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Research Article

Evaluation of the Acute and Sub-acute Toxicity of *Azanza garckeana* Aqueous Leaves Extract in Wistar Rats

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ABSTRACT

This study evaluated the safety profile of Goron Tula (*Azanza garckeana*) aqueous leaves extract (AGALE) in rats through single and 28-day oral administrations. Twenty-eight (28) adult male Wistar rats (120-160 g) were used for acute and sub-acute studies. Acute study was carried out in 2 phases using modified Lorke's method, AGALE was administered at single doses of 10, 100 and 1,000 mg/kg (n=3) and observed for 24 h for mortality or toxicity signs, while for phase II, 4 rats (n=1) were used and respectively administered with doses of 800, 600, 400 and 200 mg/kg body weight of the aqueous extract and also observed for mortality and signs of toxicity. For the sub-acute study, AGALE was administered once daily for 28 days at 50, 100 and 150 mg/kg while the vehicle was administered to control (n = 4). The results revealed that mortality was recorded at 1000 mg/kg in phase I of the acute toxicity study. In phase II, mortality was recorded at 600 and 800 mg/kg doses. The LD_{50} of AGALE in rats was determined to be 490 mg/kg b.w. The oral administration of AGALE for 28 days showed significant decrease in the levels of the electrolytes in the 50 mg/kg treated group. Histological examination of the kidney showed mild toxicity related abnormalities. There was no significant alteration in the liver function indices. Histopathology of the liver showed significant amount of immunological cells. Haematological parameters evaluated showed that there was significant increase ($P < 0.05$) in white blood cells, monocytes and lymphocytes. This study demonstrates that the LD_{50} of AGALE in Wistar rats is 490 mg/kgAGALE extracts may not exert toxic effects at doses less than 150 mg/kg.

Keywords: *Azanza garckeana, Toxicity, Liver, Kidney, Lipid profile*

INTRODUCTION

Most cultures throughout the world employ natural remedies for the treatment of diseases (Elufioye and Onoja, 2015). The discovery of novel bioactive chemicals that come from a variety of sources, including plants, fungus, bacteria, and marine species, is the product of ongoing scientific research (Jothy *et al.,* 2011). The results of Atata *et al.* (2003) suggest that there are approximately 700,000 species of tropical flowering plants. Their actions include: antibacterial, antifungal, antiviral, antihelminthic and anti-carcinogenic among others. These therapeutic effects are ascribed to specific chemical components (Oladunmoye, 2007).

Azanza, tree hibiscus, and mucous apple are only a few of the numerous English names often used to refer to the plant formally known as *Azanza garckeana*. In Botswana and South Africa, the plant species is often referred to as Morojwa and *Thespesia garckeana*, respectively. In Nigeria, the phrase used to describe this specific plant is "goron tula." The naturally occurring locations of the highly prized fruit include Michika in Adamawa State and Tula in the Kaltungo Local Government Area of Gombe State (Orwa *et al.*, 2009; Mojeremane and Tshwenyane, 2004). Other habitats for this specific species are in the Sudan, Kenya, Malawi, Mozambique, Namibia, Tanzania, Zimbabwe, and Zambia. The eastern, western, and southern regions of the continent of Africa are all covered by the distribution of *A. garckeana*.

Numerous chemical classes, including ascorbic acid, carotenoids, cyanogenic glucosides, flavonoids, lipids, phenols, saponins, and tannins, have been reported to be synthesized by *A. garckeana*. According to Maroyi (2017), *A. garckeana* exhibits properties that include antibacterial, antifungal, anti-hyperglycemic, anti-malarial and antioxidant. According to Maroyi (2017), the therapeutic qualities of *A. garckeana* encompass several plant components, including its bark, fruits, leaves, roots, and stems. This plant species' medicinal properties have been used successfully to treat and manage a wide range of illnesses and disorders across the area of its distribution. Natural remedies, especially medicinal plants, have long been relied upon by humans throughout history as a primary method of treating a variety of health problems. It is customary to do a risk assessment of a natural product extract or component in the initial stage of screening (Alkali *et al.*, 2018). Despite the traditional applications of *Azanza garckeana* (AG), detailed knowledge about the toxic effect of the leaves is scarce. Therefore, this study was carried out to evaluate the toxicity of *Azanza garckeana* in experimental animals. Acute and sub-acute oral toxicity studies of the plant's aqueous leaves extract were carried out in adult male Wistar rats.

