Niger. J. Physiol. Sci. 34 (December 2019) 131-139
www.njps.com.ng

Niger. J. Physiol. Sci.

Alterations in Gonadal Oxidative Stress Markers and Reproductive Function of Balb/C Mice Infected with Plasmodium Berghei

Ekhoye, E.I.[1], Aloamaka, C.P.[2], Nwangwa, E.K.[2].

[1]*Department of Physiology, College of Medical Sciences, Edo University Iyamho, Nigeria*
[2]*Department of Physiology, College of Health Sciences, Delta State University, Abraka. Nigeria*

Abstract: Infertility is generally regarded as a major clinical problem, and it adversely affects people both psychologically and medically. In this study, the changes in gonadal oxidative stress markers and reproductive function of BALB/c mice were investigated. Forty-eight (48) BALB/c mice acquired for this study were randomly divided into four (4) groups of eight (8) mice each. Each group was further sub-divided into male and female groups with equal number of mice. The groups were represented as thus: Group A: normal mice; Group B: mice infected with *Plasmodium berghei*; Group C: *Plasmodium berghei* infected mice treated with Artemether/Lumefantrine; Group D: *Plasmodium berghei* infected mice treated with Vitamin E. The experimental mice were inoculated with the *Plasmodium berghei*, and the parasites were confirmed in the mice four days later before the commencement of the experiments. After the experimental procedures which lasted for fourteen (14) days, the mice were sacrificed, blood samples collected for serum testosterone, estrogen and progesterone assay; semen were collected for semen analysis; and testes and ovaries were harvested for histological analyses and oxidative stress marker determination. Result show that *Plasmodium berghei* significantly ($p<0.05$) decreased the sperm count, percentage of sperm with progressive motility and percentage of sperm with normal morphology. The parasites also decreased the serum concentrations of testosterone and progesterone. *Plasmodium berghei*, also caused significant ($p<0.05$) reductions in testicular and ovarian activities of superoxide dismutase, glutathione and peroxidase catalase while significantly ($p<0.05$) increasing the malonaldehyde level. The parasites also caused marked histological distortions in the testes and ovaries of the mice. Treatment with Artemether/Lumefantrine and Vitamin E separately reversed the detrimental changes induced by the parasites by increasing the semen quality and hormonal concentrations. Treatment with Artemether/Lumefantrine and Vitamin E also decreased the oxidative stress level of the gonads and improved the histological features of the testes and ovaries of the infected mice. This study therefore showed *Plasmodium berghei* infection posed anti-fertility threat while treatment with Artemether/Lumefantrine and Vitamin E ameliorates the effect of the parasites.

Keywords*: Plasmodium berghei*, Semen Quality, Oxidative Stress, Infertility, Artemether/Lumefantrine, Vitamin E.

***Address for correspondence*:** Phone: +2347038648797, E-mail: tareonline@gmail.com

Manuscript Accepted: October, 2019

INTRODUCTION

Infertility has been established as a psychological, clinical and social problem which has proved a difficult challenge for both couples and medical professionals. Worldwide, about 10 to 15% of couples would have encountered this problem in their lifetime (Kliesch, 2014). In Nigeria, estimation from the demographic health surveys (DHS) data revealed a prevalence rate of 11% while epidemiologic and clinic-based studies suggested rates of between 14.5% and 30% (Adegbola and Akindele, 2014).

Infertility is a disease affecting the reproductive system, and it is defined as the inability or failure to achieve pregnancy after a year of regular and unprotected intercourse (Purvis and Christiansen, 1992). The male reproductive system is highly sensitive to numerous drugs, chemicals and infections which have shown deteriorating potentials on reproductive capacity under certain circumstances (Bonde, 1996). Oxidative stress generated due to the activities of free radicals triggers a range of pathophysiological changes that influence the reproductive functions generally in women and men (Said et al., 2005). Also, reactive oxygen species (ROS) has been advocated to play a huge role in infertility especially in unexplained (idiopathic) infertility (Agarwal et al., 2003). Apart from reactive oxygen species (ROS), reactive nitrogen species (RNS) are also considered to play diverse and extensive roles in many of the physiological and pathological events (Akaike and Maeda, 2000). When there is an imbalance between the pro-oxidants and antioxidants, with increase in pro-oxidants, oxidative stress (OS) arises that leads to excessive molecular damage and thrn tissue injury (Januel et al., 2006).

Oxidative stress has already been associated with progression of many ailments which include atherosclerosis, cancer, neurodegenerative diseases, rheumatoid arthritis etc (Sohail et al., 2007; Aruoma, 1998). However during malaria infection, the role of oxidative stress is closely monitored and it still remains unclear and controversial on whether it has a protective role or related to tissue pathological diseases (Becker et al., 2004; Pabon et al., 2003). These free radicals are continuously generated during normal aerobic metabolism and their production are equally removed by a variety of exogenous and endogenous antioxidants (Gutteridge, 1995).

Malaria infection induces the generation of hydroxyl radical in different organs, which is accountable for the creation of oxidative stress and possible apoptosis (Guba et al., 2006). Nitric oxide (NO•) is another molecule that have been shown to have an important role in pathogenesis of malaria (Hunt and Stocker, 1990). The malaria parasite itself has also been reported to generate large amount superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Mishra et al., 1994).

