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Original Research

Direct and residual effects of *Azanza garckeana* (Goron tula) fruit on the Growth and Internal organ histology of New Zealand White rabbit bucks

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ABSTRACT

Studies have shown *Azanza garckeana* fruit to contain appreciable amounts of alkaloids, phytosteroids and several other phytochemicals with varying pharmacological potentials capable of stimulating growths and cellular development. Thus, this study was conducted to examine the direct and residual impact of *Azanza garckeana* fruit on the internal organs. Sixty (60) New Zealand white rabbit bucks were randomly assigned to 4 treatments of 15 bucks, replicated three times in a completely randomized design. The experimental diets were formulated and supplemented with *Azanza garckeana* fruit meal at 0, 100, 200 and 300g/kg respectively. The study was conducted in two experimental phases. At the end of each phase, data were collected on the internal organs dynamics. The tissues of the liver and kidney were collected and processed for histopathology. Results showed that *Azanza garckeana* fruit did not have any deleterious direct or residual impacts on the internal organ dynamics of the bucks. The histopathology indicated that the supplementations of *Azanza garckeana* fruit meal up to 300g/kg in direct phase did not have any detrimental effect on the histology of the kidney; while the supplementations at 200 and 300g/kg impacted negatively on the histo-architecture of the liver, showing a widespread vascular degenerations of the hepatocytes. The supplementations up to 300g/kg did not have any residual impact on the histology of the liver and kidney. It is, therefore, concluded that *Azanza garckeana* fruit meal at 200 and 300g/kg in direct phase had detrimental effects on the histo-architecture of the liver of the rabbits.

Keywords: *Azanza garckeana;* growth; histology; internal organs; rabbit

INTRODUCTION

The use of pharmacologically active phyto-products and fruit meals has gradually been utilized in animal production to enhance animal physiology and reproduction (Onunkwo, *et al*., 2019; Jiwuba, *et al*., 2021; Nathaniel, *et al*., 2023). This strategy has been adopted due to some inferences and assumptions that some of these plant based products can improve growth, animal physiology and reproduction. Some plant products utilized in livestock feeding can be harmful to the liver and kidney, and detrimental to the overall physiology of an animal; if these plants contain toxic substances (Emenalom, *et al.*, 2009; Jiwuba, *et al*., 2020). As such, the depth of information on the toxicity, doses and safe limits of these plant products needs to be ascertain.

The liver is an essential organ for bile production, enzyme and hormone. The liver is also needed for metabolism and digestion of fats, proteins and carbohydrates. The kidney on the other hand plays a vital role in filtration, electrolyte balance and removal of waste products from the body (Frandson, *et al*., 2018). Therefore, any condition that damages these sensitive internal organs, thereby reduce its efficiency will be highly detrimental to life of an animal. Many factors may damage these internal organs of which malnutrition, disease and consumption of toxic substances is paramount (Jacob, *et al*, 2016; Alfred, 2017; Ansar, *et al*., 2020). There are various speculations that these plant products are safe, cheaper and readily available with less residual effects (Dikko, *et al*., 2016; Malik, *et al*., 2022). In view of the concerns of the developments of the internal organs, the scope of the biological actions of these plant products need to be ascertained. Favorably, some plant

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products have received increased attention and some have given satisfactory results (Herbert & Acha, 1995; Herbert, 1998; Ekuma, *et al*., 2017). One of such plants is *Azanza garckeana*.

Azanza garckeana is commonly consumed as fruit and used as a medicinal plant in many parts of Nigeria. Different Pharmacological studies conducted on the various plant parts of *Azanza garckeana* has demonstrated a wide range of pharmacological activities justifying some of its ethno-medicinal uses (Ochokwu, *et al.*, 2014; Esther, *et al*., 2017). These pharmacological properties includes antibacterial, antifungal, antihyperglycemic, antimalarial, antioxidant and iron absorption properties (Mshelia, *et al*., 2016; Ahmed, *et al*, 2016; Amuri, *et al.*, 2017). A plant with so many pharmacological potentials is bound to have tremendous beneficial effects on the growth, anatomy and physiology of farm animals. There is need to ascertain the safety of *Azanza garckeana* on the internal organs and on the general physiology of rabbit bucks. Therefore, this study was aimed to evaluate the impact of *Azanza garckeana* fruit on the internal organs growth, histology and the safe limits of its utilizations in rabbit production

MATERIALS AND METHODS

EXPERIMENTAL SITE

This research was conducted at the Rabbit Unit of the Teaching and Research Farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The area is located in the South-Easthern part of Nigeria on latitude $5^0 27^!$ north, longitude 7^0 32' East, an altitude of 123m above sea level with an annual rainfall of 2177mm, temperature of 22^0C – 36^0C and relative humidity of 50 – 90%. It is situated within the humid rain forest zone of West Africa, characterized by long duration of rainy season (March-October) and short period of dry season (November - February). Climatic data were collected from the Meteorological Center of National Root Crop Research Institute, Umudike, Abia State (NRCRI, 2018). The hutches were constructed with wood and wire mesh with an in-built waste trays. The condition of housing and management were similar for all the experimental animals.

