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Original Research Article

POSSIBLE KIDNEY FAILURE INDUCED BY METHANOL SEED EXTRACT OF *Azanza garckeana* IN PREGNANT ALBINO WISTAR RATS.

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Authors' contributions
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ABSTRACT
Background: Uncautious use of herbs in pregnancy could be deleterious.
Methodology: This study was designed to investigate the effect of the methanol seed extract of *Azanza garckeana* in pregnant albino Wistar rats**.** The LD50 of the fresh seed extract was determined to be 5000mg/kg body weight. Twenty (20) adult female Wister rats were randomly divided into four groups of five animals each. Group 2-4 served as the experimental groups, receiving 500 mg/kg, 1000mg/kg and 1500 mg/kg body weight of extract, respectively while Group 1(control) received 1 ml/kg body weight intraperitoneally for served as the control group and was administered with 5ml of normal saline. All administrations were carried out intraperitoneally for 12 days. At termination, body and kidney weight, and blood specimens were taken for analysis.

Result: The extract had a significant impact on the body weight of the experimental animals. There was a significant increase in the body weight of the experimental group when compared to the control group. Controversially, there was a dose-dependent decrease in body weight differences among the experimental groups as the dosage increases. Histological findings revealed dilated tubules and swollen, distorted glomeruli in all experimental groups.

Conclusion: The extract is nephrotoxic with increasing dose.

Keywords: Kidney function test, *Azanza garckeana* seeds, toxicity, atrophic glomerulus

INTRODUCTION
The use of herbal medicine and its related products continues to expand rapidly across the world with many people resorting to these products for the treatment of various health challenges. These herbal medicines are being

introduced into the market without any mandatory safety or toxicological evaluation (Ekor, 2014). Nigeria as a country is blessed with most of these medicinal plants which have shown considerable pharmacological activities such as antiviral, anti-inflammatory, antimicrobial, anticancer and anti-allergic properties. Among traditional medicinal practices, the use of herbal medicinal products, defined as formulations derived from plants that claim healing benefits, is the most prominent and most widely practiced by both the wider population and pregnant women around the world (Ernst, 2014). Pregnant women use herbal medicines for a variety of reasons such as for pregnancy-associated disorders including nausea, vomiting, and labor enhancement as well as for illnesses and diseases due to pregnancy such as fatigue, respiratory and skin issues, and nutritional benefits (Fakeye *et al.,* 2017) Additionally, pregnant women in rural areas use herbal medicines because of their wide availability, perceived better effectiveness relative to modern medicine, traditional and cultural beliefs in herbal medicines to cure diseases and relatively low cost of these medicines (Okafor *et al.,* 2017). Further research revealed that herbal products are preferred over prescription medications due to the belief that herbs are safer for the fetus than modern medicine. Despite the fact that evidence on the safety profile of herbal products is inadequate to substantiate their use in pregnancy, it is increasingly used by expectant mothers. The prevalence of herbal medicine utilization in pregnancy ranges between 7% and 55% in different geographical, social and cultural settings, and ethnic groups (Dugoua, 2013). Medications, herbs, and supplements should be used with extreme caution during pregnancy as they can result in deleterious outcomes for the mother and fetus (Bercaw *et al.,* 2012).