Malaria is transmitted by female anopheles, that is, it is a mosquito-borne infectious disease, and caused by the Plasmodium species, which include *Plasmodium vivax, Plasmodium falciparum, Plasmodium knowlesi, Plasmodium malariae* and *Plasmodium ovale.* In early 21st century, malaria was described as endemic in over 104 countries and about 3.4 billion people were at risk for contracting the infectious disease. Most of the malaria cases globally are caused by *Plasmodium vivax* and *Plasmodium falciparum* (WHO, 2013). *Plasmodium falciparum* infection is considered as life threatening and has been attributed to most of the malaria-related deaths when compared to *Plasmodium vivax* which is described as relatively benign (WHO, 2013). There are similarities in characteristic between *Plasmodium berghei* and *Plasmodium falciparum* (Sherman, 2003). Both species of Plasmodium cause pathological damages and apoptosis in liver leading to complications in liver and other systemic tissues (Sand et al., 2005; Kochar et al., 2003). Some of the induced pathological conditions include damage of vital organs such as lungs, liver, spleen etc, and possibly anaemia (Jense et al., 2006).

Since the late 20th Century, several end products of artemisinin have been synthesized and then studied. Artemisinin which is an extract of the plant *Artemisia annua* is a highly effective drug for the treatment of *P. falciparum* malaria (Adekunle et al., 2009). Artemether is one of the derivatives of Artemisinin and its tablets have also proved to be effective in treating malaria infection, these drugs have gradually replaced Chloroquine and also Quinine for the treatment of malaria (Li et al., 1994).

In recent years, the potential of dietary antioxidants to eliminate the hydrogen peroxide (H_2O_2) and superoxide (O_2^-) radicals generated during malaria infection has received increased attention (Hug et al., 2003; Murugavel and Pari, 2004). The dietary nutrients have shown promise protective capabilities in lipid-soluble antioxidants such as vitamins A and E, lycopene, α- and β-carotene in humans because of their association with membrane lipids (Matzger et al., 2001). Amongst the various Vitamins, Vitamin E (α-tocopherol) acts as the most potent lipid soluble antioxidant (Frei, 1991).

The rate of malaria infection in Sub-sahara Africa is on the increase, coupled with the rate of infertility (Ranson and Lissenden, 2016). Several studies have highlighted the adverse effects of malaria in reproduction. In past reports, controversies have been generated on the association between gonadal oxidative stress status and fertility. Some researchers have shown reductions in oxidative stress markers in gonads of infertile men (Sanocka et al., 1997; Alkan et al., 1997), many have not (Zini et al., 2000; Hsieh et al., 2002). Artemether Lumefantrine anti-fertility effect is yet to be established (Morakinyo et al., 2009), but has potency in clearing malaria parasite and reducing toxic activities of the parasites from tissues. Hence this study will also assess the ameliorative role of Artemether Lumefantrine and Vitamin E on the gonadotoxic effect of *Plasmodium berghei*.

MATERIALS AND METHODS

Drugs

Artemether/Lumefantrine in tablets (Mekophar Chemical Pharmaceutical Joint-stock Company, Vietnam) and Vitamin E (α-tocopherol) capsules (Strides-Colab Ltd., India) were both purchased from the Pharmacy of Irrua Specialist Teaching Hospital, Irrua. Artemether/Lumefantrine tablets grinded into powdered form with a glass mortar, was mixed with distilled water and administered as aqueous suspensions by oral gavage at 0.9 mL kg^{-1} b.wt according to procedure of previous studies (Morakinyo et al., 2009). The drug suspensions were continuously stirred during administration in order to avoid sedimentation and to deliver the drugs homogeneously to the animals. Vitamin E (α-tocopherol) was also given by oral gavage in its oily formulation at a dose of 0.2 mL kg^{-1} (Sheweita et al., 2001).

Animal Handling

The study protocols were reviewed by the ethics committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The *Plasmodium berghei* NK65 strain were obtained from the Biochemistry Division, Nigerian Institute of Medical Research (NIMR), Yaba, Nigeria. Forty-eight (48) BALB/c albino mice weighing 20g – 25g and 8 – 10 weeks old, used in this study were acquired and

maintained in facilities of the Animal Unit, Faculty of Basic Medical Sciences, Delta State University, Abraka. The mice were kept in fibre glass cages, with wood shavings as beddings and had access to water and food *ad libitum*

***Plasmodium berghei* Inoculation and Estimation**

The recipient mice were infected with *P. berghei* by the passage of the malaria parasite from the donor animals through intraperitoneal route as by the methods of David et al. (2004) and Peter and Anatoli (1998). Briefly, *P. berghei* infected red blood cells were first collected from the orbital vein of malaria infected mice and this was diluted with Phosphate Buffered Saline (PBS). Each of the recipient mice were injected with 0.2 mL of the diluted blood so that the infected mice contained approximately 10_6-10_7 infected red cells (parasite) per kilogram of body weight. Presence of malaria parasite was confirmed in the recipient mice after four (4) days of inoculation, this was done using thin smears of blood films made from orbital vein of mice (David et al., 2004). The thin smears were stained with 10% Giemsa at pH 7.2 for 15 min and examined under the microscope to assess level of parasitemia. After confirmation of the malaria parasite in the mice, the experiment commenced.

Study Design

The study was experimental in nature. The animals were randomly divided into four groups of twelve (12) mice, each group was further subdivided into two sub-groups of male and female mice (at proestrous stage of the estrous cycle), with equal number of animals. The respective groups were treated as follows; Group A (Control) received normal saline, Group B: mice infected with *Plasmodium berghei*, Group C: *Plasmodium berghei* infected mice treated with 56mgkg$_{-1}$bwt Artemether/Lumefantrine and Group D: *Plasmodium berghei* infected mice treated with 150mgkg$_{-1}$ bwt of vitamin E. The administration of the saline, Artemether/Lumefantrine and Vitamin E were done orally for fourteen (14) days. At the end of the experiments, the mice were sacrificed and samples collected for histological and biochemical analysis.

