HISTOPATHOLOGY OF WISTAR ALBINO RATS FED RAW AND PROCESSED *COLA ROSTRATA* (MONKEY COLA) SEED MEAL

CHRISTOPHER, Grace Idiong, EKPO, Joseph Sylvester, USORO, Ofoninyene Okon and OKON, Utibe Mfon

Department of Animal Science, Akwa Ibom State University, Obio Akpa Campus, Oruk Anam Local Government Area, Akwa Ibom State, Nigeria.

Corresponding Author: Christopher, G. I. Department of Animal Science, Akwa Ibom State University, Obio Akpa Campus, Oruk Anam Local Government Area, Akwa Ibom State, Nigeria. **Email:** graciouschristopher@yahoo.com **Phone**: +234 803 938 0371

***Received** March 22, 2024; **Revised** May 19, 2024; **Accepted** May 24, 2024*

ABSTRACT

Anti-nutritional contents of feedstuffs have deleterious effects on human/animal nutrition. Reducing the anti-nutritional content of these feedstuffs and enhancing their use in feed formulation, requires appropriate processing methods. An experiment was carried out to determine the effect of feeding processed and raw Cola rostrata (monkey cola) seed meal (CRSM) on the histopathology of some organs (liver, kidney, duodenum, jejunum and ileum), of male albino rats. The CRSM was processed using four different methods i.e. boiling only, boiling and fermenting, fermenting only, and toasting. Six experimental diets were formulated with diet T_1 (Control) containing 0% CRSM, while diets T_2, T_3, T_4, T_5 and T_6 contained, boiled, boiled and fermented, fermented, toasted and raw CRSM respectively. 90 male rats (aged, 6 – 7 weeks), were divided into six dietary treatment groups of five animals triplicated in a Completely Randomized Design (CRD). The experiment lasted for 21 days and the data collected were subjected to Analysis of Variance (ANOVA) and means separated using the Duncan's Multiple Range Test (DMRT). The results indicated that rats on T_1 and T_2 recorded no noticeable defects or abnormalities in all the organs observed. However, focal segmental sclerosis in the kidney, hepatocellular necrosis of the liver, hypercellularity and disorganisation of the mucosal glandular tissues were observed in rats from the other treatments (T_6, T_3, T_4, and T_5). It can be concluded that C. rostrata seed boiled for at least 30 minutes can be used as a feed ingredient in animal diets without any deleterious effect on their organs.

Keywords: *Cola rostrata,* Histopathology, Processed, Raw, Seed meal*,* Wistar rats

INTRODUCTION

Due to the persistently high cost of animal protein sources (meat and eggs) being a consequence of increasing prices of animal feedstuffs worldwide, considerable efforts have been put up by researchers, to identify alternative low-cost and readily available non-conventional livestock feedstuffs that could substitute for the conventional ones, especially maize (which is in serious competition as food for man feed for animals) (Christopher *et al.*, 2007). One such non-conventional feedstuff which has the potential to replace maize in livestock diets is *Cola rostrata* K. Schum (Malvales: Malvaceae) (monkey cola) seed meal.

The mesocarp of monkey cola is widely consumed in the southern parts of Nigeria and Akwa Ibom State in particular as ordinary fruits, while the seeds are often thrown away (Dunn and Agom, 1992; Christopher *et al.*, 2020). As reported by Dosumu and Eka (1989) and Christopher *et al.* (2019), the seed has 83.42% starch and therefore becomes a potential energy source in animal diets. However, due to the anti-nutrients found in it, its usage in animal feed formulation appears limited (Dosumu and Eka, 1989).

Savage (2002) reported that anti-nutritional contents of feedstuffs have deleterious effects on human/animal nutrition as it aids the risk of renal calcium absorption, causing corrosive gastroenteritis, renal damage as well as the formation of kidney stones. Therefore, using appropriate methods of processing could help in reducing the anti-nutritional contents of the feed material and enhance its usage in animal feed formulation. Studies by several authors indicated that boiling, toasting and fermenting of seeds lowered their anti-nutritional contents while increasing their crude protein (CP), nitrogen-free extract (NFE) and crude fat (CF) contents (Agiang *et al.,* 2010; Adegunwa *et al.,* 2012). The significance of evaluating the nutrient content of feed with animal assays as compared with alternative methods of analysis (i.e. laboratory chemical or microbiological) is very important. Researchers use animal feeding trials to test whether their products are safe for animal and human consumption (Olowu and Yaman, 2019). It is imperative to determine the safety of new feed material for humans and animals using the appropriate toxicity test, approved by government regulatory agencies such as the National Agency for Food and Drug Administration Control (NAFDAC), United States Food and Drug Administration (USFDA), among others. This necessitates the use of species such as albino rats, and mice that can be compared with humans with regards to metabolism, excretion, absorption and systemic transport of the test materials (Weil, 1972; Floerl *et al.*, 2020).

Albino rat is predominantly used in toxicological/pharmacological research because there are many similarities between rats and human metabolic pathways. More so, many anatomical and physiological characteristics of rats and humans are similar and therefore allow for comparison in terms of absorption, excretion and distribution (Freireich *et al.,* 1966).

Processing of *C. rostrata* seed as a potential alternative and non-conventional animal feedstuffs has received little research attention and it is for this reason, that this study was carried out to determine the effect of feeding the raw and processed (boiled only, boiled /fermented, fermented, and toasted) seed meal on the histopathology of some organs (liver, kidney, duodenum, jejunum and ileum) of male Wistar albino rats.

