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Dysregulation of Electrolyte Balance and Lipid Profile in Rats Following Oral Administration of the Methanolic Fruit Pulp Extract of Azanza garckeana (Tula Kola Nut)

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Abstract

This study investigated the effects of the methanolic fruit pulp extract of Azanza garckeana (MFEAG) on renal function through acute and subacute toxicity studies. Twenty-nine (29) apparently healthy adult male Wistar rats weighing 100-120 grams were used. For the acute study, 9 rats were randomly assigned into three groups. Group 1 was administered 300 mg/kg MFEAG once. Group 2 was sequentially administered the next dose of 2,000 mg/kg once, when there were no signs of toxicity or mortality in Group 1 at 48 hours post-treatment, while Group 3 was administered the vehicle (10% v/v Tween 80). The rats were then observed for two weeks and sacrificed. For the subacute study, 20 rats were randomly assigned into four groups and daily administered the extract (at 300, 600, or 1,200 mg/kg) or the vehicle for four weeks. No significant effects were detected on the serum kidney function markers of the treated rats, except for the increase in the serum chloride concentration and decrease in the serum triglyceride concentration after the acute study; subsequently, the serum sodium concentration increased after the subacute study. These findings indicate that MFEAG may cause some level of impairment in renal function.

Keywords: Azanza Garckeana, Pulp, Toxicity, Kidney, Rat

Introduction

For many years, plants have served as food for humans, as well as medicine for the treatment and management of various diseases [1]. Approximately 80% of the global population and 95% of those in developing countries depend on natural products of plant origin for basic and primary health care [2, 3]. This is due to the low cost of these materials [4]. Additionally, the use of herbal products is estimated to increase by 10-20% annually [5]. These medicinal plants have served as alternatives for conventional drugs because they contain many active phytochemicals that are always in demand and are considered focal points in related research [6]. They also play a vital role in both the development and production of new drugs, with up to 65% of modern drugs being directly or indirectly derived from plants [6, 7].

Azanza garckeana belongs to the Malvaceae family. It is one of the most important fruit-bearing plants in Nigeria , where it is grown mostly in Tula village of Kaltungo Local Government Area of Gombe State and where the local people who are predominantly Hausa by tribe call it 'Goron Tula', meaning 'Tula kola nut'. The generic name Azanza is derived from the word Azania and means 'black and surviving in Zanzibar. The specific name garckeana is in honor of professor August Garcke (1819-1904), a German botanist and plant collector who specialized in pharmacognosy [8, 9]. Goron Tula is an important medicinal and food plant commonly used in northern Nigeria as an herbal medicine for the treatment of various illnesses [10]. Different parts of these small trees/shrubs are used locally as herbal remedies for diseases such as cough, chest pain, infertility, menstruation

abnormalities, sexually transmitted infections and hepatic impairment [9, 11].

The fruit of Azanza garckeana is one of the most important parts of the plant, and both its traditional and therapeutic potential have been well documented [10-13]. The fruit has rough and hairy bark. It is grayish brown in color and fibrous with longitudinal fissures and brown and yellow slashes. The plant is 2.5-4 cm in diameter and is clearly divided into 5 segments, with the fleshy gummy pulp containing five seeds in each segment. The seed is hemispherical in shape with brownish and woolly floss [9].

Generally, wild edible fruits are characterized by remarkable nutrient value and are excellent sources of minerals; fibers; vitamins C, A and E; polyphenols; and ascorbic and fatty acids that add flavor and color to the diet [14-16]. Among many others, the local uses of Azanza garckeana fruit include being edible—either eaten row while slightly green or ripe, soaked in a small quantity of water to make jelly, or boiled and used as a relish or porridge [12, 13]. It has reportedly been found to have advantages in terms of nutritional content over many other food substances or other parts of the Azanza garckena [17]. It also records a use as booster for sexual performance (aphrodisiac), which is its most widely known local use [11]. The fruit has also been shown to have antioxidant, antihyperglycemic/anti-diabetic, anti-inflammatory/analgesic, antifungal, antimalarial, iron absorption and uterotonic effects in various studies [16-18].