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *Azanza garckeana* (AG) was collected from Kaltungo L.G.A, Gombe state of Nigeria and identified at the Herbarium Unit Biological Science Department, B. U. K. Kano with accession number BUKHAN 650.

Preparation of aqueous extract

The leaves were air dried at the Biochemistry laboratory Federal University Dutsin-ma, pulverized and stored in air-tight container prior to use. For the preparation of aqueous extracts, powdered leaves were macerated with distilled water for 24 hours with intermittent stirring. After filtration with Whatman No. 1 filter paper, the filtrate collected was evaporated using water bath (40ºC) and dried in a desiccator, (percentage yield = 26.8%).

Experimental animals

Experimental Wistar rats weighing about 120-160g were obtained from the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. The animals were kept under standard conditions, allowed to acclimatize to the new environment for two (2) weeks, allowed free access to water and food in accordance with the internationally accepted practices for use and care of laboratory animals as contained in US guidelines (NIH, 1992) and as approved by the Federal University Dutsin-Ma Ethical Committee on the use of experimental animals and registered as FUDMA/CEA/2023/078.

Acute oral toxicity test

LD_{50} was determined using modified Lorke's method (1983). In phase I, 9 rats were divided into 3 groups of 3 rats each. Following an overnight fast, the rats were weighed and the dose was calculated in reference to their body weight. The aqueous extract was suspended in a vehicle (distilled water). The three groups received 10, 100 and 1000 mg/kg body weight of the aqueous extract via oral gavage. The animals were observed keenly for about 30 min for any signs of toxicity or mortality. The death of animals in this phase necessitated the conduct of the second phase.

In the second phase, 4 rats were used and respectively administered with doses of 800, 600, 400 and 200 mg/kg body weight of the aqueous extract. The rats were watched for symptoms of toxicity or demise for the first four hours, then intermittently for twenty-four hours. A standard protocol including a 14-day survey of the whole rat population yielded the LD50 value.

The formula is displayed below.

$LD_{50} = \sqrt{\text{min conc. Full death} \times \text{max conc. no death}}$ (1)

Min conc. Full death is the minimum or lowest concentration that resulted in death, Max conc. no death is the maximum or highest concentration that did not result in any death

Sub-acute toxicity study

As previously described by Al-Afifi *et al.* (2018), 16 Wistar rats were grouped into four (A, B, C and D) of four rats each. Group A served as control and was administered with 0.5 mL of distilled water once daily for 28 days. Rats in groups B, C and D were administered 150, 100 and 50 mg/kg body weight respectively of the aqueous extract by oral gavage once daily for 28 days. The rats were observed daily for signs of toxicity, and their body weights were also recorded weekly throughout the experimental period. On the 28th day of the research, following an overnight fast of 8 h, all animals in various groups were anesthetized under chloroform and blood samples were collected by cardiac puncture into EDTA treated and plain bottles for haematological and biochemical investigations respectively. The liver and kidneys were excised from dissected rats and rinsed with 0.9% physiological saline. The organs were then weighed to calculate their relative organ weights (using the formula below) and observed for any gross lesions.

$$\text{Relative organ weight} = \frac{\text{Organ weights(g)}}{\text{Body weight of animal on sacrifice day}} \times 100 \quad (2)$$

The liver and kidneys were then fixed in 10% formalin for histopathological examination.

Biochemical analyses

The biochemical assay kits including alanine transaminase (ALT), aspartate transaminase (AST), total protein, albumin and electrolytes from Randox Laboratories

Limited, United Kingdom and urea and creatinine kits from Agappe Diagnostics (Switzerland).

