DOI: https://doi.org/10.4314/njbmb.v39i1.1 NJBMB (2024) 39(1):1-7

Nigerian Journal of Biochemistry and Molecular Biology

The Official Publication of the Nigerian Society of Biochemistry & Molecular Biology (NSBMB). Journal homepage: https://www.nsbmb.org.ng/journals/

e-ISSN: 2659-0042

Research Article

Antioxidant Activity and Toxicological Implications of the Aqueous Extract of *Azanza garckeana* Fruit Pulp in Female Wistar Rats

Quadri O. Nurudeen[1]*, Muhammed R. Asinmi[1], Mansurat B. Falana[2], Muhammad A. Dikwa[3], Zaharadeen M. Yusuf[1] , Muinat O. Lambe[1]

[1] *Department of Biological Sciences (Biochemistry Unit), Al-Hikmah University, Ilorin, Nigeria*
[2] *Department of Biological Sciences (Microbiology Unit), Al-Hikmah University, Ilorin, Nigeria*
[3] *Department of Microbiology and Biotechnology, Federal University, Dutse, Nigeria*

OPEN ACCESS

***CORRESPONDENCE**
Nurudeen, Q. O.
quadriolaide@yahoo.com
+234-803-427-3045

ARTICLE HISTORY
Received: 12/05/2023
Reviewed: 28/09/2023
Revised: 09/01/2024
Accepted: 30/01/2024
Published: 31/03/2024

CITATION
Nurudeen Q.O., Asinmi M. R., Falana M. B.., Dikwa, M. A., Yusuf Z. M and Lambe M. O. (2024). Antioxidant Activity and Toxicological Implications of the Aqueous Extract of Azanza garckeana Fruit Pulp in Female Wistar Rats. *Nigerian Journal of Biochemistry and Molecular Biology*. 39(1),1-7
https://doi.org/10.4314/njbmb.v39i1.1

ABSTRACT

This study examined the antioxidant activity as well as the safety profile of *Azanza garckeana* aqueous extract in female Wistar rats. Following the random distribution of 28 female Wistar rats (mean weight = 159.25 ± 3.32 g) into four (4) groups labelled (A-D) containing seven rats (animals) each, a daily administration of 0.5 mL distilled water was orally given to the rats in group A, while the aqueous extract of *A. garckeana* fruit pulp were given orally at 125, 250 and 500 mg/kg to rats in groups B, C and D respectively for 21 days. Some antioxidant activities as well as kidney and liver function indices were examined on the rat using established methods. All liver function indices assayed for showed no significant ($p > 0.05$) difference in comparison to the control. The concentration of liver enzyme revealed no significant ($p > 0.05$) difference in liver alanine aminotransferase, liver aspartate aminotransferase, lactate dehydrogenase and liver alkaline phosphatase at 500 mg/kg in comparison to the control group. Conversely, all kidney functions indices show significant increase ($p < 0.05$) following extract administration, indicating potential effects on kidney function. Significant decrease ($p< 0.05$) was observed in the level of kidney and liver reduced glutathione and kidney malondialdehyde while kidney and liver superoxide dismutase as well as liver malondialdehyde significantly increased ($p<0.05$). Overall, aqueous fruit pulp extract of *A. garckeana* shows no damaging effect on liver indices at the investigated dosage. However, it may have a significant side effect on the kidney due to the biochemical alterations observed in the kidney function indices at the investigated dosages.

Keywords: *Antioxidant activity; Azanza garckeana; Kidney functions; Liver function, Safety assessment.*

INTRODUCTION

Traditional medicine has been using medicinal plants to treat ailments for thousands of years and it is believed that such therapeutic plants are accompanied with little or no side effects (Petrovska *et al.*, 2005). Herbs including chamomile, echinacea, garlic, ginger, ginseng, ginkgo and goldenseal are frequently used medicinally. Even though prescription drugs and manufactured medications are widely used nowadays, many people still use herbal treatments since they may promote and restore both physical and mental health (Kayani *et al.*, 2014). Medicinal plants have shown high therapeutic qualities such as antibacterial, antioxidant and anti-inflammatory effects. Because of their widespread healing properties, they have been acknowledged as powerful sources of drugs with some advantages over conventional drugs (Dzoyem *et al.*, 2018; Jiang *et al.*, 2019). These plants are included in more than 90% of traditional medicine recipes and have been used in several disease prevention strategies. The creation of novel drugs has been spurred by the potential of medicinal plants for drug development. However, the lack of thorough toxicity data raises questions about their safety and

effectiveness. Herbal medicine products may be harmful due to a variety of reasons, such as adulteration, plant components or metabolites having poisonous potential and environmental variables. Even while medicinal plants have many advantages, some may pose a risk to users' health because of unfavourable effects or side effects that might result from toxicity or overdose. Numerous studies have demonstrated that some therapeutic plants can be harmful to certain organs, and skilled herbalists often provide known hazardous medicinal herbs in small doses (Ekor, 2014; Mensah *et al.*, 2019).