Despite the undeniable potential of traditional plants, it has been stressed that the use of these traditional medicines as well as the development of conventional drugs should be rationalized by evaluating their safety profile before their approval for consumption or use [19, 20]. A number of studies have attempted to evaluate the safety profile of various parts of Azanza garckeana, with one revealing that the ethyl acetate, acetone, methanol and water extracts of the plant's stem bark are toxic to brime shrimp, while its petroleum ether extract has proven to be safe [21]. A recent study by us indicated the general safety of the same type of extract at a dose of up to 2,000 mg/kg after acute and subacute oral toxicity evaluations, except for a level of toxicity in the cardiopulmonary and hepatic systems observed after subacute (4 weeks) treatment at a high dose in rats [17]. Another study reported that methanol extracts of air- and sun-dried fruit pulp cause alterations in the functional integrity of rat kidneys after two weeks of daily treatment at doses of 150, 300 and 600 mg/kg, for which it was recommended that caution be exercised during its use [22].

This study evaluated the acute and subacute effects of higher doses (300, 600, 1,200 and 2,000 mg/kg) of the methanolic fruit pulp extract of Azanza garckeana on the serum lipid profile; creatinine; urea; and electrolytes (sodium, potassium, chloride, bicarbonate and calcium) in adult male Wistar rats after one or 28 days of daily administration.

Materials and Methods

Drugs and Other Chemicals

The drugs and other chemicals used included Tween 80 (CP Execution Standard QB/440500712208) for dissolving the extract because it was jelly in nature and ketamine (midazolam injection, USP) and diazepam (Centurion Healthcare Private Limited) for anesthetizing the rats after the experiments.

Experimental Animals

Twenty-nine (29) apparently healthy growing male Wistar rats (100-120 grams) were obtained and acclimatized for two weeks before the experiments commenced. All animals were maintained on standard rodent feed and water ad libitum while being housed in the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria-Nigeria, whose laboratory was used for the experimental procedures. All the experimental protocols were carried out in compliance with standard guidelines as approved by the AHmadu Bello University Committee on Animal Use and Care (ethical approval number: ABUCAUC/2021/009).

Source of Plant Material and Extract Preparation

Azanza garckeana fruits were obtained from Tula village in the Kaltungo Local Government Area of Gombe State, Nigeria. The plant material was identified and authenticated by a botanist in the Botany Department of Ahmadu Bello University, Zaria-Nigeria, where a voucher specimen (ABU07276) was deposited. The extraction was carried out according to previously described methods [23-25]. The fruit pulp was manually separated from the seeds, air-dried, shredded into small pieces and removed for extraction. One kilogram of pulverized Azanza garckeana fruit pulp was macerated with 3,000 mL of methanol and allowed to stand for 72 hours, after which it was filtered. The filtrate was collected and concentrated in a water bath at 50°C. The concentrated jelly extract was collected and properly stored in a container before further experiments and analysis.

Experimental Design

The study was carried out according to a previously described method, and it was performed in two phases, acute and subacute, as follows [26].

Acute Toxicity Study

The extract was administered to the rats after overnight fasting by using oral gavage at a volume of 10 mL/kg body weight. The nine rats were randomly divided into 3 groups of 3 rats each. The starting dose of 300 mg/kg body weight of the extract (dissolved in 10% v/v Tween 80) was administered to Group 1. Group 2 animals were sequentially administered the next dose of 2,000 mg/kg when there was no sign of toxicity or mortality, as shown in Group 1 48 hours posttreatment. In parallel, Group 3 animals were treated with vehicle (10% v/v Tween 80) to establish a comparable normal control group according to the OECD guidelines [27].

Subacute Toxicity Study

Rats were randomly divided into four groups of 5 each. Groups 1-3 rats were administered the extract daily at different doses (300, 600 and 1200 mg/kg body weight, respectively), and Group 4 animals (control) were administered equal volumes of the vehicle (10% v/v Tween 80) via oral gavage once daily for 28 consecutive days.

