

## EVALUATION OF TOXIC POTENTIALS OF *Cola millenii* K. Schum SEED AND PULP FLOUR IN FOOD FORMULATIONS

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### ABSTRACT

Different parts of *Cola millenii* have been used in traditional medicine and available information in the literature has highlighted some nutritional potentials of *Cola millenii* seed and pulp. Previous studies on the toxicity of the seed were conducted on the extracts and conflicting evidences were obtained. Therefore, this study assesses the health risk of consuming *Cola millenii* seed and pulp flour in Wistar Albino rats.

The result obtained showed insignificant ( $p > 0.05$ ) difference in the organ weight of rats across the groups. Feeding of animals with the whole seed, pulp, and defatted seed flour showed no toxic effects on the food and water intake. Significant ( $p < 0.05$ ) increase was observed in the AST activity of group fed with whole seed and defatted seed flour while no significant ( $p > 0.05$ ) difference was observed in the ALT and GGT activity across the groups. Creatinine concentration decreased significantly ( $p < 0.05$ ) in the group fed with the defatted seed flour while urea concentration decreased significantly ( $p < 0.05$ ) in the group fed with the whole seed flour. Degenerative changes indicated by inflammation, necrosis and fibrosis were observed in the liver of group fed with the whole seed flour as well as in the kidney of groups fed with whole seed, defatted seed, and pulp of *C. millenii*. The result of this study showed that *C. millenii* seed might be slightly toxic. Therefore, there is a need for caution in the use of *C. millenii* as food.

### 1.Introduction

Plants remain a widely accepted component of Western diets and several health benefits are attributed to the consumption of plant foods (Deanna, 2019). Due to the increasing demand on valuable foods with sufficient nutrient, efforts are being made to boost food production with interest focused on the possibility of exploring the less familiar plants of the wild (Anhwange *et al.*, 2004). The tropical Africa

sub-region is home to many potentially valuable fruit species of which many are yet to be fully exploited. Therefore, there is need to promote crop and food diversifications to include neglected and underutilized species that are readily affordable and accessible to the rural and urban dwellers and most times can withstand the stresses linked to climate change (Bello *et al.*, 2008). Although some of these plants that grow in the wild are gaining attention among

researchers. One of these is *Cola millenii* K. Schum, commonly known as monkey kola. The fruit is bright red in a stellate cluster and its seed is covered with a fibrous coat with an edible kernel. The pulp has a varying characteristics sweetness which makes it useful as snack while seed is mostly appreciated for its therapeutic uses (Orisakeye and Ojo, 2013; Borokini *et al.*, 2014).

The nutritional potentials of *C. millenii* showed that the seed contain sufficient amount of carbohydrate, protein and fibre as well as essential minerals required for general wellbeing (Ibironke *et al.* (2013). In addition, phytochemical studies on the seed and pulp showed that it has medicinal potentials as it contains important phytochemicals such as saponins, terpenoids, tannins and alkaloids (Giwa *et al.*, 2012). Furthermore, the seed and pulp possess strong antimicrobial and antioxidant activity (Adeniyi *et al.*, 2004; Orisakeye *et al.*, 2013; Borokini *et al.*, 2014). Therefore, *C. milleni* is an important medicinal plant with valuable nutritional usefulness.

Although, plant foods contain essential nutritional constituents, there are increasing concerns because of their safety when consumed (Suzanne *et al.*, 2011). Currently, most toxicity studies conducted on *C. milleni* were performed on the seed and leaf extracts and a range of potential contraindications such as hepatic and renal toxicity have been identified (Oyemitan *et al.*, 2016; Itoandon *et al.*, 2016). Ubon *et al.* (2017) on the contrary reported that the ethanolic extract of the seed is non-cytotoxic, non-hepatotoxic and non-cardiotoxic. Therefore, this conflicting evidences need to be investigated. Hence, the study assessed the health risk of consumption of *C. millenii* seed and pulp flour in Wistar Albino rats.

## 2. Methods

### 2.1. Sample collection

Ripe monkey kola fruits were collected in February 2019 from (Omo Odo Agba Area, Near Baba Kekere, Osogbo) Latitude 7.762216, longitude (4.579217) Oke-Baale area, Osogbo local government in Osun State, Nigeria.