Azanza garckeana, sometimes referred to as Goron Tula, is a valued plant in traditional medicine because of its many medicinal uses. It is mostly found in Tula Village, Kaltungo Local Government Area, Gombe State, Nigeria and is a valued cash crop in the area (Ochokwu *et al.*, 2015). It can also be found in other parts of West Africa, including Ghana and Senegal (Bukar *et al.*, 2020). It is a fruit that is high in vitamins, minerals and carbohydrates (Sulieman, 2019). Important nutrients including calcium, salt, potassium and iron are also present in the fruit. It is also an excellent source of ascorbic acid, or vitamin C, which helps to build red blood cells and adds nutritious value to fruit juices. Because it is high in essential nutrients including calcium, phosphorus, ascorbic acid, iron, magnesium and potassium, the fruit is a significant nutritional supplement in the area where they are planted (Yusuf *et al.*, 2020a).

It is a local source of numerous biological benefits, such as anti-inflammatory, aphrodisiac, uterotonic, analgesic, anti-arthritic, hemostatic and wound-healing properties. It is used as a natural medicine for a number of ailments, including sexually transmitted diseases, infertility, cough and chest problems (Sulieman, 2019). Pharmacological researchers have reported several activities of the plant, including antibacterial, antifungal, antihyperglycemic, antimalarial and iron absorption (Ochokwu *et al.*, 2015; Yusuf *et al.*, 2020a). Nurudeen *et al.* (2023) also reported that *A. garckeana* was able to reverse sexual dysfunction in female rats induced with fluoxetine.

The pharmacological effects of *A. garckeana* have been the subject of several investigations; yet, there is a dearth of experimental evidence in the public scientific literature to substantiate the plant's toxicological effects on female Wistar rats. Therefore, this study investigated the antioxidant activity and safety profile of the aqueous extract of *A. garckeana* fruit pulp using female Wistar rats.

MATERIALS AND METHODS

Collection and authentication of the plant materials

The fruit pulp of *A. garckeana* were acquired at a local market in Ilorin West Local Government Area, Ilorin, Kwara State, Nigeria in April, 2022. The fruits were examined and authenticated by a botanist at the University of Ilorin Herbarium, Ilorin, Nigeria. As part of the authentication process, a voucher sample was provided and recorded under the reference number "UILH 001/1494/2022."

Experimental animals

Twenty-eight (28) female Wistar rats known as *Rattus norvegicus* were purchased from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats, weighing an average of 159.25 ± 3.32 g, were kept in clean, well-maintained cages at room temperature. Following the guidelines for the care and the use of laboratory animals throughout the investigation; unrestricted access to tap water and rat pellets were given to the rats.

Reagents and assay kits

Assay kits for serum electrolyte, bilirubin, albumin, creatinine, urea, total protein, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase are products of Sigma-Aldrich Inc., located at St. Louis, Missouri, USA. The remaining reagents used during this experiment were of analytical grade and prepared in accordance with specifications using distilled water and stored in a neat and tight reagent bottle.

Methods

Preparation of plant extracts

The fruits were sliced into small pieces and thoroughly cleaned under running water to remove the pulp. Subsequently, the sliced fruit pieces were dried for 72 hours at 40°C in an oven. Once dried, the pulps were ground using an electric blender (Chinese-made Master Chef Blender, Model MC-BL 1980) and stored in airtight containers. To initiate the extraction process, a known quantity of the powdered material (50g) was macerated in an aqueous solvent at 25°C for 48 hours which was shaken intermittently. The resulting mixture was filtered with a cheesecloth and the obtained filtrate subjected to evaporation using a rotary evaporator in order to obtain a sticky residue. Percentage yield of this extraction procedure was mathematically determined through calculations.

The residue was mixed with distilled water to make the 125, 250 and 500 mg/kg body weight. The choice of dosage preparation was based on data obtained from ethnobotanical survey (Nurudeen *et al.*, 2023).