The use of herbal medicines in pregnancy constitutes a major challenge for health care providers as most of the users are not aware of their consequences (Adams, 2011). Injudicious use of herbs or interaction of these herbs with prescribed medications can have unknown effects in pregnancy or cause serious complications in the fetus. (Holst *et al.,* 2015). Herbal medicine often used without due consideration of the adverse effects and risks imposed on vital organs like the kidneys and liver which could cause great damage to the physiologic and even anatomical state of these organs (Okaiyeto and Oguntibeju, 2021). A retrospective study that compared the differences in the neonatal development of babies born from pregnant women who took either herbal medications or pharmaceutical products for the treatment illness during pregnancy showed that the prevalence of congenital fetal abnormalities in the group of women who took herbal medication was higher, though not statistically significant (Leung *et al*., 2015). In general, the use of herbs during pregnancy is not advisable due to their possible impact on the fetal development and toxicity to mother but data on incidence of use is not available. Herbal-induced renal disease constitutes an important etiology of renal diseases in daily clinical practice. As up to 80% of the population in Africa is estimated to use herbal preparations, which are generally perceived as safe and free from adverse effects, this consumption however has been associated with 35% of all cases of acute kidney injury. Nephrotoxicity has been associated with both the herbal and non-herbal components of the remedies. Such other components may be added deliberately or inadvertently due to poor handling techniques during preparation. Consumption of potentially toxic medicinal herbs, incorrect substitution of harmless herbs with toxic herbs, contamination with toxic compounds or interactions with conventional treatments are the major problems. The source, composition and preparations of these herbs vary on the prevalent local healing practices. Most herbs contain active compounds; however, they are not tested for efficacy and safety; the ingredients are not well known and the dosage and route of administration are not standardized (Okwu, 2017). These medicines are the major causes of renal failure and are responsible for high incidence of morbidity and mortality rate in humans.

Azanza Garckeana is a deciduous shrub; the tree can grow to a height of 3-15m high depending on the climate condition and its stem diameter at a height of up to 25cm (Orwa *et al.,* 2009). Its fruit have been investigated to have anti-viral, anti-bacterial, anti-inflammatory, anti-oxidant, antifungal, antihyperglycemic antifertility, contraceptive, antitumor, antioxidant properties, (Brown *et al*., 2013; Keshmiri-Neghab & Goliaei, 2014; Yusulf *et al*., 2023; Lawal and Sani 2022). *Azanza Garckeana* have been subjected to scientific investigations for elucidating its chemical, nutritional and toxicological properties but none in pregnant rats.

MATERIALS AND METHODS

Plant Collection and Identification

Dried ripe *Azanza Garckeana* purchased from Jos, Plateau State, Nigeria were identified and authenticated by a botanist and a voucher specimen UUPH 10 (f) was assigned to it while being deposited in the herbarium Unit, Department of Botany and Ecological studies, Faculty of Science, University of Uyo, Nigeria.

Ethical approval

The Experiment was conducted in the animal house of the Faculty of Pharmacy. Ethical consent for the care and use of the animal was obtained from the Faculty's Ethical and Research Committee. All the recommendations and protocols were strictly adhered to by International Guidelines for the care and Use of Laboratory Animals and the National Health Research Ethics Committee of Nigeria (NHREC), (2014).

Preparation of extract

The ripened *Azanza garckeana* fruits were washed thoroughly with running tap water, deseeded. The seeds were pulverized using a clean and dry grinding machine. 14 grams of the grounded extract was macerated and extracted by cold percolation using a conical flask with 100% (v/v) methanol at room temperature for 72 hours, and then filtered with Whitman filter paper and concentrated at 40 °C in a water bath to dry. The concentrated extract was stored in the freezer at -4^{o}C.

Acute toxicity studies

The acute oral toxicity was conducted according to the Organization for Economic and Cultural Development for testing of chemicals guidelines and Locke's method was used for study (Nweke *et al.,* 2018). The mice were obtained from the Animal House Faculty of Pharmacy, University of Uyo, Akwa lbom State. The mice were housed and acclimatized for 2 weeks prior to the commencing the acute toxicity test. The test was performed using increasing doses of intraperitoneal 100mg to 5000mg/kg bodyweight to different mice in different phases. The group of immature albino female wistar rats were treated with , 100, 200,300,400, 500, 1500, 2000, 2500, 3000,4000, 5000mg/kg bodyweight of the extract intraperitoneally. The mortality, general behavior and toxic symptoms of the mice were observed for 24 hours.

