

Effect of Aqueous Fruit Extract of *Azanza garckeana* on Some Biochemical and Hematological Parameters in Wistar Rats

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ABSTRACT

Background and Objective: *Azanza garckeana* fruit is widely consumed in Africa both as food and as herbal medicine. This study evaluated the safety of the aqueous fruit extract of *Azanza garckeana*.

Materials and Methods: Twenty-five Wistar rats were randomly divided into five groups. Group 1 received distilled water while groups 2, 3, 4 and 5 received 200, 400, 600 and 800 mg/kg b.wt., aqueous *Azanza garckeana* fruit extract respectively for 14 days. After sacrificing the rats on the 15th day, the blood, serum liver and kidney function parameters were evaluated using standard procedures.

Results: The red blood cell (RBC) count of the rats administered aqueous fruit extract of *Azanza garckeana* significantly increased ($p < 0.05$) when compared with the control. The values of hemoglobin (HB), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) significantly increased ($p < 0.05$) at 600 and 800 mg/kg b.wt., aqueous fruit extract of *Azanza garckeana* when compared with the control. The extract significantly increased ($p < 0.05$) the level of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) when compared with the control. The values of direct bilirubin, total bilirubin and albumin at 600 and 800 mg/kg b.wt., significantly increased ($p < 0.05$) when compared with the control. The significant increase in all the liver function indices was within the normal range. All the kidney function indices assayed were not significantly different ($p < 0.05$) from the control. **Conclusion:** The aqueous fruit extract of *Azanza garckeana* is not toxic to the liver, kidney and blood.

KEYWORDS

Hematological, toxicological, biochemical, hepatic functions, *Azanza garckeana*, alanine aminotransferase, alkaline phosphatase

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INTRODUCTION

Azanza garckeana belongs to the family of Malvaceae. It is a shrub that grows up to 10 m tall. The leaves are round, alternate, 3-5 lobe and up to 20×20 cm in size¹. The flowers are purple or yellow with a dark purple or dark red center and 6 cm in diameter¹. The fruit is a round woody capsule, divided into 4 or 5 segments and red when mature. It is about 35 mm in diameter.



Azanza garckeana is one of the popular multipurpose fruit trees in West Africa. It grows in Nigeria, Kenya, Congo, Malawi, South Africa, Zimbabwe, South Sudan, Tanzania and Namibia. It has been identified by the World Agroforestry Center as one of the fruits that should be integrated into the domestication process in farming systems². Its fruits are edible and used as food additives and herbal medicines³. Wild edible fruits are characterized by remarkable nutrient values and are a good source of vitamins, minerals, fibre, polyphenols and fatty acids^{3,4}. There is a resurgence of interest in wild edible fruits because they increase the number of foods^{3,5,6}. The ripe fruit is used traditionally to treat anemia, infertility problems and malaria and is used as an aphrodisiac⁷⁻¹¹. Ahmed *et al.*¹² reported that ripe fruit inhibits alpha-amylase and angiotensin-converting enzymes and can thus be used to treat diabetes and hypertension. Therefore, the fruits of *Azanza garckeana* also serve as herbal medicines. The leaves, root and stem bark are used to treat various diseases such as diabetes, liver problems and sexually transmitted diseases and are used to induce labor¹³⁻¹⁶.

Plant parts are generally accepted to be safe especially when it is used as food but recent research has shown that some plants are toxic. Despite the use of the fruit of *Azanza garckeana* as food, its safety has not been evaluated. This study, therefore, evaluated the effect of aqueous fruit extract of *Azanza garckeana* on some biochemical and hematological parameters to elucidate its safety.

MATERIALS AND METHODS

Study area and duration: The study was conducted in Mubi, Adamawa State, Nigeria. It was conducted between July and September, 2023.

Collection and identification of plant: *Azanza garckeana* (1000 g) was purchased from a reputable dealer in a local market in Tula town, Kaltingo Local Government area of Gombe State, Nigeria. It was authenticated by a botanist in the Department of Botany, Adamawa State University, Mubi.