MATERIALS AND METHODS

Experimental Sites: The experiment was conducted at the Department of Animal Science Post Graduate Laboratory, Akwa Ibom State University, Obio-Akpa Campus, Oruk Anam Local Government Area, Akwa Ibom State, Nigeria. Obio Akpa is located within the southern zone of Nigeria at a Latitude of 4^{O}50N of the Equator and a Longitude of 7^{O}45E and 7^{O} 55E of the Greenwich Meridian. The area is in the hot humid tropics with a climate characterized by two seasons (rainy and dry seasons). The rainy season spans between April and October while the dry season spans between November and March. Temperatures are uniformly high throughout the year ranging between 26 and 28^{O}C. Solar radiation ranges from 4.11 to 4.95 mm, partly because of the high values of insulation and temperature (Christopher *et al.*, 2020).

The monkey cola seeds used in this experiment were obtained from Obio Akpa in Oruk Anam Local Government Area (LGA) in Akwa Ibom State, Nigeria. The feeding trial experiment of the rats was carried out in the Department of Animal Science, Faculty of Agriculture in the Akwa Ibom University Obio Akpa campus, while the histological studies to ascertain the effect of the seeds on the animal's organs were conducted in the Histopathological Laboratory of the University of Uyo Teaching Hospital, Akwa Ibom State, Nigeria.

Sample Preparation: Ten (10) Kg of mature *C. rostrata* seeds (yellow variety) used in this experiment were washed in clean tap water to remove dirt, chopped into sizeable chips, and sun-dried. The toxicity of the dried *C. rostrata* seed chips adapted from Odion *et al.* (2013) indicated that the seed was not toxic to rats. These chips were then divided into five batches.

The first batch was the raw sample, while the second batch was boiled for 30 minutes in water that was pre-heated to a temperature of 100^0C. The third batch was toasted (with sand that was pre-heated to 100^0C) for 30 minutes. The fourth batch was boiled at 100^0C for 20 minutes and then fermented for two days using tap water at room temperature, while the fifth batch was the raw seed fermented in plastic containers for three days using tap water at room temperature. The thermally processed seeds (boiled and toasted) were spread on a clean corrugated iron roofing sheet to cool. Thereafter, the raw and all the processed portions were sun-dried, milled and stored for feed formulation.

Feed Formulation: A total of six experimental diets were formulated as shown in Table 1. Diet 1 (T_1) which served as control was prepared without monkey cola seed meal but with maize as the major source of energy. Diet 2 (T_2) was prepared with the boiled monkey cola seed meal as the major energy source. Diet 3 (T_3) was formulated with boiled and fermented *C. rostrata* seed meal as the major energy source and Diet 4 (T_4) was prepared with fermented *C. rostrata* seed meal. Diets 5 (T_5) and 6 (T_6) were prepared with toasted and raw *C. rostrata* seed meals respectively. The protein sources used in the formulation were soybean and fish meals. All dietary ingredients and the formulated diets were assayed for their proximate composition (AOAC, 2000). All the diets were formulated to furnish 10% CP required by rats for optimum growth.

Experimental Design: 90 male albino rats, aged 6 – 7 weeks, were used in the 21-day feeding trial. The rats were allotted separately to each experimental diet in a completely randomized design (CRD) and each treatment had three replicates with five rats each. The rats were housed in stainless steel metabolic cages and had access to clean water and treatment diets *ad libitum*.

Histopathological Examination: The histological study was carried out to ascertain the effect of the diets on the major organs of the animal using two rats per treatment. At the end of the 21-day feeding trial, a rat per replicate was randomly selected and humanely sacrificed under chloroform fume anaesthesia, the rats were dissected and the liver, kidney, small and large intestines (duodenum, jejunum and ileum) were removed, rinsed with normal saline solution to remove adhering blood, and fixed in 10% buffered formalin for subsequent histopathological examination. The fixed samples were dehydrated in 70% to absolute alcohol, cleared in xylene, infiltrated with molten wax, embedded in paraffin wax, sectioned and stained with Haematoxylin and Eosin stain (Drury *et al.,* 1967; Bancroft and Gamble, 2008). The stained tissues sectioned were examined under a light microscope as described by Helena *et al.* (2020). Cellular details, tissue organization and pathological changes commonly specific to rat tissues were observed and documented. Photomicrographs were taken to observe these features and compare them with the normal rat tissue histology.

Histopathological Data Analysis: The observed histological features were interpreted in the context of the normal rat's health and histopathological conditions (Treuting *et al.,* 2017). The presence of abnormalities, inflammation, necrosis, and other pathological changes were identified and characterized (Treuting *et al.,* 2017). The histopathological results obtained were quantified and compared with standard rats' histological atlases (Treuting *et al.,* 2017; Helena *et al.,* 2020) to establish the effects of monkey cola seed meal on the histology of albino rats.

RESULTS AND DISCUSSION

Histopathology of Experimental Rats: Although no mortality was recorded throughout the trial period, the histopathological examination results as presented in Figures 1 – 5 and summarized in Table 2, indicated abnormalities in some organs of the rats that were fed with raw, fermented and toasted *Cola rostrata* seed meals. The organs examined were the liver, kidney, duodenum, jejunum and the ileum. In the control (T_1) and boiled seeds (T_2) treatment groups, there were no noticeable defects or abnormalities in all the organs observed.