Animal grouping and administration of plant extracts

Following a two-week period of acclimatization, a total of twenty-eight female rats were distributed randomly into four (4) groups (A, B, C and D), with each containing seven (7) rats. As the control group, group A rats received 0.5mL of distilled water orally. On the other hand, rats belonging to groups B, C and D, which were identified as the experimental groups, were given an equivalent volume of *A. garckeana* fruit pulp aqueous extract, equivalent to dosages of 125, 250, as well as 500 mg/kg. The different animal groups were treated as described above using a plastic oropharyngeal cannula, once daily for 21 days.

Tissue and serum supernatants preparation

The tissue and serum supernatants were prepared following the procedure from the report of Nurudeen and Yakubu (2016). On Day 22, the rats were rendered unconscious, sedated with diethyl ether fumes and the jugular veins were incised. After collecting blood into clean and dry centrifuge tubes, they were centrifuged for 10 minutes at 894 × g after being given 15 minutes to coagulate. Pasteur's pipette was used to aspirate the resultant sera, which were then refrigerated before further biochemical investigation. The animals were then quickly dissected in order to gently collect the liver and kidney, which were then blotted and kept in an ice-cold solution of 0.25M sucrose. Separation and homogenisation of the organs in an ice-cold 0.25M sucrose (1:5 w/v) solution was done. The supernatants were then used to measure several biochemical parameters after the organs were centrifuged for 10 minutes at 1789 x g.

Determination of biochemical parameters

The determination of biochemical parameters was carried out according to established protocols. The methods of Jendrassik and Grof (1938) for total and conjugated bilirubin, Veniamin and Verkirtzi (1970) for urea, Bartels and Bohmer (1972) for creatinine, Doumas *et al.* (1971) for albumin and Gornall *et al.* (1949) for total protein. The technique outlined by Tietz (1995) was used to calculate the electrolyte concentrations (Na^+ and K^+). GSH and MDA levels were measured using the protocols described by Ohkawa *et al.* (1979) and Ellman (1959), in that order. Following reputable guidelines, the activities of glutathione reductase (Prabhu *et al.*, 2004), lactate dehydrogenase (Wróblewski and La due, 1955), alkaline phosphatase (Aebi, 1974), catalase (Reitman and Frankel, 1957), alanine aminotransferase (Beutler, 1984), aspartate aminotransferase (Wright *et al.*, 1972), glucose-6-phosphate dehydrogenase (Goldberg and Spooner, 1992) and superoxide dismutase (Fridovich, 1995) were also determined.

Data analysis

To determine statistical significance, the mean and standard error of the mean were calculated using seven sets of duplicate data. Additionally, a one-way Analysis of Variance (ANOVA) was performed. Statistical analysis was performed using GraphPad Prism 6.01 (GraphPad Software, Inc., San Diego, California, United States). At $p<0.05$ (significance level), the results were deemed statistically different.

RESULTS

The extract administered at dosages of 125, 250 and 500 mg/kg body weight did not significantly ($p>0.05$) alter the concentrations of liver and serum total protein, total bilirubin, albumin and direct bilirubin in contrast to control group (Table 1). Similarly, the concentrations of liver and serum aspartate aminotransferase, lactate dehydrogenase and serum alkaline phosphatase were not significantly altered ($p>0.05$) following the administration of the extract when compared to the control group. However, the concentrations of serum alanine aminotransferase and that of glucose-6-phosphate dehydrogenase showed significant increment ($p < 0.05$) after the ingestion of the extract, particularly at 500 mg/kg body weight (the maximum dosage) in contrast to the control group (Table 2). The administration of the extract at doses of 125, 250 and 500 mg/kg body weight resulted in significant increment ($p < 0.05$) in the concentrations of urea, creatinine, sodium ion, potassium ion and kidney protein in contrast to the control group (Table 3).

Furthermore, kidney and liver catalase level did not differ significantly ($p>0.05$) from control when the extract was given at the maximum dosages of 500 mg/kg. The concentrations of kidney and liver superoxide dismutase and liver malondialdehyde significantly increased ($p<0.05$) following the use of the extract at 500, 250 and 125 mg/kg body weight dosages in contrast to the control group. Conversely, the concentrations of kidney and liver reduced glutathione and kidney malondialdehyde decreased ($p < 0.05$) significantly after the extract was given to the group at varying dosages in comparison to the control group. (Table 4).