Preparation of aqueous extract: The pulp was removed, washed and carefully crushed into small pieces. It was then dried under shade to constant weight. Afterward, the dried pulp was ground to a fine powder using a pestle and mortar. As 400 g of the powder were soaked in 1.6 L of water. It was filtered using a conical flask and Whatman filter paper. The extract was evaporated to dryness in a vacuum evaporator at 50°C.

Experimental animals: As 25 male Wistar rats weighing between 120-150 g were obtained from the National Veterinary and Research Institute (NVRI), Vom, Plateau State. The animals were handled under ethical conditions as approved by the Adamawa State University, Mubi Institutional Animal Care and Ethics Committee. The animals were fed with standard feed pellets and water *ad libitum*. They were left for two weeks to acclimatize to the environment before the commencement of the experiment.

Experimental design: As 25 Wistar rats were randomly divided into five groups. Group 1 received distilled water and groups 2, 3, 4 and 5 received 200, 400, 600 and 800 mg/kg b.wt., aqueous *Azanza garckeana* fruit extract respectively for 14 days.

Collection of blood samples: The animals were sacrificed on the 15th day using ether anesthesia. Blood was collected in EDTA bottles as well as plain sample bottles. Blood in the plain bottles was then centrifuged at 3,000 rpm for 10 min and serum was collected and used for biochemical analysis. The blood in the EDTA bottle was used for hematological analysis.

Hematological analysis: The Packed Cell Volume (PCV), White Blood Count (WBC), Red Blood Cell (RBC), Hemoglobin (Hb), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined according to the method described by Ochei and Kolhatkar¹⁷.

Biochemical analysis: Serum protein, albumin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), direct bilirubin (DB) and total bilirubin (TB) were done using standard methods described by Weichselbaum¹⁸, Doumas *et al.*¹⁹, Reitman and Frankel²⁰, Shephard *et al.*²¹ and Levitt and Levitt²², respectively. The serum sodium, potassium, chloride, bicarbonate, urea and creatinine were done according to the method described by Wooten *et al.*²³, Fawcett and Scott²⁴ and Evans²⁵, respectively.

Statistical analysis: All data was subjected to one-way ANOVA followed by Duncan's multiple *post hoc* test using SPSS version 26. The $p < 0.05$ was considered significant. All values are expressed as Mean \pm Standard Error of mean (SEM).

RESULTS

Table 1 shows the result of the hematological parameters of rats administered aqueous fruit extract of *Azanza garckeana*. The levels of PCV, WBC and MCH of rats administered *Azanza garckeana* were not significantly different from the control. The red blood cell (RBC) count of rats administered aqueous fruit extract of *A. garckeana* significantly increased ($p < 0.05$) when compared with the control. As 600 and 800 mg/kg b.wt., of *A. garckeana* aqueous fruit extract significantly increased ($p < 0.05$) the level of hemoglobin when compared with the control. The level of MCHC in rats administered 400, 600 and 800 mg/kg b.wt., of aqueous fruit extract of *A. garckeana* significantly increased ($p < 0.05$) when compared with the control.

The serum liver function indices of rats administered aqueous fruit extract of *A. garckeana* are shown in Table 2. The extract at all doses significantly increased the level of ALT, AST and ALP when compared with the control. The level of direct and total bilirubin was significantly increased by administration of 400, 600 and 800 mg/kg b.wt., aqueous fruit extract of *A. garckeana*. The concentration of albumin in rats administered 600 and 800 mg/kg b.wt., aqueous fruit extract of *A. garckeana* significantly increased when compared with the control.

The serum kidney function indices of rats administered aqueous fruit extract of *Azanza garckeana* are shown in Table 3. All the kidney function indices assayed for were not significantly different from the control.