Determination of Estrous Cycle

A vaginal swab was collected from a restrained female mouse using a cotton tipped swab that was wetted with physiological saline and inserted into the vagina of the animal. This swab was gently rolled against the vaginal wall with minimal animal discomfort and then removed. The cells acquired from the vagina were transferred to a dry glass slid, this was done by rolling the swab across the slide. The slide dried and then stained with 400 µL of stain (Accustain, Sigma-Aldrich, St. Louis, MO). The stained slides were rinsed with water, then covered with a coverslip, and viewed immediately at magnification of x200 under bright illumination. Mice whose vaginal swab contained more of nucleated and few cornified epithelial cells, and leukocytes were confirmed to be at proestrus stage (Felicio et al, 1984). Only female mice at proestrus stage of estrous cycle were selected for this study.

Histological Study

Testis and Ovaries were harvested and immediately preserved by placing in 10% formalin solution and later transferred into Bouin's fluid for longer fixation period. After fixation, the tissues were placed into separate ascending grades of alcohol with the purpose of dehydration. After dehydration, the tissues were cleared in two different changes of xylene, and then finally embedded in paraffin wax. With a rotary microtome, specimens were sectioned at 5µm and sections were mounted on clear slides and stained with haematoxylin and eosin. The slides were air dried and examined under the microscope using a magnification of x100 objective.

Semen Analysis

Immediately after dissection of the mice's abdomen, the testes were harvested and then semen were collected via aspiration from the epididymis. The procedure involved making minor incisions in the caudal portion of right ductus deference of the testis. Drops of semen were placed on the microscope slide and drops of warm 2.9% sodium citrate for every drop of semen were added. This mixture was then covered with the cover slip and examined under the microscope using a magnification of x40 objective with reduced light. Sperm counts, percentage of sperm with normal morphology and percentage of sperm with progressive motility were carried out using the new improved Neubeur's haemocytometer counting chamber.

Serum Hormonal Analysis

Serum was obtained from blood sample collected into plain bottles and assayed for testosterone, estrogen and progesterone concentrations. The hormone, testosterone was measured by using DRG Diagnostics testosterone kit, Germany, while estrogen and progesterone concentrations were analyzed using Monobind CA kit, USA. The three hormones were analysed according to the protocols of the manufacturer's kit.

Analysis of Biochemical Parameters

Lipid-peroxidation was ascertained by measuring the Malonaldehyde activities (MDA) using the procedure of Varshney and Kale (1990) and the values were expressed as nanomolar (nmol) of malondialdehyde (MDA) per gramme tissue. The level of superoxide dismutase (SOD) activity was assessed using the method of Mistra and Fridovich, (1972). Catalase activity was also following the guidelines of Sinha (1972). Glutathione peroxidase activity (GPx) was

measured as prescribed by the method of Rotruck et al. (1973).

Statistical Analysis

Data generated in this study were presented as mean ± SEM for four mice per group. One-way Analysis of Variance (ANOVA) was used to compare means while a post hoc test (Least Significant Difference) was further carried out used to assess the statistical significance of the data. A value of p-less than 0.05 was considered to be statistically significant. IBM SPSS (version 20) software was used to analyse the data

RESULTS

Changes in Semen Quality due to *Plasmodium berghei* Infection

Plasmodium berghei adversely affected spermatogenic activities as significant ($p<0.05$) decrease in the mean sperm count, percentage of sperm with progressive motility and percentage of sperm with normal morphology was observed in the infected mice depicted in Table 1. Artemether/Lumefantrine was observed to inhibit the anti-fertility effect of the *Plasmodium berghei*.

It was also observed that Artemether/Lumefantrine increased the percentage of sperm with progressive motility and percentage of sperm with normal morphology respectively. The increase in percentage of sperm with progressive motility was also significant ($p<0.05$) when compared with *Plasmodium berghei* malaria infected mice. Similarly improvement in semen quality was also observed in *Plasmodium berghei* infected mice treated with Vitamin E, with significance ($p<0.05$) in percentage of sperm with progressive motility and normal morphology when compared with the values of *Plasmodium berghei* infected mice.

Effect of *Plasmodium berghei* infection on serum levels of reproductive hormones

Table 2 shows that *Plasmodium berghei*, caused an adverse effect on testosterone secretion as a decrease in serum concentration of testosterone in male mice was observed when compared to control. Minimal decrease in serum estrogen level of the female mice was observed following infection from *Plasmodium berghei*. Data from Table II also showed that *Plasmodium berghei* caused a significant ($p<0.05$) reduction in the serum concentration of progesterone in the female mice. These decrease in serum testosterone and progesterone concentrations were countered with Artemether/Lumefantrine and Vitamin E treatment in the infected mice. Despite the ameliorative effects of Artemether/Lumefantrine and Vitamin E treatments, statistical significance were not recorded.

Alteration in the level of Oxidative stress markers of *Plasmodium berghei* infected mice.

Data from Table III show the changes in oxidative stress markers of testis and ovary in mice infected with *Plasmodium berghei*. It was observed that there was significant ($p<0.05$) increase in the oxidative stress level in the testes and ovaries of malaria infected mice. Data show that *Plasmodium berghei*, significantly ($p<0.05$) decreased the activities of superoxide dismutase, catalase and glutathione peroxidase, while increasing the malonaldehyde activities with significance ($p<0.05$) when compared with the oxidative stress markers in control's testes and ovaries. Further, treatment with Artemether/Lumefantrine and Vitamin E decreased the testicular and ovarian oxidative stress level. Artemether/Lumefantrine increased the gonadal oxidative stress markers of mice infected with *Plasmodium berghei* with no statistical significance. On the other hand, treatment with Vitamin E significantly ($p<0.05$) increased the superoxide dismutase and catalase activities of the testis. It also increased the ovarian superoxide dismutase and glutathione peroxidase level while decreasing the malonaldehyde activities with significance ($p<0.05$) when compared with oxidative stress markers of *Plasmodium berghei* infected mice.