Sacrifice of Animals and Collection of Blood Samples

At the end of each experiment (15th day for acute studies and 29th day for subacute studies), the rats were anesthetized by in-

traperitoneal injection of 75 mg/kg ketamine and 5 mg/kg diazepam. Blood samples (4-5 mL) were then collected into plain bottles via cardiac puncture. The blood samples were allowed to stand for 20-30 minutes before they were allowed to coagulate and then centrifuged at 3,000 rpm for 10-15 minutes. The serum was subsequently decanted into clean specimen tubes and used for biochemical assessment of serum kidney function markers and lipid profiles, which were performed spectrophotometrically using an Audicum autoanalyzer and Surechem spectrophotometer.

Data Analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Turkey's post hoc multiple comparison test to compare the effects across the groups. P values less than 0.05 ($P < 0.05$) were considered to indicate statistical significance. SPSS (Statistical Package for Social Science) version 24.0 was used for the analysis. The results are expressed as the mean ± standard error of the mean (SEM).

Results

Acute Study Results

As shown in Table 1, there were no statistically significant changes ($P \geq 0.05$) in the serum sodium, potassium, bicarbonate, calcium, urea or creatinine levels of the animals after the acute study. However, a significant increase ($P < 0.05$) in the serum chloride concentration was observed after treatment with a high dose of 2,000 mg/kg compared to the control.

Table 1: Acute effects of Azanza garckeana fruit pulp extract on rats' serum kidney function markers.

Treatment	Creatinine (mEq/L)	Urea (mg/dL)	Na+ (mMol/L)	K+ (mMol/L)	Cl- (mg/dL)	CO32- (mg/dL)	Ca2+ (mg/dL)
10% v/v Tween 80	1.07±0.12	16.8±5.2	62.0±10.6	10.7±0.9	30.7±2.3	80.7±2.3	1.50±0.3
300 mg/kg extract	3.27±2.37	48.3±19.2	68.8±11.9	14.6±1.7	31.3±1.9	85.3±5.2	1.07±0.2
2,000 mg/kg extract	1.13±0.09	9.77±2.7	61.5±14.2	16.6±2.3	40.3±0.3ab	91.7±1.2	1.5±0.4

The superscripts 'a' and 'b' indicate statistically significant differences ($p < 0.05$) from the 10% v/v Tween 80- and 300 mg/kg extract-treated groups, respectively.

Additionally, the results in Table 2 below show no significant differences in serum total cholesterol (T/Chol), high-density lipoprotein cholesterol (HDL) or low-density lipoprotein cholesterol (LDL) between the treated rats and the control rats ($P \geq 0.05$) after the acute study. However, treatment of rats with 2,000 mg/kg of the extract caused a significant decrease in triglyceride (TG) levels compared to those in the control group ($P < 0.05$).

Table 2: Acute effects of Azanza garckeana fruit pulp extract on the serum lipid profile of rats.

Treatment	T/Chol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
10% v/v Tween 80	61.67±15.81	126.73±27.09	7.07±1.41	29.27±12.07
300 mg/kg extract	94.27±22.14	77±9.47	10.77±0.62	68.03±23.84
2,000 mg/kg extract	70.9±15.4	51.37±8.04a	7.13±1.59	53.50±14.83

T/CHol: total cholesterol; TG: triglycerides; HDL: high-density lipoproteins; LDL: low-density lipoproteins; superscript 'a' indicates a statistically significant difference compared to the 10% v/v Tween 80-treated group.

Subacute Study Results

The results obtained after the subacute study (Table 3) indicated no significant changes ($P \geq 0.05$) in the serum levels of potassium, chloride, bicarbonate, urea or creatinine compared to those in the control group. However, a significant increase ($P < 0.05$) in the serum level of sodium ions was observed after treatment with 1,200 mg/kg of the extract compared to the control.

Table 3: Subacute effects of Azanza garckeana fruit pulp extract on rat serum kidney function markers.