Haematological analyses

Haematological parameters were analyzed by using a Diatron Diagnostic Abacus Junior automatic hematology analyzer.

Histopathological examination

Paraffin wax was used to embed tissue samples, and 5 mm thick sections were cut from the organs. Slices that had been stained with haematoxylin and eosin were placed on glass slides and examined using a regular light microscope.

Statistical analysis

The results were presented as mean ± standard error of mean. SPSS (version 20; Chicago, Illinois, USA) was used to conduct an ANOVA. Significance was attributed to data with a P-value of less than 0.05 when compared to the control group.

RESULTS

Oral administration of aqueous extract of *Azanza garckeana* aqueous leaves extract (AGALE) lead to the mortality of 1 rat from the group administered 1000 mg/kg 15hrs after administration of the extract in the phase I of this study (Table 1). In the phase II, mortality was recorded at 800 mg/kg on the 3rd day and at 600 mg/kg on the 4th day (Table 2). The animals showed dose dependent signs of toxicity such as sluggishness, diarrhea and labored breathing (Table 3), however those that received 10 mg/kg regained their pre-treatment stamina and activities after a couple of days. The animals that died were observed to have bled through the nose, eyes and mouth. No significant disparity in terms of body and organ weight was seen between the groups treated with the extract and the control groups.

The body weight of the rats in control group showed gradual increase during the 28-day treatment period up to 42.6% of weight gain. Administration of extract at 150, 100 and 50 mg/kg per day showed respectively, 23.1%, 23.2% and 20.1% increase in body weight (Table 4). There was no significant difference in average organs (kidney and liver) weight between control and extract treated groups at the tested doses (Table 5)

Table 1. Dose and Mortality Recorded Following a 24 hour Administration of *Azanza garckeana* Aqueous Leaves Extract (Phase 1)

Phase 1 (groups)	Number of animals	Dose (mg/kg)	Mortality recorded
1	3	10	0
2	3	100	0
3	3	1000	1 (15HRS)

Table 2. Dose and Mortality Recorded Following a 24 hour Administration of *Azanza garckeana* Aqueous Leaves Extract (Phase II)

Phase II (groups)	Number of animals	Dose (mg/kg)	Mortality recorded
1	1	800	1 (DAY 3)
2	1	600	1 (DAY 4)
3	1	400	0
4	1	200	0

$LD_{50} = \sqrt{600*400} = \sqrt{240000}$

$LD_{50} = 490$ mg/kg

Table 3. Toxicity Signs Observed in Rats Following a 24 Hour Administration of *Azanza garckeana* Aqueous Leaves Extract

Dose (mg/kg b.w)	Signs of toxicity				
	Sluggishness	Respiratory distress	Bleeding	Diarrhea	Death
10	-	-	-	-	-
100	-	-	-	-	-
1000	+	+	+	+	+
200	+	-	-	-	-
400	+	+	-	+	-
600	+	+	+	+	+
800	+	+	+	+	+

Key: - means negative, = means positive

Table 4. Relative Weight Change during Subacute Toxicity Study of *Azanza garckeana* Aqueous Leaves Extract (AGALE)

Group	Weight (day0)	Weight (day 7)	Weight (day 14)	Weight (day 21)	Weight (day 28)	Percentage weight gain
Group A	141±31.5	162±23.1	179±24.3	194±26.1	201±24.7	42.6%
Group B	173±24.2	188±25.6	199±27.8	211±29.7	213±28.7	23.1%
Group C	177±17.1	184±16.6	200±20.7	215±25.2	218±27.8	23.2%
Group D	164±10.7	167±11.5	180±10.6	193±14.2	197±16.9	20.1%

Values are expressed as the mean ± SEM (n=4).