Table 1:
Effect of Plasmodium berghei on Semen Quality

	Control	Pb infection	Pb + ACT	Pb + Vitamin E
Sperm Count (x10$_6$ cells/mm$_3$)	75.00 ± 5.00	49.17 ± 8.41	56.67 ± 6.15	60.00 ± 5.77
Sperm with progressive Motility (%)	65.83 ± 3.52	25.00 ± 3.65*	42.50 ± 3.10*$_+$	60.83 ± 2.71$_+$
Sperm with normal morphology (%)	77.50 ± 3.10	50.83 ± 7.79*	62.50 ± 4.79*	73.33 ± 2.47*

*: $p < 0.05$ compared with Control group; +: $p < 0.05$ compared with *Plasmodium berghei* infected group (n = 6)

Table 2:
Effect of *Plasmodium berghei* on male Testosterone and female Estrogen and Progesterone

	Control	Pb Infection	Pb + ACT	Pb + Vitamin E
Testosterone (g/dL)	1.02 ± 0.09	0.77 ± 0.09	0.87 ± 0.09	0.97 ± 0.15
Estrogen (pg/mL)	75.00 ± 5.00	49.17 ± 8.41	56.67 ± 6.15	60.00 ± 5.77
Progesterone (ng/mL)	1.05 ± 0.07	0.75 ± 0.13*	0.93 ± 0.05	0.92 ± 0.10

*: $p < 0.05$ compared with Control group (n = 6)

Effect of *Plasmodium berghei* infection on the Histology of Testis

Fig. IA showed normal features of the testis were observed in the control mouse testis, but infection by *Plasmodium berghei*, caused distortion of the seminiferous tubules and interstitial cells of Leydig (Fig. IB). This detrimental change caused arrest of spermatogenic development in the testis. In Fig. 1C, Artemether/ Lumefantrine reversed the detrimental effect of the parasite, with minimal spermatogenesis and mild interstitial degeneration. Vitamin E treatment caused an improvement in the cellular structures of the testis in *Plasmodium berghei* infected mice with defined seminiferous tubules and essentially normal Sertoli cells and interstitial cells of Leydig (Fig. ID).

Effect of *Plasmodium berghei* infection on the histology of ovary: Micrograph in Fig. II shows blood vessels, corpus luteum, maturing oocytes, granulosa cells and corona radiata, follicular antrum and granulosa cells in ovary of control female mouse. It was observed in Fig. IIB that *Plasmodium berghei* infection caused degenerated oocytes, also degenerating granulosa cells were also observed in the ovary of *Plasmodium berghei* infected mice. Artemether Lumefantrine reduced the effect of *Plasmodium berghei* infection as mild congestion observed in the ovary (Fig. IIC) compared with the severe derangement of the histo-architecture of ovary in *Plasmodium berghei* infected mice (Fig. IIB). These derangements in ovary induced by *Plasmodium berghei*, appeared to be healed or reversed by Vitamin E treatment (Fig. IID) as the histology of the ovary showed similar features with control.

Table 3:
Effect of Plasmodium berghei on gonadal oxidative stress level

	Gonad	Control	Pb infection	Pb + ACT	Pb + Vitamin E
SOD (U/mgHb)	**Testes**	74.30 ± 6.09	32.88 ± 5.62*ь	40.49 ± 5.68	57.63 ± 5.35
	Ovaries	63.63 ± 4.80	30.49 ± 6.88*ь	42.26 ± 9.15	56.98 ± 3.78
Catalase (U/mgHb)	**Testes**	21.62 ± 3.04	9.26 ± 1.38*ь	11.97 ± 2.45	16.41 ± 1.58
	Ovaries	18.52 ± 3.03	11.81 ± 2.01*	13.15 ± 1.69	15.27 ± 1.58
GPx (U/mgHb)	**Testes**	17.22 ± 2.21	9.07 ± 2.08*	13.74 ± 3.59	15.86 ± 2.19
	Ovaries	18.35 ± 0.79	7.40 ± 0.99*ь	13.18 ± 1.53	16.31 ± 0.41
MDA (nmol/gm tissue)	**Testes**	110.70 ± 5.45	178.76 ± 27.40*	147.75 ± 15.22	126.47 ± 22.53
	Ovaries	95.58 ± 9.82	168.35 ± 12.62*ь	142.11 ± 17.86	71.82 ± 13.74

*: $p < 0.05$ compared with Control group; ь: $p < 0.05$ compared with Vitamin E group (n = 6)