Table 1. The Levels of Liver Function Indices in Female Rats after Aqueous Extract of *A. garckeana* Fruit Pulp was Administered

Parameters	Control	Dose(mg/kg body weight)		
		125	250	500
Liver total protein (mg/dL)	186.27 ± 4.62 [a]	185.48 ± 1.95 [a]	188.07 ± 2.84 [a]	183.35 ± 2.79 [a]
Serum total protein (mg/dL)	70.02 ± 0.23 [a]	77.13 ± 3.41 [a]	72.16 ± 1.59 [a]	71.40 ± 0.47 [a]
Albumin (mg/dL)	20.31 ± 1.30 [a]	21.36 ± 0.25 [a]	18.37 ± 0.84 [a]	21.42 ± 0.03 [a]
Total bilirubin (mg/mL)	64.78 ± 3.04 [a]	58.32 ± 1.75 [a]	60.98 ± 1.07 [a]	62.07 ± 1.98 [a]
Direct bilirubin (mg/dL)	30.59 ± 2.70 [a]	30.85 ± 1.21 [a]	27.29 ± 0.19 [a]	25.71 ± 1.21 [a]

Values represent mean of seven determinations ± standard error of mean, results for each parameter having distinct superscripts (a-d) in separate rows are deemed statistically different at $p<0.05$.

Table 2. The Levels of Certain Liver Enzymes in Female Rats after Aqueous Extract of *A. garckeana* Fruit Pulp was Administered

Parameters	Control	Dose(mg/kg body weight)		
		125	250	500
Serum alanine aminotransferase (U/L)	28.96 ± 0.49 [a]	37.21 ± 1.19 [b]	38.04 ± 0.82 [b]	34.79 ± 1.78 [b]
Liver alanine aminotransferase (U/L)	5.23 ± 0.28 [a]	6.64 ± 0.41 [b]	6.75 ± 0.16 [c]	5.74 ± 0.33 [a]
Liver aspartate aminotransferase (U/L)	42.39 ± 3.47 [a]	53.64 ± 3.99 [a]	51.43 ± 3.48 [a]	45.02 ± 4.78 [a]
Serum aspartate aminotransferase (U/L)	48.54 ± 1.32 [a]	45.93 ± 3.91 [a]	45.78 ± 2.76 [a]	48.27 ± 0.28 [a]
Liver alkaline phosphatase (U/L)	2.05 ± 0.24 [a]	2.71 ± 0.22 [b]	2.97 ± 0.18 [b]	3.00 ± 0.73 [a]
Serum alkaline phosphatase (U/L)	5.67 ± 0.02 [a]	5.13 ± 0.48 [a]	5.15 ± 0.23 [a]	5.74 ± 0.17 [a]
Lactate dehydrogenase (U/L)	59.16 ± 1.48 [a]	61. 41 ± 2.38 [a]	64.93 ± 3.43 [a]	62.94 ± 5.92 [a]
Glucose-6-phosphate dehydrogenase (U/L)	21.41 ± 1.30[a]	19.31 ± 1.39 [a]	18.81 ± 1.74 [a]	39.28 ± 1.61 [b]

Values represent mean of seven determinations ± standard error of mean, results for each parameter having distinct superscripts (a-d) in separate rows are deemed statistically different at $p<0.05$.

Table 3. The Levels of some Kidney Function Indices of Female Rats after Aqueous Extract of *A. garckeana* Fruit Pulp was Administered

Parameters	Control	Dose (mg/kg body weight)		
		125	250	500
Urea (mg/dL)	100.53 ± 1.26 [a]	104.84 ± 1.57 [a]	114.30 ± 2.56 [b]	122.31 ± 1.34 [c]
Creatinine (mg/mL)	1.33 ± 0.11 [a]	1.69 ± 0.11 [b]	3.23 ± 0.46 [c]	3.64 ± 0.24 [c]
Sodium ion (mg/dL)	71.97 ± 0.03 [a]	78.90 ± 0.75[b]	86.26 ± 3.78 [c]	80.97 ± 0.06 [b]
Potassium ion (mg/dL)	8.22 ± 0.19 [a]	9.98 ± 0.16 [b]	9.38 ± 0.13 [c]	10.04 ± 0.04 [b]
Kidney Protein (mg/dL)	132.44 ± 0.76 [a]	126.71 ± 3.17 [a]	140.23 ± 3.90 [a]	150.56 ± 5.75 [b]

Values represent mean of seven determinations ± standard error of mean, results for each parameter having distinct superscripts (a-d) in separate rows are deemed statistically different at $p<0.05$.