Group A: received 1ml distilled water (control), Group B: received 150 mg/kg AGALE, Group C: received 100 mg/kg AGALE, Group D: received 50 mg/kg AGALE

Table 5. Effect of *Azanza garckeana* Aqueous Leaves Extract (AGALE) on Relative Organ Weights of Wistar Rats after Subacute Oral Toxicity Studies.

Group	Liver (%)	Kidney (%)
Group A	3.27±0.62[a]	0.80±0.10[a]
Group B	2.77±0.18[a]	0.60±0.06[a]
Group C	2.83±0.28[a]	0.63±0.03[a]
Group D	2.67±0.03[a]	0.60±0.06[a]

Values are expressed as the mean ± SEM (n=4). Values with different letters along a column are significantly ($p<0.05$) different from each other. Group A: received 1ml distilled water (control), Group B: received 150 g/kg AGALE, Group C: received 100 mg/kg AGALE, Group D: received 50 mg/kg AGALE.

The results of this study showed that the oral administration of AGALE for 28 days did not produce significant alteration in the renal function indices except for the levels of Na, Cl and HCO_3 which were significantly lower $P < 0.05$) in the group treated with 50 mg/kg compared to the other groups (Figure 1).

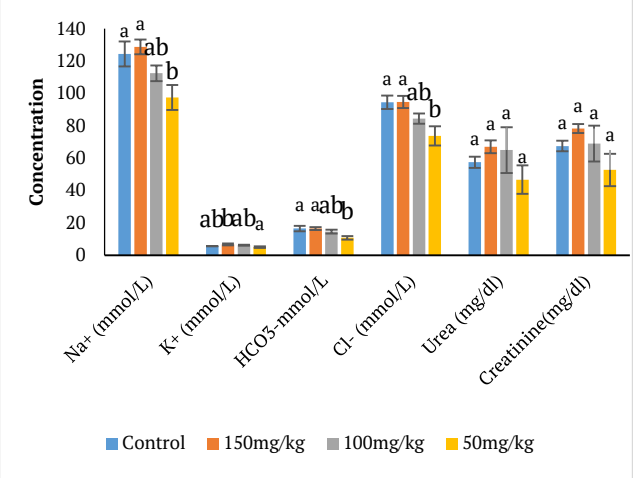

Figure 1. Serum Kidney Function Indices Levels of Rats Treated with Oral Doses of *Azanza garckeana* Aqueous Leaves Extract

Values are expressed as the mean ± SEM (n=4), values with different letter over the bars for a given parameter are significantly ($p<0.05$) different from each other (a = not statistically different from each other, b = statistically different from a)

The results of the tests on liver function indices of experimental animals treated with the plant extract and control group are summarized in (Figures 2 and 3). The oral administration of AGALE for 28 days showed that there were no significant differences ($P > 0.05$) in the level of liver enzyme and plasma protein levels when AGALE treated groups were compared with the control group.

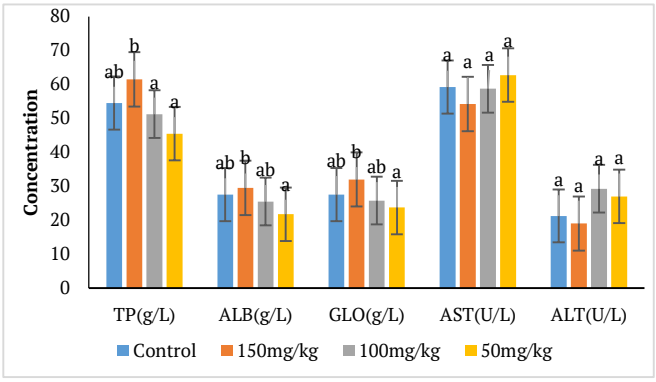

Figure 2. Indices of Liver Function in Rats Treated with Oral Doses of *Azanza garckeana* Aqueous Leaves Extract.