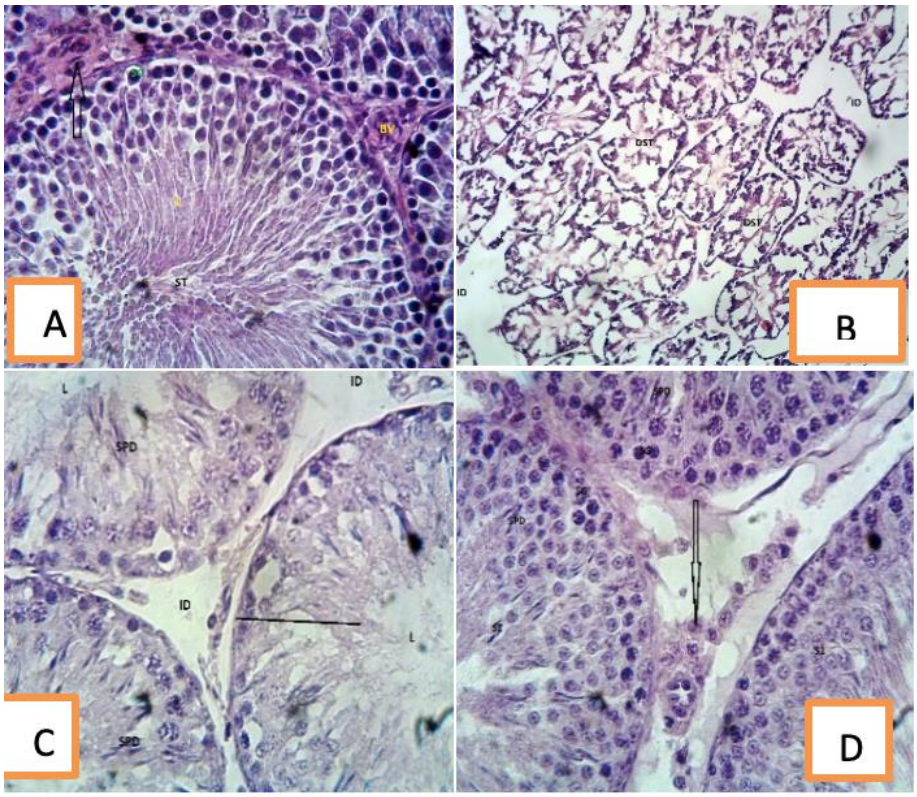

Figure 1:
Cross sections of histology of testis in control mouse (A), *Plasmodium berghei* infected mouse (B), *Plasmodium berghei* infected mouse treated with Artemether/Lumefantrine (C) and *Plasmodium berghei* infected mouse treated with Vitamin E. Stains: Haematoxylin & eosin, Magnification: x400.

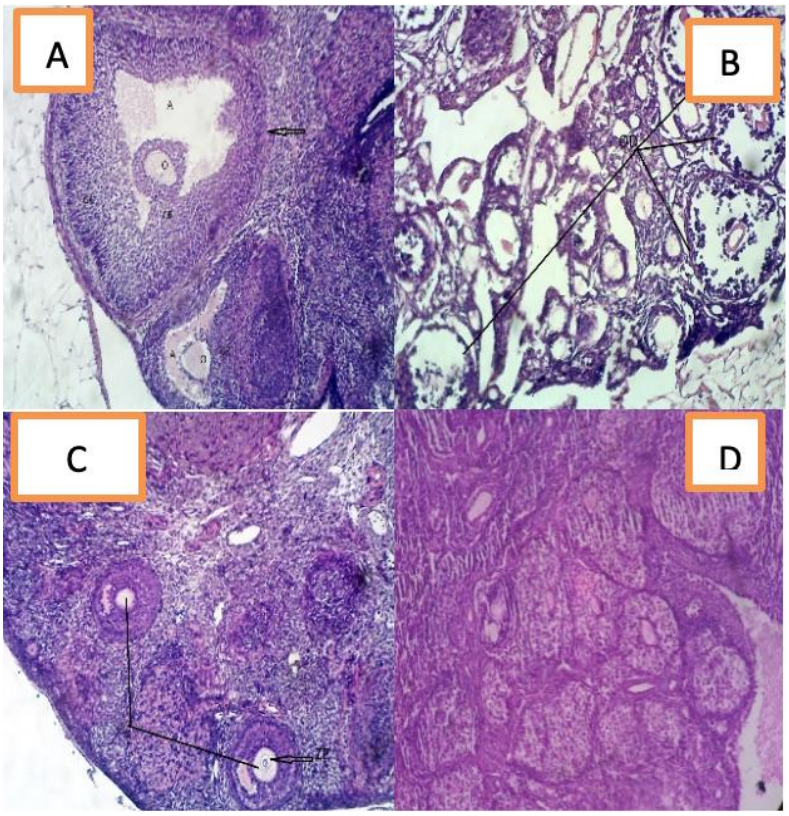

Figure 2:
Cross sections of histology of ovary in control mouse (A), *Plasmodium berghei* infected mouse (B), *Plasmodium berghei* infected mouse treated with Artemether/Lumefantrine (C) and *Plasmodium berghei* infected mouse treated with Vitamin E. Stains: Haematoxylin & eosin, Magnification: x100

DISCUSSION

Malaria is one of the leading cause of mortality and morbidity especially in developing countries, and this remains a serious public health issue in malaria endemic area of the World (Breman et al., 2004). Oxidative stress has been linked with pathogenesis of numerous disease conditions amongst which include malaria. The present study was carried out to examine the effect of *Plasmodium berghei* infection on male and female reproductive system (Agarwal et al., 2005).

In this present study, increase in oxidative stress level was established as *Plasmodium berghei* decreased the gonadal concentrations of superoxide dismutase, catalase and glutathione peroxidase, whilst increasing the malonaldehyde activities. The increase in MDA level is attributed to the insufficient antioxidant defence system that is manifested by decrease in the activities of testicular and ovarian catalas, superoxide dismutase and glutathione peroxidase. Findings from Kulkarni et al. (2003) reported that the decrease in levels of antioxidants during malaria infections and this is responsible for increase in oxidative stress. Conversely, Pabón et al. (2003) showed that elevated oxidative stress during malaria infection is also responsible for the increase in lipid peroxidation rather than from antioxidant decrease.

Plasmodium berghei, induced pathological changes in the testes and ovaries of the mice. In Fig. IB, degenerative changes caused by *Plasmodium berghei*, showed altered structural integrity of seminiferous tubules and interstitial cells of Leydig of the testis. This adverse changes is attributed to the increase in oxidative stress markers as recorded in Table III. This assertion was further confirmed by Sharma et al. (2006) whose study showed attenuating and toxic changes of *Plasmodium berghei* leading to disruption of antioxidant and pro-oxidant balance, by continuous generation of reactive oxygen species (ROS). In support to the findings of this study, Sibmooh et al. (2006) also showed that oxidative stress is a common phenomenon in acute malaria infection, hence the alteration in testicular histo-architecture observed in this present study, was induced by increased oxidative stress markers following infection from *Plasmodium berghei*.