Treatment	Creatinine (mEq/L)	Urea (mg/dL)	Na+ (mMol/L)	K+ (mMol/L)	Cl- (mg/dL)	CO32- (mg/dL)
10% v/v Tween 80	1.02±0.09	54.2±12.8	82.6±8.8	9.9±1.7	37.0±2.3	100.6±5.7
300 mg/kg extract	0.81±0.14	22.9±2.5	86.9±8.1	10.2±1.4	35.7±3.1	105.6±5.8
600 mg/kg extract	0.96±0.11	28.7±9.7	87.6±6.9	38.3±28.0	26.6±4.4	97.4±2.7
1,200 mg/kg extract	1.90±1.03	81.8±16.8bc	144.7±20.6ac	18.0±3.0	27.1±1.9	95.4±3.9

The superscripts 'a', 'b' and 'c' indicate statistically significant differences ($p < 0.05$) from the 10% v/v Tween 80-, 300 and 600 mg/kg extract-treated groups, respectively.

Furthermore, the results in Table 4 indicate that, compared with the control, the extract had no significant effect on the serum levels of any of the lipids ($P \geq 0.05$) after the subacute study.

Table 4: Subacute effects of Azanza garckeana fruit pulp extract on the serum lipid profile of rats.

Treatment	T/Chol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
10% v/v Tween 80	36.44±7.69	49.24±11.24	7.22±1.29	19.66±6.38
300 mg/kg extract	45.44±5.46	66.75±9.39	9.77±1.16	22.24±4.54
600 mg/kg extract	31.14±3.99	77.36±14.05	9.36±0.73	6.32±2.88
1,200 mg/kg extract	57.60±6.36c	97.09±12.80	8.97±1.06	30.43±6.85c

T/CHol: total cholesterol, TG: triglycerides, HDL: high-density lipoproteins, LDL: low-density lipoproteins; superscript 'c' indicates a statistically significant difference ($p < 0.05$) from the 600 mg/kg extract-treated group.

Discussion

The safety of herbal medicines is usually questioned due to reports of certain adverse effects, illnesses and even fatalities, particularly related to nephrotoxicity [5, 28]. The kidney receives the largest amount of blood flow per gram due to its ability to filter large amounts of toxins and drug metabolites from the blood. These toxins and metabolites can concentrate within the kidney's renal tubules and cause various degrees of damage [29]. Hence, evaluating the safety of herbal medicines for the kidney is highly important [5, 28]. Thus, this study evaluated the safety of the methanolic fruit pulp extract of Azanza garckeana (MFEAG) on the kidney function of adult male Wistar rats through acute and subacute oral toxicity evaluations. Both an initial assessment using an acute method and establishing a safety margin using subacute or chronic multiple administrations of different doses of extracts of medicinal plants in laboratory animals, in whom it is almost only possible, are essential in safety screening of these medicines [30, 31]. Therefore, the effects of one-time administration of MFEAG and multiple subacute administrations of the extract on kidney function were assessed in this study in rat models.

Despite the deficiency of the glomerular filtration rate (GFR) in the assessment of true tubular health, the assessment of kidney function is considered to be more appropriate using the GFR. However, due to the invasiveness and time consumption of determining the GFR, certain markers of kidney function, such as serum or urine, are usually used to indirectly assess kidney function and diagnose kidney damage/dysfunction despite the imperfections of not fully fulfilling certain criteria for representing the GFR [32-35]. Therefore, the serum creatinine, urea and electrolyte levels are the major markers routinely used for the assessment of renal function [32, 35].