Values are expressed as the mean ± SEM (n=4), values with different letter over the bars for a given parameter are significantly ($p<0.05$) different from each other (a = not statistically different from each other, b = statistically different from a).
Key: TP-Total protein, ALB: Albumin, GLO: Globulins, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

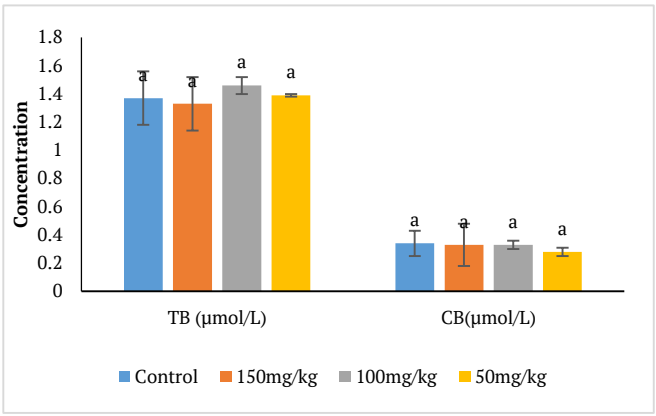

Figure 3. Bilirubin Levels of Rats Treated with Oral Doses of *Azanza garckeana* Aqueous Leaves Extract.

Values are expressed as the mean ± SEM (n=4), values with different letter over the bars for a given parameter are significantly ($p<0.05$) different from each other (a = not statistically different from each other, b = statistically different from a).
Key: TB- Total Bilirubin, CB- Conjugated Bilirubin

The results of the tests on lipid profile of experimental animals treated with the plant extract and control group are summarized in Figure 4. The results show that administration of AGALE increased the serum level of cholesterol significantly ($P < 0.05$), compared to the control group. The triglyceride level was significantly lower at the highest dose of 150 mg/kg. There was also a significant increase in HDL levels while LDL only increased at the 150 mg/kg group.

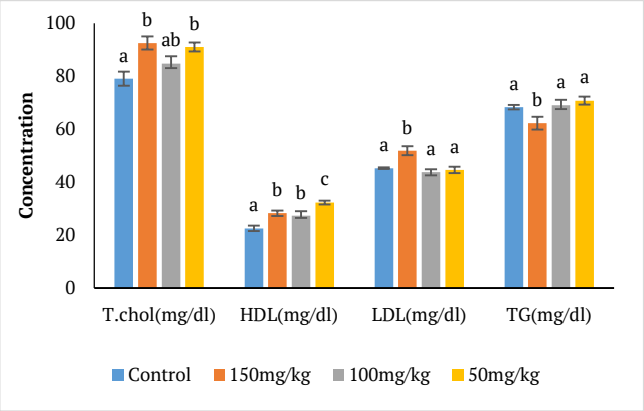

Figure 4. Serum Lipid Profile of Rats Treated with Oral Doses of *Azanza garckeana* Aqueous Leaves Extract.

Values are expressed as the mean ± SEM (n=4), values with different letter over the bars for a given parameter are significantly ($p<0.05$) different from each other (a = not statistically different from each other, b = statistically different from a).
Key: T. chol: Total cholesterol, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, TG: Triglycerol

The results of the tests on haematological indices of experimental animals treated with the plant extract and control group are summarized in Table 6. The results show that after 28 days administration of AGALE, haematological parameters (red blood cell, neutrophils, basophils, haemoglobin, and platelets) evaluated were not significantly different ($P > 0.05$) from the control, however there was significant difference ($P < 0.05$) in white blood cells, monocytes, eosinophil lymphocytes and haematocrit levels.