Table 4. The Level of Enzymatic and Non-Enzymatic Antioxidant Parameters after Aqueous Extract of *A. garckeana* Fruit Pulp was Administered

Parameters	Control	Dose (mg/kg body weight)		
		125	250	500
Kidney Catalase (mmol/min/mg)	2.50 ± 0.01 [a]	3.34 ± 0.03 [b]	2.89 ± 0.03 [b]	2.95 ± 0.36 [a]
Liver Catalase (mmol/min/mg)	1.78 ± 0.04 [a]	2.93 ± 0.11 [b]	2.15 ± 0.13 [a]	2.07 ± 0.17 [a]
Kidney Superoxide dismutase (mmol/min/mg)	0.55 ± 0.01 [a]	0.47 ± 0.02 [b]	0.48 ± 0.09 [a]	1.66 ± 0.24 [b]
Liver Superoxide dismutase (mmol/min/mg)	0.71 ± 0.01 [a]	0.71 ± 0.08 [a]	0.57 ± 0.04 [b]	0.87 ± 0.07 [b]
Kidney Reduced glutathione (mmol/mL)	0.67 ± 0.01 [a]	0.67 ± 0.02 [a]	0.59 ± 0.03 [b]	0.57 ± 0.02 [b]
Liver Reduced glutathione (mmol/mL)	0.47 ± 0.03 [a]	0.39 ± 0.02 [b]	0.39 ± 0.04 [b]	0.41 ± 0.01 [b]
Kidney Malondialdehyde(mmol/mL)	4.75 ± 0.46 [a]	3.79 ± 0.01 [b]	2.67 ± 0.12 [c]	2.53 ± 0.10 [c]
Liver Malondialdehyde(mmol/mL)	4.11 ± 0.16 [a]	4.04 ± 0.36 [a]	4.86 ± 0.41 [b]	4.51 ± 0.24 [b]

Values represent mean of seven determinations ± standard error of mean, results for each parameter having distinct superscripts (a-d) in separate rows are deemed statistically different at $p<0.05$.

DISCUSSION

The use of herbal products has significantly increased due to the belief that they are more natural and safer than conventional pharmaceuticals (Latha *et al.*, 2010). Still, there are concerns about their toxicity, safety and effectiveness. Even while herbal remedies are frequently thought to carry a smaller risk than synthetic medications, toxicity or unfavourable consequences are still a possibility. Herbal products should have their safety and effectiveness assessed before being used for therapeutic reasons, as they are not inherently safe (Woo *et al.*, 2012; Mensah *et al.*, 2019). Like conventional medications, herbal medicines have the ability to affect the body and cause harm if not taken appropriately. They also have the ability to alter the body metabolism and interact with the body's physiology, which can have serious harmful effects. In order to be sure that the benefits of taking herbal products outweigh any potential risks, it is crucial to assess their efficacy and safety. One of the primary concerns with using herbal products is how they affect the liver and kidney, which are the two main organs involved in detoxification and biotransformation.

Drug metabolism is mostly dependent on the liver, which is the primary organ for drug biotransformation and excretion from the body. Since many herbal medications have the potential to injure the liver, hepatotoxicity connected to herbal medicines is a developing issue. Herbal products are being used more often; thus, before employing them for therapeutic reasons, their safety should be assessed.

Liver function tests are blood tests used to assess the health and function of the liver. Certain enzymes and blood proteins, such as albumin, liver and serum total protein and direct and total bilirubin are all important markers of hepatic function and are measured by different assays (Roy, 2012). Furthermore, liver damage or dysfunction is associated with higher levels of liver enzymes, including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) (Teschke *et al.*, 2014). The fact that serum total protein, direct and total bilirubin and albumin levels did not significantly increase (p>0.05) following the administration of the maximum dose (500 mg/kg) of *A. garckeana* fruit pulp extract suggests that the extract is safe in terms of liver tolerance. For general health and well-being, the liver's structural and functional integrity must be preserved. The liver is essential for a number of processes, such as, drug metabolism and excretion, synthesis of proteins (e.g fibronectin and IGF-I that influence bone health) and the body's reaction to hormones that stimulate bone formation and the generation of cytokines that influence bone resorption (Nakchbandi, 2014).