Similarly, the membrane degeneration observed in testicular and ovarian tissue distortion is as a result of increased lipid peroxidation induced by *Plasmodium berghei,* this claim was established with increase in tissue malonaldehyde (MDA), an important lipid peroxidation marker (Cabrales et al., 2011). Apart from lipid peroxidation, *Plasmodium berghei* effect in decreasing other antioxidants such as catalase, gluathatione peroxide and superoxide dismutase in the testis and ovaries is another mechanism for gonadal histo-architectural damage. Evidence from reports of previous studies have shown that the activities of SOD and other antioxidants are decreased due to the infection in *Plasmodium berghei*-infected cells (Rodrigues and Gamboa. 2009; Farombi et al,. 2003). Studies from Sibmooh et al. (2000) and Das et al. (1993) have also previously shown that during malaria

infection, the presence of oxidative stress includes increased plasma lipid peroxidation, depletion of antioxidants and alteration of erythrocyte membrane flexibility.

This present study showed that *Plasmodium berghei* decreased the production of testosterone in male mice. This can be understandable with the testosterone synthesis function of interstitial cells of Leydig (Miller and Auchus, 2011) and due to the parasite inducing histological distortions in the interstitial cells of Leydig, hence impairing the testosterone producing function of the testis. The decreased serum progesterone level in female mice following *Plasmodium berghei* infection is also a reflection on damage on the ovarian integrity with increase in oxidative stress. Data generated from Reddy, Mahipal and Subhashini, (2006) showed that oxidative stress during *Plasmodium berghei* infections were related with momentary generation of pro-inflammatory mediators such as inducible nitric oxide synthase, interleukin 1β and cyclo-oxygenase-2. Also, Lalita et al., (2012) also showed the presence of apoptotic cells in tissues with increased oxidative stress and infected with *Plasmodium berghei*

Plasmodium berghei significantly ($p<0.05$) decreased the sperm count, percentage of sperm with progressive motility and percentage of sperm with normal morphology. Testosterone is required in large local concentrations to maintain the process of spermatogenesis. Decrease in testosterone may hamper the production of sperm cells. Malaria parasites decreased the testosterone level in report from Muawia and Nabiela (2009), another possible reason for the decrease in sperm parameters due to *Plasmodium berghei* from this study. The distortion in testicular histo-architecture would result to decrease in sperm production (Orth, 1993).

Treatment with Artemether/Lumefantrine inhibited the damaging effects of *Plasmodium berghei* as the mean sperm count, percentage of sperm with progressive motility and percentage of sperm with normal morphology in the malaria infected mice remained within control limits. This was in line with Akinlolu et al. (2007) that rats exposed to separate doses of Artemether Lumefantrine for seven (7) days showed normal morphological structures of the testis with evidence of spermatogenesis occuring. The semen quality also remained intact or within control range because of the normal level of testosterone in Plasmodium berghei infected mice treated with Artemether Lumefantrine. Similar findings from Morakinyo, Oludare, Ojulari et al. (2009), showing that serum testosterone level remained normal after administration of Artemether/Lumefantrine. Artemisinin-Combination Therapies ACT for which Artemether Lumefantrine is one, is known for its rapid parasite clearance (Price, Nosten, Luxemburger et al., 1996). This malaria parasite clearing property of Artemether Lumefantrine inhibited the increase in gonadal oxidative stress and hence limited the destructive influence of Plasmodium berghei on the testis and ovary.

Similarly, Vitamin E administration reversed the effect of malaria parasite in the mice by decreasing the oxidative stress level, improving the semen quality and increasing the serum concentration of the reproductive hormones. The reduction in oxidative stress level due to the effect of Vitamin E could play a huge role in the recovery of the fertility potential of the Plasmodium berghi infected mice. Akpotuzor et al. (2007) showed that antioxidant concentrations in malaria patients were lower than the levels for the control, suggesting that the reduction in antioxidant vitamins during malaria is attributed to increased function of the host's serum antioxidants caused by the malaria parasites to counteract oxidative injuries. This further confirms the beneficial role of Vitamin E in preventing the detrimental effect of *Plasmodium berghei* on reproductive function in this study.

The observation in this study confirms that *Plasmodium berghei* impairs gonadal function. The separate use of antioxidants supplements and anti-malaria drug, Artemether Lumefantrine were effective in ameliorating the anti-fertility of *Plasmodium berghei* through the decrease in gonadal oxidative stress level.

REFERENCES

Adegbola, O. and Akindele, M. (2014). The pattern and challenges of infertility management in Lagos, Nigeria . Afr. Health Sci. 13(4):1126 - 1129.

Adekunle, A.S., Agbedana, E.O., Oyewopo, O., Adedeji, A.L. and Adebisi, A.J. (2009). Antispermatogenic effects of artemether: an animal model. Toxi. And Environ. Chem. 91(3): 511-519.

Agarwal, A., Gupta, S. and Sharma, R.K. (2005). Role of oxidative stress in female reproduction. Reprod. Biol Endocrinol. 14: 3:28.

Agarwal, A., Saleh, R.A. and Bedaiwy. M.A. (2003). Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil. Steril. 79: 829-843.

Akaike, T. and Maeda, H. (2000). Nitric oxide and virus infection. Immunol 100: 300 - 308.

Akinlolu, A.A., Otulana, J.O., Olatunde, O., Adebayo, B.E. and Akinola, O.B. (2007). The antispermatogenic and antifertility effects of arthemether on the testis of adult wistar rats. Pak. J. Pathol. 18(2): 64 - 67.

