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Sub-Acute Toxicity Studies of Methanol Root Bark Extract of *Azanza Garckeana* (F Hoffm.) Exell And Hillc. in Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Background: Plants are widely used for various medicinal purposes without careful consideration of potential adverse effects on the system. The fruit of *Azanza garckeana* is eaten as food, while the other parts are shown to have different medicinal uses and serve as treatments in managing several diseases and ailments throughout its geographical coverage. The study is a necessary/relevant addition in the ongoing efforts to increase awareness about the potential dangers of *Azanza garckeana* that is presently in use for multiple purposes without prior toxicity studies.
Objectives: This study aimed to determine the sub-acute toxicity of the methanol root bark extract of *A. garckeana* (MEAG).
Methods: The Organization for Economic Co-operation and Development **(OECD) test guidelines (TG) 425 and 407** were followed for conducting acute oral toxicity and sub-acute toxicity study of MEAG, respectively.
Results: In the overall acute toxicity test carried out on MEAG, there were no observed signs and symptoms of toxicity, morbidity or mortality and the LD_{50} was estimated to be greater than 5000 mg/kg in the oral route of administration. Sub-acute toxicity study, revealed a significant increase ($p<0.05$) in direct (conjugated) bilirubin at 400 and 800 mg/kg as well as in serum urea at 200 and 400 mg/kg in the MEAG treated groups respectively when compared to the control group. Also, histology of the rats' organs such as liver, kidney, brain and the heart revealed some level of tissue damage such as, moderate hepatic and slight periportal necrosis, slight glomerular necrosis, as well as slight neuronal necrosis and slight myocardial necrosis, respectively.
Conclusion: Prolonged administration of methanol root bark extract of *Azanza garckeana* appears to be toxic to Wistar rats.

Keywords: *Azanza garckeana* root bark: Sub-acute toxicity: OECD

INTRODUCTION

The use of medicinal plants has gained much attention over the years and this is indicated by the increase in the scientific exploration of their therapeutic potentials and safety (Chaachouay and Zidane, 2024). *Azanza garckeana* is found to be abundant in the North-eastern part of Nigeria, precisely Tula, Kaltungo Local Government Area of Gombe State. It is commonly known as *Goron Tula* in Hausa (kola of Tula), and it belongs to the family Malvaceae (Yusuf *et al.*, 2020). The fruit is eaten as food, while the other parts such as the stems, leaves, bark, and roots are shown to have different medicinal uses and serves as treatments in managing several diseases and ailments throughout its geographical coverage (Maroyi, 2017). The root infusion is used orally for chest pains, painful menstruation and cough in Nigeria, Kenya and Zimbabwe (El-Hadi Sulieman, 2019). It is also used as ear drop for earache in Zimbabwe (Maroyi, 2011;

Maroyi, 2013; El-Hadi Sulieman, 2019). The root or leaf decoction is use orally for oedema and membrane rupture in the Democratic Republic of Congo (DRC) (Amuri *et al.,* 2017; Khang *et al.,* 2017). Also, the root decoction is mixed with roots of *Sterospermum kunthianum* Cham. and is taken orally for asthma in Malawi (Bioltif *et al.,* 2020).

Plate I: Different parts of *A. garckeana*

A. Mature fruits; B. Roots bark; C. Leaves and flowers; D. Whole tree
(Pictures taken from its natural habitat at Tula, Kaltungo Local Government, Gombe State)

The use of herbs is generally accepted by the populace because of the belief that they do not cause any deleterious effects when used for treating various diseases (Anywar *et al.,* 2020). Although some of these plant preparations are relatively safe, majority of them utilized as medicines have been found to be highly toxic when taken for a short or long duration (Anywar *et al.,* 2020). No matter how important a substance is medicinally, it cannot be freely used if its safety to the living cells and tissues has not been evaluated which is a very important component of preclinical studies conducted on any potential investigational new drug (IND) (Biala *et al.,* 2023). Therefore, the safety profile of medicinal plants can only be established upon carrying out a toxicity study of their extracts and *A. garckeana* is not an exception (Madhav *et al.,* 2024). This study was based on the potential sub-acute toxicity of prolonged administration of methanol root bark of *Azanza garckeana* (F Hoffm.) Exell and Hillc. in rats.

METHODOLOGY

Collection, identification and preparation of plant material

Collection and identification of plant material

The root bark of *A. garckeana* was obtained, from Shawa, Tula Wange, Kaltungo Local Government, Gombe State, Nigeria, in July, 2021. A verbal permission was obtained from the elders of a particular family clan, before the collection was made on their private land. The plant was identified by Mal. Isah Umar and authenticated by Dr. Namadi Sanusi at the Herbarium Unit of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria where a voucher specimen was deposited with the voucher number 68039 for future reference.