SOURCING AND PREPARATION OF *Azanza garckeana* FRUIT MEAL

The *Azanza garckeana* fruit were sourced from the rural and urban markets in Abia State. The *Azanza garckeana* fruit were dried at room temperature to a constant weight. *A. garckeana* fruit were ground to a fine powder using a mechanical grinding machine to produce *Azanza garckeana* fruit meal. The ground *Azanza garckeana* fruit meal were used as the test ingredients for the experiment. **The ground** *A. garckeana* fruit meal were mixed with the feed and offered to the rabbit bucks daily between 7.00am and 8.00am local time.

EXPERIMENTAL ANIMALS AND MANAGEMENT

A total number of 60 growing New Zealand white rabbit bucks aged 2-3 months were used for this experiment. The rabbits were purchased from a rabbit farms in in Uyo, Akwa Ibom State, and Umuahia, Abia state, Nigeria. Prior to the arrival, hutches were washed and disinfected. A quarantine period of 2 weeks pre- experimental trial were allowed during which the animals were treated against ecto and endo-parasite using Ivomectin and Levamisole (0.1ml/kg body weight), respectively. On arrival, the bucks were weighed. The 60 experimental bucks were divided into 4 treatments groups and replicated 3 times. Care were taken when placing the rabbits into treatment groups in order to balance the groups such that there were no significant differences between the rabbits on the basis of age and weight. The experimental rabbits were kept in single colony hutches for ease of identification throughout the experimental period. Experimental diets and clean water were offered to the rabbits *ad-libitum.* All routine management practices were strictly adhered throughout the experimental period. All weight measurements were taken with a 5kg digital weighing scale (Camry EK 5055 Digital Scale) of 0.01 sensitivity. The field work lasted for 16 weeks.

EXPERIMENTAL DESIGN

Phase 1: *Azanza garckeana* fruit meal administration Phase

This experimental phase lasted for 8 weeks. In this phase, the ground *Azanza garckeana* fruit were given between 7.00 and 8.00am local time daily for 8 weeks to the rabbit bucks. The ground *Azanza garckeana* fruit meal were mixed with the feed and offered to the rabbit bucks daily. At the end of this phase, data were collected, evaluated, and analyzed. The compositions of the experimental diets for the Direct and residual phase are presented in Table I.

This experimental phase lasted for another 8 weeks. In this phase, no ground *Azanza garckeana* fruit meal were administered. These were done to examine for possible side effects or residual effects of the test ingredients after the first 8 weeks administration of the test ingredients. At the end of this phase, data were collected, evaluated, and analyzed. The compositions of the experimental diets for the residual phase of the experiment are presented in Table II.

The design for the study was a completely randomized design (CRD) experiment with 4 treatments consisting of T_1, T_2, T_3 and T_4 respectively. T_1 served as the control. Fifteen (15) growing rabbits bucks were randomly assigned to each treatment, balanced in weights and replicated 3 times, with 5

Table 1: **Gross Composition and Calculated Nutrients of Experimental Diets for Rabbit Bucks in Phase 1**

Ingredients	**T_1**	**T_2**	**T_3**	**T_4**
Maize	44.94	44.94	44.94	44.94
Soya beans	17.31	17.31	17.31	17.31
Rice husk	32.00	32.00	32.00	32.00
Fishmeal	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00
Limestone	2.00	2.00	2.00	2.00
Vit/min Premix*	0.25	0.25	0.25	0.25
Salt	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Ground *Azanza garckeana* (g/kg feed)	0.00	100.00	200.00	300.00
Calculated nutrients				
Crude Protein (%)	17.00	17.00	17.00	17.00
Metabolizable Energy (ME) (Kcal/kg diet)	2505.42	2505.42	2505.42	2505.42
Crude fiber (%)	11.36	11.36	11.36	11.36
Lysine (%)	0.514	0.514	0.514	0.514
Methionine (%)	0.199	0.199	0.199	0.199

*Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg.

Table II. **Gross Composition and Calculated Nutrients of Experimental Diets for Rabbit Bucks in Phase 2**

Ingredients	**T_1**	**T_2**	**T_3**	**T_4**
Maize	44.94	44.94	44.94	44.94
Soya beans	17.31	17.31	17.31	17.31
Rice husk	32.00	32.00	32.00	32.00
Fishmeal	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00
Limestone	2.00	2.00	2.00	2.00
Vit/min Premix*	0.25	0.25	0.25	0.25
Salt	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated nutrients				
Crude Protein (%)	17.00	17.00	17.00	17.00
Metabolizable Energy (ME) (Kcal/kg diet)	2505.42	2505.42	2505.42	2505.42
Crude fiber (%)	11.36	11.36	11.36	11.36
Lysine (%)	0.514	0.514	0.514	0.514
Methionine (%)	0.199	0.199	0.199	0.199

*Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg.

rabbits per replicate. The ages of the rabbits were between 2-3 months. The rabbits in T_1 (Control) were given no *Azanza*

The residual effects of *Azanza garckeana* fruit meal on the *garckeana* fruit meal. Each rabbit in T_2 were given 100g/kg of *Azanza garckeana* fruit meal per kilogram of feed, the rabbits in T_3 were given 200g/kg of *Azanza garckeana* fruit meal per kilogram of feed, and T_4 rabbits were given 300g/kg of *Azanza garckeana* fruit meal per kilogram of feed. The *Azanza garckeana* fruit meal were mixed with the feed and offered to the rabbit bucks between 7.00am and 8.00am local time daily.

The experimental model were as follows:

$\mathbf{Y_{ij} = \mu + Ti + e_{ij}}$

Where:

$\mathbf{Y_{ij}}$ = individual observation on the rabbit characteristics.