Table 1: Hematological indices of rats administered aqueous fruit extract of *Azanza garckeana*

Group	PCV	WBC	RBC	HB	MCV	MCH	MCHC
1	42.33 \pm 0.88 ^a	66.33 \pm 1.76 ^a	6.86 \pm 0.08 ^a	13.99 \pm 0.38 ^a	55.66 \pm 1.20 ^a	19.06 \pm 0.12 ^a	35.26 \pm 0.31 ^a
2	40.00 \pm 0.57 ^a	63.00 \pm 2.08 ^a	7.26 \pm 0.14 ^b	13.22 \pm 0.11 ^a	57.00 \pm 1.15 ^a	18.60 \pm 0.11 ^a	33.53 \pm 0.14 ^a
3	41.67 \pm 0.66 ^a	66.33 \pm 0.45 ^a	7.06 \pm 0.08 ^b	13.67 \pm 0.33 ^a	56.00 \pm 1.15 ^a	18.46 \pm 0.14 ^a	36.13 \pm 0.08 ^b
4	43.00 \pm 1.15 ^a	62.33 \pm 0.88 ^a	7.53 \pm 0.08 ^c	14.22 \pm 0.48 ^c	62.00 \pm 1.15 ^b	18.03 \pm 0.08 ^a	35.13 \pm 0.17 ^b
5	43.66 \pm 0.88 ^a	75.67 \pm 0.88 ^c	7.73 \pm 0.06 ^c	14.55 \pm 0.29 ^c	66.66 \pm 0.88 ^b	19.70 \pm 0.11 ^a	36.53 \pm 0.08 ^b

Values with different superscripts down the column are statistically different from each other ($p < 0.05$), PCV: Packed Cell Volume, WBC: White Blood Cell, RBC: Red blood cell, HB: Hemoglobin, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin and MCHC: Mean Corpuscular Hemoglobin Concentration

Table 2: Serum liver function indices of rats administered aqueous fruit extract of *Azanza garckeana*

Group	Protein (mg/dL)	Albumin (mg/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	DB (mmol/L)	TB (mmol/L)
1	5.43 \pm 0.18 ^a	3.50 \pm 0.05 ^a	13.33 \pm 0.88 ^a	61.33 \pm 0.88 ^a	53.33 \pm 1.20 ^a	0.04 \pm 0.01 ^a	0.06 \pm 0.01 ^a
2	6.36 \pm 0.06 ^b	3.73 \pm 0.08 ^a	22.33 \pm 0.33 ^b	75.66 \pm 1.20 ^b	64.00 \pm 1.73 ^b	0.06 \pm 0.01 ^a	0.07 \pm 0.01 ^a
3	6.36 \pm 0.08 ^b	3.96 \pm 0.06 ^a	25.66 \pm 0.33 ^b	79.33 \pm 0.33 ^c	75.00 \pm 1.15 ^c	0.08 \pm 0.01 ^b	0.09 \pm 0.01 ^b
4	6.53 \pm 0.06 ^b	4.26 \pm 0.06 ^b	32.33 \pm 0.88 ^c	82.33 \pm 0.66 ^c	93.00 \pm 1.73 ^d	0.09 \pm 0.01 ^b	0.09 \pm 0.01 ^b
5	6.60 \pm 0.05 ^b	4.56 \pm 0.03 ^b	34.66 \pm 0.66 ^c	88.33 \pm 0.66 ^d	97.00 \pm 1.15 ^d	0.10 \pm 0.01 ^b	0.10 \pm 0.01 ^b

Values with different superscript down the column are statistically different from each other ($p < 0.05$), ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, DB: Direct bilirubin and TB: Total bilirubin

Table 3: Serum kidney function indices of rats administered aqueous fruit extract of *Azanza garckeana*