Akpotuzor, J.O., Udoh, A.E. and Etukudo, M.H. (2007). Total antioxidant status, vitamin A, C and ß-carotene levels of children with Plasmodium falciparum in University of Calabar Teaching Hospital (UCTH) Calabar. Pak. J. Nutr. 6(5): 485 - 489

Alkan, I., Simsek, F., Haklar, G., Kervancioglu, E., Ozveri, H., Yalcin, S. and Akdas, A. (1997). Reactive oxygen species production by the spermatozoa of patients with idiopathic infertility: relationship to seminal plasma antioxidants, J Urol. 157: 140 - 143.

Aruoma, O.I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc. 75: 199 – 212.

Becker, K., Tilley, L., Vennerstrom, J.L., Roberts, D., Rogerson, S. and Ginsburg, H. (2004). Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. Int J Parasitol, 34: 163 - 189.

Bonde J.P. (1996). Environmental factors. In Comhaire, F.M. (ed.), Male infertility, clinical investigation, cause evaluation and treatment. Chapman and Hall, London. 267-284

Breman, J.G., Alilio M .S. and Mills, A. (2004). Conquering the intolerable burden of malaria: what's new, w hat's needed: a summary. Am. J. Trop. Med. Hyg. 71: 1-15.

Cabrales, P., Zanini, G.M., Meays, D., Frangos, J.A. and Carvalho, L.J. (2011). Nitric oxide protection against murine cerebral malaria is associated with improved cerebral microcirculatory physiology. J. Infect. Dis. 203(10): 1454 - 1463

Das, B.S. Patnaik, J.K. Mohanty, S. Mishra, S.K. Mohanty, D. Satpathy, S.K. and Bose, T.K. (1993). Plasma antioxidants and lipid peroxidation products in falciparum malaria. Am. J. Trop. Med. Hyg. 49(1993): 720-725.

David, A.F. Philip, J.R. Simon, R.C. Reto, B. and Solomon, N. (2004). Antimalarial drug discovery: efficiency models for compound screening, Nature Rev. 3: 509–520.

Farombi, E.O. Shyntum, Y.Y. Emerole. G.O. (2003). Influence of chloroquine treatment and Plasmodium falciparum malaria infection on some enzymatic and nonenzymatic antioxidant defense indices in humans. Drug. Chem. Toxicol. 26(1): 59 - 71.

Felicio, L.S., Nelson, J.F. and Finch, C.E. (1984). Longitudinal studies of estrous cyclicity in aging C57BL/6J mice: II. Cessation of cyclicity and the duration of persistent vaginal cornification. Biol. Reprod. 31: 446 – 453.

Frei, B. (1991). Ascorbic acid protects lipids in human and low-density Lipoprotein against oxidative damage. Am. J. Clin. Nutr. 54: 135 - 185.

Guba, M., Kumar, S., Choubey, V., Maity, P. and Bandyopadhyay, U. (2006). Apoptosis in liver during malaria: role of oxidative stress and implication of mitochondrial pathway. FASEB J 20: 1224-1226.

Gutteridge, J.M.C. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin. Chem. 41: 1819 -1828.

Hsieh, Y.Y., Sun, Y.L., Chang, C.C., Lee, Y.S., Tsai, H.D. and Lin, C.S. (2002). Superoxide dismutase activities of spermatozoa and seminal plasma are not correlated with male infertility. J. Clin. Lab. Anal. 16: 127 - 131.

Hug, N.T. Seradi, S. Trang D.T. and Kondo, Y. (2003). Neutralization of toxic haem by plasmodium falciparium Histidine- rich protein. J. Biochem. 1333: 693-698.

Hunt, N.H. and Stocker, R. (1990). Oxidative stress and the redox status of malaria-infected erythrocytes. Blood Cells. 16: 499 - 526.

Januel, C. E., Hentati, F.Z., Carreras, M., Arthur, J.R., Calzada, C., Lagarde, M. and Vericel, E. (2006). Phospholipid-hydroperoxide glutathione (GPx-4) localization in resting platelets, and compartmental change during platelet activation. Biochim Biophys Acta. 1761: 1228 - 1234.

Jense, C.J., Ramesa, R.J. and Waters, A.P. (2006). High-efficacy transfection and drug selection of genetically transformed blood stages of the rodent malaria parasite *Plasmodium berghei*. Nature Protocols. 1: 346-356.

Kliesch, S. (2014). Diagnosis of Male Infertility: Diagnostic Work-up of the Infertile Man. Eur. Urol. Suppl. 13:10.

Kochar, D.K., Agarwal, P., Kochar, S.K., Jain, R., Rawat, N., Pokharna, R.K., Kachhawa, S. and T. Srivastava, Hepatocytes dysfunction and hepatic encephalopathy in Plasmodium falciparum malaria. Int. J. Med. 96(2003): 505 –512.

Kulkarni, A.G., Suryakar, A.N., Sardeshmukh, A.S. and Rathi, D.B. (2003). Studies on biochemical changes with special reference to oxidant and antioxidants in malaria patients. Indian. J. Clin. Biochem. 18(2).136 - 49.

Lalita, S,. Jagdeep K. and Geeta, S. (2012). Role of Oxidative Stress and Apoptosis in the Placental Pathology of Plasmodium berghei Infected Mice. PLoS One. 7(3): 1 - 8

Li, G.Q., Guo X.B., Fu, L.C., Jian H.X., and Wang. X.H. (1994). Clinical trials of artemisinin and its derivatives in the treatment of malaria in China. Transaction of Royal Soc. of Trop. Med. Hyg. 88(l): 1: 5- 6.

