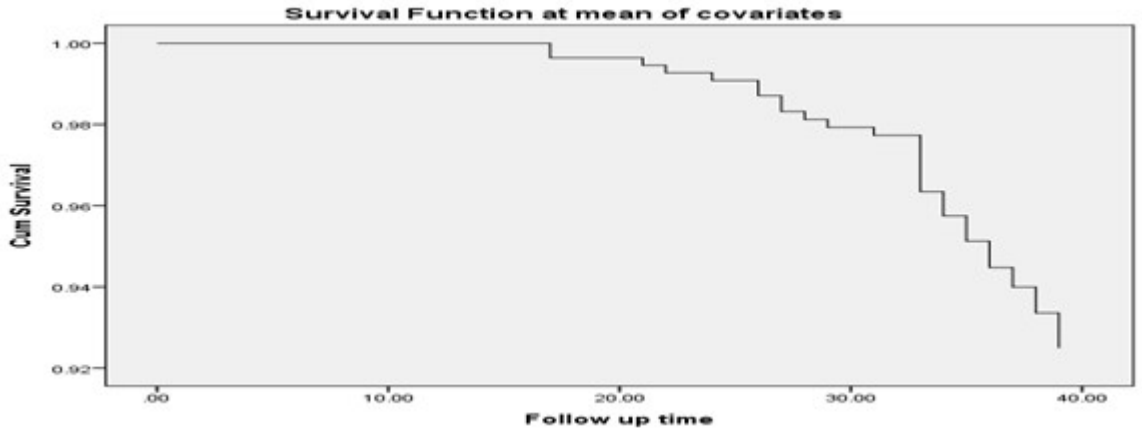

Fig.4: Survival function for un-adjusted previous history of hypertension

Discussion

In this study, 34 pregnant women developed different types of HDP out of the 487 pregnant women whose pregnancy outcomes were known with a prevalence of 7.2%. Ten of these women with a prevalence of 2.1% developed the HDP in the second trimester of pregnancy while 24 with a prevalence of 5.1% were in the third trimester of pregnancy. This observation is in line with the report of Khong *et al*., [13] that early onset of HDP occurs less frequently (between 0.4-1%) than late-onset of HDP.

Preeclampsia is the most common type of hypertensive disorder of pregnancy followed by pregnancy induced/gestational hypertension [8]. In this present study, the different types of HDP developed were 19 (55.9%) preeclampsia-eclampsia, 12 (35.3%) pregnancy induced/gestational hypertension, 2 (5.9%) chronic hypertension and 1 (2.9%) preeclampsia superimposed on chronic hypertension respectively. The prevalence among the study participants were preeclampsia-eclampsia 4.0%, gestational hypertension 2.6%, chronic hypertension 0.4% and preeclampsia superimposed on chronic 0.2%. Other studies also reported highest prevalence of HDP in preeclampsia and gestational/ pregnancy induced hypertension than chronic and preeclampsia superimposed on chronic hypertension (56%; 31.4%, 78.8%; 19.2%, 60.5%; 18% and 63%; 21.1%) [4-6, 8].

Maternal age has an important influence on the incidence of hypertensive disorders of pregnancy [4]. In the present study, the highest incidence of HDP occurred among those aged 29 to 40 years. This is statistically significant when compared with normotensive pregnant women ($p<0.02$). The age distribution in this study is similar to other reports in and outside Nigeria. Peter *et al.* [14], South East Nigeria, reported higher age with high blood pressure

in pregnant women. Ebeigbe and Aziken [15] also reported highest incidence of HDP in the age group of 30-34years in Benin City, Nigeria. Other studies reported risk of developing hypertension in pregnancy to be more amongst women older than 30 years and same is indicated in our study [4, 8]. In contrast, Singh *et al.,* [3] in Northern part of Nigeria reported highest incidence of HDP in the age range of 25-29years. This might be due to early marriage and high low level of girls 'education (high illiteracy) which is prevalent in the Northern part of Nigeria [16].

The caesarean section rate in women with HDP was significantly higher when compared with normotensive women (85.3% vs. 35.7%) in our study. Higher caesarean section rates have been reported from other studies and this supports the high incidence of caesarean delivery in our study [3, 5, 6, 12, and 14]. This high incidence indicates that most of the hypertension cases were severe or rapidly progressive necessitating immediate delivery [12].

Meanwhile, previous medical history of hypertension was found to be a significant risk factor for developing HDP in this study ($p<0.001$) which is in accordance to the findings of other studies [3, 17]. Gongora and Wenger, [17], Karakilic and Karakilic [19] in their studies described previous history of HDP as a principal risk factor in the development of HDP. This is consistent with the hypothesis that immune maladaptation might play a role in triggering the development of HDP [3].

Seventeen (4.6%) of the women who developed HDP delivered at a gestational age greater than or equal to 37 weeks which is term gestation compared to 350 (95.4%) normotensive women at the same gestational age. This is similar to the findings of an earlier study in a University Teaching Hospital, South-South area of Nigeria [12].

Only few women developed HDP at an early gestational age (17-26 weeks). Highest number of hypertension was between 32-36 weeks (50.0%) and just 6 of the women (17.6%) had the disease in the term gestation. Our finding is in accordance with findings of Borade *et al.,* [8] and Singh *et al.,* [3].
It is noteworthy that two third of the women who developed HDP in this study were nulliparous (67.6%) with lowest percentage in the primiparous (11.8%). Risk of developing hypertension during pregnancy in nullipara is extreme because pregnancy in these women is maternal first exposure to chorionic villi- specifically to trophoblast of fetal origin, to which the body respond with strong immunological reaction in the form of hypertension during pregnancy [8]. The etiology of HDP is diverse. It is believed that immune maladaptation of the primigravida is responsible for the higher incidence of preeclampsia in this group. This mal adaptation is lost in subsequent pregnancies, hence the decreasing incidence of preeclampsia in the multipara [12]. Our findings agree with previous studies [3, 4, 12 and 20]. Our findings agree with previous studies that reported insulin levels as significant predictors of hypertensive disorders in pregnancy

Conclusion

In this study, the prevalence of HDP was 7.2%. Out of 34 hypertensive pregnant women 55.9% were diagnosed as pre-eclampsia-eclampsia, 35.3% as gestational hypertension, 5.9% as chronic hypertension and 2.9% as preeclampsia superimposed on chronic hypertension. Maternal age ≥31years, previous history of hypertension, 3rd trimester of pregnancy and primigravida are few epidemiological risk factors associated with HDP. Early diagnosis and treatment through regular antenatal checkup is a key factor to prevent HDP and its complications.

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Challenges of gynaecological cancer care in Nigeria – a review article

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Abstract
*Background***:** Gynaecological cancers and their management in the tropics constitute a big challenge to the gynaecological oncologist considering the overwhelming economic burden of care on patients and their relatives. These challenges are numerous and present at different levels vis-a-vis of prevention, diagnosis, treatment of the disease and patients' follow up. The main thrust of this review was to illustrate the challenges affecting gynaecological cancer care in Nigeria and proffer potential opportunities for their early identification while making recommendations that may be beneficial in ameliorating their effects and impacts.
*Methodology***:** Electronic search of local and international literatures was conducted in major databases including PubMed, Web of Science, Sciencedirect, EMBASE, SpringerLink, Scopus, JSTOR, JaypeeDigital, and Google Scholar using appropriate MESH terms either individually or in combination. All relevant peer–reviewed articles and publications were identified, retrieved and reviewed.
Results: Challenges of management of gynaecological cancers are enormous and diverse. They vary at different levels of care and included poverty, lack of access to health care, inadequate or absence of basic infrastructure for cancer care and lack of political will. Delay at different levels of care leading to late presentation remains a leading factor that negatively contributes to survival.
Conclusion: Gynaecological cancer care in the tropics is a big challenge to health care providers. Efforts should be intensified in prompt identification of these challenges and offering solutions that will help in improving the health of our women.

Keywords: *burden, care, challenges, gynaecologic cancer, Nigeria*

Résumé
Contexte: Les cancers gynécologiques et leur prise en charge dans les tropiques constituent un grand défi pour l'oncologue gynécologique compte tenu du fardeau économique accablant des soins pour les patients et leurs proches. Ces défis sont nombreux et présents à différents niveaux vis-à-vis de la prévention, le diagnostic, le traitement de la maladie et la suivie des patientes. L'objectif principal de cette revue était d'illustrer les défis qui affectent les soins gynécologiques contre le cancer au Nigeria et de proposer des opportunités potentielles pour leur identification précoce tout en faisant des recommandations qui pourraient être bénéfiques pour améliorer leurs effets et leurs impacts.
Méthodologie **:** La recherche électronique des littératures locales et internationales a été menée dans des bases de données majeures telles que PubMed, Web of Science, Sciencedirect , EMBASE, SpringerLink , Scopus, JSTOR, JaypeeDigital et Google Scholar en utilisant les termes MESH appropriés individuellement ou en combinaison. Tous les articles et publications pertinents évalués par des pairs ont été identifiés, récupérés et examinés.
Résultats: Les défis de la prise en charge des cancers gynécologiques sont énormes et diversifiés. Ils varient selon les différents niveaux de soins et comprennent la pauvreté, le manque d'accès aux soins de santé, l'insuffisance ou l'absence d'infrastructures de base pour les soins contre le cancer et le manque de volonté politique. Retard à différents niveaux de soins menant à la présentation tardive reste un facteur majeur qui contribue négativement à la survie.
Conclusion: Les soins gynécologiques contre le cancer dans les tropiques représentent un défi important pour les fournisseurs de soins de santé. Les efforts devraient être intensifiés pour identifier rapidement ces défis et offrir des solutions qui aideront à améliorer la santé de nos femmes.