Table 1: Composition of experimental diets fed to the albino rats

Ingredients	Processing methods and treatments					
	Control	Boiled	Boiled and fermented	Fermented	Toasted	Raw
Maize	91.49	0.00	0.00	0.00	0.00	0.00
Cola rostrata	0.00	88.26	87.90	88.04	87.90	87.79
Soybean	1.41	3.99	4.28	4.17	4.28	4.37
Fish meal	0.35	1.00	1.07	1.04	1.07	1.09
Palm oil	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix*	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated nutrients						
Crude protein (%)	10.00	10.00	10.00	9.99	10.00	10.00
Metabolizable energy (Kcal/g)	3239.90	3256.52	3177.48	3177.14	3194.50	3175.04
Analysed nutrients (%)						
Crude protein	10.09	10.02	9.95	9.97	10.08	9.91
Ether extract	2.18	3.11	2.98	2.90	3.02	2.83
Crude fibre	1.36	1.08	1.27	1.16	1.33	1.87
Calcium	0.97	1.07	0.99	0.95	0.86	0.94
Phosphorus	2.04	2.57	2.31	1.93	2.84	2.15
Nitrogen free extract	77.19	78.93	76.18	76.04	75.86	75.73
Metabolizable energy (Kcal/g)	3078.90	3427.16	3316.30	3305.53	3313.03	3286.58

**Each 2.5kg of grower vitamins and mineral premix contains 800,000 IU of Vitamin A, 1600,000 IU of Vitamin D3; 5,000 IU of Vitamin E; 2000 mg of Vitamin K; 1500 mg of B1; 4000 mg of B2; 80g of manganese, 50 g of Zinc; 20 g of Iron; 5 g of Copper, 15000 mg of Niacin; 10 mg of B12; 5000 mg of Pantothenic acid, 5000 mg of Folic acid, 20 mg of Biotin, 125 mg of Antioxidant; 200 g of Selenium; 200 mg of Cobalt and 200 mg of Choline chloride*

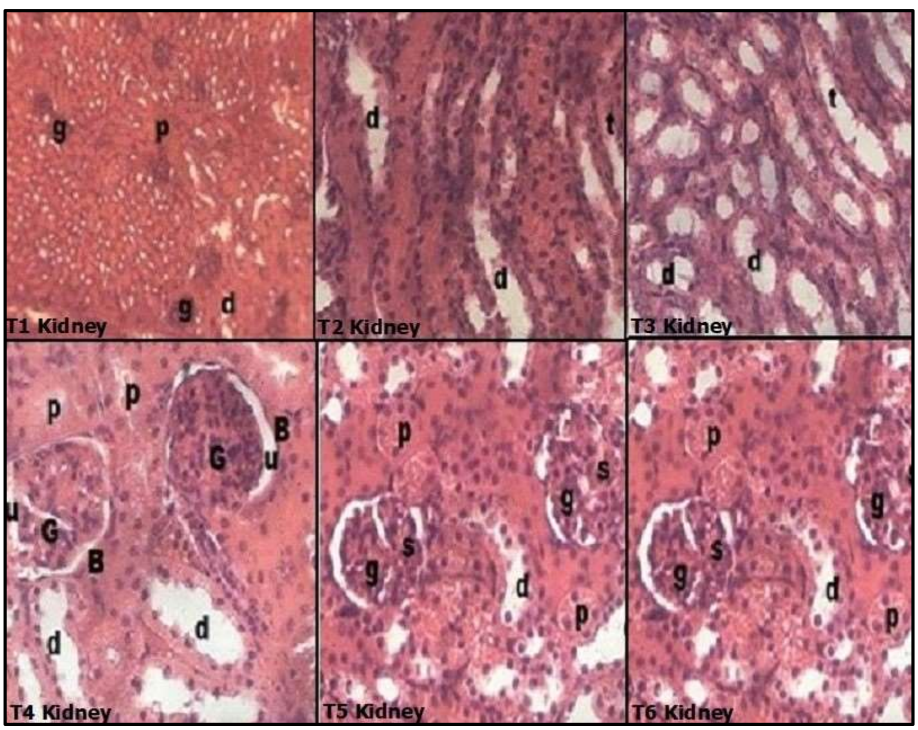

Figure 1: Photomicrographs of kidney cells of albino rats fed raw and processed *Cola rostrata* seed meal*. Key:* *showing the glomeruli (G, g), tubule (t), proximal tubule (p), distal tubule (d), Bowman's capsule (B), intervening urinary space (u), sclerosis (s) H&E, Mag. x400*