Preparation of plant material

The collected root bark was washed, cut into smaller pieces, and dried under the shade until a constant weight was obtained. It was crushed into powder using mortar and pestle. About 979.4g of the powdered dried root bark was extracted with 8.5 litres of 70% v/v methanol using cold maceration. The powder was soaked with some quantity of the solvent and was shaken vigorously. This was allowed to stand for 72hrs and was decanted. The same procedure was repeated 2 more times using fresh quantity of the solvent on each occasion. The whole extract obtained after decanting for 3 consecutive times was appropriately filtered using Whatmann filter paper and the filtrate obtained was concentrated using a rotary evaporator, with the digital water bath set at 60 $^{\circ}$C. The concentrate obtained was transferred into an evaporating dish and placed on a water bath set at 50-60 $^{\circ}$C. This was allowed until all the solvents evaporated leaving behind the dried root extract on the dish. This was gently scraped off with the help of a spatula into a clean, dried glass container, and labelled as the methanol root bark extract of *A. garckeana* (MEAG). The dried root bark extract was weighed and the percentage yield of MEAG was calculated as thus:

$$\% \text{ yield} = \frac{\text{Weight of dried extract obtained}}{\text{Weight of crude powder sample}} \times 100$$

Experimental animals

Adult Wistar rats of weight range of 150-200 g of both sexes were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, and were used for this study. The animals were maintained under standard laboratory conditions at room temperature and fed with standard pellet diet and tap water, *ad libitum*. All animal experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85-23, revised 1985) and in accordance with the Ahmadu Bello University Ethics Committee on the use of Laboratory Animals. The protocol was approved by the University Ethics Committee on the use of Laboratory Animals with the number ABUCAUC/2023/033.

Acute toxicity

Female rats (5) which are nulliparous and non-pregnant were randomly selected for the study. Each animal was fasted overnight by withholding food but not water prior to treatment and for further 3-4 hrs after the treatment. The procedures for carrying out a limit test at 5000 mg/kg body weight were followed. This is because; there is substantial information on the toxicity profile of other parts (leaves and fruit) of the test substance. The animals were dosed one at a time sequentially. The first animal was weighed and was administered 5000 mg/kg dose of MEAG. Observations were made for signs and symptoms of toxicity, morbidity and mortality in the first 30 minutes after administration, then periodically for 48 hrs. After 48 hrs, the first animal survived and thereafter, two additional animals were dosed at the same time with the same 5000 mg/kg dose of MEAG. These were also observed for signs and symptoms of toxicity, morbidity and mortality for the next 48 hrs. At the end of 48 hrs, there was no death recorded at 5000 mg/kg dose and so the test was terminated as recommended by the guideline. However, observations were carried out on these animals for 14 days without further dosing of animals. The weight of these animals was taken at least once a week (OECD, 2001b).

Sub-acute toxicity (repeated dose 28-day oral toxicity study)

Here, as previously described by Alkali *et al.,* (2018), twenty-four rats of either sex were randomly selected, weighed and divided into four groups of six rats each (3 males and 3 females). First group were given 10 ml/kg distilled water orally. The second, third and fourth groups were given orally, graded doses (200 mg/kg, 400 mg/kg, and 800 mg/kg) of MEAG respectively, daily for 28 days. All the animals were allowed access to food and water *ad libitum* throughout the duration of the experiment. Observation was made on a daily basis for general signs and symptoms of toxicity, morbidity and

mortality. Weights of the animals were taken on a weekly basis. On the 29th day with overnight fasting, under ketamine anaesthesia (100 mg/kg dose) the animals were euthanized. Blood samples were collected in EDTA and plain containers for haematological and biochemical tests respectively. The heart, liver, kidney, spleen and brain of the animals both in the treatment and in the control groups were harvested and preserved in 10 % formalin and histopathological examination were carried out on the heart, liver, kidney, and the brain (OECD,2008), modified by Alkali *et al.,* (2018).

RESULTS

Extraction Yield of Methanol Root Bark Extract of *Azanza garckeana*

The extraction of 979.40 g of the dried crude root bark powder of *Azanza garckeana* with 70 % methanol yielded 73.00 g of a dark brown, granular and woody/resinous smelling residue, which is equivalent to 7.45% w/w yield.

The yield of the root bark extract of *A. garckeana* (7.45 % w/w) may be considered to be low. This is in concordance with another study conducted on the same plant part which showed a low yield of 6.26 % w/w (Chanda *et al.,* 2020). Obtaining low yield with root of plants is common. This could be because root contains lesser amount of extractable substance than other plant parts (Tegaboue *et al.,* 2021), as another study on the root of *Cyphostemma adenocaule* indicated low yield of 6.0 % w/w upon extraction with 70 % ethanol as the solvent (Abebe, *et al.,* 2022). However, extraction of another part (fruit pulp) of *A. garckeana* was found to produce higher yield of 58.03 % (Bukar *et al.,* 2020), 27.80 % (Ahmad *et al.,* 2022) and 49.2 % (Dawud *et al.,* 2023).

Acute toxicity study

The oral median lethal dose (LD_{50}) of MEAG was estimated to be greater than 5000 mg/kg. The animals (rats) did not show any changes in general appearance during the 14-day observation period. Morphological characteristics of the skin, eye, fur, and nose appeared normal throughout the period. No tremor, convulsion, diarrhoea, lethargy, or any other behaviour was observed. Also, there was no death recorded (Table 1).