Measuring blood and tissue enzyme activity has been proposed by Ajiboye *et al.* (2014) as a means of evaluating the safety of herbal remedies. Enzyme activity in tissues and serum may be assessed using a variety of methodologies and procedures to detect toxicity at the cellular level. One of the most important aspects of evaluating drug-induced liver damage (DILI) is the evaluation of enzyme activity. Since DILI can cause rapid liver failure and even death, it is a severe issue. An essential element of assessing liver health and function is measuring blood enzyme activity, such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and alanine aminotransferase (ALT). These tests are used to determine the presence and level of liver damage, identify distinct types of liver problems, detect the existence of liver illness and track the effectiveness of therapy. Regarding the safety profile, it is comforting to note that there is no significant (p<0.05) difference in the levels of ALT, AST, ALP, LDH and AST, especially at the maximum dosage when compared to untreated rats. This indicates the absence of liver injury or damage (Ajiboye *et al.*, 2014).

Several herbal remedies include substances like aristolochic acids that have been linked to kidney harm, including cancer and renal failure; there has been worry regarding the possible nephrotoxicity of these products when used (Teschke *et al.*, 2014; Gardiner *et al.*, 2017). This is because the kidney plays a critical role in the excretion of drugs and their metabolites (Nwanjo *et al.*, 2005; Luyckx and Naicker, 2008). The safety of herbal kidney medications is in doubt and the most toxicological evaluations should be used to assess the botanicals' possible nephrotoxicity. Serum creatinine, urea and ion levels can be used to measure kidney function; these values rise in situations of renal disease or injury (Ramachandran, 2006). A spike in serum creatinine can only be seen after around 50% of kidney function has been lost. Serum creatinine is a late indication of acute renal damage. Both acute and chronic renal illness are associated with higher serum urea values.

Urea and creatinine are frequently employed as markers of renal function since they are waste products of protein metabolism that the kidneys eliminate (Levey *et al.*, 2003). In addition, the levels of potassium and sodium present in the blood are very important indicators of renal function because they are absolutely necessary for the maintenance of the body's electrolyte balance. These renal function test indicators are used to evaluate the health and function of the kidneys. Their levels can be used to determine the degree of known kidney damage, monitor the effectiveness of treatment, identify the existence of kidney disease and differentiate between various types of kidney illnesses. Thus, a decrease in the excretion of nitrogenous waste products and a reduction in glomerular filtration rate may be indicated by the significant (p<0.05) increase in the levels of urea, creatinine, sodium, potassium and kidney protein, which might have adverse consequences on the nephron (Nwanjo *et al.*, 2005; Luyckx and Naicker, 2008). The observed threat to the kidney in this study may be attributed to the dosage of the extract as the increase in the kidney indices are dose dependent.

Highly reactive molecules known as reactive oxygen species (ROS) are produced as byproducts of regular cellular metabolic processes. ROS have the ability to oxidatively damage DNA, proteins and membrane lipids in cells, as well as play a role in the aetiology of a number of illnesses. In addition, ROS have the potential to be important players in the underlying processes of anticancer treatment as well as the pathophysiology of chronic human disorders like cancer. Nevertheless, phagocytes and other cell types can potentially deploy ROS as lethal weapons against infections. For cells to remain in a homeostasis and avoid oxidative damage, the generation of reactive oxygen species (ROS) and antioxidant defense systems must be in balance. Enzymes that are known to be antioxidants, such catalase, glutathione peroxidase and superoxide dismutase, help shield cells from the harmful effects of reactive oxygen species (ROS) (Valko *et al.*, 2007; Sies and Jones, 2020). The extract may provide protection against oxidative damage, as evidenced by the significant (p<0.05) decrease in kidney malondialdehyde (MDA) and reduced glutathione (GSH). This emphasizes the extract's possible therapeutic use in the treatment of oxidative stress-related illnesses and the cellular damage they cause. It is also necessary to protect cells from oxidative damage and maintain redox balance. The Lower levels of malondialdehyde (MDA) also signify a reduction in lipid peroxidation (Halliwell and Gutteridge, 2015). An antioxidant enzyme called catalase is also essential for shielding cells from reactive oxygen

species' harmful effects (ROS). The fact that the concentrations of liver and kidney catalase did not significantly (p<0.05) change further corroborates the extract's antioxidant modulatory action.

The research is consistent with other research suggesting that, despite the fact that herbal treatments have been used for many years to treat a wide range of disorders, herbal medications are not always completely safe and can have negative effects on certain body organs (Ekor, 2014). Hence, it is crucial to weigh the advantages and disadvantages of employing these extracts in medicinal applications.