Mots clés: *fardeau, soins, défis, cancer gynécologique, Nigéria*

Introduction

Gynaecological cancers are a diverse group of diseases with different forms of presentations, natural histories and response to treatment. They result from malignant changes affecting the female reproductive system. They are named according to the organ or part of the body where they first develop and include: ovarian, endometrial, cervical, vaginal,

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vulval, fallopian tubes cancers and gestational trophoblastic diseases. Cervical, endometrial and ovarian cancers are the most common cancers in the sub-Sahara Africa while vulva, vagina and choriocarcinoma are less common. The symptoms of these cancers depend on the type of cancer, the size of the tumour, rate of tumour growth and clinical stage of presentation [1]. Symptoms of gynaecological cancers may include irregular bleeding (postmenopausal, inter-menstrual and/or post-coital), unusual vaginal discharge, pain or discomfort in the abdomen, abdominal swelling, change in bowel and bladder habits, dyspareunia, vulvar itching, burning or soreness, lumps or wart-like growth [2,3].

The definitive causes of most gynaecological cancers are not understood but a number of risk factors are associated with their development – some of which are modifiable while others are not. These risk factors include: increasing age, strong family history, identified gene mutations, reproductive history such as child-bearing, nulliparity, exposure to hormones – intrinsic or extrinsic, intrauterine exposure to diethylstilbestrol (DES), viral infections such as human papilloma virus (HPV), lifestyle factors such as smoking and those leading to obesity. Exposure to high risk HPV infections has been associated with cervical cancers.

Optimum management of gynaecological cancers requires co-ordinated teamwork between the different levels of care – primary, secondary and tertiary care centres. It is expected that all women should have access to specialist care as gynaecological cancer care requires a comprehensive and multidisciplinary team-based approach for maximum benefit [4]. It entails prevention of cancer by vaccination, screening for premalignant lesions, ensuring early diagnosis by comprehensive history taking, detailed examination, investigations such as biopsies and examination under anaesthesia. Treatment could be in form of surgery, chemotherapy, radiotherapy but most favoured is a combination of any or all of these modalities. The multidisciplinary dimension of care is usually provided by the gynaecological oncologist, oncologist nurse, surgeons, pathologists, radiologists, palliative care team, physical therapists, medical oncologists, radiation oncologists, clinical psychologists (or psycho-oncologists where available), physicians, anaesthetists and any other professional that may be necessary at any point in time.

Gynaecological cancer care in the tropics is thus a big challenge to the health care providers knowing that it often adversely affects those in the low socio-economic group such that the economic burden on these patients and their relations are usually overwhelming. Challenges of management of gynaecological cancers are enormous and could be from various levels such as that of prevention, diagnosis, treatment of the disease or follow up. Efforts should be made at identifying these challenges and offering lasting solutions to improve the health of women.

Materials and methods

The information contained in this review were obtained through electronic search of local and international literatures conducted in major databases including PubMed, Web of Science, Sciencedirect, EMBASE, SpringerLink, Scopus, JSTOR, JaypeeDigital, and Google Scholar. We used the following MESH and free terms either individually or in combination: cancer, tumour, gynaecological cancer, gynaecological tumour, cancer in Nigeria, management of gynaecological cancer, gynaecological cancer burden, economic burden of gynaecological cancer, cervical cancer, ovarian cancer, endometrial cancer, corpus cancer, vulval cancer, vaginal cancer, choriocarcinoma. All relevant peer–reviewed articles and publications were identified, retrieved and reviewed. Relevant bibliographies from the literature obtained in the primary search were also retrieved and reviewed.

Burden of gynaecological cancer

The burden of gynaecological cancers in Nigeria continues to defy current interventions. Repeatedly, several studies have shown high prevalence in our society especially for the vaccine-preventable cervical cancer with most patients presenting at very late stages [3, 5–17]. In Northern Nigeria, a study conducted on gynaecological malignancies in Aminu Kano teaching hospital between October 2008 and September 2011 showed an overall prevalence rate of 10.7% for gynaecological cancers out of which 48.6% was cervical cancer, 30.5% was ovarian, 11.25% was endometrial while 3.6% was vulval cancer [10]. In Zaria, Oguntayo *et al* (2016) reported organ-specific rate of 71% for cervical cancer, 16.5% for ovarian cancer, 5.1% for choriocarcinoma, 4.2% for endometrial cancer, 2.6%, for vulval cancer, 0.27% for vaginal cancer, and 0.09% for fallopian tube cancers [7].

Another study conducted at the University of Nigeria Teaching hospital, Enugu, Nigeria on the frequency and pattern of female genital tract malignancies in 2013 revealed a similar distribution: cervical cancer (66.3%), ovarian cancer (21.1%), corpus uteri cancer (9%) and vulval cancer (3.6%)

[8]. Moreover, this study reported that the most common gynaecological cancers (cervical and ovarian) presented in advanced stages of the diseases, and too late for curative interventions to be undertaken such that the patients were only treated symptomatically. In Abakaliki, Agboeze *et al* (2015) reported similar pattern for female genital tract malignancies with 60.6% having cervical cancer, 19.2% ovarian cancer, 10.1% endometrial cancer, 7.1% vulval cancer, and 3% choriocarcinoma [5].

In a 28-year review of gynaecological cancers performed in Ibadan, Okolo *et al*, in 2013, also reported similar distribution of prevalence for gynaecologic cancers with over four–fifth, 80.5%, having cervical cancer, ovarian cancer occurred in 10.3% while 6.4%, 1.4% and 1.3% had uterine, vaginal and vulval cancers respectively [9]. A study done at the University College Hospital, Ibadan, on the characteristics and management of ovarian cancer showed that ovarian cancer still has the highest fatality rate among all gynaecological cancers mostly because of lack of effective screening methods and the non-specific early warning symptoms with subsequent late presentation [6]. In a review of 2059 women who presented in Sagamu over a period of 10 years by Adefuye *et al,* a prevalence rate of 8.7% was reported for gynaecologic cancers with the most common being cervical cancer, 51.6%, followed by ovarian cancer (35.4%), endometrial (9.9%), and choriocarcinoma (1.9%) [11]. The authors also noted that presentations were at late stages and recommended a need for the attending physicians to improve on their indices of suspicions as regards endometrial and ovarian cancers. This pattern of presentation in which cervical cancer was the commonest followed by ovarian cancer was repeatedly found in other studies conducted in other parts of the country [11–25].

Gynaecological conditions such as gestational trophoblastic diseases are equally becoming more prevalent than previously reported either because the disease condition is increasing in its rate of occurrence or rather that there are now better facilities for making diagnosis. The reported prevalence of gestational trophoblastic disease was 4.5 per 1000 deliveries in Northern Nigeria which is comparable to 4.7 per 1000 deliveries in Nnewi and 3.58 per 1000 deliveries in Abakaliki from Southeast Nigeria [26]. Gestational trophoblastic diseases (GTD) are relatively uncommon in Nigeria compared to other cancers of the female genital tracts [1,5,7,8,10,11,15,17,21,27]. GTD occur most commonly among the younger age group when they present with abnormal vaginal bleeding but generally has good prognosis when early diagnosis, proper management with good follow-up are instituted [26,27]. Adequate follow up and regular monitoring of the serum beta-human chorionic gonadotrophin (â-hCG) level, quantitatively, are important in offering the best of health care services to these patients [15,26,27][11].

There are few regional peculiarities in the management of gynaecological cancers such as preference for alternative treatments, religious influence, inaccessibility due to terrain or topography of some parts of the country, recurrent displacement and selective overpopulation especially in rural-urban migrations [1,3,28–38]. These peculiarities notwithstanding, health systems are often unprepared for adequate response to cancer care [39–42]. Sociocultural issues of the husband having the final say have precluded many women from having access to screening. In management of most patients with gynaecological cancers, three additional main problems were identified – lack of coordination between the surgical team and general practitioners in terms of early referral to facilitate prompt diagnosis, serious post-operative morbidity that caused additional pain and suffering as well as poor pain management. The lack of continuity between the acute and primary care settings and inadequate management of pain are acknowledged problems in health care [43][12].

Standard of care

The standards of care for gynaecological cancers are well defined. In several countries with established processes, there are guidelines for sorting, making diagnosis (including frozen sections), treatment and following up of patients peculiar to each level of care. In addition, there are specialized gynaecologic cancer centres with well-equipped human and non-human facilities that foster opportunities for early diagnosis and prompt management. Moreover, out-of-pocket payment for cancer care has been eased out with patients having opportunities to benefit from national health insurance schemes and/or from clinical trials. There are well established cancer registries which enable adequate provision for advanced care planning, budgeting for cancer care and research network on ways of improving cancer care.

In Nigeria, there are several reports of patients being unaware of available preventive and screening techniques which result in low uptake of such facilities where available. Other factors negatively affecting the standard of care include late presentation, lack of competent personnel and appropriate basic infrastructure for making diagnosis as well as non-availability or non-affordability of

treatment measures including surgical intervention and chemotherapy [1,30,44–48].