Table 1: OECD 425 (2001b) Limit Test at 5000 mg/kg body weight for the Oral Median Lethal Dose of Methanol Root Bark Extract of *Azanza garckeana*

Sequential dosing	Number of animals dosed at a time	Dose(mg/kg)	Observation period	Signs of toxicity	Mortality
First dosing	1	5000	48 hrs	None	0/1
Second dosing	2	5000	48 hrs	None	0/2

LD_{50} > 5000 mg/kg body weight

Acute oral toxicity (LD_{50}) refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours (OECD, 2001b). It is the first step in toxicological investigations of an unknown substance, (Lorke, 1983). In the overall acute toxicity test carried out on MEAG, there were no observed signs and symptoms of toxicity, morbidity or mortality and the LD_{50} was estimated to be greater than 5000 mg/kg via oral route of administration. This corresponded to the value obtained using the fruit pulp extract of the plant in the work reported by Bukar *et al.,* (2020) and Ahmad *et al.,* (2022). According to the Category 5 Global Harmonized Classification System for chemical substances and mixtures (GSH) as adopted by the OECD, the plant extract could be considered as a non-toxic substance (OECD, 2001a). Based on the fact that Erhirhie *et al.,* (2018), stated that a conclusion can be made on the toxicity status of the test substance from the report of acute toxicity studies, it can be concluded that MEAG is relatively non toxic.

Sub-acute toxicity (repeated dose 28-day oral toxicity study)

Effect of MEAG on biochemical parameters in rats

The effect of MEAG on biochemical parameters was evaluated. There was an observed dose-dependent increase in the liver enzymes alanine transaminase (ALT) and alkaline phosphatase (ALP) in the groups treated with the extract. However, these were not significant ($p > 0.05$) when compared with the control. There was also an increase in direct bilirubin, which was significant ($p < 0.05$) at 400 and 800 mg/kg when compared with the control, even though the increase was not dose dependent. MEAG was also shown to increase significantly ($p < 0.05$) the urea level in rats when given at 200 and 400 mg/kg. There was also an increase in the urea level of 71.95 ± 9.27 at 800 mg/kg (MEAG), but this was seen not to be significant ($p > 0.05$) when compared with the control (Table 2).

Table 2: Effect of 28-day Daily Oral Administration of Methanol Root Bark Extract of *Azanza garckeana* on Biochemical Parameters in Rats

Biochemical parameters	Treatment groups (mg/kg)			
	DW(10ml/kg)	MEAG (200)	MEAG (400)	MEAG (800)
ALT(IU/L)	7.33±1.80	8.00±1.24	28.33±11.56	44.50±19.56
AST(IU/L)	24.00±2.09	22.00±3.56	23.83±4.24	21.00±0.97
ALP(IU/L)	26.87±2.15	29.72±4.56	30.18±3.92	39.22±2.63
Total Protein(mg/dL)	14.10±1.18	13.73±0.69	13.40±0.55	17.07±0.76
Albumin(mg/dL)	2.57±0.13	3.45±0.29	2.97±0.21	3.25±0.26
Direct Bilirubin(mMol/L)	4.53±0.60	6.53±0.58	9.75±0.78***	7.12±0.32*
Total Bilirubin(mMol/L)	13.72±0.91	16.52±1.18	19.27±0.96	17.98±2.09
Urea(mg/dL)	49.23±14.39	91.12±5.14*	97.55±7.77**	71.95±9.27
Creatinine (mMol/L)	1.05±0.11	0.95±0.04	0.97±0.07	1.07±0.08
Cholesterol (mg/dL)	59.70±8.44	61.97±3.57	66.97±3.83	80.58±5.45

Values are Mean ± SEM (n=6); *, **, ***, represents statistical significance at $p<0.05$, $p<0.01$, and $p<0.001$compared to the control group. Result was analysed using one-way ANOVA followed by Bonferroni post hoc test, DW= Distilled water, MEAG = Methanol root bark extract of Azanza garckeana, ALT = Alanine Transaminase, AST = Aspartate Transaminase, ALP = Alkaline Phosphatase, IU = International Unit

Effect of MEAG on histopathology of some organs in rats

The histology of the heart, brain, liver and kidney of the animals were also evaluated for signs of toxicity. Histopathological changes were observed on the brain, heart, liver, as well as the kidney of the extract treated groups. Pathological changes showed on the brain of MEAG treated rats were; slight neuronal necrosis and slight hyperplasia of inflammatory cells (Plate II). Pathological changes showed on the heart of MEAG treated rats were; sinusoidal congestion, slight hyperplasia of inflammatory cells and slight myocardial necrosis (Plate III). Pathological changes showed on liver of MEAG treated rats were; hepatic necrosis, slight peri-portal necrosis, hyperplasia of inflammatory cells, Kupfer cell hyperplasia, vascular congestion, and Slight vacuolation and necrosis, (Plate IV). Pathological changes showed on the kidney of MEAG treated rats were; slight tubular necrosis, moderate tubular adhesion, hyperplasia of inflammatory cells, and slight glomerular necrosis (Plate V).