Matzger, A., Mukasa, G., Shanker, A.H., Ndeezi, G., Melikian G. and Samba, R.D. (2001). Antioxidant status and acute malaria in children in Kampala, Uganda. Am. J. Trop. Med. Hyg. 65: 115-119.

Miller, W.L. and Auchus, R.J. (2011). The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr. Rev. 32: 81–151.

Mishra, N.C., Kabilan, L. and Sharma, A. (1994). Oxidative stress and malaria infected erythrocytes. Indian J Malariol 31: 77-87.

Mistra H.P. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry 247(10). 3170-3175.

Morakinyo, A., Olufemi, O. G. O., Ojulari, S. and Afolabi, A. O. (2009). Effects of Short Term Administration of Artemether –Lumefantrine on Testicular Functions and Antioxidant Defence in the Rat. Res. J. Med. & Med. Sci. 4(2): 165-170.

Muawia A.A. and Nabiela, M.E. (2009). Effect of falciparum malaria on some plasma proteins in males: With special reference to the levels of testosterone and cortisol. Afr. J. Biochem. Res. 3(11): 349 - 355.

Murugavel P. and Pari, L. (2004). Attenuation of chloroquine induced renal damage by α-lipoic acid: possible antioxidant mechanism. Renal Failure. 26: 515-522.

Orth, J.M. (1993). Cell and Molecular biology of the testis (eds. Desjardins,C, Ewing). University Press, New York, pp 3 - 43

Pabón, A., Carmona, J., Burgos, L.C. and Blair, S. (2003). Oxidative stress in patients with non-complicated malaria. Clin Biochem. 36(1): 71 - 78.

Peter I.T. and Anatoli, V.K. (1998). The current global malarial situation. Malaria parasite biology, Pathogenesis and protection ASM Press W.D.C. pp 11 - 22.

Price, R.N., Nosten, F., Luxemburger, C., Paiphun, L., Chongsuphajaisiddhi, T. and White, N.J. (1996). Effects

of artemisinin derivatives on malaria transmissibility. Lancet. 347: 1654–1658.

Purvis, K. and Christiansen, E. (1992). Male infertility: Current concepts. Annual Med. 24: 259-272.

Ranson, H. and Lissenden, N. (2016). Insecticide resistance in African anopheles mosquitoes: A worsening situation that needs urgent action to maintain malaria control. Trends Parasitol. 32:187–196.

Reddy, M.M. Mahipal, S.V. and Subhashini, J. (2006). Bacterial lipopolysaccharide -induced oxidative stress in the impairment of steroidogenesis and spermatogenesis in rats. Reprod. Toxicol. 22: 493-500.

Rodrigues, J. and Gamboa, N. (2009). Effect of dequalinium on the oxidative stress in *Plasmodium berghei*-infected erythrocytes. Parasitol. Res. 104(6): 1491-1496.

Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hoekstra, W.G. (1973). Selenium: Biochemical role as a component of glutathione peroxidase. Science. 179: 588-590.

Said, T.M., Agarwal, A. and Sharma, R.K. (2005). Impact of sperm morphology on DNA damage caused by oxidative stress. Fertil Steril. 83: 95-103.

Sand, C. Hortsmann, S. Schmidt, A. Sturn, A. Bolte, S. Krueger, A. Lutgehetmann, M. Pollok, J.M. Libert, C. and Heussler, V.T. (2005). The liver stage of *Plasmodium berghei* inhibits host cell apoptosis. Mol. Microbiol. 58(3): 731-742.

Sanocka, D., Miesel, R., Jedrzejczak, P., Chelmonska-Soyta, A.C. and Kurpisz, M. (1997). Effect of reactive oxygen species and the activity of antioxidant systems on human semen; association with male infertility, Int. J. Androl. 20: 255-264.

Sharma, J.B., Sharma, A., Bahadur, A., Vimala, N., Satyam, A. and Mittal, S. (2006). Oxidative stress markers and antioxidant levels in normal pregnancy and pre-eclampsia. Int. J. Gynaecol. Obstet. 94(1): 23 - 27

Sherman, I.W. (2003). Reflections on a century of malaria biochemistry, In vivo and in vitro models. Adv. Parasitol. 67: 25 - 47.

Sheweita, S.A., Abd El-Gabar, M. and Bastawy, M. (2001). Carbon tetrachloride-induced changes in the activity of phase II drug-metabolizing enzyme in the liver of male rats: Role of antioxidants. Toxicol. 165: 217 - 224.

Sibmooh, N. Pipitaporn, B. Wilairatana, P. Dangdoungjai, J. Udomsangpetch, R. Looareesuwan, S. and Chantharaksri, U. (2000). Effect of artemisinin on lipid peroxidation and fluidity of the erythrocyte membrane in malaria. Biol. Pharm. Bull. 23: 1275 - 1280.

Sibmooh, N., Yamanont P. and Krudsood, S. (2004). Increased fluidity and oxidation of malarial lipoproteins: relation with severity and induction of endothelial expression of adhesion molecules. Lipid in Health & Dis. 1476: 1-11.

Sinha, A.K. (1972). Colorimetric assay of catalase. Analytical Biochem. 47: 389 - 394.

Sohail, M., Kaul, A., Raziuddin, M. and Adak, T. (2007). Decreased glutathione-S-transferase activity: Diagnostic and protective role in vivax malaria. Clin. Biochem. 40: 377 - 382.

Varshney, R. and Kale, R.W. (1990). Effects of cadmium antagonist on radiation induced lipid peroxidation in microsomes. Internat. J.Rad. Biol. 58: 733 - 743.

World Health Organization (2013). Introduction. In: World malaria report. WHO Library Cataloguing-in-Publication Data. 1 – 2.

Zini, A., Garrels, K. and Phang, D. (2000). Antioxidant activity in the semen of fertile and infertile men. Urol. 55: 922 - 926.