Challenges of gynaecologic cancer care

Globally, several studies have been conducted on the burden and epidemiological distribution of gynaecological malignancies but little attention has ever been paid to challenges of caring for these patients. It has been said that diagnosis of cancer for one person in a family will eventually affect the entire family either directly or indirectly [49, 50]. The challenges involved in management of gynaecologic cancers are multiple and vary according to the level of care. These challenges have been made worse by non-availability of insurance coverage or funded research work and clinical trials that could be directly beneficial to the patients and indirectly to the society at large [28, 51–55].

An overview of the challenges includes competition of cancer care with infectious diseases and other health needs for limited resources; under-reporting of the true incidence as a result of absence of standardized central registries; educational, religious, and economic barriers to care; absence of high-quality laboratories for tissue processing and interpretation as well as shortage of specialized diagnosis and treatment centres coupled with non-availability of a computerized database that can facilitate prompt and proper follow-up of incident cases [56]. For ease of discussion, the challenges have been divided into those encountered at the level of prevention of the disease condition, level of making diagnosis especially with respect to investigations and surgery as may be necessary, level of treatment of the disease as well as at the level of patients' follow-up and discussions on genetic risk assessment.

Challenges of prevention

Although it is generally known and agreed that prevention is the best and cheapest form of care, gynaecologic cancers faced myriads of challenges when it comes to their prevention. Factors such as national awareness, lack of political will as evidenced by continuous reduction in budgetary allocations to the health sector which in turn adversely affect policy formulation and implementation as well as religious beliefs and alternative care providers are main obstacles to successful implementation of gynaecological cancer prevention strategies [1,3,4,37,39,46,48].

Cervical cancer is preventable by vaccinating young females against human papilloma virus before sexual debut. The main challenges with prevention centered around screening and vaccination and most often have to do with awareness, accessibility and affordability [2,47,48,57]. Most people in Nigeria are not aware of vaccination and screening for cervical cancer and this has been a major challenge while those who are aware do not have easy access to screening and vaccination against human papilloma virus (HPV) which appears basic in the prevention of cervical cancer [34,36,47,58]. In addition, affordability is a major challenge for cancer preventive measures. Although HPV vaccine is licensed for use in many developing countries like Nigeria, the cost of the vaccine makes it generally unaffordable. In Nigeria it costs about USD 100.00, (36,500 naira), to complete the HPV vaccine - a country where over 70% of the population lives on less than USD 1.0 (365 naira) per day [3]. HPV detection and typing are expensive and also require highly skilled manpower which are not adequately available in Nigeria which has made cytology screening an only alternative in spite of its shortcomings. Full cervical cytology screening appears restricted to tertiary care centre only in many places with its attendant challenge of access and cost.

In addition, screening and treatment of premalignant stages are important parts of preventive measures for cervical cancers involving cytology, colposcopy and treatment of non-invasive lesions. Unfortunately, few centres in Nigeria can provide adequate colposcopic services while the "see and treat" approach following visual inspection with acetic acid (VIA) or visual inspection with Lugol's iodine (VILI) had had significantly lower uptake than envisaged.

The burden of ovarian cancer in developing countries like Nigeria appears to be rapidly increasing with a recent study suggesting greater burden than in developed countries [1,8,15,30,42,59]. The key to control of ovarian cancer practically remains an early detection and treatment at stages when cure may be possible. Despite being unrealistic, screening for ovarian cancer is done using the serum assay of CA–125 and transvaginal ultrasonography. In Nigeria, availability of reagent for CA 125 is a big challenge thus making its usage for screening at an early stage, and also for monitoring of treatment progress for the disease, a major issue. The importance of family history, clinical screening and risk of malignancy index scoring with pelvic ultrasound has been explored with minimal yield [60]. Tumour markers and genetic counselling with or without prophylactic oophorectomy are other options for prevention of

ovarian cancers but strong aversion for surgery has little potential for cultural acceptability.

Endometrial cancer is the third most common gynaecological cancer in developing countries. Population screening for endometrial cancer is not yet recommended, although early detection with transvaginal ultrasound using endometrial thickness measurement in symptomatic women with or without endometrial biopsy has been found useful but this is also a challenge in Nigeria because of late presentation.

Prevention of vaginal and vulval cancers remains an important challenge as most patients present to non-specialists who are often not suspicious of possible malignancy until the cancer stage has progressed to an advanced stage. Gestational trophoblastic neoplastic diseases are best prevented by having high index of suspicion as more than half arise from benign hydatidiform moles and this can be achieved by adequately managing and following up such patients [27].

Challenges of diagnosis

The diagnosis and management of gynaecological cancers are still challenging in low- and middle-income countries. It is a well-known fact that delays at any point increase the risk of morbidity and mortality of any disease condition. For gynaecological malignancies, most patients present initially to non-specialists who manage them for other conditions without suspecting or evaluating them for possible gynaecologic cancers thereby contributing to late presentation to specialist and subsequently late diagnosis. In the same vein, since most of the gynaecological cancers occur among postmenopausal and older women, presentation in younger, premenopausal women may lead to missed diagnosis as symptoms might be mistaken for other disease conditions.

Poor cancer awareness and knowledge among primary health-care providers in sub-Saharan Africa have been documented with its negative effect on accurate diagnosis at the primary care level, causing delays in referrals to specialists and subsequent late diagnosis [45]. Knowing fully well that substantial time would have been spent by the patient before deciding to seek medical attention (first level delay) and then making transportation arrangement to get to the health facility (second level delay), it becomes extremely important to reduce the third level delay in terms of arriving at the diagnosis and instituting management.

Early diagnosis will facilitate prompt management which in turn has the potential to yield excellent results in terms of management outcome, survival and quality of life. High index of suspicion is a sine qua non for early diagnosis and should be the ideal for every health care provider. Late presentation in respect to ovarian cancer is partly due to the anatomical location of the ovaries and this is responsible for the non-specific symptoms of early ovarian cancer, leading to late diagnosis [1,6].

Histopathological service is mostly provided in tertiary health institutions. Unfortunately, these teaching hospitals are too often affected by different types of industrial actions! Worse still, it is important that doctors wait and review the histological diagnosis rather than act on their clinical evaluation and diagnosis alone [57]. It is imperative to note that delays in obtaining histopathology results is alien to many developed nations that have been able to successfully remove unnecessary bottlenecks in making diagnosis of gynaecologic cancers. In addition, non-availability of facilities for frozen sections (except in few specialized centres such as the University College Hospital, Ibadan) negatively affects the ability to make intraoperative diagnosis and optimize surgical care as may be necessary.

Other challenges of diagnosis include lack or inadequacy of diagnostic materials and specialists. Although most teaching hospitals and federal medical centres (with the aid of the public–private partnership initiative) now have some basic radiological facilities, several centres in Nigeria still lacked the necessary facilities for specific investigations such as computed tomography, magnetic resonance imaging, serum CA-125 and HPV DNA among other specialized investigations. In few centres where some of these specialized facilities are available, they are hardly affordable for most patients as payments are usually done out-of-pocket [1].

Challenges of treatment

Optimal treatment of patients with gynaecological cancers is premised on many factors including the stage of disease at presentation. As mentioned earlier, late presentation is a major challenge to successful treatment of cervical, ovarian and endometrial cancers which are the most common in this environment. Most patients are offered symptomatic treatment because they present in the advanced stage of the disease. Late presentation of gynaecological cancers in developing countries tends to be multifactorial ranging from lack of insight into the implications of the disease condition to the outright

rejection of treatment options based on cultural or religious beliefs [41].

Another factor responsible for late presentation in Nigeria is the health-seeking behaviour which is still pervaded by ignorance and rooted in traditional beliefs despite exposure to western civilization although these are often fueled by poverty, ignorance and illiteracy. One of such belief is that cancers are caused by witchcraft and are not amendable to medical care coupled with the fact that the herbalists tend to offer cheaper care than orthodox treatment. Other factors that contribute to delayed presentation for care in the hospital is the subordinate role of women in traditional African societies, which limits the capacity of women to express themselves and report symptoms related to genital tract or seek medical attention without the approval of the husband or the husband's family. Lack of economic empowerment for the women in terms of being able to fund treatment equally contributes to late presentation.

Another major challenge is the non-availability or inadequacy of facilities and expertise for proper treatment. In a scarce health resource country like Nigeria where there are multiple challenges in battling infectious diseases such as malaria, human immunodeficiency virus, tuberculosis and Lassa fever such that cancers care is hardly a priority. There is a paucity of radiotherapy centres in government institutions; the few available are poorly maintained. Facilities in private sectors are limited and inaccessible to the common man due to high cost of treatment. For instance, at the moment, there is no single functioning radiotherapy facility in the entire country of Nigeria with a population of over 180 million people and patients have to travel to other countries for radiotherapy. In sub-Saharan Africa generally, there is a significant dearth of facilities for radiotherapy [28,45,46,61,62]. In addition, laboratory reagents for biochemical parameters such as serum cancer antigen (CA)–125 assay are not readily available in many centres such that patients have to be referred to other centres which is quite stressful [39,40,42,45,48].

In Nigeria, as in many other resource-limited countries, there is no policy or action plan regarding cancer care. Cancer care is excluded in the National Health Insurance Scheme (NHIS) in Nigeria and this contributes a huge financial burden on the patient and relatives [1,3,28,30,38,41,51,63]. In the absence of research funds or clinical trials that cancer patients can directly or indirectly benefit from, it is believed that provision of insurance coverage for them will encourage early presentation with prompt, optimal management and better outcomes [43,45,48,51,64–67,54,53,68,52,69]. A potential for overtreatment has been speculated in cases managed with VIA and VILI. However, it is imperative to ensure an uptake of the screening program in the first instance, be able to measure its negative and positive predictive values before introducing further measures to address the challenges of overtreatment.