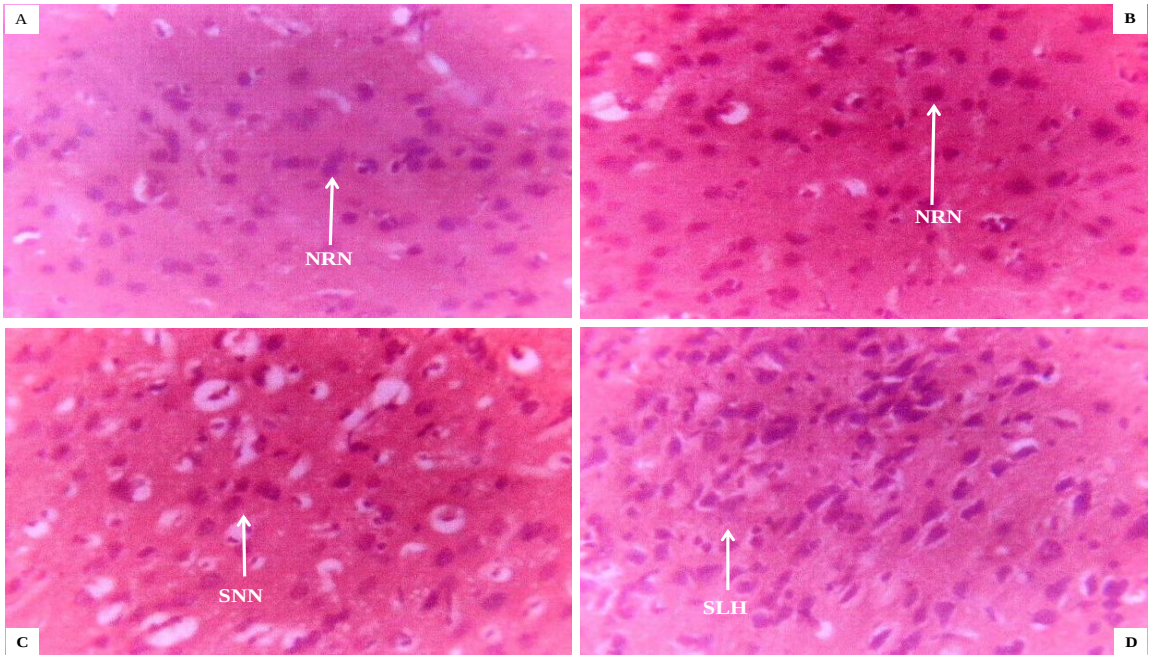

Plate II: Photomicrographs of Brain Sections of Rats following 28-day Daily Oral Administrations of Methanol Root Bark Extract of *A. garckeana* (Hematoxylin and Eosin Stain, Magnification x 400)

A) Control (Distilled water), B) MEAG (200 mg/kg), C) MEAG (400 mg/kg), D) MEAG (800 mg/kg), NRN = Normal neurons, SNN = Slight neuronal necrosis, SLH = Slight hyperplasia of inflammatory cells, MEAG = Methanol root bark extract of *Azanza garckeana.*

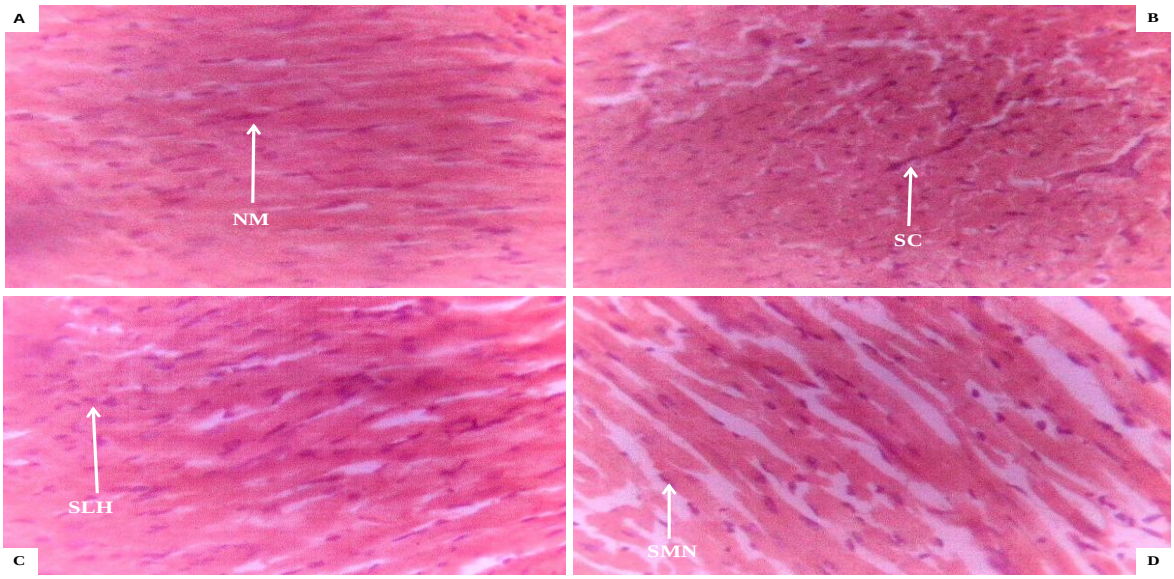

Plate III: Photomicrographs of Heart Sections of Rats following 28-day Daily Oral Administrations of Methanol Root Bark Extract of *A. garckeana* (Hematoxylin and Eosin Stain, Magnification x 400)