Experimental Animals

Twenty (20) mature female albino rat weighing between 150g – 250g were used for the study. The rats were obtained and kept in the Animal House of the college of Health Sciences, University of Uyo, Uyo, Akwa lbom State. They were housed in 15 cages (40cm × 35cm) with adequate space to encourage free movement. The animals were kept at a room temperature of 27^{o}C – 30°C. They were fed standard rat pelletized diet (Vital Feed, Grand Cereal Nigeria) and water. The animals were acclimatized for one week. The weight of the animals was noted. This experiment followed the guidelines for the care and use of laboratory animals (National Institute of Health, 2011).

Preparation of Test sample and Dosing

A stock solution of the extract was prepared based on the LD50 using a clean dried beaker and normal saline.

Experimental design.

Experimental animals were randomly divided into 4 groups with 5 animals each. Group 1 (Control) received 1 ml/kg body weight of normal saline while experimental groups received 500 mg/kg , 1000 mg/kg, 1500 mg/kg body weight of methanol seed extract of *Azanza garckeana* respectively, intraperitoneally for 12 days. At the end of the experiments, the rats were weighed and anaesthetized with 0.6ml ketamine intraperitoneally. The abdomen was carefully dissected and the kidney was removed carefully, cleaned of peritoneal fat and fixed in 10% buffered formalin. The tissue obtained where weighed. Blood was collected via the cardiac puncture into the appropriate EDTA bottles for analysis of kidney function test.

Tissue processing

After preservation kidneys in 10% buffered formalin to prevent enzyme autolysis and bacterial decay, tissues were processed following the stages: dehydration, clearing, infiltration, embedding, sectioning, staining and mounting. The processed tissues were then subjected to microscopy. (Carson, 1990).

Statistical analysis

Data was analyzed using SPSS version 25. Evaluation of significant difference between the means of the different experimental groups and the control group was obtained using one-way analysis of variance (ANOVA). Values were expressed as mean ± SEM. The level of significance considered was a P-value less than 0.05 ($P<0.05$).

RESULTS

Effect of methanol seed extract of *Azanza garckeana* on body weight

Statistical results show that the extract administered had little significant impact on the body weight of the experimental animals.

Table 1: The Effect of Methanol Seed Extract of *Azanza garckeana* on the Body Weight of Experimental Animals.

Group	Initial Weight	Final weight	Kidney Weight Difference
Control + Normal Saline	167.8 ± 10.36	176.4 **±** 10.20	13.00 ± 6.44
E.G + A.G (500 mg/kg)	164.0 **±** 16.77	187.8 **±** 11.00	23.80 ± 23.80
E.G + A.G (1000 mg/kg)	184.2 **±** 4.03	194.4 **±** 7.14	10.20 ± 7.12
E.G + A.G (1500 mg/kg)	180.0 **±** 14.46	205.0 **±** 19.58	22.40 ± 8.72

E.G: Experimental Group; A.G: *Azanza garckeana*;
Values represent Mean ± SEM; $P < 0.05$

Table 2: Concentrations of Kidney function test in experimental groups in methanol seed extract of *Azanza garckeana*

Group	**Urea**	**Creatinine**	**Potassium**	**Sodium**	**Chloride**	**HCO^{3-}**
Control + Normal Saline	2.8 ± 0.06	64.3 ± 2.34	4.2 ± 0.50	132.7 ± 5.36	47.3 ± 0.33	24.0 ± 1.15
E.G + A.G (500 mg/kg)	2.9 ± 0.34	69.0 ± 6.00	4.3 ± 0.95	130.7 ± 12.20	50.3 ± 3.75	27.0 ± 1.00
E.G + A.G (1000 mg/kg)	3.9 ± 0.73	85.7 ± 11.80	4.0 ± 0.95	126.7 ± 10.91	49.0 ± 2.31	23.7 ± 1.76
E.G + A.G (1500 mg/kg)	2.8 ± 0.29	85.7 ± 3.00	4.1 ± 0.10	134.0 ± 1.00	45.0 ± 1.73	23.3 ± 0.66

E.G: Experimental Group; A.G: *Azanza garckeana*;
Values represent Mean ± SEM; $P < 0.05$
Source: Computed by the researcher, 2025