In addition, limitation in the number of specialists in gynaecological oncology is also a challenge to care of gynaecological cancers. There is still exists a huge unmet need for trained gynaecological oncologists, oncology nurses, radiotherapists and interventional radiologists as well as intensive care facilities [45].

Challenges of follow up

Patients often say that the aftermaths of cancer treatment are tougher than the treatment itself. Moreover, the cancer care system in Nigeria should look to members of the gynaecological care team to care for patients in this follow-up and rehabilitative phase of care. The goals of follow up include: health promotion and prevention (to lower the risk of new and recurrent cancers), monitoring for recurrent cancer and other late effects (such as depression and vocational challenges), management of symptoms resulting from cancer and its treatment (fatigue, peripheral neuropathy, sexual dysfunction) as well as discussions on the need for possible genetic risk assessment, counselling and testing.

Abandonment of treatment and follow up is a major challenge encountered repeatedly in gynaecological cancer care and could be ascribed to several factors such as inability to sustain funding for treatment and prescribed drugs, lack of clinical improvement following initial treatment and inability of patients to tolerate side effects of cytotoxic drugs [1,70,71]. Supportive care for the side effects of chemotherapy could be hampered by the high cost of antiemetics, erythropoietin and blood transfusion facilities [1].

An uncommon challenge for many long-term gynaecological cancer survivors is lack of access to the records documenting the treatment they received. For example, in situations involving the need for a change of doctor due to various reasons, the new doctor may not have full information about the treatment the patient has had so far as most of our patients are not literate and thus due follow up is hampered. This is worse in situations in which chemotherapy regimens are repeatedly changing as a result of advancement and breakthroughs in medical world.

Psychological challenge

As patients recover from gynaecological cancer, there may be emotional distress and difficulties requiring continued coping. Persisting emotional distress from the trauma of diagnosis, treatment and generally altered quality of life may occur in about 5-7% of cancer patients [72]. Some patients actually rate the physical sequelae of treatment as the most significant challenge of survivorship. In addition to the cancer patient, the stress may become significant for her caregivers such as spouse or children. Problems facing the family members of a cancer patient include loneliness, isolation, and role overload [72].

Gynaecologic cancer survivors also reported significantly higher anxiety scores than all other patients, and higher depression scores and lower well-being scores than gastrointestinal and urologic cancer survivors [50,67,72–74]. Recurrence of cancer and frequent complication from disseminated disease such as pain and fear of death also contribute to the psychological challenge of gynaecological cancer care thus necessitating the need for psychologists and the palliative care team in the management of gynaecological cancers [72].

Psychosocial factors affecting survival included provision of frank details about the disease condition and its prognosis, treatment choices, medication use and available alternative options. Availability and use of adequate medications for pain relief, provision of spiritual care and availability of palliative care services can interfere in patient management. In addition, the patients' autonomy as well as family and community participation in care, right mental attitude, social support, end of life issues and preservation of fertility, where feasible, are also big issues that determine the course of gynaecological cancer care [31,49,72-77].

Recommendations

It is beyond doubt that gynaecological cancers are of significant public health interest in spite of the daunting challenges associated with them. In Nigeria, concerted efforts should be made at identifying these challenges and offering lasting solutions to improve the health of our women. Based on the epidemiology, risk factors and challenges of gynaecological cancer in Nigeria, the following recommendations will go a long way in reducing the effects and impact of these challenges.

i. Prevention strategies: according to the World Health Organization, generally 43% of all cancers are preventable using primary, secondary or tertiary measures. Primary measures are aimed at reducing or eliminating exposure to risk factors or carcinogens. Secondary measures aimed at early detection of cancer or screening for pre-malignant stages while tertiary measures are treatment or palliative care given to diagnosed cancer cases to avoid complications and improve quality of life [78].

a. Primary prevention include health education and awareness of these gynaecological cancers, outreaches should be organized in the community so as to educate them on the early warning signs of gynaecological cancers, who is at risk, screening and vaccination, when and where to present for treatment. People should also be informed (through community awareness and the media) about health promotion through dietary control, consumption of fruits and vegetable, avoiding sedentary life styles, exercise, tobacco and alcohol control. By doing such, most of the modifiable risk factors of cancers would have been avoided.

b. Vaccination is another important factor that helps in reducing the burden of cervical cancer. Though cervical cancer is deadly but it is also preventable by vaccinating young male and female against human papilloma virus before sexual exposure. Government should subsidize the cost of vaccination in order to make it affordable. Human papilloma virus vaccination should also be integrated as part of the preventive measures for cervical cancer.

ii. Early detection and diagnosis will also go a long way to prevent complications from advanced stages of gynaecological cancers. The cervical cancer screening should be coordinated and emphasis should be shifted to using rapid screening tests like Visual Inspection with Acetic acid (VIA) or Visual Inspection with Lugol's Iodine (VILI) for screening at community levels at high coverage (78), and this is quite accessible and affordable, cytology screening can be left for the teaching hospitals.

a. Histological diagnosis should also be looked into, equipment that process tissue faster should be made available so patients can get histological diagnosis early enough and start treatment.

b. HPV DNA testing is a cost-effective measure which should be made feasible for all in Nigeria by the government.

iii. National Policy on Cancer Care: It is extremely important to form a concise national policy on cancer care in resource-limited setting like Nigeria. This would make cancer a priority disease, and would ensure adequate resource allocation for its control. Putting cancer care on the National health insurance plan would have a huge impact [57].

iv. Investment in health care systems in Nigeria: For oncology, this includes developing a sustainable

supply of trained oncology professionals, expanding the supply of treatment modalities, improving drug supply, physical infrastructure and organizational infrastructure for cancer control [57]. This will offer definitive positive impact on gynaecological cancer care in Nigeria.

v. Establishment of specialized cancer centres and cancer registries: It has been observed that the establishment of national obstetric fistulae centres across the country has contributed in no small measure to reduction in the prevalence of obstetrics fistulae in the country. As a corollary, it has become imperative to establish specialized cancer centres at different regions of Nigeria. Cancer centres are unique in facilitating evidenced-based and gold standard care to patients. Moreover, pooling of cases into specified centres help in further entrenching expertise in subsequent case managements as well as in data collection. Risk assessment, genetic counselling and testing as well as prophylactic care are better handled in specialized cancer care centres. It also becomes feasible to participate in local and internationally-organized clinical trials with subsequent positive impact in the overall services rendered to cancer patients. Data on management outcomes, including records of death, will be easier to gather in specialized cancer care centres.

vi. Training and retraining of gynaecological oncologists. This will ensure a sustainable supply of trained oncology professionals. Encouraging specialists also in the area of research will give opportunity to know the epidemiology of the disease and the new trend of the disease. Multidisciplinary and translational research is essential to improve our understanding of the modifiable risk factors for cancer in African populations, and for the development of evidence-based prevention and treatment interventions to reduce cancer-associated morbidity and mortality [57]. Establishment of regional centres for coordination of cancer care has been advocated [45].

vii. Psychological supports/Survivor groups: Psychological services should be offered to both patients and their relatives with the aim of helping them cope with emotional and psychosocial stress. Support groups for gynaecological cancer survivors should be created so they can share their experiences and this could also help recovery. Moreover, most of the survivor group members are able to facilitate additional funds for care of the new patients in forms of donations, grants and procurement of equipment for further care.

Conclusion

In resource-constrained countries without specialized services, experience has shown that much can be done to prevent and treat cancer by deployment of primary and secondary caregivers, use of off-patent drugs, and application of regional and global mechanisms for financing and procurement. Furthermore, several middle-income countries have included cancer treatment in national health insurance coverage with a focus on people living in poverty. These strategies can reduce costs, increase access to health services, and strengthen health systems to meet the challenge of cancer and other diseases [53,78]. Advanced care planning including palliative care for gynaecological cancer patients is an important element of care package that should be embraced by all and adapt into routine care for these patients [48,54,67,79].

Management of gynaecologic cancers will yield better outcomes when substantial efforts are placed on primary preventive mechanisms, early diagnosis and prompt management. Primary prevention remains the goal standard to pursue in our environment and includes putting concerted efforts on health education and awareness of the gynaecological cancers, approaches to screening and vaccination as well as genetic counselling and prophylactic measures where feasible.

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Views and preferences of patients attending a tertiary hospital in Nigeria on use of saliva for clinical or laboratory tests

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Abstract

Aim: The purpose of this study was to assess the knowledge and views of patients on the use of saliva for clinical or laboratory analysis.

Methods: This was a cross sectional survey of 189 patients attending one primary and one tertiary oral health facility in Nigeria. Information was obtained from participants using pretested structured questionnaires and SPSS version 23 to analyze the data. Tests of associations between variables were determined using Chi-square and level of significance set at < 5%.

Results: One hundred and fifty-two (80.4%) respondents were aware of the use of saliva for clinical or laboratory test. The majority 152 (80.4%) agreed that saliva is easier to collect than other body fluids while 63 (33.3%) preferred to give saliva sample to blood 54 (28.6%) and urine 51 (27%) for clinical or laboratory tests. Only 20 (10.6%) had given saliva for tests before. Ninety-nine (52.4%) indicated strong interest in donating saliva for research while only 4 (2.1%) had given saliva as samples for research work before. Age, educational status and occupational class were significantly associated with awareness of use of saliva as investigative specimen ($p < 0.05$).