A) Control (Distilled water), B) MEAG (200 mg/kg), C) MEAG (400 mg/kg), D) MEAG (800 mg/kg), NM = Normal myocardium, SC = Sinusoidal congestion, SLH = Slight hyperplasia of inflammatory cells, SMN= Slight myocardial necrosis, MEAG = Methanol root bark extract of *Azanza garckeana*

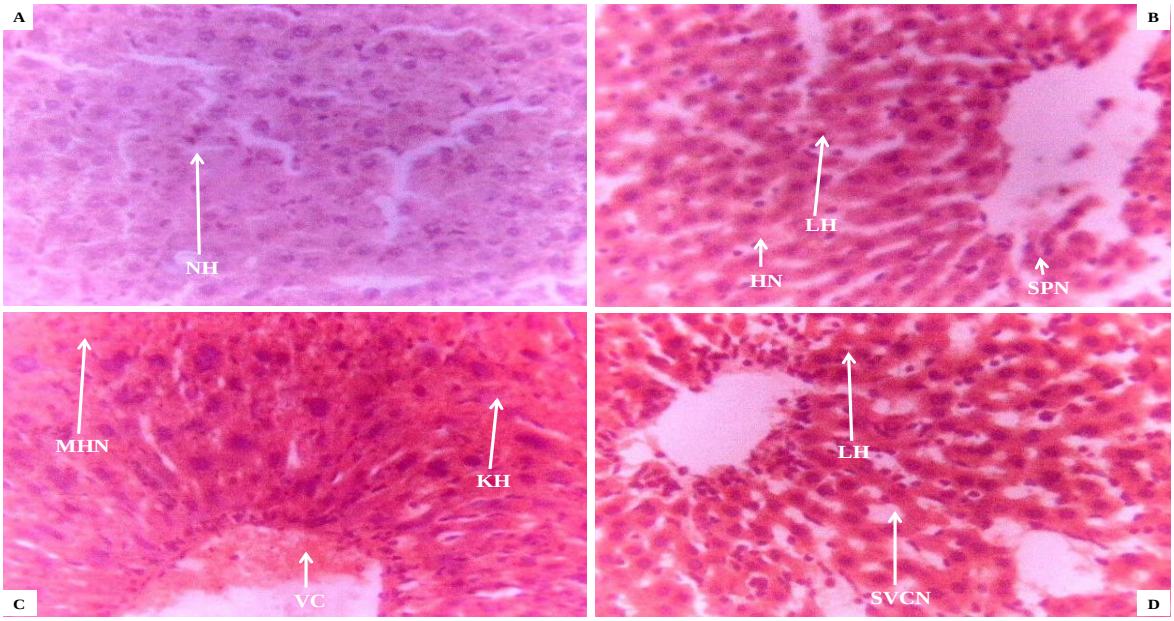

Plate IV: Photomicrographs of Liver Sections of Rats following 28-day Daily Oral Administrations of Methanol Root Bark Extract of A. garckeana (Hematoxylin and Eosin Stain, Magnification x 400)

A) Control (Distilled water), B) MEAG (200 mg/kg), C) MEAG (400 mg/kg), D) MEAG (800 mg/kg), NH= Normal hepatocites, HN=Hepatic necrosis, MHN = Moderate hepatic necrosis, SPN= Slight periportal necrosis, LH = Hyperplasia of inflammatory cells, KH=Kupfer cell hyperplasia, VC=Vascular congestion, SVNC=Slight vacuolation and necrosis, MEAG = Methanol root bark extract of Azanza garckeana

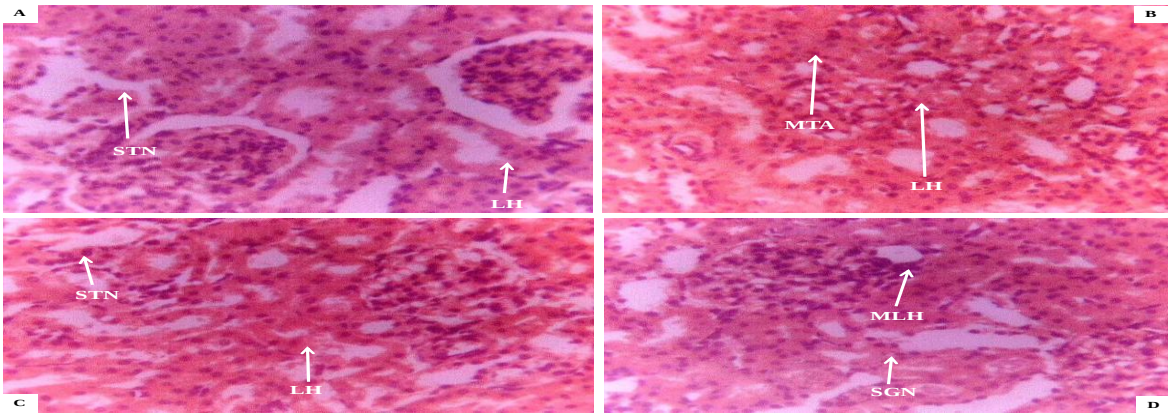

Plate V: Photomicrographs of Kidney Sections of Rats following 28-day Daily Oral Administrations of Methanol Root Bark Extract of A. garckeana (Hematoxylin and Eosin Stain, Magnification x 400)