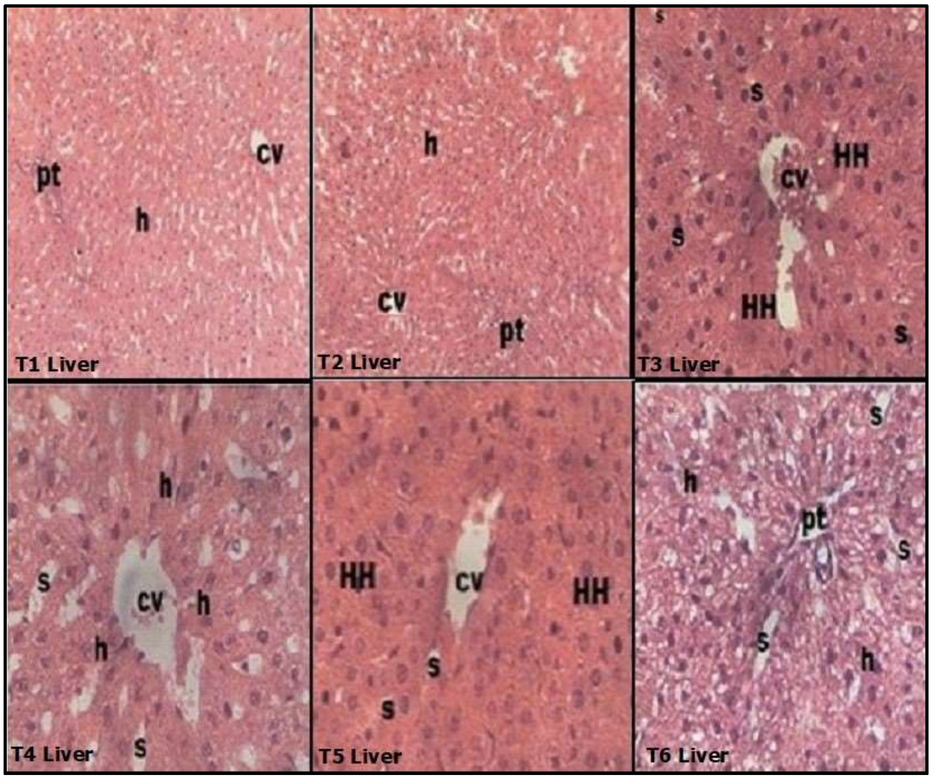

Figure 2: Photomicrographs of liver cells of albino rats fed raw and processed *Cola rostrata* seed meal. ***Key:*** *showing the central vein, (cv), portal tract (pt), and hepatocellular hypertrophy (HH) with some obliteration of the sinusoids (s) in some treatments* T_3, T_4, T_5 *and* T_6. *H&E, Mag. x400*

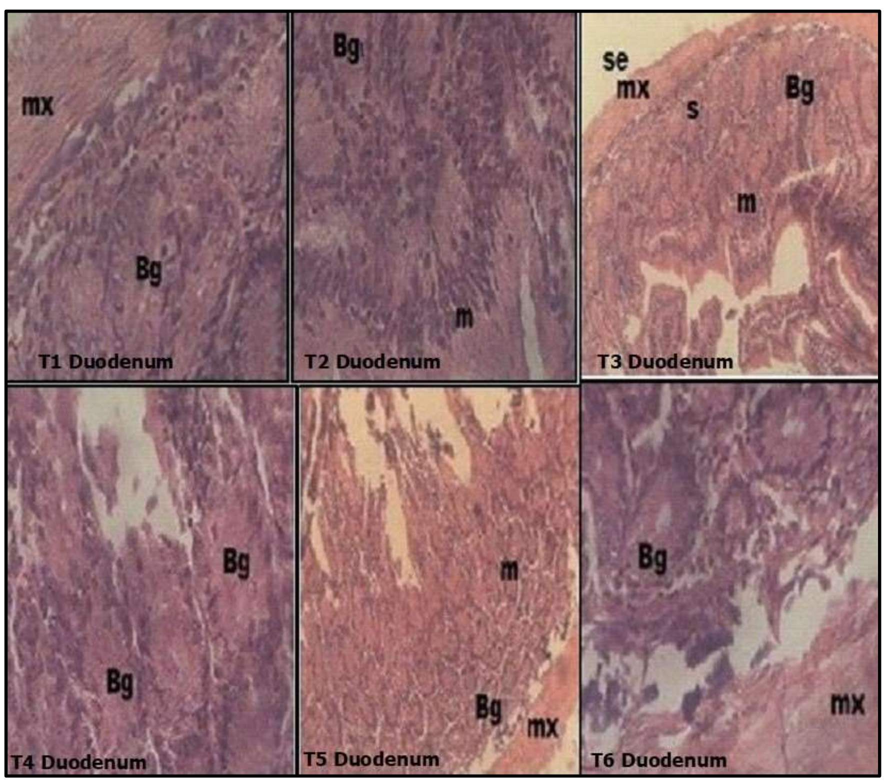

Figure 3: Photomicrographs of the duodenum of albino rats fed raw and processed *Cola rostrata* seed meal. ***Key:*** *showing Brunner glands. (Bg), muscularis externa (mx), mucosa (m) submucosa (s), serosa (se), H&E, Mag. x400*

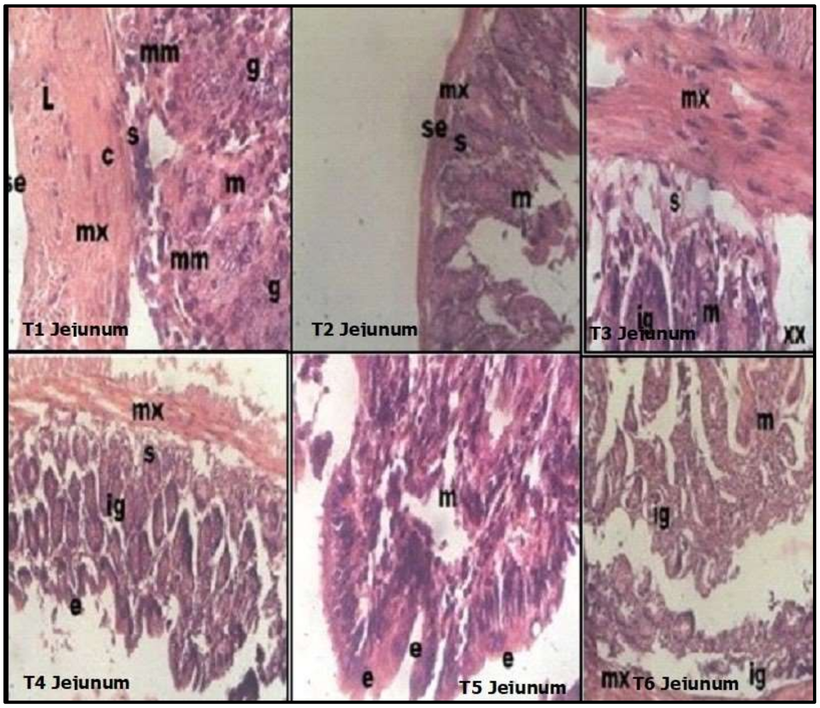

Figure 4: Photomicrographs of the Jejunum of albino rats fed raw and processed *Cola rostrata* seed meal. ***Key:*** *showing the serosa (se) muscularis externa (mx) comprising of the inner circular cell (c) and outer longitudinal layer (L), the submucosa (s) and the inner part of the mucosa (m) which is seen to be atrophied in T5, the glandular epithelial cell (g), mild loss of mucosal tissues (xx), erosion of epithelial cell (e), H&E, Mag x400*