Conclusions: This survey revealed that majority of the patients were aware of the use of saliva as well as its advantages over other body fluids for clinical or laboratory tests. Very few indicated previous saliva sampling for clinical and laboratory tests. Thus there is need for development of precise, cheap and accessible saliva tests for patient-centered diagnostic testing and disease monitoring.

Keywords: *Awareness; clinical testing; diagnosis; laboratory testing; patients; saliva*

Résumé

But: Le but de cette étude était d'évaluer les connaissances et les points de vue des patients sur l'utilisation de la salive pour l'analyse clinique ou laboratoire.

Méthodes : Ceci fut une enquête transversale de 189 patients fréquentant un établissement primaire et un établissement tertiaire de santé bucco-dentaire au Nigeria. L'information a été obtenue des participants en utilisant des questionnaires structurés pré testés et SPSS version 23 a été utilisé pour analyser les données. Les tests d'associations entre variables ont été déterminés en utilisant le Chi-carré et le niveau de signification fixé à <5%.

Résultats : Cent cinquante-deux (80,4%) répondants étaient au courant de l'utilisation de la salive pour des tests cliniques ou laboratoire. La majorité des répondants (80,4%) ont indiqué que la salive était plus facile à recueillir que les autres fluides corporels, 63 (33,3%) préféraient donner des échantillons de salive que de sang 54 (28,6%) et d'urine 51 (27%) pour des tests cliniques ou laboratoire. Seulement 20 (10,6%) avaient donné de la salive pour les tests avant. Quatre-vingt-dix-neuf (52,4%) ont manifesté un vif intérêt pour le don de salive à des fins de recherche alors que seulement 4 (2,1%) avaient donné de la salive comme échantillons pour des travaux de recherche auparavant. L'âge, le niveau d'éducation et la classe professionnelle étaient significativement associés à la connaissance de l'utilisation de la salive comme spécimen d'investigation ($p <0,05$).

Conclusions: Cette enquête a révélé que la majorité des patients étaient conscients de l'utilisation de la salive ainsi que de ses avantages par rapport aux autres fluides corporels pour des tests cliniques ou laboratoire. Très peu ont indiqué que des échantillons de salive ont été prélevés auparavant pour des tests cliniques et laboratoire. Il est donc nécessaire de développer des tests de salive précis, à bas prix et accessibles pour les tests de diagnostic centrés sur le patient et la surveillance des maladies.

Mots clés: *Sensibilisation; essais cliniques; diagnostic; essais laboratoire; patients; salive*

Introduction

Several clinical conditions can be assessed by using saliva as a diagnostic biofluid. For example, data are available that correlate levels of specific salivary proteins or RNAs with parameters of oral cancer [1-3] and breast cancer [4,5]. Oral fluid based tests also exist or are being developed to detect a variety of

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infectious diseases including HIV, parvovirus, acute hepatitis, dengue fever and malaria, as well as to detect alcohol, drug use and steroid hormone levels [1,6,7].

Although, changes in salivary composition can provide insight into disease pathogenesis, in a review of saliva's premise as a diagnostic tool, it was emphasized that if no one uses the test then the test is not useful [6]. It has been reported in the literature that saliva collection/testing will become accepted for diagnostic procedures only if a greater focus is placed on diagnosis and disease susceptibility rather than immediate treatment [8].

One of the key presumed advantages of using saliva as a diagnostic tool is that it is easier to collect and avoids the invasiveness and discomfort associated with collecting blood and the inconvenience associated with collection and the occasional inability to collect urine. However, a literature search of medical and dental databases (PubMed, HINARI and Cochrane collaboration), revealed sparse patient data on the knowledge and practices of the use of saliva and other body fluids (blood and urine) for clinical testing and to support the assumption that saliva offers some advantages over other traditional diagnostic fluids for clinical or laboratory testing. Without acceptability or demand from patients for salivary testing, saliva is unlikely to be used as often as blood and urine are for diagnostic testing. This could be due to lack of evidence or dearth of research into this field; hence the need for this study.

In addition, there is a need for data to evaluate the advantages or disadvantages of saliva compared to other traditional diagnostic fluids for clinical testing from the patients' perspectives. The findings from this study may help elucidate the level of awareness of and receptivity toward saliva-based tests among patients. This will offer insights on this issue and move our understanding from mere conjecture to the realm of empirical evidence. Thus, this study aimed at assessing the knowledge and opinions of patients on the use of saliva for clinical and laboratory tests.

Materials and methods

This was a pilot descriptive cross sectional survey carried out at a Primary Oral Health Care Centre and a Tertiary Dental Centre; both of the University College Hospital, Ibadan, Nigeria. Consecutive adult patients aged 16 years and above attending the clinics, for the first time, during the period of the study were recruited. Information on biodata of the participants and their views as it relates to the use of saliva in clinical and laboratory testing was obtained through a structured questionnaire. The questions assessing the biodata of the participants evaluated their age, gender, marital status, tribe, occupation and level of education. The occupational class of respondents was classified based on a modification of the classification from the Office of Population Census and Survey (OPCS) into skilled workers, unskilled workers and dependants [9]. The questionnaire also assessed the knowledge of use of saliva in clinical and laboratory testing, sources of this knowledge, the diseases that it could be used to investigate and diagnose, their preferred choice of sample donation as it relates to saliva and other body fluids, perceived advantages of saliva over other body fluids and if they would prefer to give saliva for research purposes. The questionnaire was pretested among 30 patients in the two clinics, before the onset of the study, to validate the questionnaire and determine the ease of answering the questions as well as its comprehensiveness. Prior to administration of the questionnaire, the purpose of the study was explained to the patients and only those who consented were recruited for the study. Patients who participated in the pre-testing of the questionnaire were excluded from the main study. Patients in pain and those below the age of 16 years were excluded from the study. Ethical approval was obtained from the Joint University of Ibadan/ University College Hospital Ethics Review Committee (UI/EC/13/0420). All statistical analyses were carried out using SPSS Version 23. Categorical data were displayed as frequencies and percentages and compared with Chi Square while quantitative data were displayed as mean ± standard deviation (SD). For the purpose of analysis and to reduce the number of empty cells; age was dichotomized around the mean age as less than or equal to 34 years and above 34 years. Educational qualification was also constructed as a binary variable; less than tertiary and tertiary qualification. Statistical significance was accepted when $p < 0.05$.

Results

A total of 189 patients participated in the study. The age of the participants ranged from 16 to 78 years and the mean age was 33.9 (± 13.1) years. There were 92 (48.7%) male, many 139 (73.5%) of the respondents were of the Yoruba tribe and 82 (43.4%) were dependants (Table 1).

Knowledge of use of saliva in clinical and laboratory tests

One hundred and fifty-two respondents knew saliva could be used for clinical and laboratory testing and mass/social media was the major source of

knowledge 45 (29.6%). Other sources of knowledge included; training 43 (28.3%), journals/scientific publications and conferences 28 (18.4%), other sources such as internet, friends, families, hospital 14 (9.2%) while others 22 (14.5%) could not remember the source of information. The majority 108 (57.1%) mentioned that saliva could be used for diagnosis of disease condition. The diseases mentioned included oral diseases 121 (64.0%), systemic diseases 59 (31.2%) and HIV 33 (17.5%), Fig.1.

Table 1: Socio-demographic characteristics of study participants

Variable	Frequency	%
Age (years)		
≤ 20	25	13.2
21-40	119	63.0
41-60	35	18.5
> 60	10	5.3
Sex		
Male	92	48.7
Female	97	51.3
Marital status		
Single	86	45.5
Married	100	52.9
Widowed	3	1.6
Educational qualification		
None	7	3.7
Primary	5	2.6
Secondary	26	13.8
Post-secondary	56	29.6
Tertiary	95	50.3
Occupational class		
Skilled	62	32.8
Unskilled	45	23.8
Dependants	82	43.4

Advantages, preference and convenience of saliva and other body fluids

The majority strongly agreed or agreed that saliva has some advantages over other body fluids (Table 2), which included; ease of collection 152 (80.4%), elimination of fear of prick 130 (68.8%), lower cost of sample collection 135 (71.4%) and reduced risk of infection 122 (64.6%). Of the three body fluids (saliva, urine and blood); saliva was the most preferred specimen to give as sample for tests by the respondents 63 (33.3%), followed by blood 54 (28.6%), urine 51 (27.0%) and none was preferred by 21 (11.1%). With regards to most convenient and comfortable specimen to give; saliva was the most frequently mentioned 100 (52.9%) followed by urine 52 (27.5%) and blood 25 (13.2%) while 12 (6.3%) were indifferent.

Interest in giving samples for research purposes

Responses to questions on giving of samples for research purposes showed that 136 (72.0%) respondents were interested in donating saliva, 27 (14.3%) were not interested and 26 (13.8%) were undecided. A significant number 128 (67.7%) were interested in giving urine, 33 (17.5%) were not interested and 28 (14.8%) were undecided. One hundred and four (55.0%) were interested in giving blood for research, 59 (31.2%) were not interested and 26 (13.8%) were undecided.