A) Control (Distilled water), B) MEAG (200 mg/kg), C) MEAG (400 mg/kg), D) MEAG (800 mg/kg), STN= Slight tubular necrosis, MTA= Moderate tubular adhesion, MLH = Moderate hyperplasia of inflammatory cells, LH = Hyperplasia of inflammatory cells, SGN= Slight glomerular necrosis, MEAG = Methanol root bark extract of Azanza garckeana

Metabolism in mammals is largely regulated by the liver and kidney. While the renal system is mainly involved in the excretion of waste product out of the body as well as maintaining homeostasis by reabsorbing vital electrolytes. The liver removes toxic substances that are harmful to the body. Therefore, any observable change in the normal function of the kidney and the liver after exposure over a period of time to a test substance may be indicative of the possible toxic effects rendered by the substance in question (Amarasiri *et al.,* 2020).

It is worthy of note that, urea and creatinine are the major indicators of renal toxicity. While serum urea accumulation is used as the acute marker, serum creatinine accumulation on the other hand is used in detecting chronic renal toxicity (Alkali *et al.,* 2018). In the study conducted, MEAG showed a significant increase in serum urea at 200 and 400 mg/kg ($p < 0.05$ and $p < 0.01$, respectively) when compared to the control group. However, creatinine levels were not significantly ($p > 0.05$) affected. This indicated that the kidney function in the test animals (rats) may have been affected by the sub-acute (28-day) repeated MEAG oral administration, which showed that the extract may have some level of toxicity on the kidney. Also, photomicrographs of the histological sections of the rats' kidneys in the treatment groups (200, 400 and 800 mg/kg MEAG) indicated the presence of slight tubular necrosis (STN), moderate tubular adhesion (MTA) as well as slight glomerular necrosis (SGN) which pointed to the harmful effect of the extract on the kidney. The slight tubular necrosis (STN) and hyperplasia of inflammatory cells (LH) observed in the control group may be coincidental as a result of other factors. This finding agreed with the work of Dawud *et al.,* (2023), which also revealed some level of damage to the kidney due to the sub-acute oral administration of the fruit pulp extract of the same plant.

An elevation in the serum concentration of liver enzymes such as ALT, AST and ALP is a reliable indicator of liver toxicity. These changes are brought about by alterations in hepatic cellular permeability or necrosis and cellular injury as these enzymes are released due to liver injury (Alkali *et al.,* 2018). Bilirubin is also a well-established marker that is commonly included in biochemical tests to ascertain liver dysfunction or any other condition (Ruiz *et al.,* 2021). In this study, oral administration of MEAG over a period of 28 days showed no significant increase ($p > 0.05$) in the levels of the liver enzymes when compared to the control. This concurred with the report of Dawud *et al,* (2023) except for ALT which was shown to be increased significantly ($p < 0.05$) with the fruit pulp extract of the same plant. However, there was a significant increase ($p < 0.001$ and $p < 0.05$) in the level of direct bilirubin both at 400 and 800 mg/kg body weight doses respectively. This may be indicative of the possible toxic effects of the MEAG extract on the liver which may have impacted on the ability of the liver to excrete the conjugated bilirubin. This is because conjugated (direct) hyper bilirubinemia has a high level of specificity for indicating liver damage (Ruiz *et al.,* 2021). Also, photomicrographs of the histological sections of the rats' liver in the treatment groups (200, 400 and 800 mg/kg MEAG) following the 28-day repeated oral administrations indicated the presence of slight and moderate hepatic necrosis (MHN), slight vacuolization and necrosis (SVNC) as well as slight periportal necrosis (SPN) which pointed to the harmful effect of the extract on the liver. Histology report on the liver of rats in Dawud *et al,* (2023) study, also showed some level of damage to the rats' liver when given the methanol fruit pulp extract of the same plant.

Effect of MEAG on haematological parameters in rats

Administration of MEAG at all the doses given (200, 400, 800 mg/kg) did not produce significant ($p > 0.05$) effect in all the haematological parameters evaluated (Table 3).

Table 3: Effect of 28-day Daily Oral Administration of Methanol Root Bark Extract of *Azanza garckeana* on Haematological Parameters in Rats

Haematological parameters	Treatment groups(mg/kg)			
	DW(10ml/kg)	**MEAG (200)**	**MEAG (400)**	**MEAG (800)**
WBC($\times10^9$/L)	4.98±0.41	4.83±0.26	4.78±0.33	4.85±0.32
LYMPH (%)	65.62±1.47	61.35±1.79	58.63±1.51	60.58±2.65
GRAN (%)	30.63±1.19	33.13±1.95	35.88±1.89	35.32±2.46
RBC($\times10^{12}$/L)	6.15±0.38	6.05±0.62	6.08±0.60	5.75±0.17
HGB(g/dL)	13.12±0.46	12.53±0.42	15.50±1.73	12.72±0.75
HCT (%)	39.50±1.54	38.00±1.79	44.17±3.24	38.00±1.65
MCV(fL)	87.95±2.42	86.02±1.54	88.58±0.77	87.10±1.59
MCH(pg)	31.95±1.73	29.33±0.74	31.47±1.53	30.27±0.75
MCHC(g/dL)	32.67±0.60	33.68±1.23	32.78±0.43	34.53±0.69
PLT($\times10^9$/L)	199.52±20.61	193.17±9.59	173.33±7.89	177.33±6.14

Values are Mean ± SEM (n=6); DW= Distilled water, MEAG = Methanol root bark extract of *Azanza garckeana*, WBC = White blood cell count, LYMPH % = Percentage of lymphocytes, GRAN % = Percentage of granulocytes, RBC = Red blood cell count, HGB = Haemoglobin, HCT = Haematocrit, PLT = Platelet, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration.