Creatinine is a product of muscle metabolism. Because it is not normally reabsorbed by renal tubules after filtration, its serum concentration usually increases when the kidney fails to excrete it as a result of damage, especially during chronic kidney injury [33, 35]. Serum creatinine is the most commonly used marker of kidney function because of its advantages of being inexpensive and quick to assess, although its level is often influenced by an individual's muscle mass [34-36]. It is an integrator of both intrarenal and extrarenal kidney functions/injuries and indicates a balance between its generation and excretion [37]. In this study, the serum creatinine level was not affected by treatment with MFEAG. This finding is in contrast to the finding of the study of Yusuf et al. , where the air-dried methanol fruit pulp extract of the plant decreased the serum creatinine level after two weeks of daily treatment, thereby indicating renal damage [22]. However, a decrease in the serum creatinine level does not truly indicate impaired renal function because the creatinine level usually increases (rather than decreases) when renal function is impaired, which affects the glomerular filtration rate (GFR) [33, 34]. Therefore, the decrease in the serum creatinine concentration observed in the study of Yusuf et al. might have occurred through other mechanisms unrelated to renal function. The observation that MFEAG did not affect the serum creatinine level in rats treated at acute or subacute levels or at a dose of up to 1,200 mg/kg in this study (which is relatively greater than the doses of 150, 300 and 600 mg/kg used in the study of Yusuf et al. further highlights that the fruit of Azanza garckeana may not have any ability to affect the excretion of creatinine through inflicting renal damage [22].

Like creatinine, urea is also a product of muscle metabolism and is considered a nonprotein nitrogenous (NPN) waste. Its concentration is determined by renal excretion as well as by the intake and metabolism of proteins. Unlike creatinine, it is not excreted only by the kidneys; hence, it is not as good as creatinine for use as a marker of renal function. Nonetheless, its serum concentration is usually increased, especially in acute renal injuries [33,34]. Moreover, the serum urea concentration was not affected by acute or subacute treatment with MFEAG in these rats. These findings contrast with those of Yusuf et al., in which the serum urea concentration was significantly decreased by all doses of both the air-dried and sun-dried fruit pulp extracts of Azanza garckeana [22]. Nevertheless, a decrease in the serum urea level, rather than an increase, is also seldom observed in kidney injuries but rare inherited disorders of the urea cycle and advanced liver disease; hence, a decrease in the serum urea level is considered to be of less clinical significance and, therefore, does not actually reflect renal injury [33, 34]. This further stress the safety of the fruit on renal function.

Kidney damage can lead to the accumulation of electrolytes such as sodium, potassium, calcium, magnesium and bicarbonate in the body because the kidneys are not able to properly excrete

them, leading to, for instance, hypernatremia. Therefore, these electrolytes are similar to the "canary in the coal mine"; they can signal a problem with the kidneys before other symptoms appear. Therefore, abnormalities in these electrolytes are associated with reduced kidney function, as shown in a number of studies [35, 38]. In the present study, MFEAG had no effect on the serum potassium, bicarbonate or calcium ion concentration. This finding is in contrast to the findings of Yusuf et al., where bicarbonate was found to increase following a two-week daily administration of the methanolic fruit pulp extract of the plant. However, after the acute treatment period, the serum concentration of chloride ions was significantly increased by the 2,000 mg/kg dose of MFEAG, which contradicts the findings of Yusuf et al., who reported no effects on electrolytes from the same type of extract [22]. Again, after the subacute study, MFEAG was found to significantly increase the serum sodium ion concentration at a dose of 1,200 mg/kg, which is in line with the findings of Yusuf et al., in which the sodium level was also increased by the fruit extract. Since an increase in the serum sodium (hypernatremia) and chloride (hypercholeraemia) concentrations is indicative of kidney injury, the effect of MFEAG may be the result of possible damage to the kidney that caused a failure to excrete excess sodium and chloride ions [38, 39].