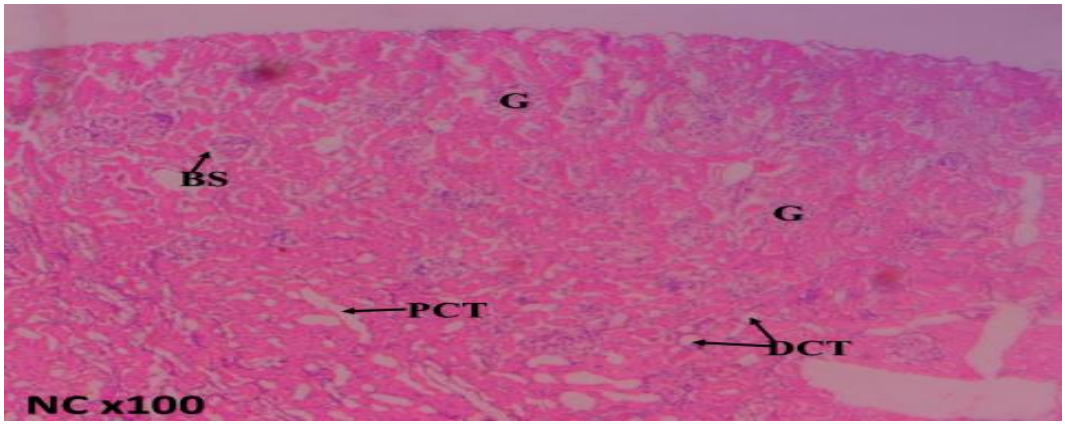

Fig 1: Photomicrograph of the kidney histology of the control group () showing normal features; G= Glomerulus, BS= Bowman's space, DCT= Distal convoluted tubules, PCT= proximal convoluted tubule. H&E, 100 x

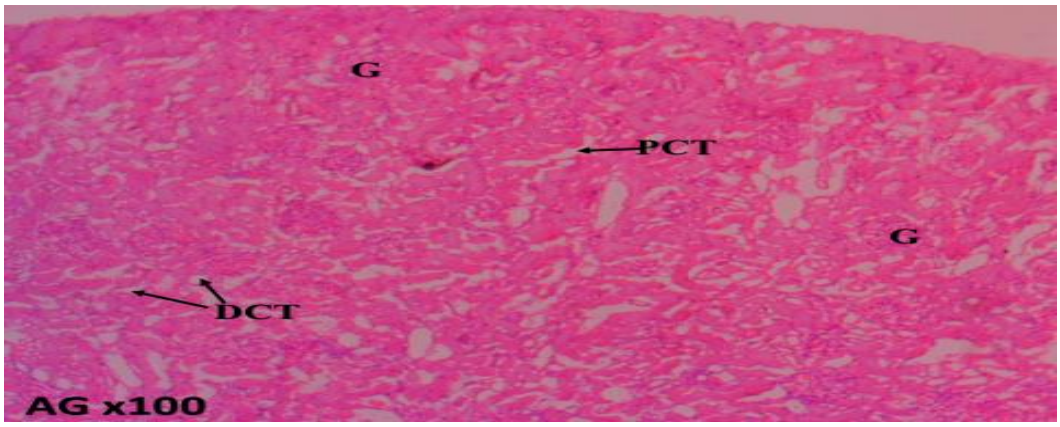

Figure 2: Section of the kidney cortex receiving 500 mg/kg body weight of methanol seed extract of *Azanza garckeana* (low dose group) showing G= swollen glomerulus, BS= Bowman's space, DCT= Distal convoluted tubules, PCT= proximal convoluted tubule. H&E Mag 100