Haematological analysis is one of the key laboratory investigations recommended to be carried out at the end of sub-acute toxicity studies (OECD, 2008). This is to ascertain the effects of the test substances on vital blood parameters such as platelet count (PLT), haemoglobin content (HGB), differential WBC such as lymphocytes e.t.c.

Lymphocytes represent around 20 to 40% of WBC. It is a type of white blood cell that is divided into two principal groups and a null group; B-lymphocytes which produces antibodies in the humoral immune response, T-lymphocytes, which participates in the cell-mediated immune response and the null group, which contains natural killer cells, cytotoxic cells that participate in the innate immune response (Erhabor *et al.*,2021).

It is worthy of note that white blood cell (WBC) differentials such as neutrophils, lymphocytes, monocytes, or ratio of neutrophil to lymphocyte counts (N/L) has been marked as an easy, simple, inexpensive, and reliable means of determining host immunity and for prognostic purpose (Erhabor *et al.*,2021). From the findings of this study, it could be seen that MEAG at all the doses given (200, 400, and 800 mg/kg body weight), did not have any significant effect on any of the haematological parameters tested. This corroborates the findings of Dawud *et al.*, (2023) which showed no significant ($p > 0.05$) effect on any of the haematological parameters with the fruit pulp extract of *Azanza garckeana.* This may be suggestive of the fact that the extract (MEAG) generally may have no toxic effect on haematological parameters.

Effect of MEAG on body weight and relative organ weight in rats

The effect of the extract on the relative organ weight of the animals (spleen, liver, kidney, heart and the brain) was also ascertained with no significant effect ($p > 0.05$) seen (Figure 1). The weekly changes in body weight of the animals were also taken note of throughout the administration period and MEAG showed no significant ($p > 0.05$) increase in body weight of the rats between and within the groups (Figure 2).

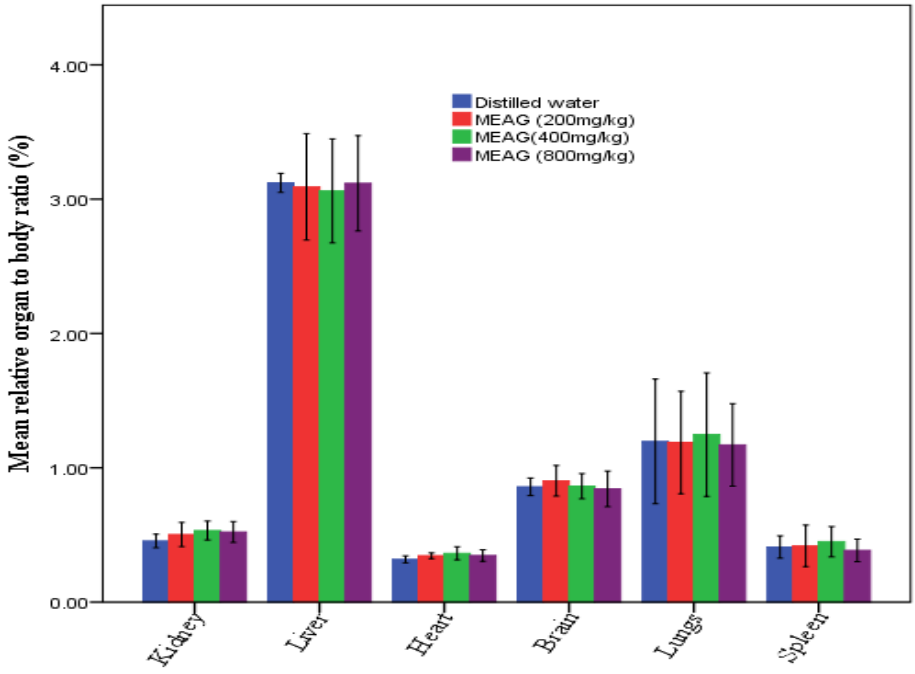

Figure 1: Effect of 28-day Daily Oral Administration of Methanol Root Bark Extract of *Azanza garckeana* on Relative Organ Weights in Rats

Data are presented as Mean ± SEM (n=6); MEAG = Methanol root bark extract of *Azanza garckeana*