Table 6. Serum Haematological Profile of Rats Treated with Oral Doses of *Azanza garckeana* Aqueous Leaves Extract

Group	WBC $10^3/\mu l$	LYM $10^3/\mu l$	MON %	NEUT %	EOS %	BAS %	RBC $10^6/\mu l$	HGB g/dl	HCT %	PLT $10^3/\mu l$
Control	6.6 ± 0.2^a	83.1 ± 1.3^a	2.2 ± 0.3^a	5.8 ± 0.6^a	6.6 ± 0.5^a	1.8 ± 0.2^a	7.7 ± 0.2^a	15.2 ± 0.2^a	44.3 ± 0.3^a	418.3 ± 5.6^a
150 mg/kg	9.7 ± 0.5^b	88.3 ± 0.9^b	4.0 ± 0.1^b	2.9 ± 0.7^b	2.7 ± 0.1^b	2.1 ± 0.2^a	8.1 ± 0.2^a	15.7 ± 0.3^a	46.3 ± 1.1^{bc}	504.5 ± 35.9^b
100 mg/kg	8.8 ± 0.8^b	86.1 ± 1.1^{ab}	3.8 ± 0.3^b	4.7 ± 0.6^{ab}	3.4 ± 0.7^b	2.1 ± 0.5^a	7.9 ± 0.1^a	15.8 ± 0.4^a	47.1 ± 0.4^c	477.0 ± 20.1^{ab}
50 mg/kg	9.1 ± 0.4^b	87.8 ± 0.6^b	3.4 ± 02^b	4.4 ± 0.8^{ab}	2.4 ± 0.3^b	2.1 ± 0.2^a	8.1 ± 0.1^a	15.7 ± 0.2^a	44.9 ± 0.1^{ab}	494.7 ± 26.3^{ab}

Values are expressed as the mean ± SEM (n=4), values with different letter in each column are significantly ($p<0.05$) different from each other (a = not statistically different from each other, b = statistically different from a). WBC (White blood cells) $10^3/\mu l$, MON (Monocytes) %, NEUT (Neutrophils) %, EOS (Eosinophils) %, BAS (Basophils) %, RBC (Red blood cells) $10^6/\mu l$, HGB (Hemoglobin) g/dl

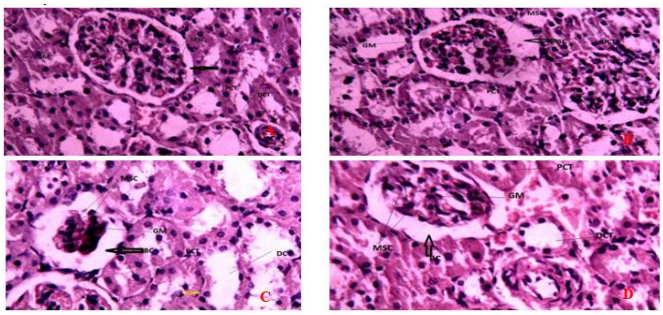

Figure 5. Histopathology of kidney
Group A: Control, Group B: received 150 mg/kg AGALE, Group C: received 100 mg/kg AGALE, Group D: received 50 mg/kg AGALE. (H & E: X 250). GM= Glomerulus, BC= Bowman capsule, MSC=Mesangial cells, PCT=Proximal convoluted tubule, DCT=Distal convoluted tubule.

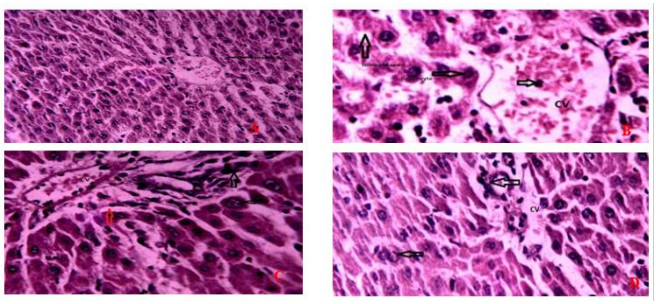

Figure 7. Histopathology of Liver
Group A: Control, Group B: received 150 mg/kg AGALE, Group C: received 100 mg/kg AGALE, Group D: received 50 mg/kg AGALE. (H & E: X 250). CV= Central vein

DISCUSSION

Based on Hodge and Sterner scale (CCOHS, 2005), the aqueous extract of AG leaf could be declared moderately toxic as the LD_{50} was determined to be 490 mg/kg. A test drug administered orally is considered extremely toxic at ≤ 1 mg kg^{-1}, highly toxic at 1-50 mg kg^{-1}, moderately toxic at 50-500 mg kg^{-1}, slightly toxic at 500-5000 mg kg^{-1}, practically nontoxic at 5000-15,000 mg kg^{1} and relatively harmless at $\geq 15{,}000$ mg kg^{-1}. Also, according to the O.E.C.D. guidelines for oral toxicity tests, AGALE can be classified as Category 4.