Only 20 (10.6%) patients had given saliva sample for clinical or laboratory tests. The respondents that had previously given saliva as sample for clinical or laboratory tests collected their sample in form of spitting 9 (45.0%), use of cotton wool 8 (40.0%) and with the use of mechanical

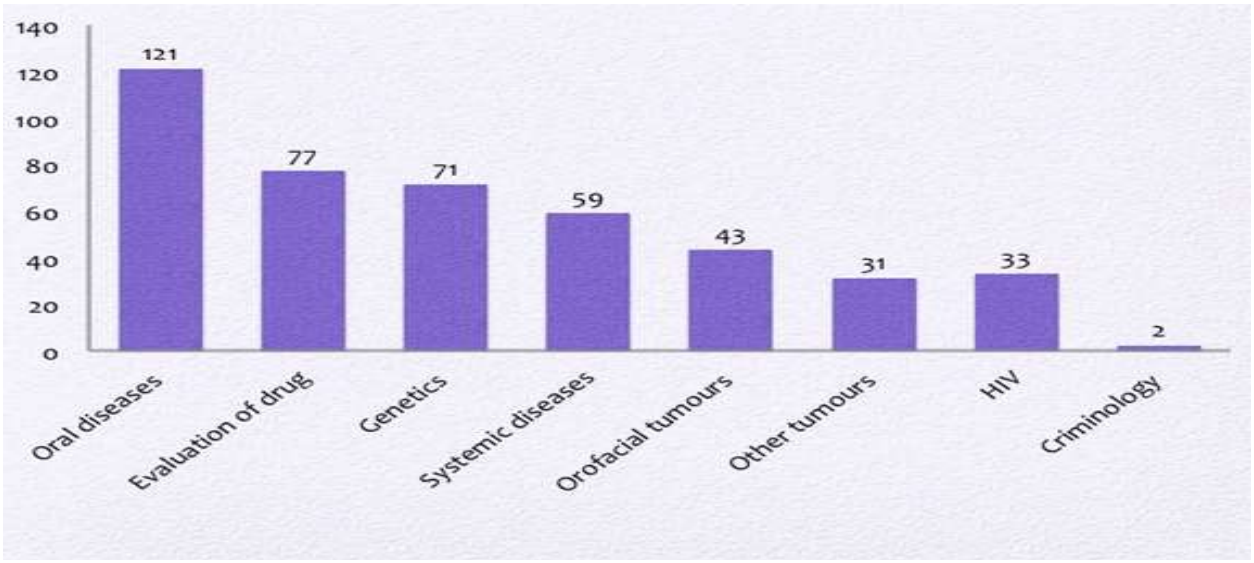

Fig. 1: Responses from the patients on the use of saliva for clinical and laboratory testing

collector 2 (10.0%) while 1 (5.0%) could not remember how it was collected for the investigation. The site of sample collection included the hospital 16 (80.0%), home 2 (10.0%) and laboratory 2 (10.0%). The saliva sample was used for disease diagnosis 9 (45.0%), research purposes 4 (20.0%), treatment monitoring 2 (10.0%) and other things 5 (25.0%) such as genetic tests and DNA analysis.

The specific investigations the saliva sample was used for included; HIV tests 2 (10.0%), oral diseases 6 (30.0%), systemic diseases 2 (10.0%), drug evaluation 2 (10.0%), genetic tests 6 (30.0%), and others such as investigating tuberculosis 2 (10.0%).

Sociodemographic characteristics and knowledge of saliva as clinical and laboratory specimen

The younger age group (≤ 34 years) knew more than those > 34years that saliva could be used as an investigative specimen for clinical or laboratory tests (p = 0.02). A higher proportion of those with tertiary education also mentioned that saliva could be used as an investigation specimen (p = 0.01). Those in the skilled occupational class also had a greater awareness than others that saliva could be used as an investigation specimen (p = 0.001). There was no association between other sociodemographic variables and knowledge of saliva as a specimen for clinical and laboratory tests (Table 3).

Table 2: Advantages of saliva over other body fluids as specimen

Advantages of saliva	Views of respondents			
	Strongly agree n (%)	Agree n (%)	Strongly disagree/ Disagree n (%)	Don't know n (%)
Ease of collection	104 (55.0)	48 (25.4)	4 (2.1)	33 (17.5)
Elimination of prick	75 (39.7)	55 (29.1)	14 (7.4)	45 (23.8)
Lower cost of sample collection	70 (37.0)	65 (34.4)	13 (6.9)	41 (21.7)
Reduced risk of infection	66 (34.9)	56 (29.6)	21 (11.1)	46 (24.3)
Does not require special skills	64 (33.9)	46 (24.3)	32 (16.9)	47 (24.9)

NB: There were very few strongly disagree "responses" hence "strongly disagree" and "disagree" were merged to reduce empty cells.

Table 3: Socio-demographic characteristics and knowledge of saliva as clinical and laboratory specimen

Variable	Knowledge of saliva as diagnostic specimen		Total	X^2	p value
Age (years)	Yes (%)	No (%)	n (%)		
≤ 34	107 (84.9)	19 (15.1)	126 (100.0)	5.9445	0.015*
>34	44 (69.8)	19 (30.2)	63 (100.0)		
Total	151 (79.9)	38 (20.1)	189 (100.0)		
Sex					
Male	75 (81.5)	17 (18.5)	92 (100.0)		
Female	76 (78.4)	21 (21.6)	97 (100.0)		
Total	151 (79.9)	38 (20.1)	189 (100.0)	0.296	0.359
Educational qualification					
Tertiary	83 (87.4)	12 (12.6)	95 (100.0)	6.643	0.010*
Less than tertiary	68 (72.3)	26 (27.7)	94 (100.0)		
Total	151 (79.9)	38 (20.1)	189 (100.0)		
Occupational class					
Skilled	53 (85.5)	9 (14.5)	62 (100.0)	14.579	0.001*
Unskilled	27 (60.0)	18 (40.0)	45 (100.0)		
Dependants	71 (86.6)	11 (13.4)	82 (100.0)		
Total	151 (100.0)	38 (100.0)	189 (100.0)		

Discussion

The use of saliva in diagnostics has been introduced since the second half of the 20th century [10]. Its main advantage is easy and non-invasive sample collection compared to peripheral blood. Generally salivary analysis has shown promising suitability in two important areas: early detection of some diseases and monitoring the course of the disease as well as the treatment outcome [11-14]. In addition, saliva has shown significant application in the detection of addictive drugs [15,16]. However, despite all the attributes and achievements in salivary diagnostics, its use for clinical testing is still subject to its acceptability by the patients. Within the context of our environment, in a developing country, where research as well as health promotion is of paramount need, the knowledge and preference of the populace on the use of saliva as an alternative to other body fluids (especially blood and urine) for clinical or laboratory tests are essential.

In this study, majority of the participants (80.4%) knew that saliva could be used for clinical or laboratory testing, which is an indicator that the awareness is good. The high level of awareness may be explained by the socioeconomic status of the participants. Majority of the participants had tertiary education and the findings indicated that the major source of awareness was print/electronic media.

The majority (80.4%) agreed that saliva is easier to collect than other body fluids while 33.3% preferred to give saliva sample to blood (28.6%) and urine (27%) for clinical or laboratory tests. Although the percentage of participants that indicated preference for saliva sampling in our study is lower than those reported in previous studies [8,17], this finding indicates that among the three body fluids, saliva was most preferred to give for clinical or laboratory test by the participants. Similarly, McCall *et al*., in their assessment of patients' preferences for drug testing methods and comparison of the acceptability of urine testing versus oral fluid testing within a hospital setting, reported that majority (85%) of the respondents indicated preference for oral fluid testing [18]. Also, they showed that the majority of patients and staff rated oral fluid as more comfortable and easier to give.

Questions on giving of samples for research purposes showed that majority (72.0%) were interested in donating saliva for clinical or laboratory tests. However, only 20 (10.6%) had given saliva sample for clinical or laboratory tests before the study. This finding is similar to previous report by Dhima *et al.*, which showed that only 11 (10.6%) of their respondents had ever given saliva samples for medical appointments or research studies [17]. This suggests that the use of saliva for clinical or laboratory test is still poor in our environment, although whether this has improved in the developed countries is not known. Among those that indicated previous saliva sampling, the specific use indicated were disease diagnosis (47.4%), research purposes (21.1%), and treatment monitoring (10.5%). This shows that despite the awareness and the advantages of saliva over other fluids (blood and urine) as well as the respondents' preference of its use for clinical or laboratory testing, very few have previously given saliva for different purposes. One of the factors that may account for the low prevalence of previous use of saliva for clinical or laboratory tests may be non-availability of point of care saliva based tests in our environment.

One limitation of this study is the inclusion of patients attending the hospital setting, which may limit the applicability of the finding to the general population. For the purpose of early disease detection or screening exercise, a survey in a non-hospital setting would have been more appropriate. Another limitation is that the sample is relatively small to make generalized submissions.

Conclusion

This study evaluated knowledge and opinions of patients attending dental care settings in Nigeria on the use of saliva, urine and blood samples for clinical or laboratory testing. The majority was aware of the use of saliva and also indicated preference for saliva sampling over other body fluids (blood and urine). Very few indicated previous saliva sampling for clinical and laboratory tests. Thus there is need for the development of precise, cheap and accessible saliva-based tests for patient-centered diagnostic testing and disease monitoring.