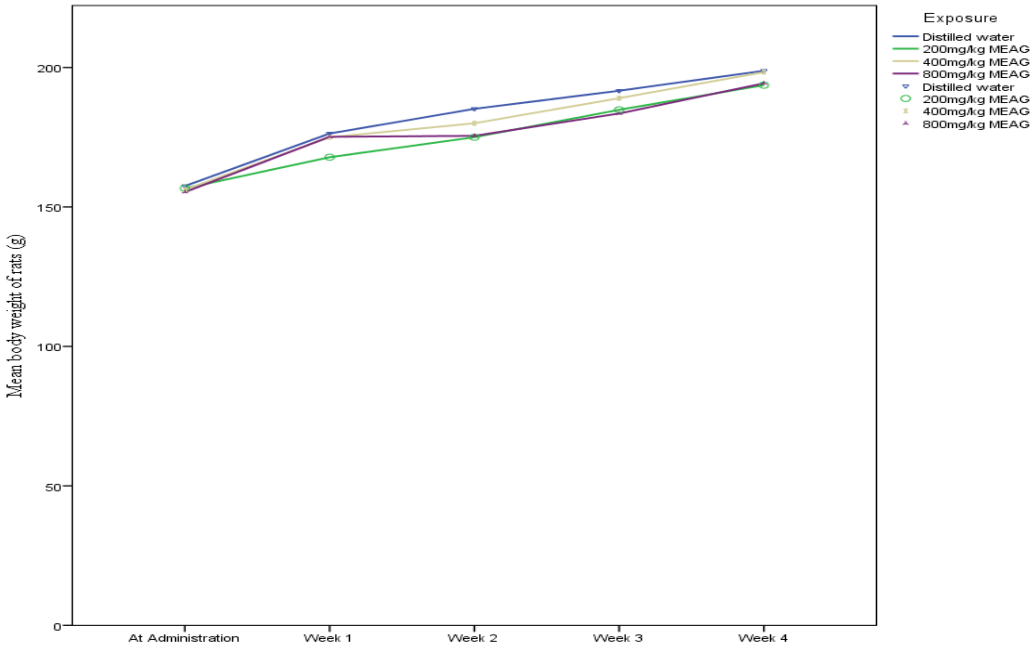

Figure 2: Effect of 28-day Daily Oral Administration of Methanol Root Bark Extract of *Azanza garckeana* on Weekly Body Weight in Rats

Data are presented as mean ± SEM (n = 6); values are significant when $p < 0.05$ within and between group (Split-plot ANOVA), MEAG = Methanol root bark extract of *A. garckeana*

Sub-acute administration of MEAG produced no observable change in the behaviour of the animals, and or morphological characteristics of the skin, eye, fur, and nose within the period of study. Also, there was no significant ($p > 0.05$) increase in the body weight of the rats between and within the groups. This is consistent with the report of Dawud *et al.*, (2023) which showed no significant change in the body weight of rats given the methanol extract of the fruit pulp of *A. garckeana.* The non- significant increase ($p > 0.05$) in the body weight in all the animals between and within the groups could be due to the normal metabolic process in the animals as a result of food and water intake with time. However, this increase in the mean body weight of the rats occurred in a haphazard manner between the groups. The increase in the weight was higher in the group that received distilled water, followed by the groups that received MEAG at 400 and 800 mg/kg body weight respectively, and lastly by the group that received MEAG at 200 mg/kg body weight. It is worthy of note that within the groups, there was an observed steady increase in the body weight of the animals as time elapsed except at 800 mg/kg, where the initial body weight increase after the first week was maintained in the second week followed by a steady increase in the third and fourth weeks respectively. The reduced rate, at which the rats' body weight increased weekly as time elapsed in the MEAG treated groups, when compared with the distilled water group, may be indicative of an early sign of toxicity. This is because, reduction in the body weight of test animals as well as abnormal weight gains in sub-acute toxicity studies is indicative of the toxic effects of the test substance (OECD, 2008; Amarasiri *et al.*, 2020). However, changes may occur due to normal physiological response to the plant extract and accumulation of fat instead of toxicity potentials of the chemical substances (Nfozon *et al*., 2019).

Relative organ weight is an important index of the physiological and pathological status in animals (Prasanth *et al*., 2015). Its changes are sensitive indicators of toxicity, effects on enzymes, physiologic disturbances and target organ injury (Chinenye *et al*., 2019). According to this study, there was no significant difference ($p > 0.05$) on the relative organ (liver, kidney, lungs, heart, brain and spleen) weight between the test and control groups. This outcome also agreed with the findings of Dawud *et al.*, (2023), which also showed an insignificant ($p > 0.05$) effect of the fruit pulp extract of *A. garckeana* on relative organ weight, except for its significant effect ($p < 0.05$) on the lungs and the heart. This may be suggestive of the fact that MEAG is relatively less toxic on these organs. However, it is worthy of note that organ weight data is usually interpreted in an integrated manner with gross pathology, clinical pathology and histopathology findings (Chinenye *et al*., 2019).

CONCLUSION

Given the widespread use of the plant parts of *Azanza garckeana* both as food and drug; it was necessary to draw attention to the potential hazards of prolonged consumption/use of the plant parts for food or as medicine. The logical conclusion derivable from the results and discussion taken together indicate that signs of hepatic and renal damage resulted from the 28-day sub-acute administration of methanol root bark extract of *Azanza garckeana*.

Ethical approval for the use of Laboratory animals: The protocol used to conduct this study was in accordance with the Ahmadu Bello University Ethics Committee on the use of Laboratory Animals. This was approved by the University Ethics Committee with the number ABUCAUC/2023/033.

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