$\boldsymbol{\mu}$ = overall mean

$\mathbf{T_i}$ = treatment effect

$\mathbf{e_{ij}}$ **= Experimental error.**

DATA COLLECTION AND EVALUATION

WEIGHT DETERMINATION

At the end of each phase of the experiment, 3 rabbit bucks randomly sampled from each replicate were used to determine the internal organ dynamics. The rabbits were weighed before and after slaughtering. The rabbits were eviscerated and all the internal organs removed and weighed. The Heart, Kidney, Liver, Lungs, Spleen, Stomach, small and large Intestine were carefully trimmed of adhering fats and weighed using a 5kg digital weighing scale (Camry EK 5055 Digital Scale) of 0.01 sensitivity. Thereafter, the tissues of the liver and kidney were collected for histological studies.

.INTERNAL ORGAN DYNAMICS

At the end of the experiment, organ histology was conducted at the Histopathology laboratory of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The tissues of the kidney and liver were processed for histology. The tissues of these organs for histological study were embedded in paraffin wax, dehydrated in an alcoholic solution of different concentration. Clearing and impregnation were done using xylene and paraffin wax respectively. The tissue were cut (Sectioned) using a microtome (Rotary Kepee Model KD 202A), stained with hematoxylin and eosin; and examined using a light microscope of different magnification according to the procedure described by Bancroft & Layton, (2013) and Oguike, *et al*., (2019) for histological studies. The slides were examined for histological indicators to observe for possible degenerative changes on the tissues structure using a microscope connected to a computer system. A photomicrographic software - Phoenix Micro Image Analysis (2003) version 1.33 were used to project the slides on the computer for clear assessment. The slides were subsequently captured and printed for interpretation and documentation.

HISTOLOGICAL STUDY

At the end of the experiment, organ histology was conducted at the Histopathology laboratory of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The tissues of the kidney and liver were processed for histology. The tissues of these organs for histological study were embedded in paraffin wax, dehydrated in an alcoholic solution of different concentration. Clearing and impregnation were done using xylene and paraffin wax respectively. The tissue were cut (Sectioned) using a microtome (Rotary Kepee Model KD 202A), stained with hematoxylin and eosin; and examined using a light microscope of different magnification according to the procedure described by Bancroft & Layton, (2013) and Oguike, *et al*., (2019) for histological studies. The slides were examined for histological indicators to observe for possible degenerative changes on the tissues structure using a microscope connected to a computer system. A photomicrographic software - Phoenix Micro Image Analysis (2003) version 1.33 were used to project the slides on the computer for clear assessment. The slides were subsequently captured and printed for interpretation and documentation.

STATISTICAL ANALYSIS

Data collected on the different parameters were subjected to Analysis of Variance (ANOVA) using a software package (SPSS version 23). Significant means were separated using Duncan's Multiple Range Test at 5% level of significance (Duncan, 1955). All statistical analyses were in accordance with the methods of Steel and Torie, (1980).

RESULTS AND DISCUSSION

The direct effects of *Azanza garckeana* fruit meal on the internal organs of New Zealand white rabbit bucks are shown in Table III.

The result showed that no significant differences ($p>0.05$) were observed on the internal organ weights of the New Zealand white rabbit bucks following the supplementation of *Azanza garckeana* fruit meal in their diets. All the internal organs measured were statistically similar ($p>0.05$) with the control group. This indicated that the *Azanza garckeana* fruit meal up to 300g/kg in the diets of New Zealand white rabbit bucks did not have any detrimental direct effects on the internal organ weights of the rabbit bucks.

The residual effects of *Azanza garckeana* fruit meal on the internal organs of New Zealand white rabbit bucks are shown in Table IV. The result in this table showed that no

Table 3: Internal organ weights of New Zealand White rabbit bucks administered Azanza garckeana fruit meal

Parameter (g)	T_1 (0.0g/kg)	T_2 (100g/kg)	T_3 (200g/kg)	T_4 (300g/kg)	SEM
Heart	3.08	3.80	3.57	3.70	0.11
Kidney	8.57	9.30	8.87	8.03	0.40
Liver	38.00	39.67	36.33	36.73	2.27
Lungs	8.97	9.27	8.70	9.13	0.41
Spleen	0.77	0.72	0.77	0.90	0.05
Stomach	113.01	110.87	109.03	115.47	2.82
Small Intestine	52.40	53.13	49.33	47.63	2.01
Large Intestine	126.02	120.83	132.23	124.80	6.10

Table IV: Residual effects of *Azanza garckeana* fruit meal on the internal organ weights of New Zealand White rabbit bucks

Parameter (g)	T_1 (0.0g/kg)	T_2 (100g/kg)	T_3 (200g/kg)	T_4 (300g/kg)	SEM
Heart	4.00	3.83	4.23	4.10	0.08
Kidney	9.03	8.93	8.10	8.44	0.25
Liver	54.37	47.93	42.97	43.27	2.28
Lungs	8.69	7.62	8.20	9.30	0.40
Spleen	0.60	0.51	0.48	0.73	0.05
Stomach	104.37	100.67	115.40	104.82	3.08
Small Intestine	39.73	48.31	48.83	45.71	1.80
Large Intestine	124.63	139.50	134.97	133.60	4.03

significant differences ($p>0.05$) were also observed on the different internal organ weights of the New Zealand white rabbit bucks measured at the residual phase compared with the control group. They are all statistically similar ($p>0.05$) with the control group. This observation inferred that the different supplementation levels of *Azanza garckeana* fruit meal up to 300g/kg in the diets of the New Zealand white rabbit bucks did not have any residual effect on the internal organs weights of the rabbit bucks. The addition of the internal organs produces the overall body weight of an animal. Therefore, these results are contrary to the reports of Yussuf *et al*. (2023) who reported increase in body weight across the same three dosage treatments 100, 200 and 400 mg/Kg of *Azanza garckeana* fraction in a diabetes-induced dyslipidemia, hepatopathy, and nephropathy in rats. The difference is reasonable comparing species (rabbit versus ray) and physiological state of the experimental animal (normal versus induced-disease).