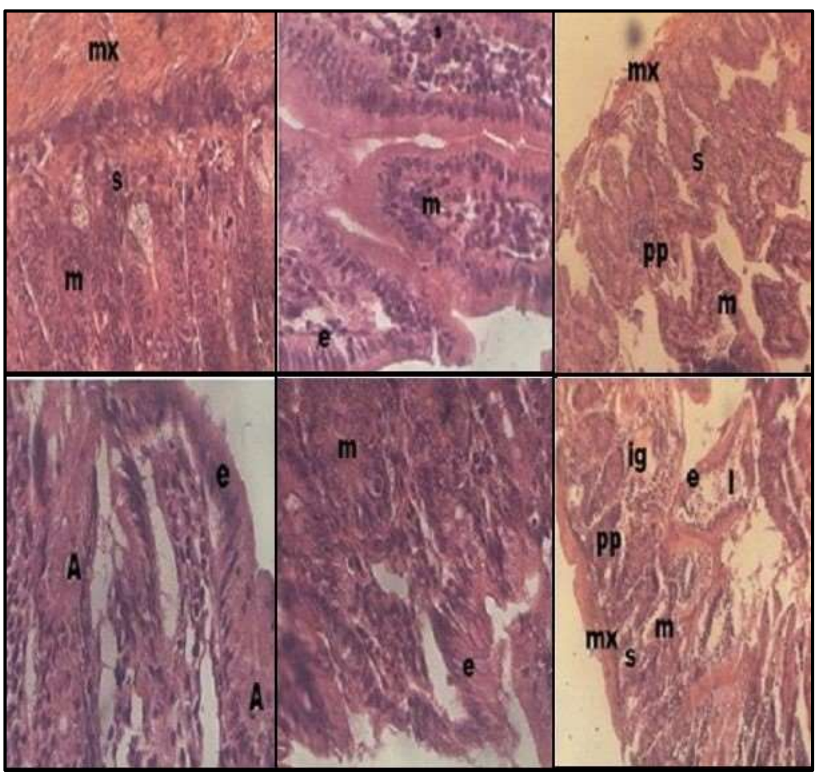

Figure 5: Photomicrographs of the Ileum of albino rats fed raw and processed *Cola rostrata* seed meal. ***Key:*** *showing muscularis externa (mx), submucosa (s) mucosa (m) epithelial cells (e), Peyer's patches (pp) within the submucosa of the apoptotic tissues (A) and the lymphoid tissue (L) and the intestinal glands (Ig) in T6 seem to be hypertrophied, H&E, Mag. x400*

Table 2: Histopathological lesions of some organs of rats fed experimental diets

Dietary Treatment	Organs Liver	Kidney	Duodenum	Jejunum	Ileum
T_1	Normal	Normal	Normal	Normal	Normal
T_2	No abnormality detected	No abnormality detected	No abnormality detected	No abnormality detected	No abnormality detected
T_3	Mild hepatocellular hypertrophy	Glomeruli hypoplasia	No abnormality detected	No abnormality detected	No abnormality detected
T_4	Mild hepatocellular hypertrophy, sinusoidal dilatation with evidence of stasis	Loss of brush borders in kidney cells detected	No abnormality detected	Moderate erosion of mucosal glandular tissue	No abnormality detected
T_5	Hepatocellular hypertrophy and sinusoidal collapse	Focal glomeruli cell loss and glomeruli hypoplasia	No abnormality detected	Erosion of epithelial cells and mucosal atrophy	No abnormality detected
T_6	Fatty degenerated changes, periportal and sinusoidal fibrosis and general hepatocellular necrosis	Focal segment sclerosis with loss of podocytes in the cortex	No abnormality detected	Hypercellularity and disorganization of the mucosal glandular tissue	Moderately hypertrophied intestinal glands

Treatments T_1, T_2 and T_4 had normal kidney cells. The kidney functions in filtration, ion homeostasis, and blood pressure control. Treatments T_1, T_2 and T_4 did not interfere with these functions that rely on multiple cell types and anatomical structures. T_3 and T_5 had glomeruli hypoplasia and focal glomeruli cell loss. Focal segment sclerosis with loss of podocytes in the cortex was observed in kidney cells from T_6 treatment (Figure 1). Renal hypoplasia may be one of the most misused terms in renal developmental anomalies. Historically, many authorities considered all small kidneys as hypoplastic, thus, the literature is cluttered with a host of diseases, both congenital and acquired, that have in common small overall renal size or limited amount of renal parenchyma, such as pyelonephritic, hydronephrotic, and dysplastic kidneys, as well as, truly hypoplastic kidneys. This condition interferes with the filtration function of the kidney (Bonsib, 2020).

The kidney cells of rats in T_5 showed a loss of foci cells. Foci cells are specialized cells found in the kidney that also help the kidney to function optimally, and their loss can disrupt the normal functioning of this organ. Loss of podocytes and focal segmental sclerosis in the kidney was observed in rats fed with the raw (T_6) monkey cola seed meal.

The podocytes are specialized cells in the glomerulus responsible for maintaining the filtration barrier by preventing the leakage of protein and other essential nutrients from the blood into the urine. Also, the portal tract of a healthy kidney will facilitate the entry and exit of blood vessels and the ureter thereby enabling the kidney to carry out its essential functions in filtering blood and producing urine (Arif and Nihalani, 2013). On another hand, sclerosis which is the scarring of the glomeruli can lead to impaired filtration of the blood. All the abnormalities noted in the kidney (loss of brush borders, loss of foci cells, loss of podocytes) can probably be attributed to the presence of oxalate as reported by Gemede and Ratta (2014).