CONCLUSION

This research has shown that, at doses of up to 500 mg/kg body weight, the aqueous extract of *A. garckeana* fruit pulp may not have a substantial toxic impact on the liver, but it does pose some risks to the kidneys. In addition, further research is necessary since it's critical to pinpoint the exact mechanisms by which the extract exerts its effects on the liver and kidney.

AUTHORS' CONTRIBUTIONS

The research was conceptualized by author QON who also participated in the writing of the manuscript and in editing the final copy. Authors MRA, MBF and MAD carried out the research and result analysis. Author ZMY helped with the laboratory analysis while author MOL participated in the result analysis as well as preparation of the manuscript and submission of same for publication. All authors approved the final version for publications.

FUNDING STATEMENT

The research work received no funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors are grateful to Mr. Dele Aiyepeku (Phytomedicine, Toxicology and Reproductive Biochemistry Research Laboratory, Department of Biochemistry, University of Ilorin, Ilorin 24003, Nigeria) for the technical assistance provided during this study.

REFERENCES

Aebi, H. (1974). Catalases. Methods of Enzymatic Analysis, 2: 673-684.

Ajiboye, T. O., Adeleye, A. O., Salau, A. K., Ojewuyi, O. B., Adigun, N. S., Sabiu, S. and Sunmonu, T. O. (2014). Phenolic extract of *Parkia biglobosa* fruit pulp stalls aflatoxin B1–mediated oxidative rout in the liver of male rats. *Revista Brasileira de Farmacognosia*, 24: 668-676.

Bartels, H. and Bohmer, M. (1972). Kinetic determination of creatinine concentration. *Clinica Chimica Acta*, 37: 193-197.

Beutler, E. (1984). Red cell metabolism: a manual of biochemical methods. Grune and Stratton. *Incorporated Orlando*, FL,32887, 248-251.

Bukar, B. B., Tsokwa, N. E. and Orshi, O. D. (2020). Ameliorative and fecundity potentials of aqueous extract of *Azanza garckeana* (T. Hoffm) fruit pulp in formalin-induced toxicity on male albino mice. *Journal of Pharmacy and Bioresources*, 17(2): 164-173.

Doumas, B. T., Watson, W. A. and Biggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta*, 31(1): 87-96.

Dzoyem, J. P., McGaw, L. J., Eloff, J. N. and Stevens, J. (2018). The potential of South African plants against Mycobacteriuminfections. *Journal of Ethnopharmacology*, 219(3): 235-260.

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4: 177-185.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82(1): 70-77.

Fridovich, I. (1995). Superoxide radical and superoxide dismutases. *Annual Review of Biochemistry*, 64(1): 97-112.

Gardiner, P., Phillips, R. and Shaughnessy, A. F. (2017). Herbal and dietary supplement-drug interactions in patients with chronic illnesses. *American Family Physician*, 96(2): 101-107.

Goldberg, D. M. and Spooner, R. J. (1992). Glutathione reductase. *Methods in Enzymatic Analysis*, 258-265.

Gornall, A. G., Bardawill, C. J. and David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, 177(2): 751-766.

Halliwell, B. and Gutteridge, J. M. C. (2015). *Free Radicals in Biology and Medicine*. Oxford University Press.

Jendrassik, L. and Grof, P. (1938). Quantitative determination of total and direct bilirubin in serum and plasma. *Biochemistry*, 297: 81-89.

Jiang, Y., David, B., Tu, P. and Barbin, Y. (2010). Recent analytical approaches in quality control of traditional Chinese medicines—a review. *Analytica chimica acta*, 657(1): 9-18.

Kayani, S., Ahmad, M., Zafar, M., Sultana, S., Khan, M. P. Z., Ashraf, M. A. and Yaseen, G. (2014). Ethnobotanical uses of medicinal plants for respiratory disorders among the inhabitants of Gallies Abbottabad, Northern Pakistan, *Journal of Ethnopharmacology*, 156: 47-60.

Latha, B. P., Reddy, R. M., Ismail, S. M. and Vijaya, T. (2010). Medicinal plants and their derivatives as potential source in treatment of obesity. *Asian Journal of Experimental Biological Sciences*, 1(4): 719-727.

Levey, A. S., Bosch, J. P., Lewis, J. B., Greene, T., Rogers, N. and Roth, D. (2003). A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Annals of Internal Medicine*, 130(6): 461-470.

Luyckx, V. A. and Naicker, S. (2008). Acute kidney injury associated with the use of traditional medicines. *Nature Clinical Practice Nephrology*, 4(12): 664-671.