Assessment of body and organ weights are sensitive indicators in determination of early toxicity of chemicals. The normal weight increment observed from day 1 to day 28 of the study (Table 4) could mean that the extract did not have any adverse effect on the normal physiological functions of the animals. Kifayatullah *et al.* (2015) stated that scientific evidence has proved that increase or decrease in the body weights are accompanied with accumulation of fats and physiological adaptation responses to the plant extracts rather than to the toxic effects of the chemicals or drugs that lead to decrease appetite and as a result lower calorie intake by the test animal. The weight of organs may change owing to toxicity, and this might offer evidence of organ-specific toxicity. (Jothy *et al.,* 2011). Changes in organ weight may reveal target organ toxicity. It may not be evident after acute toxicity test, but usually seen after a sub-chronic or chronic tests (Dawud *et al.,* 2022). The results of

this study revealed that the weights of vital organs such as liver and kidney were not adversely affected throughout the treatment period by the extract which could indicate that the extract may not be toxic to those organs at the tested doses to cause a significant alteration in their weights. Sub-acute toxicity study of methanol fruit pulp extract of *Azanza garckeana* in adult male wistar rats found that the relative organ weights of the liver and kidney were not significantly affected when compared to those of the control (Dawud *et al.,* 2022), this agrees with the finding of this study.

One of the most important metrics for assessing the safety of plant extracts is kidney function. Elimination of metabolic waste, preservation of internal fluid balance, and generation of hormones that may be used to gauge renal health are the three major duties of the kidneys. When the kidneys fail, acutely or chronically, the end product of nitrogen metabolism builds up, increasing non-protein nitrogen levels which can be expressed in the form of elevation of blood urea nitrogen and serum creatinine (Kaid *et al*., 2019). Renal damage is measured using indicators like urea and creatinine. In this study, the oral administration of AGALE for 28 days did not produce significant alteration in the renal function indices except for the levels of the electrolytes Na, K and HCO_3 which were significantly lower in the group treated with 50 mg/kg. These changes might be associated with functional alteration in the proximal tubules due to its nephrotoxic effect (Tizhe *et al.,* 2014). Due to limited literature regarding the evaluation of kidney function parameters of *A. garckeana*, information was gathered about other plants of the Malvaceae family such as *A, moschatus* whose sub-acute toxicity was evaluated in wistar rats showed that the administration of the extract did not cause any changes in the kidney function parameters (Amarasiri *et al.,* 2020). The histological examination of the kidneys showed some mild and patchy glomerular retraction, some congested spaces and extravasated blood cells (RBC) in the interstitium as well as a reduction in size and cellularity of the glomeruli and a thinning of their lining epithelium, and swelling of the mesangial cells when compared to the control group which could indicate some level of nephrotoxicity of the extract.

The degree of liver damage induced by a chemical substance can be evaluated by determining the level of certain markers of the liver function such as Aspartate transaminase (AST) and alanine transaminase (ALT), two indicators of liver function, these enzymes are released as a result of liver injury, especially damage to mitochondria of liver cells. Elevation of the level of these enzymes can be an indication of cellular damage, leakage and collapse of functional integrity of hepatic cell membrane (Alkali *et al.,* 2018). Plasma proteins are produced in the liver. In case of liver damage, production of these proteins is reduced or completely ceased. Plasma proteins interact with virtually all body tissues or cells and are ultimately related to protein metabolism in the liver (Chukwudoruo *et al.,* 2020).The result of this study reveals that there was no significant differences in the level of liver enzyme and plasma protein levels when AGALE treated groups were compared with the control group. Dawud *et al.* (2022) reported that the 28 day administration of methanol extract of *the fruit pulp of A. garckeana* in male adult wistar rats did not cause any change in total protein and AST levels, but at the high dose ALT and albumin levels were increased after the sub-acute treatment. This agrees with the results of this study. Histopathology of the liver showed significant amount of immunological cells in the liver cells of rats in all three test groups. This may indicates that the extract may have caused an inflammatory reaction. This is in contrast to the result of Dawud *et al*. (2022) who reported no treatment-related gross abnormalities in the tissue histology of the liver or rats treated with the methanol fruit pulp extract of *A. garckeana*.