Dyslipidemia, or abnormal levels of lipids in the blood, can have an impact on renal function. The two main types of lipoproteins are high-density lipoprotein (HDL) and low-density lipoprotein (LDL). HDL is often called "good" cholesterol because it helps to remove excess cholesterol from the body, while LDL is often called "bad" cholesterol because it can deposit cholesterol in the walls of blood vessels. The balance between HDL and LDL is an important factor in cardiovascular health and thus other organs, such as the kidney, are dependent on this balance. Changes in this balance can also affect the levels of triglycerides and cholesterol in the body. High levels of triglycerides and LDL as well as lower levels of HDL have been found to cause glomerulosclerosis, resulting in a decline in the glomerular filtration rate (GFR) [40, 41]. In this study, no effects were observed on total cholesterol or high- or low-density lipoprotein levels after treatment of rats with MFEAG at either acute or subacute levels. However, a significant decrease in triglyceride levels was observed in the 2,000 mg/kg MFEAG-treated rats after the acute study. This finding is in contrast to the findings of a recent study in which no significant effects were observed on the serum triglyceride concentration after 12 weeks of treatment with the same type of extract at a dose of 500 mg/kg in rabbit bucks [42]. There is evidence that high triglyceride levels can be associated with impaired renal function, as highlighted above, but it is unclear whether lower triglyceride levels could also have a negative impact on the kidneys. In fact, there is some evidence that lowering triglyceride levels with certain medications, such as statins, can actually improve renal function [43]. This finding implies that the decrease in triglyceride levels induced by MFEAG observed in this study may not indicate renal damage; rather, it could be considered beneficial to the body by eliminating harmful lipids.

Many plant species have been reported to exert their pharmacological activities through their phytoconstituents [44]. For example, excessive dosages of several phytochemicals, e.g., anthraquinones in laxatives, cardiac glycosides such as digoxin, alkaloids, saponins, steroids and flavonoids, have been shown to cause electrolyte imbalance and nephrotoxicity [45-50]. Anthraquinones and tannins have been shown to inhibit certain chloride channels that are involved in chloride ion secretion [51-53]. Furthermore, certain steroids, such as glucocorticoids, which cause sodium retention via various pathways [54]; aldosterone, which stimulates sodium and chloride ion absorption via the epithelial Na+ channel (ENaC); Na+–K+–2Cl- cotransporter 2 (NKCC2); and the flavonoid quercetin, which activates NKCC1 to inhibit the excretion or increase the absorption of sodium and chloride ions in the renal tubules, thereby increasing the body's levels of these electrolytes [55]. The increase in the serum sodium and chloride ion concentrations observed as a result of treatment with MFEAG in this study might have been the result of the effects of these phytochemicals, which were found to be present in the extracts in our previous study, or extracts of other parts of the plant [17, 8, 11, 15, 56, 57].

Again, certain phytochemicals present in the fruit of Azanza garckeana have also been implicated in lipid metabolism. For instance, flavonoids and saponins increase the activity of lipoprotein lipase (LPL), which is an enzyme that helps in degrading triglycerides into free fatty acids and glycerol, thereby playing an important role as a regulator of the serum triglyceride level [58-62]. Although it is found in many other organs, LPL is also expressed in the kidneys, where it plays a role in triglyceride metabolism [62,63]. It is possible that the extract increases the activity of LPL through the presence of these phytochemicals, thereby increasing the metabolism of triglycerides and decreasing their level. A study reported that LPL appears to have a minor role in the kidney [64]. Therefore, the effect of MFEAG on triglyceride levels may not necessarily be related to its effect on renal function.

Suffice it to say that, while there is no direct evidence yet, there is some indication that Azanza garckeana fruit pulp might affect certain chloride channels or enzymes involved in sodium and chloride transport as well as triglyceride metabolism through its active phytochemicals, thereby increasing the retention of these electrolytes and the metabolism of triglycerides, hence affecting their levels in the body.

Conclusion

From the findings of this study, it can be deduced that MFEAG may confer some level of toxicity to the kidney owing to its ability to increase the serum sodium and chloride levels in rats. However, further studies may be required to investigate the effects of the fruit or its active ingredients on certain molecular targets that are involved in renal handling of electrolytes and lipid metabolism.

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Compliance with Ethical Standards

Competing Interests

The authors declare that they have no competing interests.

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Ethics approval and Consent to Participate
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study protocol was reviewed and approved by the Ahmadu Bello University Committee on Animal Use and Care, with approval number ABUCAUC/2021/009.

Availability of Data and Materials
The data supporting the findings of this study are available upon reasonable request from the corresponding author.

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