Furthermore, the glomerulus is a tiny, ball-shaped cluster of blood vessels which is usually located at the beginning of a structure in the kidney called the nephron. The proximal tubule is a major part of the nephron saddle with the responsibility of reabsorbing about 65 – 70% of filtered sodium and water, as well as glucose, amino acids, and other nutrients out of the filtrate collected from the Bowman's capsule. The distal tubule is a part of the nephron that is also designated for further reabsorption of filtered electrolytes and water (Murray and Paolini, 2023).

Rats on T_4. showed loss of brush borders in their kidneys. The brush border is the tiny villi which increase the surface area of the kidney cells thereby allowing for their efficient reabsorption of important substances (glucose, amino acids, and ions) from the filtrate back into the bloodstream (Basile *et al.,* 2012).

Normal hepatocytes were observed in treatments T_1 and T_2. Mild hepatocellular hypertrophy was observed in T_3 and T_4, with sinusoidal dilatation with evidence of stasis in T_4. T_5 had hepatocellular hypertrophy and sinusoidal collapse, while T_6 had fatty degenerated changes, periportal and sinusoidal fibrosis and general hepatocellular necrosis (Figure 2). Hepatocytes in a normal kidney contain enzymes that metabolize toxins making them less harmful and easier for the animals' body to eliminate. On the other hand, the central vein will enable nutrient exchange, and waste elimination and regulate the flow of blood within the liver (Dutta *et al.,* 2021).

In addition, hepatocellular hypertrophy and obliteration of the sinusoids were noted in the liver of rats on T_3. Hepatocellular hypertrophy involves a series of processes which eventually result in the enlargement of hepatocytes in response to various stimuli. The obliteration of the sinusoids in the liver is the narrowing or blocking of these specialized blood vessels within the liver lobules. Sinusoids are the smallest blood vessels in the liver and are lined with endothelial cells.

Observable sinusoidal dilatation (enlargement of the sinusoids) of the liver cells of rats in T_4 may be due to the demand for more nutrients and blood flow. Evidence of stasis was also noticed in the liver of the rats on T_4. Stasis is the slowing down or stopping of normal blood flow that can lead to the formation of blood clots (thrombosis) (Garmo *et al*., 2023). Sinusoidal collapse (narrowing of sinusoids) was observed in the liver of rats on T_5. This implies a distortion and damage of the liver cells. Periportal and Sinusoidal fibrosis (a condition of excessive accumulation of fibrous tissue (scar tissue) around the portal tracts and the sinusoids) in the liver of rats on T_6 were also observed. In summary, injuries observed in the liver of the rats used in this study may be attributed to the rats' ingestion of some reasonable amount of phytate, tannin and oxalate which were incidents in the test diets. Marrone *et al.* (2016) reported that phytate, oxalates and tannin can cause low nutrient uptake and nutrient deficiencies. Ingestion of high content of oxalate can cause liver damage.

Hepatocyte hypertrophy is commonly associated with microsomal enzyme induction secondary to exposure to certain xenobiotics. It most frequently affects centrilobular hepatocytes, depending upon the xenobiotic and the dose administered, although the hypertrophy can extend into the middle of the hepatic lobule or become panlobular. This condition interferes with the functions of the hepatocyte (Le Roy *et al.,* 2018). Hepatocellular necrosis of the liver depicts the death of hepatocytes and other hepatic cells of the liver and is a characteristic feature of liver diseases/injury (Amaral *et al.,* 2009; Abu-Amara *et al.,* 2010).

No abnormality was detected in the duodenum of rats exposed to treatments T_1 to T_6 (Figure 3). This means that the various treatments did not affect the function of the duodenum. The small intestine is crucial for digestion and absorption of nutrients. The duodenum, the first segment, has Brunner glands that secrete mucus for digestion. Its wall, along with the jejunum and ileum, contains the muscularis externa for material flow. The mucosa, the innermost layer, aids absorption and digestion, while the sub-mucosa supports the tissue framework. The serosa, the outermost layer, secretes fluid for smooth organ movement (Collins *et al*., 2023). After ingestion of foods, the food mixes with stomach acid and moves into the duodenum, where it mixes with digestive juices from the pancreas and bile from the gallbladder. The absorption of minerals, vitamins, and other nutrients begins in the duodenum (Lopez *et al*., 2023).

The small intestine, including the jejunum, contains intestinal glands in its mucosa, aiding in digestion and nutrient absorption. The normal histological layers observed include the muscularis externa, mucosa, serosa, and submucosa in rats from T_1 and T_3 treatments (Figure 4). However, mucosal tissue loss occurred in T_3, likely due to ingestion of chemical or antinutrients causing irritation and damage to the jejunum lining (Figure 4). Erosion of epithelial cells of rats in T_4 and T_5 suggests inflammation from dietary toxin exposure, leading to mucosal atrophy, potentially due to prolonged inflammation. Conditions like inflammatory bowel disease (IBD) can result in chronic duodenal mucosa inflammation, leading to atrophy (Sonnenberg *et al.,* 2011).

Hypercellularity and disorganization of mucosal glandular tissues observed in T_5 and T_6 groups may result from oxalate-induced inflammation disrupting normal gland structure in the small intestine. The intestinal glands are found in the mucosa of the small intestine, including the jejunum. These glands assist in the digestive process by secreting various substances that aid in digestion and absorption of nutrients.