Mensah, M. L., Komlaga, G., Forkuo, A. D., Firempong, C., Anning, A. K. and Dickson, R. A. (2019). Toxicity and safety implications of herbal medicines used in Africa. *Herbal medicine*, 63: 1992-0849.

Nakchbandi, I. A. (2014). Osteoporosis and fractures in liver disease: relevance, pathogenesis and therapeutic implications. *World Journal of Gastroenterology*, 20(28); 9427.

Nurudeen, Q.O., Asinmi, M.R., Falana, M.B. and Dikwa, M.A. (2023). Aphrodisiac Potentials of Aqueous Extract of *Azanza garckeana* Fruit Pulp in Fluoxetine-induced Sexually-impaired Female Rats. *Journal of Science and Technology*, 15(2): 11 -22.

Nurudeen, Q. O. and Yakubu, M. T. (2016). Aqueous extract of Phyllanthus amarus leaves restores sexual competence in female rats induced into sexual dysfunction by fluoxetine. *Nigerian Journal of Biochemistry and Molecular Biology*, 31(1): 1-14.

Nwanjo, H.U., Okafor, M.C and Oze G.O. (2005). Changes in Biochemical parameters of kidney function in rats co-administered with chloroquine and aspirin. *Journal of Clinical Sciences*, 2(1): 10-12

Ochokwu, I. J., Dasuki, A. and Oshoke, J. O. (2015). *Azanza garckeana* (Goron Tula) as an edible indigenous fruit in North Eastern Part of Nigeria. *Journal of Biology, Agriculture and Healthcare,* 5(15): 26-31.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2): 351-358.

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy Reviews*, 6(11): 1-5.

Prabhu, K. S., Reddy, P. V., Jones, E. C., Liken, A. D. and Reddy, C. C. (2004). Characterization of a class alpha glutathione-S-transferase with glutathione peroxidase activity in human liver microsomes. *Archives of Biochemistry and Biophysics*, 424(1): 72-80.

Ramachandran, S.V. (2006). Biomarkers of cardiovascular Disease molecular basis and practical considerations. *Circulation*, 113(2): 2335-2362.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28(1): 56-63.

Roy, S. D., Das, S., Shil, D. and Dutta, K. N. (2012). Herbal hepatoprotective agents: a review. *World Journal of Pharmaceutical Research*, 1(2): 87-99.

Sies, H. and Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews in Molecular Cell Biology*, 21(7): 363-383.

Sulieman, A. M. (2019). *Azanza garckeana*: Distribution, Composition, Nutritive Value and Utilization. Wild Fruits: Composition, *Nutritional Value and Products,* 25(2): 379-393.

Teschke, R., Wolff, A., Frenzel, C. and Schulze, J. (2014). Herbal hepatotoxicity–an update on traditional Chinese medicine preparations. *Alimentary Pharmacology and Therapeutics*, 40(1): 32-50.

Tietz, N. W. (1995). Clinical guide to laboratory tests. *Clinical guide to laboratory test*s, 1096-1096.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, 39(1): 44-84.

Veniamin, M. P. and Vakirtzi-Lemonias, C. (1970). Chemical basis of the carbamid diacetyl micro method for estimation of urea, citrulline and carbamyl derivatives. *Clinical Chemistry*, 16(1): 3-6.

Woo, C. S. J., Lau, J. S. H. and El-Nezami, H. (2012). Herbal medicine: toxicity and recent trends in assessing their potential toxic effects. *Advances in Botanical Research*, 62, 365-384.

Wright, P. J., Leathwood, P. D. and Plummer, D. T. (1972). Enzymes in rat urine: alkaline phosphatase. *Enzymologia*, 42(4): 317-327.

Wróblewski, F. and Ladue, J. S. (1955). Lactic dehydrogenase activity in blood. *Proceedings of The Society for Experimental Biology and Medicine*, 90(1): 210-213.

Yusuf, A. A., Garba, R., Alawode, R. A., Adesina, A. D., Oluwajobi, I., Ariyeloye, S. D. and Berinyuy, B. B. (2020a). Effect of drying methods and extractants on secondary metabolite compositions of *Azanza garckeana* pulp and shaft. *Noble International Journal of Agriculture and Food Technology*, 2(1): 01-07.

Yusuf, A. A., Lawal, B., Sani, S., Garba, R., Mohammed, B. A., Oshevire, D. B. and Adesina, D. A. (2020b). Pharmacological activities of *Azanza garckeana* (Goron Tula) grown in Nigeria. *Clinical Phytoscience*, 6(1): 1-8.