The direct and residual effects of *Azanza garckeana* fruit meal on the Histology of the liver of New Zealand white rabbit bucks are shown in **Plate I** and **Plate II** respectively.

Acute toxicity study was not done in this study based on results of previous studies that have shown *Azanza garckeana* was relatively safe and tolerable up to 5000 mg/kg body weight (Ochokwu et al., 2015, Itodo et al., 2023).

OVERALL OBSERVATION ON THE LIVER

The histology of the liver of the rabbits in T1 and T2 are normal. This means that the administration of *Azanza garckeana* fruit meal up to 100g/kg on the diets of New Zealand white rabbit bucks did not have any adverse effect on the histo-architecture of the liver. Moreover, the histology of the liver of the rabbit bucks in T3 and T4 were negatively affected by the administration of the test ingredients. The administration of *Azanza garckeana* fruitp meal at 200g/kg and 300g/kg on the diets of rabbit bucks showed a mild to widespread vacuolar degeneration of the hepatocytes. As such, the administration of *Azanza garckeana* fruit meal at 200g/kg and 300g/kg impacted negatively on the histo-architecture of the liver. Furthermore, the supplementation of *Azanza garckeana* fruit meal at 100g/kg, 200g/kg and 300g/kg in the direct phase did not have any residual effect on the histo-architecture of the liver of the New Zealand white rabbit buck. All the histo-architecture of the liver on the control group and the treatment groups were all histologically normal.

The direct and residual effects of *Azanza garckeana* fruit meal on the Histology of the kidney of New Zealand white rabbit bucks are shown in **Plate III** and **Plate IV** respectively.

Previous studies have implied improvement in liver histomorphology by AG and melatonin in a bishephenol A induced changes of the organs ((Itodo et al., 2023).

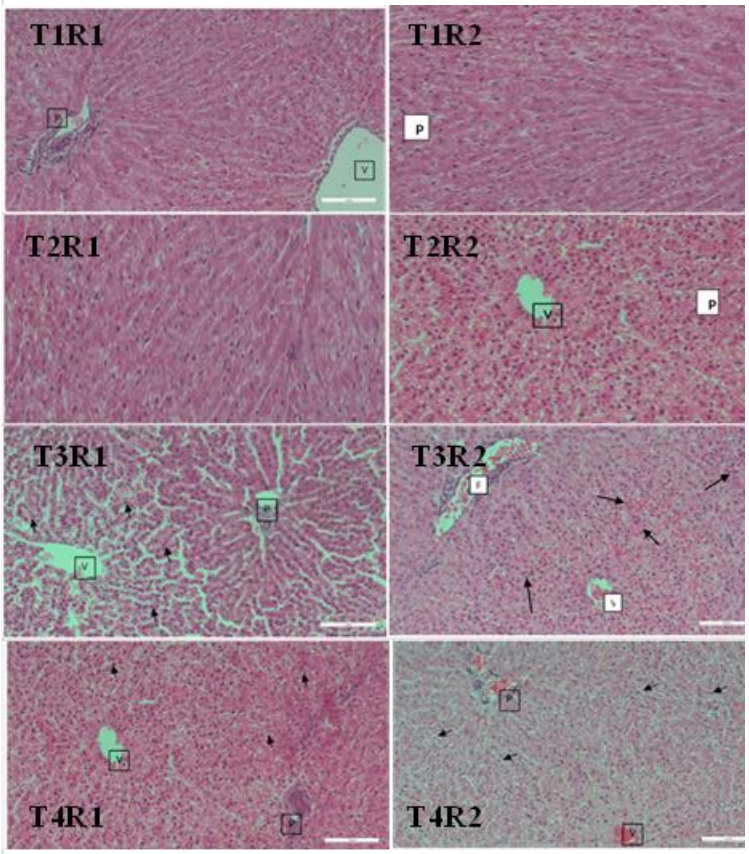

Plate I: Histology of the Liver of New Zealand white rabbit bucks administered *Azanza garckeana* fruit meal (H&E; ×400).

T1R1 & T1R2 LIVER: Sections of the liver presented in this group showed the normal hepatic histomorphology. The liver showed normal hepatocytes arranged in interconnecting cords around the central veins (V). Normal structures of the portal area (P) were also observed.

T2R1 & T2R3 LIVER: The sections of the liver presented in this group showed a normal hepatic histo-architecture. The liver showed normal hepatocytes arranged around the Central veins (V), with normal structures of the Portal area (P).

T3R1 LIVER: The sections of the liver presented in this group showed a mild to widespread vacuolar degeneration of the hepatocytes. The hepatocytes showed clear cytoplasmic vacuoles (arrow). Central vein (V); Portal area (P).