The jejunum is the middle of the three parts of the small intestine between the duodenum and ileum. Its arterial supply is provided by the jejunal arteries, while the innervation by the celiac and superior mesenteric plexi together with the vagus nerve. It plays an important role in digestion as 40% of the whole small intestine is jejunum. Its functions include absorbing water and nutrients. The hypercellularity and disorganisation of the mucosal glandular tissues of the jejunum disrupt these functions (Karunaharamoorthy and Mytilinaios, 2023).

No abnormality was detected in the ileum of rats exposed to all treatments except for T_6 which had moderately hypertrophied intestinal glands (Figure 5). Hypertrophy of the intestinal gland is an enlargement of this gland which may be due to overworking. Ortiz *et al.* (1994) reported an enlargement of the intestinal gland due to ingesting anti-nutrients. The ileum is the final portion of the small intestine, measuring around three meters, and ends at the cecum. It absorbs any final nutrients, with major absorptive products being vitamin B12 and bile acids (Collins *et al.*, 2023).

The damage to the liver, kidney and gastrointestinal tract may be caused by the anti-nutrients (tannin, oxalate and phytate) in the seed. Several authors have reported that phytic acid and tannin cause damage to the liver and kidney (Hirose *et al.*, 1991; Chung *et al.,* 1998; Nath *et al.*, 2022).

Conclusion: *C. rostrata* seed contains anti-nutrients (tannin, oxalate and phytate) and caused damage to the liver, kidney and gastrointestinal tract of the rats that were fed the raw seed meal. However, processing the seed by boiling for at least 30 minutes engenders the reduction of these anti-nutrients and therefore would allow for its use as a replacement for maize in animal diets without deleterious effects on the animal's organs and tissues.

ACKNOWLEDGEMENTS

The authors are grateful to the Tertiary Education Trust Fund (TETFUND) for funding this research, and the Department of Animal Science, Akwa Ibom State University for allowing us to use their laboratory for this study.

REFERENCES

ABU-AMARA, M., YANG, S. Y., TAPURIA, N., FULLER, B., DAVIDSON, B. and SEIFALIAN, A. (2010). Liver ischemia/reperfusion injury: processes in inflammatory networks – a review. *Liver Transplantation*, 16(9): 1016 – 1032.

ADEGUNWA, M. O., ADEBOWALE, A. A. and SOLANO E. O. (2012). Effect of thermal processing on the biochemical composition, anti-nutritional factors and functional properties of Beniseed (*Sesamum indicum*) flour. *American Journal of Biochemistry and Molecular Biology,* 2(3): 175 – 182.

AGIANG, M. A., UMOH, I. B., ESSIEN, A. I. and ETENG, M. U. (2010). Nutrient changes and antinutrient contents of beniseed and beniseed soup during cooking using a Nigerian traditional method. *Pakistan Journal of Biological Sciences: PJBS*, 13(20): 1011 – 1015.

AMARAL, J. D., VIANA, R. J., RAMALHO, R. M., STEER, C. J. and RODRIGUES, C. M. (2009). Bile acids: regulation of apoptosis by ursodeoxycholic acid. *Journal of Lipid Research*, 50(9): 1721 – 1734.

AOAC (2000). *Official Methods of Analysis.* 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA.

ARIF, E. and NIHALANI, D. (2013). Glomerular filtration barrier assembly: An insight. *Postdoc Journal: A Journal of Postdoctoral Research and Postdoctoral Affairs*, 1(4): 33 – 45.

BANCROFT, J. D. and GAMBLE, M. (2008). *Theory and Practice of Histological Techniques*. Elsevier Health Sciences, Amsterdam, Netherlands.

BASILE, D. P., ANDERSON, M. D. and SUTTON, T. A. (2012). Pathophysiology of acute kidney injury. *Comprehensive Physiology*, 2(2): 1303 – 1353.

BONSIB S. M. (2020). Renal hypoplasia, from grossly insufficient to not quite enough: consideration for expanded concepts based upon the author's perspective with historical review. *Advances in Anatomic Pathology,* 27(5), 311 – 330.

CHRISTOPHER, G. I., OFFIONG, S. A. and IDIONG, I. C. (2007). Effect of the replacement of maize with wheat offal in broiler finisher diets on growth performance and feed cost. *Journal of Central European Agriculture*, 8(1): 33 – 38.

CHRISTOPHER, G. I., OGUNDU, E. C., UMOREN, U. E. and UDOFE, M. J. (2019). Performance of growing African Giant Snails (*Archachatina marginata*) fed forage mixtures and maize-based concentrate supplemented with monkey cola (*Cola rostrata*) seed meal. *Asian Journal of Advances in Agricultural Research*, 8(4): 1 – 7.

CHRISTOPHER, G. I., ONUKAK, C. E., SAM, I. M., EVANS, E. I. and UMOREN, E. U. (2020). Haematological and serum biochemical indices of male Wistar albino rats fed raw and processed monkey cola (*Cola rostrata*) seed meal. *Nigerian Journal of Animal Science*, 22(3): 48 – 55.

CHUNG, K. T., WEI, C. I. and JOHNSON, M. G. (1998). Are tannins a double-edged sword in biology and health? *Trends in Food Science and Technology*, 9(4): 168 – 175.

COLLINS, J. T., NGUYEN, A. and BADIREDDY, M. (2023). *Anatomy, Abdomen and Pelvis, Small Intestine*. *In:* StatPearls [Internet], StatPearls Publishing, Treasure Island Florida, USA. https://www.ncbi.nlm.nih.gov/books/NBK459366/

DOSUMU, M. and EKA, O. U. (1989). Chemical evaluation of the nutritive value of C*ola rostrata*. *Nigerian Journal of Science,* 23(1-2): 85 – 87.