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Behavioural responses of medical students on exposure to cadaver dissection

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Abstract

Objective: Medical Students react differently when exposed to cadaver dissection for the first time. Reactions range from fear to anxiety, conjunctiva irritation, nausea and vomiting. Long term effects include loss of appetite, insomnia, headaches, to mention a few despite its importance in the training of Medical students. This study therefore seeks to determine the influence of cadaver dissection on behavioural patterns and its effect on the consumption of meat among Medical students.

Method: This cross sectional survey includes 240 volunteer first year Medical and Paramedical students of the Delta State University, Abraka, Nigeria, who correctly filled and returned administered questionnaires with thirteen statements of problems. The students were to choose either YES or NO for each statement put forward.

Results: Result reveals 57.9% were excited at first exposure, 61.7% experienced emotional shock, 47.5% experienced fear while virtually all respondents 93.0% experienced eye irritation. A later observation revealed 44.2% experienced headache, 57.5% dizziness, 48.0% experienced disturbed sleep, 27.1% experienced loss of appetite, 23.8% experienced dislike for meat and 19.2% experienced unusual thirst.

Conclusion: Most of their experiences could be associated with improper orientation before contact with cadaver, smell of formalin and its constituents. However most students believed cadaver dissection is important in their training. Therefore, proper orientation before first contact with cadaver could help reduce some of the behavioural responses observed.

Keywords: *Cadaver, dissection, behavioural response*

Résumé

Objectif : Les étudiants en médecine réagissent différemment lorsqu'ils sont exposés à la dissection de cadavre pour la première fois. Les réactions vont de la peur à l'anxiété, l'irritation conjonctive, des nausées et vomissements. Les effets à long terme inclure la perte d'appétit, l'insomnie, des maux de tête, pour n'en citer que quelques-uns en dépit de son importance dans la formation des étudiants en médecine. Cette étude cherche donc à déterminer l'influence de la dissection des cadavres sur les comportements et leurs effets sur la consommation de viande parmi les étudiants en médecine.

Méthode : Cette enquête transversale comprend 240 bénévoles étudiants en première année de médecine et paramédical de l'Université de l'Etat de Delta, à Abraka, au Nigéria, qui ont correctement rempli et renvoyé les questionnaires administrés avec treize déclarations de problèmes. Les étudiants devaient choisir OUI ou NON pour chaque énoncé proposé.

Résultats : Le résultat révèle que 57,9% ont été excités à la première exposition, 61,7% ont éprouvé un choc émotionnel, 47,5 % ont eu peur et pratiquement tous les répondants 93,0% ont éprouvé une irritation des yeux. Une observation ultérieure a révélé que 44,2% ont eu des maux de tête, 57,5% d'étourdissements, 48,0% de sommeil perturbé, 27,1% de perte d'appétit, 23,8% d'aversion pour la viande et 19,2% de soif inhabituelle.

Conclusion : La plupart de leurs expériences pourraient être associées à une mauvaise orientation avant le contact avec le cadavre, l'odeur de formol et ses constituants. Cependant, la plupart des étudiants croient que la dissection du cadavre est importante dans leur entraînement. Par conséquent, une bonne orientation avant le premier contact avec le cadavre pourrait aider à réduire certaines des réactions comportementales observées.

Mots-clés: *Cadavre, Dissection, Réponse comportementale*

Introduction

Anatomy which deals with the structure of the body is vital to the training of medical student. It is one of the three core subjects taught at the preclinical level. Traditionally, gross anatomy is taught in various medical schools with the aid of cadaver or cadaver specimen, this is accepted universally [1] . Cadaver, as a teaching and demonstrating tool is also accepted in Nigeria. Most medical students look forward to

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dissecting cadaver for the first time. Dissection tends to bring about familiarity with the human body. All association of anatomy clearly state cadaver dissection provides an essential building block of knowledge for medical students [2] .

Previous studies revealed cadaver dissection contributes to the ritual transformation of lay people to medical practitioners [2]. 'Dissection of cadaver is a daunting experience and is regarded as the first rite of passage in medical training'[3]. Dyer and Thornlike (2000) revealed the persistence and universality of cadaver dissection as features of medical education [4]. For over five hundred years, human cadaver has been constantly used as a learning tool [5-7]. Cadaver dissection seems to be the most accepted universal tool for training of medical students. The first medical student to dissect a cadaver was Andreas Vesalius [8].

In the last few years however, concern and controversies have ensued in the effectiveness and appropriateness of using humans as learning tools. Some researchers feel prosected bodies or models are as effective as dissection. [5,9,10]

Aziz *et al*., (1999) admit cadaver is without doubt an unwieldy tool in teaching anatomy to medical students despite his promotion of re-evaluation of cadaver as teaching tool [11]. He claimed anatomy dissection exposes students to high level of formaldehyde and diseases from fixation resistant viruses. Dubhashi *et al*., (2011) also revealed that students, during dissections are exposed to high level of formaldehyde which is well documented for its toxic effects [12]. Older (2004) reveals students may experience anxiety and stress when exposed to cadaver dissection [13]. Some researchers claim prosected bodies are as effective as traditional dissection in the study of anatomy [5, 9]. Some also believed cadaver dissection is archaic and should be replaced with anatomical models and electronic media. About 25-48% of medical student in the USA and UK see dissection as challenging [14,15]. Despite the shortcoming associated with dissection, it is still a major teaching tool in most medical schools as most medical student look forward to it with great excitement. This study therefore seeks to determine student's behavioural responses towards cadaver dissection and its effect on meat consumption among Medical and Paramedical students of Delta State University, Abraka, Nigeria.

Materials and method

Type of study

This is a descriptive questionnaire-based cross sectional study. The purpose of this study was explained to all first year medical and paramedical students of Delta State University, Abraka, Nigeria who take part in cadaver dissection.

Study population

Two hundred and forty volunteer Medical and paramedical students of Delta State University, Abraka correctly filled and returned structured questionnaires with thirteen statements of problems anonymously.

Method of data collection

Questionnaires that were not properly filled were discarded. Students' excitement at first contact, emotional shock, eye irritation, fear, unusual thirst, nausea, vomiting, tiredness, disturbed sleep, headache, dizziness, loss of appetite, hatred for any meat were accessed. Respondents were to either tick Yes or No, regarding each statement. The questionnaire had two sections.

Section A: experience at first encounter
Section B: experience on a latter date

Analysis of data

Data obtained were analyzed using SPSS Version 21 for descriptive statistics. Results are presented in frequency and percentages of responses for each item of the questionnaire. Results were then compared with available literature.

Results and discussion

On first contact with cadaver a total of 139 (57.9%) students were excited, 148 students (61.7%) were shocked, 114 (47.5%) students became afraid of cadaver and virtually all the student experienced eye irritation (93%). Also, 48 students felt like vomiting (20.2%), 5 (2.08%) actually vomited and 138 (57.5%) felt dizzy. Result also revealed that students experienced the following after some weeks of dissection. 44.2% experienced headache, 57.5% tiredness, 20% disturbed sleep, 27.1% experienced loss of appetite, 23.8% developed hatred for meat and 19.2 % unusual thirst. Our study has established the influence of cadaver dissection on students' behavioural responses to fear, shock, excitement, eye irritation as well as its effect on meat consumption.

Results reveal cadaver dissection is an interesting part of learning anatomy as students looked forward to it with eagerness. First dissection experience was exciting and interesting to students as shown in table 1. A study by Rajkumari *et al*., (2007) also showed certain similarities. (16) 61.7% experienced emotional shock despite their initial

Table 1: Behavioural response experienced on first entry into dissecting room

Questions		Yes (%)	No (%)
1.	Did you find your first visit to dissecting room exciting	139 (57.9)	101(42.1)
2.	Did you experience emotional shock at first exposure to cadaver	148 (61.7)	92 (38.3)
3.	Did you experience eye irritation at first exposure to cadaver	224 (93)	16 (7)
4.	Were you afraid on first exposure to cadaver	114 (47.5)	126 (52.5)
5.	Did you feel like vomiting on seeing a cadaver for the first time	48 (20.2)	192 (79.8)
6.	Did you vomit at first exposure to cadaver	5 (2.08)	235 (98)
7.	Did you feel dizzy at first exposure to cadaver	138 (57.5)	102 (42.5)

Table 2: Symptoms experienced on later date after exposure to dissecting room

Questions		Yes (%)	No (%)
1	Do you feel headache after exposure to dissections	106 (44.2)	134 (55.8)
2	Do you feel tired after exposures to dissection	138 (57.5)	102 (42.5)
3	Do you experience disturbed sleep after exposures to dissection	48 (20.0)	192 (80.0)
4	Do you experience loss of appetite after exposures to dissection	65 (27.1)	175 (72.9)
5	Do you have any hatred for meat after exposures to dissection	57 (23.8)	183 (76.3)
6	Do you experience unusual thirst after exposures to dissection	46 (19.2)	194 (80.8)

excitement; Izunya (2010) reported a similar trend in his study [5]. Face to face contact with cadaver gave emotional shock to 61.7% of the student studied and brought fear into the heart of about 114 students (47.5%). These could be attributed to some cultural beliefs, norms and folktales associated with the dead and handling the dead. It was obvious that some were seeing the dead for the very first time and the fact that they had to dissect the human bodies seem unpleasant to some. Tiredness/dizziness, vomiting and nausea experienced could be attributed to component of the preserving solution. Some students claimed they experienced disturbed sleep, this could also arise from cultural beliefs, norms and folktales. The nightmares experienced could be a replay of their activities during the day.