T3R2 LIVER: Sections of the liver presented in this group showed mild degeneration of the hepatocytes. The hepatocytes showed multiple tiny and clear cytoplasmic vacuoles (arrow). Central veins (V); Portal area (P).

T4R1 LIVER: The sections of the liver presented in this group showed a mild vacuolar degeneration of the hepatocytes in the centrilobular areas of the hepatic lobules and multiple tiny and clear cytoplasmic vacuoles (arrow). Central vein (V); Portal area (P) within the hepatocytes

T4R2 LIVER: The sections of the liver presented in this group showed a widespread vacuolar degeneration of the hepatocytes. The hepatocytes showed clear cytoplasmic vacuoles (arrow). Central vein (V); Portal area (P).

The rabbits in T_1 (Control) were given no *Azanza garckeana* fruit meal. Each rabbit in T_2 were given 100g/kg of *Azanza garckeana* fruit meal/ Kg of feed, the rabbits in T_3 were given 200g/kg of *Azanza garckeana* fruit meal per kilogram of feed, and T_4 rabbits were given 300g/kg of *Azanza garckeana* fruit meal/Kg of feed.

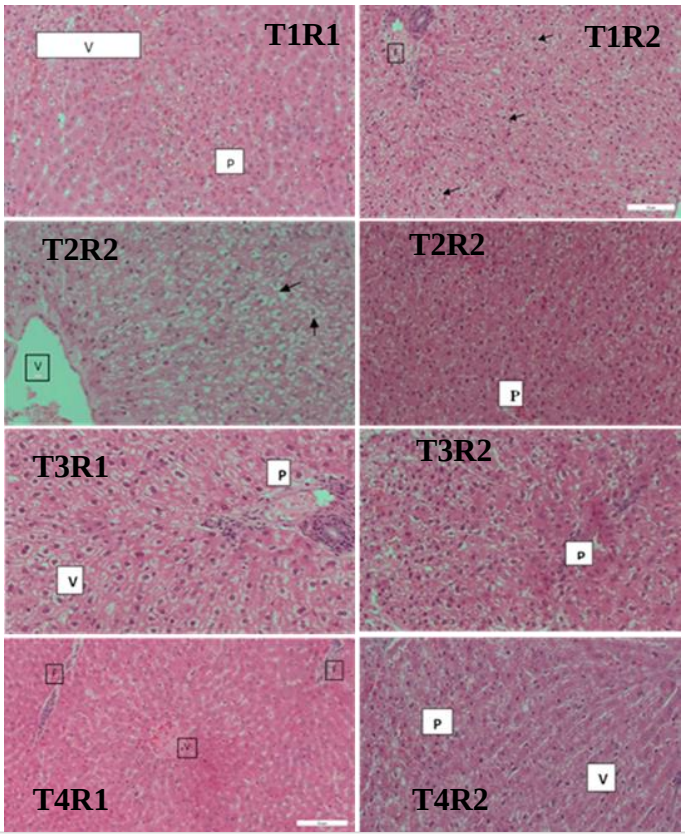

Plate II: Residual effects of *Azanza garckeana* fruit meal on the Histology of the Liver of New Zealand White rabbit bucks (H&E; ×400)

T1R1 & T1R2: Sections of the liver presented in this group showed normal hepatocytes, with normal central veins (V); and portal area (P); Hepatocytes (Black Arrows).

T2R2: The liver showed a normal hepatocyte, with normal structures of the Portal area (P).

T3R2 & T3R3: The liver presented in this group showed the normal hepatic histomorphology. The liver showed normal hepatocytes are arranged in interconnecting cords around the central veins (V); with Normal structures of the portal area (P).

T4R1 & T4R2: Sections of the liver presented in this group showed a normal hepatic histomorphology. The liver showed normal hepatocytes with normal Central veins (V) and portal area (P).