DRURY, R. A., WALLINGTON, A. and CAMEROUN, S. R. (1967). *Carlleton's Histological Techniques*. Oxford University Press, New York.

DUNN, J. and AGOM, D. (1992). The tree uses in Igonigoni and Abo Mkpang, Cross River State Nigeria: A comparison of two villages located in areas with different vegetation types. G*lobal Ecology and Biogeography Letters,* 2(6): 196 – 206.

DUTTA, S., MISHRA, S. P., SAHU, A. K., MISHRA, K., KASHYAP, P. and SAHU, B. (2021). Hepatocytes and their role in metabolism. *In:* DUNNINGTON, K. (Ed.). *Drug Metab*olism. Intech Open, London. https://doi.org/10.5772/intechopen.99083

FLOERL, S., KUEHNE, A. and HAGOS, Y. (2020). Functional and pharmacological comparison of human, mouse, and rat organic cation transporter 1 toward drug and pesticide interaction. *International Journal of Molecular Sciences*, 21(18): 6871. https://doi.org/10.3390/ijms21186871

FREIREICH, E. J., GEHAN, E. A., RALL, D. P., SCHMIDT, L. H. and SKIPPER, H. E. (1966). Quantitative comparison of toxicity of anticancer agents in mice, rat, hamster, dog, monkey, and man. *Cancer Chemotherapy Reports*, 50(4): 219 – 244.

GARMO, C., BAJWA, T. and BURNS, B. (2023). Physiology, Clotting Mechanism. *In:* StatPearls [Internet]. StatPearls Publishing, Treasure Island, Florida, USA. https://www.ncbi.nlm.nih.gov/books/NBK507795/

GEMEDE, H. F. and RATTA, N. (2014). Antinutritional factors in plant foods: Potential health benefits and adverse effects. *International Journal of Nutrition and Food Sciences*, 3(4): 284 – 289.

HELENA, V., VASCONCELOS-NÓBREGA, C., GAMA, A., FERREIRA, R., OLIVEIRA, P. A. and FAUSTINO-ROCHA, A. I. (2020). Histopathological features of organs in a rat model of mammary carcinogenesis: a reference database. *REDVET - Revista Electrónica de Veterinaria,* 21(4): 12 – 24.

HIROSE, M., OZAKI, K., TAKABA, K., FUKUSHIMA, S., SHIRAI, T. and ITO, N. (1991). Modifying effects of the naturally occurring antioxidants gamma oryzanol, phytic acid, tannic acid and n-triacontan-16, 18-dione in rat wide spectrum organ carcinogenesis model. *Carcinogenesis,* 12(10): 1917 – 1921.

KARUNAHARAMOORTHY, A. and MYTILINAIOS, D. (2023). *Jejunum: Anatomy, Histology and Function*. Ken Hub, https://www.kenhub.com/en/library/anatomy/the-jejunum

LE ROY, B., DUPRÉ, A., GALLON, A., CHABROT, P., GAGNIÈRE, J. and BUC, E. (2018). Liver hypertrophy: underlying mechanisms and promoting procedures before major hepatectomy. *Journal of Visceral Surgery*, 155(5): 393 – 401.

LOPEZ, P. P., GOGNA, S. and KHORASANI-ZADEH A. (2023). Anatomy, Abdomen and Pelvis: Duodenum. *In: StatPearls [Internet].* StatPearls Publishing, Treasure Island, Florida, USA. https://www.ncbi.nlm.nih.gov/books/NBK482390/

MARRONE, G., SHAH, V. H. and GRACIA-SANCHO, J. (2016). Sinusoidal communication in liver fibrosis and regeneration. *Journal of Hepatology*, 65(3): 608 – 617.

MURRAY, I. V. and PAOLINI, M. A. (2023). *Histology, Kidney and Glomerulus. In:* StatPearls [Internet]. StatPearls Publishing, Treasure Island, Florida, USA. https://www.ncbi.nlm.nih.gov/books/NBK554544/

NATH, H., SAMTIYA, M. and DHEWA, T. (2022). Beneficial attributes and adverse effects of major plant-based foods anti-nutrients on health: A review. *Human Nutrition and Metabolism*, 28: 200147. https://doi.org/10.1016/j.hnm.2022.200147

ODION, E. E., POH, C. F., FALODUN, A. and ADELUSI, S. A. (2013). *Cola rostrata*: Phytochemical and toxicity studies. *Journal of Applied Sciences and Environmental Management*, 17(4): 603 – 607.

OLOWU, O. O. and YAMAN, S. (2019). Feed evaluation methods: Performance, economy and environment. *Eurasian Journal of Agricultural Research*, 3(2): 48 – 57.

ORTIZ, L. T., ALZUETA, C., TREVINO, J. and CASTANO, M. (1994). Effects of faba bean tannins on the growth and histological structure of the intestinal tract and liver of chicks and rats. *British Poultry Science*, 35(5): 743 – 754.

SAVAGE, G. P. (2002). Oxalates in human foods. *Proceedings of the Nutrition Society of New Zealand,* 27: 4 – 24.

SONNENBERG, A., MELTON, S. D. and GENTA, R. M. (2011). Frequent occurrence of gastritis and duodenitis in patients with inflammatory bowel disease. *Inflammatory Bowel Diseases*, 17(1): 39 – 44.

TREUTING, P. M., DINTZIS, S. M. and MONTINE, K. S. (2017). *Comparative Anatomy and Histology: A Mouse, Rat, and Human Atlas*. Academic Press.

WEIL, C. S. (1972). Guidelines for experiments to predict the degree of safety of a material for man. *Toxicology and Applied Pharmacology*, 21(2): 194 – 199.