Group	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	HCO ³⁻ (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
1	140.00 ± 0.57 ^a	4.43 ± 0.08 ^a	101.33 ± 0.88 ^a	30.33 ± 0.33 ^a	16.33 ± 0.88 ^a	0.56 ± 0.06 ^a
2	144.33 ± 0.88 ^a	4.50 ± 0.17 ^a	98.33 ± 0.33 ^a	26.33 ± 0.66 ^a	15.76 ± 0.66 ^a	0.66 ± 0.33 ^a
3	141.66 ± 1.20 ^a	4.56 ± 0.08 ^a	103.33 ± 0.66 ^a	27.00 ± 1.15 ^a	14.66 ± 0.33 ^a	0.56 ± 0.03 ^a
4	140.00 ± 0.66 ^a	4.26 ± 0.14 ^a	101.33 ± 0.33 ^a	30.33 ± 0.33 ^a	15.66 ± 0.88 ^a	0.63 ± 0.03 ^a
5	142.00 ± 0.57 ^a	4.66 ± 0.14 ^a	103.33 ± 0.88 ^a	30.00 ± 0.57 ^a	17.33 ± 0.66 ^a	0.66 ± 0.03 ^a

Values with different superscript down the column are statistically different from each other (p < 0.05)

DISCUSSION

The non-significant difference in WBC suggests that the extract does not have immunological properties which can boost the defense system of the animals. White blood cells (WBC) fight infections, defend the body against invasion by foreign bodies and produce antibodies in immune response.

Elevated levels of MCHC indicate that the extract contains phytochemicals that can stimulate the secretion of erythropoietin which stimulates the stem cells in the bone marrow to produce RBC. This might be the reason for the observed elevated RBC count. An increase in red blood cells, hemoglobin and MCHC suggests that the extract has hematopoietic properties and thus promotes erythropoiesis in animals.

There was a significant increase in the values of ALT, AST and ALP. Alanine Aminotransferase (ALT) is an enzyme found predominantly in the liver^{11,12} while AST is present in the liver, kidney, skeletal muscle, pancreas and heart. High levels of serum ALT indicate liver damage. The primary pathological marker of liver dysfunction is ALT, AST is used to supplement ALT²⁶. Alanine aminotransferase is found in the cytosol of the liver. The enzyme leaks out of the cytosol into the serum when there is damage to the plasma membrane of the liver. The reference ranges of AST, ALT and ALP in rats are 50-150, 10-40 and 30-130 IU/L, respectively²⁷. Though there was a significant increase in the values of these enzymes, the values are still within the normal range which indicates that the extract is not toxic to the liver.

The values of the total and direct bilirubin are within the normal range of bilirubin. This suggests that the extract did not affect the excretory function of the liver. This is because bilirubin is a useful index of the excretory functions of the liver²⁸. It passes through the liver and is excreted out of the body.

Though there was a significant increase in the level of albumin at high doses, the level is within the normal range of 3.4-4.8 mg/dL. This indicates that the synthetic capacity of the liver is not affected by the plant because the liver synthesizes albumin. Albumin levels are used to evaluate the synthetic capacity of the liver. The non-significant difference in all kidney function parameters assayed indicates that the extract is not toxic to the kidney.

CONCLUSION

The aqueous fruit extract of *Azanza garckeana* caused some alterations in the liver function indices assayed for, but these alterations were within the normal range. The extract did not cause any alterations in the kidney and blood parameters. This indicates that the extract is not toxic to the liver, kidney and blood at the doses and number of days tested for. However, higher doses for a prolonged time should be used with caution. Prolonged usage at higher doses may further increase the liver function indices beyond the normal range leading to toxicity of the fruit.

SIGNIFICANCE STATEMENT

Azanza garckeana fruit is widely consumed in the Northeastern part of Nigeria. Medicinal plants are usually assumed to be safe but current research has shown that some are not safe. Despite the wide consumption of *Azanza garckeana* fruits, its safety has not been authenticated. This study therefore evaluated the safety of *Azanza garckeana* fruit. The study found that aqueous *Azanza garckeana* fruit

extract significantly increased all the liver function indices assayed for, but this increase was within the normal range. The levels of PCV, WBC, MCH and the kidney function indices of rats administered *Azanza garckeana* were not significantly different from the control. This suggests that *Azanza garckeana* fruit is not harmful to the blood, liver and kidneys.

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