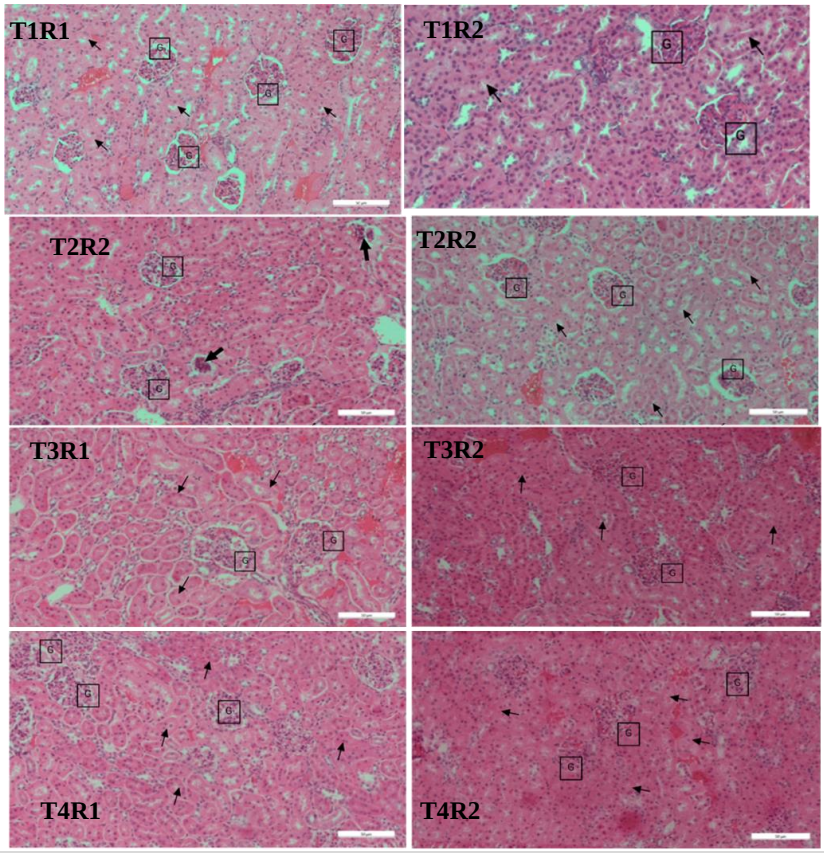

Plate III: Histology of the Kidney of New Zealand White rabbit bucks administered *Azanza garckeana* fruit meal (H&E; ×400)

T1R1 & T1R3 KIDNEY: Sections of the kidney presented in this group showed the normal renal histomorphology. Normal Glomeruli (G) in their respective Bowmans capsules were observed, surrounded by multiple normal renal tubules (arrow).

T2R1 & T2R3 KIDNEY: Sections of the kidney presented in this group showed the normal renal histomorphology. Normal Glomeruli (G) in their respective Bowman's capsules were observed. Normal renal tubules (arrow) were also observed.

T3R1 & T3R2 KIDNEY: Sections of the kidney presented in this group showed the normal renal histomorphology. Normal Glomeruli (G) in their respective Bowman's capsules were observed. Normal renal tubules (arrow) were also observed.

T4R1 & T4R2 KIDNEY: Sections of the kidney presented in this group showed the normal renal histomorphology. Normal Glomeruli (G) in their respective Bowman's capsules and normal renal tubules (arrow) were also observed.

The rabbits in T_1 (Control) were given no *Azanza garckeana* fruit meal. Each rabbit in T_2 were given 100g/kg of *Azanza garckeana* fruit meal per kilogram of feed, the rabbits in T_3 were given 200g/kg of *Azanza garckeana* fruit meal per kilogram of feed, and T_4 rabbits were given 300g/kg of *Azanza garckeana* fruit meal per kilogram of feed.

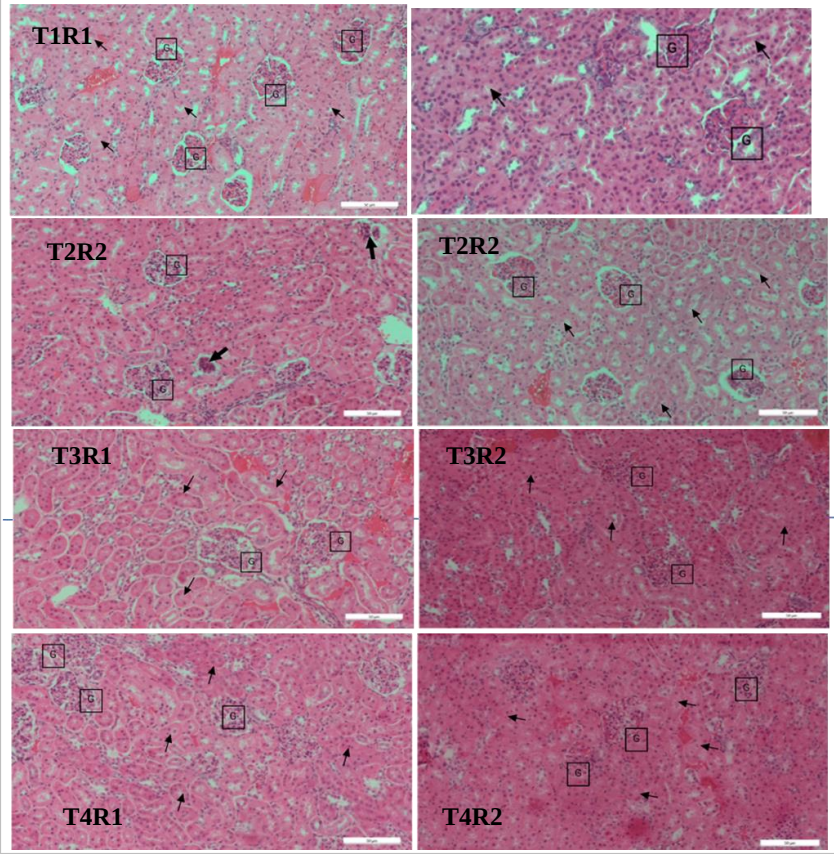

Plate IV: Residual effects of *Azanza garckeana* fruit meal on the Histology of the kidney of New Zealand White rabbit bucks. (H&E; ×400)

T1R1 & T1R3 KIDNEY: Sections of the kidney presented in this group showed the normal renal histomorphology. Normal Glomeruli (G) in their respective Bowmans capsules were observed, surrounded by multiple normal renal tubules (arrow).

T2R1 & T2R3 KIDNEY: Sections of the kidney presented in this group showed the normal renal histomorphology. Normal Glomeruli (G) in their respective Bowman's capsules were observed. Normal renal tubules (arrow) were also observed.

T3R1 & T3R2 KIDNEY: Sections of the kidney presented in this group showed the normal renal histomorphology. Normal Glomeruli (G) in their respective Bowman's capsules were observed. Normal renal tubules (arrow) were also observed.