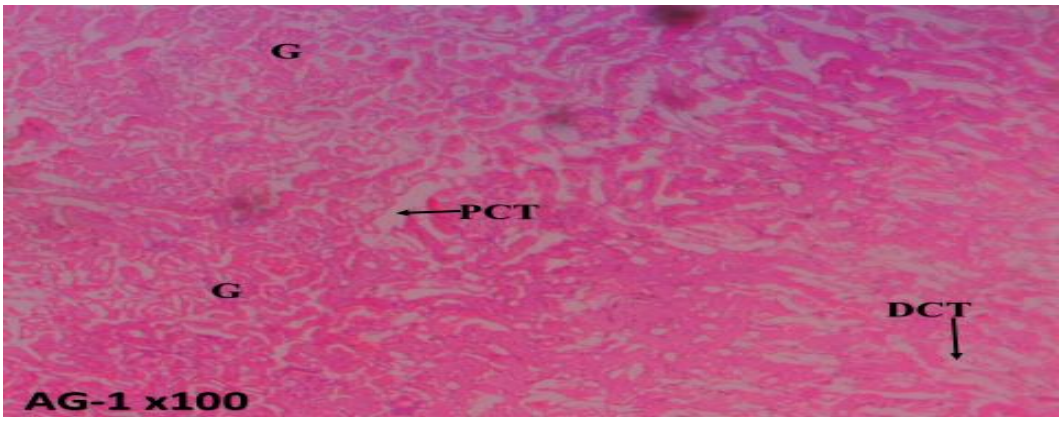

Fig 3: Photomicrograph of the kidney cortex administered with 1000 mg/kg of methanol seed extract of *Azanza garckeana* showing G= swollen glomerulus, DCT= dilated distal convoluted tubules, PCT= dilated proximal convoluted tubule. H&E; 100 x

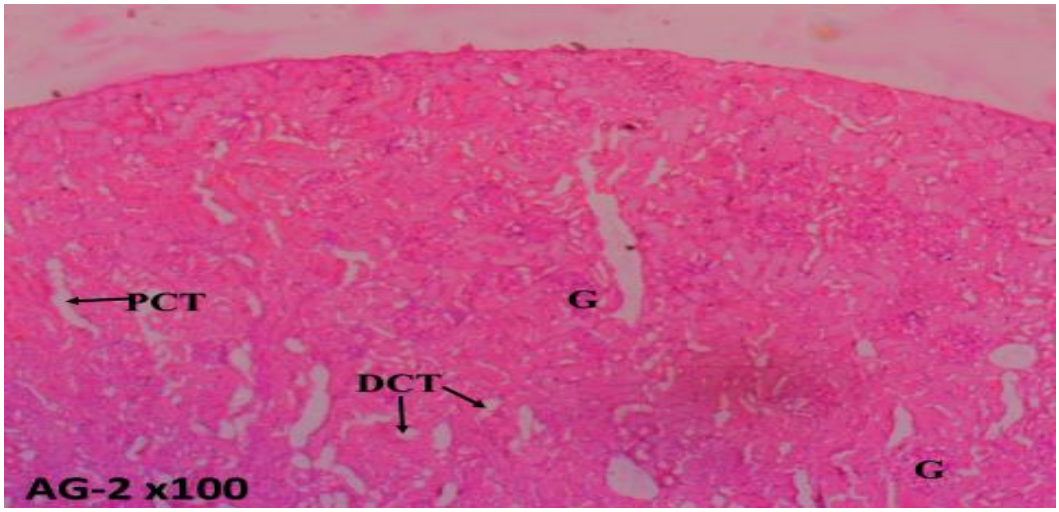

Fig 4: Photomicrograph of the kidney cortex administered with 1500 mg/kg of methanol seed extract of *Azanza garckeana* showing G= swollen glomerulus, DCT= dilated distal convoluted tubules, PCT= dilated proximal convoluted tubules. H&E; mag 100 x

DISCUSSION

Investigation of the acute toxicity is often the initial stage in all screening of unknown potential medicinal herbal substances. The acute toxicity data are used to predict the safe dose limit in clinical application because some plants extract exert toxic effects even at a very low dose (El-shaq *et al.,* 2019). In the current study of the acute toxicity of the methanolic extract of *Azanza garckeana* seeds, the extract was administered intraperitoneally at the dose levels of 500 mg/kg – 5000 mg/kg body weight and no abnormal changes in the general appearance and behavioral characteristics like writhing, aggression was observed in the animals, also no mortality were recorded after 24 hours of administration. A research study carried out by Hodge and Stenner (2017), the results obtained from the acute toxicity study showed that the crude extract and ethyl acetate and n-hexane fractions of *Azanza garckeana* pulp demonstrated a high degree of safety since the animals tolerated up to 5000 mg/kg body weight of the extracts with no mortality recorded and therefore should be tagged a harmless substance. This also agrees with another report that the *Azanza garckeana* fruit is relatively safe (Ochokwu *et al*., 2015).