It is interesting to note that majority of students 147 (61.3%) agreed that cadaver dissection is considered important and indispensable in anatomical studies, and they would prefer such dissection sessions in the future., This is also in agreement with other studies which reported that dissection gives students a better appreciation of the three-dimensional view in human anatomy. Moreover, removal of cadaver dissection in learning anatomy will impair the students ability to apply scientific method during diagnosis [11]. From this study, cadaver dissection had no negative effect on dietary choice, although a few developed hatred for meat (beef) because of its resemblance to cadaver tissues but quickly substitute it with alternatives. Cadaver is still a powerful means of presenting and learning anatomy.

Conclusion

Cadaver as an educational tool teaches medical student how to use their hands and help develop touch based skills. However, students should be given proper orientation to prevent psychological stress they undergo at first exposure. Prosectors should also be present at each dissection to guide the students.

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Infective endocarditis following prolonged umbilical catheterisation in an extreme preterm: A case report

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Abstract
Care of extreme preterm newborns remains a challenge in resource poor settings as management is fraught with many constraints. Use of indwelling umbilical catheters is part of their routine management but these catheters can predispose to risk of thrombus formation and subsequent infection. Infective endocarditis is rare in them. We describe a case of infective endocarditis in an extreme preterm following prolonged umbilical catheterization which resolved after use of sensitive antimicrobial.

Keywords: *Preterm neonate, Catheterisation, Infective endocarditis, Echocardiography*

Résumé
La prise en charge des nouveau-nés prématurés extrêmes reste un défi dans les milieux pauvres en ressources vu que la gestion est offusquée à de nombreuses contraintes. L'utilisation de cathéters ombilicaux domiciliant fait partie de leur gestion de routine, mais ces cathéters peuvent prédisposer au risque de formation de thrombus et de subséquentes infections. L'endocardite infectieuse est rare chez ces derniers. Nous décrivons un cas d'endocardite infectieuse chez un prématuré extrême suite à un cathétérisme ombilical prolongé qui s'est résolu après l'utilisation d'un antimicrobien sensible.

Mots-clés: *Nouveau-né prématuré, cathétérisme, endocardite infectieuse, échocardiographie*

Introduction

Preterm infants are at risk of thrombi formation which commonly follows use of indwelling umbilical or other vascular catheters which results in obstruction of flow, endothelial damage and low blood flow [1]. Formation of intracardiac thrombi is not unexpected when catheters are placed in the cardiac chambers even in the absence of a cardiac lesion [2].Dissolution of vegetations is usually difficult in extreme preterms despite prolonged use of antimicrobials and removal of offending catheters [2,3].

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Most cases of infective endocarditis in preterm infants are associated with use of central venous catheters and most of the vegetations are in the right atrium [2].*Coagulase negative staphylococcus, staphylococcus aureus and candida* are the common isolates in such cases [2-4]. Marks et al in their review reported a survival rate of 60% [2] and clinical course was complicated by prolonged sepsis, multiorgan dysfunction and progressive cardiac failure [2]. Most cases reviewed were managed using prolonged course of anti-infective agents [2,4]. Surgical removal of thrombus especially for fungal agents was associated with high mortality [5,6], few cases however reported use of recombinant tissue plasminogen activator [2,7-9].

We describe a case of infective endocarditis in an extreme preterm following prolonged umbilical catheterization which resolved after use of sensitive antimicrobial agents. The echocardiographic diagnosis, the type of organism and the sensitivity pattern as well as response to therapy are documented.

Case history

A female neonate was admitted into the Special Care Baby Unit of University College Hospital, Ibadan at 8 hours of life following referral from a peripheral centre. Her delivery was at a gestational age of 27weeks via spontaneous vaginal delivery vertex presentation and her birth weight was 900grams. Mother did not receive antenatal care, delivery was at a Primary Health Care centre and no documentation of APGAR score.

She was found to be dyspnoeic at presentation, with a respiratory rate of 64/min and vesicular breath sounds. Pulses were of normal volume, heart rate was 148/min, heart sounds were normal with no murmur. Ballard score was compatible with gestational age of 26-28 weeks. She was managed on admission as a case of extreme prematurity with respiratory distress syndrome and neonatal sepsis. She had umbilical venous catheterization for intravenous infusions and antibiotic- ampicillin-*sulbactam* and *amikacin*.

Initial electrolyte, urea and creatinine showed hypocalcemia of 7.9mg/dl which was

corrected. Full blood count was essentially normal. On 7th day of life, clinical condition deteriorated as child had worsening respiratory distress, apnoea and reduced activity. Blood culture revealed methicillin resistant staphylococcus *aureus* sensitive to vancomycin and gentamycin and resistant to ampicillin/*sulbactam, cefoxitin, ceftazidime*, ciprofloxacin. Antibiotics were changed to the sensitive drugs. She commenced oral diuretics and an ACE inhibitor on the 15th day of life on account of heart failure secondary to possible patent ductus arteriosus (PDA). She did not improve appropriately and was therefore evaluated for infective endocarditis and had bedside echocardiography on the 27thday of life which showed small PDA, secundum ASD and vegetation in the right atrium**.** Repeat blood culture yielded methicillin resistant Staphylococcus *aureus* sensitive to vancomycin. She was subsequently continued on Vancomycin at 15mg/kg/dose 12hourly for 6 weeks.

Repeat echocardiography after completion of antibiotics on the 61st Day of life showed no vegetation and the infant was subsequently discharged home for follow up at the neonatology out-patient clinic.

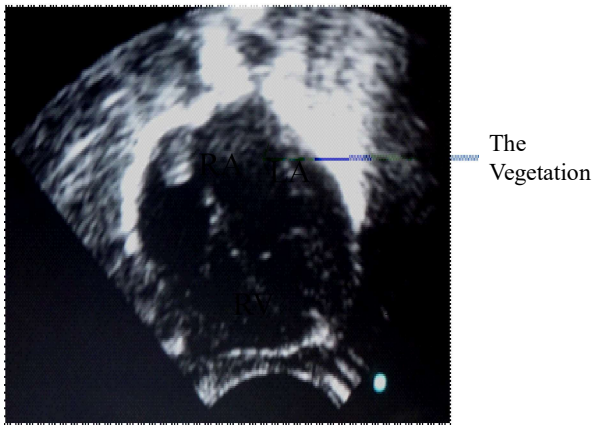

Fig.1: Echocardiographic findings of an atrial thrombus

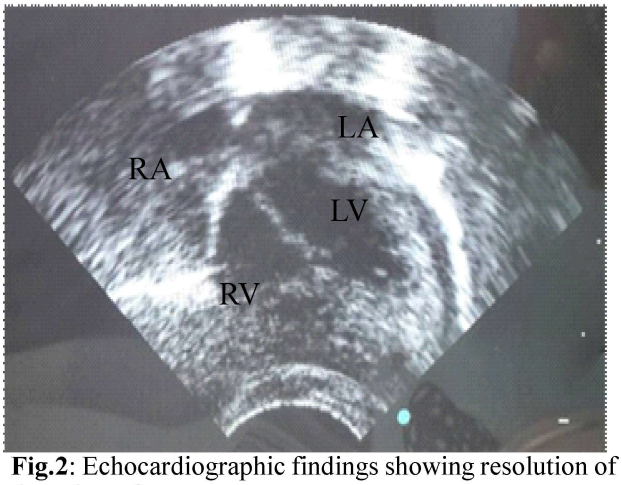

Fig.2: Echocardiographic findings showing resolution of thrombus after treatment

Discussion

A major contributor to improved survival among extreme low birth weight infants is the incorporation of central venous catheter in the management, nevertheless the presence of these catheters increases the risk of thrombus formation [1]. Cases reported confirm that infective endocarditis is a consequence of catheterisation even though the catheters are not in-situ at the time of onset of symptoms. However, in this case report, the infective endocarditis occurred while the catheter was in-situ. The pathogenesis is related to endothelial damage caused by the catheter which acts as a focus for formation of thrombus and hence the pathology is usually in the right side of the heart especially the right atrium as described in this case. A great proportion would be prevented by appropriate positioning of the tip of catheters in the great vessels and not the right atrium. The positioning was clinically appropriate here, using the formula (2 X weight) + 5 cm, though could not be confirmed by bedside ultrasonography as this is not a routine practice in the centre. Initial signs and symptoms are non-specific, new onset murmurs and prolonged positive cultures may give a clue as to the possibility of infective endocarditis. However, diagnosis is usually made by echocardiography as in this patient. In the patient described, there was worsening of signs, and positive culture. The most important causative organisms are coagulase negative staphylococcus, Staphylococcus *aureus* and candida species [2]. The identified organism here was methicillin resistant Staphylococcus *aureus*,. The patient's positive response to treatment might have been due to correct diagnosis aided by bedside echocardiography and appropriate use of culture sensitive antibiotic administered for the correct duration of time required for dissolution of the thrombus as it has been shown that high dose antibiotics for 4-6 weeks are associated with a survival rate of 67-87% in premature infants with staphylococcus endocarditis [2].

Conclusion

Prolonged use of umbilical venous catheters i.e. catheter stay exceeding 7 days should be discouraged in the management of extreme preterms as it is a risk factor for development of infective endocarditis, in view of the potential high antibiotic cost, long duration of treatment and admission as well as potential high mortality associated with development of infective endocarditis. The availability of bedside echocardiography is essential in early diagnosis and monitoring of treatment of infective endocarditis in neonates.

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