T4R1 & T4R2 KIDNEY: Sections of the kidney presented in this group showed the normal renal histomorphology. Normal Glomeruli (G) in their respective Bowman's capsules were observed. Normal renal tubules (arrow) were also observed.

OVERALL OBSERVATION ON THE KIDNEY

The renal histology of the rabbit bucks from T1 to T4 are all normal. This suggests that the supplementation of *Azanza garckeana* fruit meal up to 300g/kg in the diets of New Zealand white rabbit bucks did not have any deleterious effect on the histo-architecture of the kidneys. Furthermore, the supplementation of *Azanza garckeana* fruit meal at 100g/kg, 200g/kg and 300g/kg in the direct phase did not have any residual effect on the histology of the kidney of all the rabbit bucks. All the renal histo-architecture of the control group and the treatment groups were all histologically normal. The non-detrimental effects of *Azanza garckeana* fruit meal up to 300g/kg in the diets at the direct and residual phase may have indicated that the varying supplementations of *Azanza garckeana* fruit meal in the diets were mild and moderate to the renal physiology of the rabbits. Also, the podocytes, glomeruli Bowmans capsules, renal tubules and nephrons of the kidneys were not affected; suggesting that the liver may have efficiently handled any detoxification and the toxic substances did not escape the hepatocytes and infiltrate to the kidney. These findings in the present study agrees favourably with the reports of Itodo *et al.* (2023) who documented nephro-restorative effects of methanolic extracts of *Azanza garckeana* fruit pulp and melatonin at higher doses on New Zealand rabbit bucks; and Yusuf *et al*. (2023) on the renal protective, nephropathy and hepatopathy of diabetic rats.

CONCLUSION

Based on the results and observations from this study, it is concluded that the supplementations of *Azanza garckeana* fruit meal at 100g/kg, 200g/kg and 300g/kg in the diets of New Zealand white rabbit bucks did not have any direct or residual effect on the internal organ dynamics of the rabbit bucks. The histology of the kidney and duodenum in all the treatment groups were all normal. Furthermore, the supplementation of *Azanza garckeana* fruit meal at 200g/kg and 300g/kg had adverse effects on the liver, impacting negatively on the histo-architecture of the liver. It showed widespread vascular degenerations on the hepatocytes. On the residual phase, *Azanza garckeana* fruit meal up to 300g/kg in the diets did not have any residual effects on the histo-architecture of the liver and kidney.

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REFERENCES

Ahmed, R.H., El Hassan M.S. & El Hadi, H.M. (2016). Potential capability of *Azanza garckeana* fruits aqueous extract on enhancement of iron absorption in Wistar albino rats. *International Journal of Advanced Research in Biological Sciences*, 3, 245-250.

Alfred, M. (2017). *Azanza garckeana* Fruit Tree: Phytochemistry, Pharmacology, Nutritional and Primary Healthcare applications as Herbal medicine: A Review. *Research Journal of Medicinal Plants*, 11, 115 - 123.

Amuri, B., Maseho, M., Simbi, L., Okusa, P. Duez P. & Byanga, K. (2017). Hypoglycemic and antihyperglycemic activities of nine medicinal herbs used as antidiabetic in the region of Lubumbashi (DR Congo). *Phytotherapy Research.,* 31, 1029-1033.

Ansari R.A., Rabiu K.M., Ayuba V., & Omolabake O.O. (2020). Review on *Jatropha tanjorensis* (Hospital too far); significance as an anti-anaemia plant. *International blood research and reviews,* 11(4), 1–7.

Bancroft, J. D., & Layton, C. (2013). The Hematoxylin and eosin. In: Theory & Practice of histological techniques. In S. K. Suvarna, C. Layton, & J. D. Bancroft (Eds., 7th ed., Ch. 10 and 11, pp. 179-220). Philadelphia: Churchill Livingstone of El Sevier.

Dikko, Y.J., Khan, M.E., Tor-Anyiin, T.A., Anyam J.V. & Linus, U.A. (2016). In vitro antimicrobial activity of fruit pulp extracts of *Azanza garckeana* (F. Hoffm.) Exell and Hillc. and isolation of one of its active principles, Betulinic acid. *British Journal of Pharmaceutical Research*, 14, 1-10.

Duncan, D. B. (1955). Multiple Range and Multiple f-test. Biometrics 11, 1-2.

Ekuma, B. O., Amaduruonye, W., Onunkwo, D. N. & Herbert, U. (2017). Influence of garlic (*Allium sativum*) and vitamin E on semen characteristics, Reproductive performance and histopathology of rabbit bucks. *Nigerian Journal of Animal Production,* 44(3), 117 – 128.

Emenalom, O. O., Esonu, B. O., Etuk, E. O. & Anaba, C. (2009). Effect of *Mucuna Pruriens* (Velvet Bean) leaf meal on performance and Blood Composition of Finisher Broiler Chickens. *Nigerian Journal of Animal Production*, 36 (1), 52-60.

Esther, K.I., Kleph, K.Z., Mariette K.K., Sylvie Y.N. & Andre, W.O.T. (2017). Plants used by pregnant women at Kipushi city in democratic republic of Congo: Prevalence and indications. *Open Access Library Journal*, Vol. 4. 10.4236/oalib.1103390.