The result of the differences in the body weight analysis showed a significant increase in the *Azanza garckeana* extract group treated at low dose when compared with the control group. *Azanza garckeana* has been reported to be rich in fibre, total carbohydrates, and protein level (Nkafamiya, *et al*., 2016). Sirajo, *et al*., 2022 also reported that the *Azanza garckeana* can be an excellent source of fat. These high nutritional contents of this extract must have contributed significantly to the body weight of the group treated with the extract when compared to the control group. Controversially, there was a significant dose-dependent decrease in the body weight differences in the extract treated groups when compared amongst low-high dose extract. The phytochemical composition of *Azanza garckeana* contains about 0.22% tanin, 1.72% saponin amongst others (Michael, *et al*., 2015). Tannins can bind to digestive enzymes, preventing the breakdown of food molecules, leading to decreased absorption of food nutrients, potentially amounting to reduced weight gain. It was reported that high levels of sorghum tannins have been shown to reduce dry matter and protein digestibility of chick diets (Zdunczyk *et al*., 2018; Amesa and Asfaw, 2018). This explains the significant decrease in the body weight differences as the dose increases. Saponins can also reduce the absorption of dietary fats by inhibiting pancreatic lipase, which helps in the breakdown of fats, hence, contributing to the weight loss significantly as the dose increases (Marrelli, *et al*., 2018).

The photomicrograph of the kidney in figure 4.1 shows the normal microscopic histological architecture of the kidney, with the glomerulus and the glomerular capsule supporting the glomerulus, presence of the distal and proximal convoluted tubules and capillaries. The glomerulus capillaries form a central tuft of looped capillaries

located in the center of the renal corpuscle. The Bowman's capsule shows visceral and inner visceral layer which completely encircles the glomerular capillaries. It is comprised of specialized stellate epithelial cells termed podocytes. The outer or parietal layer of Bowman's capsule is a single layer of simple squamous epithelium. It is into the space between these two layers into which urine is filtered. The proximal convoluted tubules shows the presence of small lumen which are lined by columnar cells. The nuclei are central and euchromatic.

The general histological examination of the kidneys of experimental groups administered with *Azanza garckeana* showed some mild and patchy glomerular retraction, presence of hemorrhage, dilation of the proximal and distal convoluted tubules, some congested spaces in the interstitium as well as a reduction in size and cellularity of the glomeruli and a thinning of their lining epithelium when compared to the control group which could indicate some level of nephrotoxicity of the extract. Figure 4.2 shows a section of the kidney tissue which were administered with 500mg kg of *Azanza garckeana* showed a disruption of the glomerulus, and haemorrhage but a normal histology for the distal and proximal convoluted tubule, indicating a distortion of the histological features when compared to the control group. Hemorrhagic findings may indicates that *Azanza garckeana* may contain compounds that could directly damage the blood vessels, causing bleeding and a corresponding initiation of inflammatory response in the kidney (Ahmed and Goodluck, 2024). A dilated proximal and distal convoluted tubules and a swollen glomerulus may result from cellular proliferation and increase in intrarenal pressure, oxidative stress and tubular damage, which could cause chronic kidney disease (Adedapo *et al.,* 2017).

CONCLUSION

The methanol seed extract of *Azanza garckeana* has a dose-dependent nephrotoxic effects and should be used with caution.

Limitations

This study is limited to biochemical and basic histomorphological staining method.

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Conflicts of interest.

The authors declare no conflict of interest.

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