Triglyceridaemia, liver obstruction, primary and secondary hyperlipoproteinaemia, and fatty liver disease are all routinely diagnosed using the lipid profile. Sutrisni *et al.* (2019) established a correlation between raised levels of triglycerides and cholesterol and an increased susceptibility to atherosclerosis and cardiovascular disease. In this study, measurements were conducted on the serum level of cholesterol, high density lipoprotein, low density lipoprotein and triglyceride. The results show that administration of AGALE significantly increased the serum level of cholesterol, HDL and LDL. The observed increase in LDL-cholesterol, shows lipid abnormalities and is synonymous with increased risk of atherosclerosis as reported by Oyewole *et al.* (2008). Iyojo *et al*. (2022) also reported an increase in levels of TGs, HDL and LDL concentrations after the administration of aqueous extracts of *A. garckeana* fruit on New Zealand white rabbit bucks, which agrees with the findings of this study.

The haematopoietic physiology is sensitive to toxins, it is possible for toxins to affect the physiological processes that underlie haemopoiesis. Therefore, the physiological or pathological status of an animal after exposure to hazardous chemicals may be ascertained by analyzing haematological data (Kabiru *et al*., 2010). More so, the analysis of haematological parameters is important in risk evaluation as the haematological system being the main medium of transport of drugs and xenobiotics has a high prognostic value for toxicity of drugs (Dawud *et al*., 2022). Administration of AGALE for 28 days showed that the extract had no effect on the levels of red blood cell, neutrophils, basophils, haemoglobin, and platelets. However there was significant increase in white blood cells, monocytes, lymphocytes and haematocrit levels. As a consequence of the immune system's reaction to acute and chronic infections as well as exposure to irritating and dangerous chemicals, white blood cells (WBCs) may be used as a general biomarker of inflammation (Kabat *et al.,* 2004). Since there was no significant difference in RBC and Hb, it could be an indication that neither the RBC synthesis rate nor the RBC depletion rate had changed appreciably, this could be interpreted that AGALE does not stimulate the release of erythropoietin from the renal system, which is a humoral regulator of RBC production (Alkali *et al.,* 2018). A similar result was reported by Iyojo *et al.* (2022) which reported an increase in lymphocytes, white blood cells and platelet counts following the administration of crude and

aqueous extracts of *A. garckeana* fruit on New Zealand white rabbit bucks. In contrast, a study by Dawud *et al.* (2022) reported that no significant changes in all the haematological parameters after both acute and sub-acute periods of testing with the methanol extract of *the fruit pulp of A. garckeana* in male adult wistar rats.

CONCLUSION

In conclusion, this study provides very important data on the acute and sub-acute toxicity profile of *Azanza garckeana* aqueous leaf extract. LD_{50} was determined to be 490 mg/kg. At high doses, the extract caused elevation of some serum biochemical parameters and produce histologic changes in in the liver and kidney, which are thought to be the target organs of toxicity. Although there is exciting medical potential for the plant specimen in the pharmaceutical sector, excessive usage may be linked to mild organ damage.

AUTHORS' CONTRIBUTIONS

Author MI, ASI and AIG designed the study and helped with data interpretation; MI carried out the laboratory investigations and drafted the manuscript. All authors proofread and approved the manuscript.

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CONFLICT OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper

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None

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