Frandson, R. D., Lee, W. W., & Anna, D. F. (2018). Anatomy of the Digestive System. In: Anatomy and physiology of farm animals, 8th edition, Wiley Blackwell publishers. Pp. 335-391.

Herbert, U. & Acha, C.O. (1995). Preliminary observations on the seminal characteristics of rabbits raised in two locations in south-Eastern Nigeria. *Nigerian Journal of Animal Production*, 22, 108-112.

Herbert, U. (1998). Reproductive performance of rabbit does fed diets containing *Leaucaena leucocephala* and *Gliricidia sepium* leaf meal from conception through weaning of kits. *Nigerian Journal of Animal Production*, 25(1), 163-168.

Jacob, C., Shehu, Z, Danbature, W.L. & Karu, E. (2016). Proximate analysis of the fruit *Azanza garckeana* ("Goron Tula"). *Bayero Journal of pure and applied sciences*, 9(2), 221 – 224.

Itodo, J. I., Jolayemi, K. O., Sani, D., Ocheja, J. O., Ibrahim, S. & Ojeamiren, M. T. (2023). Effects of Azanza garckeana and melatonin on serum biochemical and histopathological changes induced by chronic bisphenol-A administration in New Zealand rabbit bucks. *Emerging Animal Species*, *9*, 100031.

Jiwuba, P.C., Amaduruonye, W.N., & Akazue, R.C. (2020). Effect of Gmelina leaf meal diets on productive and physiological parameters of West African dwarf goats. *Agricultural Science and Technology*, 12(4), 318-323.

Jiwuba, P.C., Ilo S.U., Amaduruonye, W., Azodo, N.L., & Uzoma, C. (2021). Blood Profile of Growing Rabbits Fed Pro-Vitamin A Fortified Cassava Peel Meal Based Diets. *Agricultura*, 18, No 1-2: 1-8.

Malik, A. A., Busari, L. B., Idundun, S. O., Adebowale, Z. A. & Okoye, A. O. (2022). Effect of feeding *Jatropha tanjorensis* leaf meal as a growth promoter on the performance, carcass characteristics and sensory properties of broiler chicken. *Nigerian Journal of Animal Production*, 49(2), 140-149.

Mshelia, E.H., Watirahyel, E.M., Maigari, A.U., Yohanna C. & Ismail, F. (2016). Cytotoxicity and antioxidant activity of stem bark extracts of *Azanza garckeana* (kola of Tula). *European Journal of Pure and Applied Chemistry*, 3, 16-24.

Nathaniel, J., Akinsola, K.L., Ibrahim, Y., Amaduruonye, W., Gabriel, G.O. & Enwereji, A. V. (2023). Performance and Carcass Characteristics of Rabbit Bucks Fed Diets Containing Varying Levels of Tiger Nut Meal. *Nigerian Journal of Animal Science and Technology*, 6(3), 94 -107.

NRCRI. (2018). Agro-meteorological Unit, National Root Crop Research Institute, Umudike, Umuahia, Nigeria.

Oguike, M. A., Onuta, S. C., Amaduruonye, W., & Akpan, I. Ochokwu, I.J., Onyia, L.U., & Ajijola, K.O. (2014). Effect of *Azanza garckeana* (Goron Tula) Pulp Meal Inclusion on Growth Performance of *Clarias gariepinus* Broodstock. .*Nigeria Journal of Tropical Agriculture*, 14, 134 - 146.

Ochokwu, I. J., Dasuki, A., & Oshoke, J. O. (2015). Azanza arckeana (Goron Tula) as an edible indigenous fruit in North Eastern Part of Nigeria. *Journal of Biology, Agericulture and Healthcare*, *5*(15), 26-31U. (2019). Impact of *Aspilia africana* on Semen and Testicular Characteristics of Rabbit Bucks. *Journal of Advanced Agricultural Technologies*, 6(2), 144 -149.

Onunkwo, D.N., Nathaniel, J., Ezike, J.C., Amaduruonye, W., & Daniel-Igwe, G. (2019). Effects of garlic rhizome (*Allium sativum*) powder supplemented diets on the hematology and serum biochemistry of broiler chickens at starter and finisher phases. *Nigerian Journal of Animal Production*, 46(1), 149 – 156.

NRCRI (2010). Agro-meteorological Unit, National Root Crop Research Institute, Umudike, Umuahia, Nigeria Ochokwu, I.J., Onyia, L.U., and Ajijola, K.O., (2014). Effect of *Azanza garckeana* (Goron Tula) Pulp Meal Inclusion on Growth Performance of *Clarias gariepinus* Broodstock. .*Nigeria Journal of Tropical Agriculture*, 14: 134-146.

Steel R.D. and Torie, J.H., (1980). Principles and Proceedings of Statistics. A Biochemical Approach, 2nd ed., New York. USA: McGraw-Hill Book co. Inc.

Yusuf, A.A., Lawal, B., Alozieuwa, U.B., Onikanni AS, Lukman, H.Y., Fadaka, A.O., Olawale, F., Osuji, O, Sani, S., Owolabi, M.S., Adewuyi, A.H., Yusuf, D.H., Batiha, G.E., Ataya, F.S., Fouad, D. (2023). Attenuating effects of *Azanza garckeana* fractions on glycemo-impaired-associated dyslipidemia, hepatopathy, and nephropathy. *American Journal of Translational Research*, 15(10), 5997-6014.