### 2.2. Sample preparation

The ripe fruits were washed and left to dry off at room temperature. The fruit's endocarp was cut open with the use of a knife and then the clustered seeds were removed. The pulp (mesocarp) was removed from the seed itself and was left to air-dry separately at room temperature. After drying, the seeds and pulp were ground separately using a using a blender (Power Deluxe CB 8231 N model) until a fine homogenous sample was obtained and the ground sample was kept an air-tight container. A portion of the ground seed sample was defatted by weighing into a filter paper and inserted into the thimble of a Soxhlet apparatus. 200 ml of n-hexane was measured into the round bottom flask of the soxhlet setup. The condenser was coupled and the setup was heated to reflux using a heating mantle. The oil sample was extracted continuously for 4 hours. The cake was then placed in oven at less than 60°C for 2 hours until uniform weight was obtained. The deffated seed (seed cake) was then kept in an air-tight container for animal feeding.

Commercial stock rat diet was obtained from Glory veterinary, Oritanaira Area, Ogbomoso, Oyo State. Diets were prepared by mixing 95 % of the commercial diet with 5 % of pulp, cake and seed of *C. milleni* separately every day (Datta *et al.*, 2011).

### 2.3. Experimental Design

Adult Swiss male Albino rats (120-180g) used in this study were divided into 4 groups of 5 animals. Group A received the normal commercial diet, Group B received feed mixed with 5 % pulp flour, Group C received diet mixed with 5 % defatted seed flour while Group D received diet mixed with 5 % seed flour. The animals were maintained in Basket Cages with wood shave as beddings and kept under standard condition of relative humidity and temperature. Standard protocol on animal handling and care were followed throughout the study whereas approval and permission for animal use were obtained prior to the commencement of the study.

## 2.4. Acute Toxicity Study

After grouping of animals, they were fed with diet containing 5 % of *C. milleni* seed, defatted seed, and pulp respectively for 21 days. Daily food and water intake and weekly body weights were monitored (Datta *et al.*, 2011). At the end of the 21 days of experiment, animals were fasted overnight and sacrificed by cervical dislocation. The blood was collected via heart puncture into plane sterile bottles while the liver and kidney were harvested and fixed in formalin buffer for histopathological analyses. Blood sample was allowed to clot and then centrifuge at 4000 rpm for 10 minutes. The serum was then separated and used for further biochemical studies.

### 2.4.1. Liver and renal function Analysis

All biochemical analysis Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Gamma Glutamyl Transferase (GGT), Urea and Creatinine) were performed with Fortress Assay Kit, Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS (United Kingdom) following the standard procedure in the manufacturer guide.

#### 2.4.1.1 Estimation of AST, ALT and GGT activities

The AST and ALT activity was determined by the method described by Tietz, (1995). The AST activity was estimated using the equation,  $\text{AST activity} = \Delta A / \text{min} \times 1908$ .

While the ALT activity was estimated using the equation,  $\text{ALT activity} = \Delta A / \text{min} \times 1746$ . GGT activity on the other hand was determined by the method of Szasz, (1976). Its activity was estimated using the equation,

$\text{GGT (U/L)} = 1158 \times \Delta A$  405nm/min where  $\Delta A$  405nm/min is the change in absorbance

#### 2.4.1.2 Determination of Serum creatinine and Urea concentration

The serum creatinine concentration was determined by the method of Tietz, (1995). While the serum urea concentration was determined by the method described by Fawcett and Scott, (1962). The serum creatinine concentration was estimated using the equation,

$$\frac{\text{Serum creatinine concentration}}{\frac{\Delta \text{abs sample}}{\Delta \text{abs standard}}} \times 177 = \quad (1)$$

Urea concentration was estimated using the equation,

$$\text{Serum urea concentration} = \frac{\Delta \text{abs sample}}{\Delta \text{abs standard}} \times \text{concentration of standard} \quad (2)$$

### 2.4.2. Histological Analysis

The histo-pathological analysis was performed by the hematoxylin and eosin staining (Pearse, 1980). Firstly, the samples were dehydrated through ascending grades of alcohol, cleared in xylene, and then impregnated in paraffin wax of melting point between 55°C-56°C for infiltration. Tissue sects were mounted on slides and then stained with hematoxylin and eosin (H & E). The stained tissues were then observed under microscope.

## 2.5. Statistical Analysis

Data were expressed as mean  $\pm$  SEM and analyzed by One-way Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS) 21.0. The level of significance was determined at 95 % confident level and separation between mean was determined by Duncan multiple test.

## 3. Results and Discussions

### 3.1. Results

#### 3.1.1. Acute toxicity of *Cola milleni* Seed, Pulp, and Cake on Albino Rats

Table 1 showed the weekly food and water intake of rats fed with the seed, pulp, defatted seed, and commercial diet. Incorporation of 5 % of seed sample showed no effects on the food intake of the treatment groups. The animals showed comparable food and water intake with the control (commercial diet). Figure 1 showed the body weight gain of rats during the period of study. The result showed total body weight increase across the groups. Feeding of the animals with *C. milleni* had no negative impact on the growth and body weight. In table 2, the

effects of feeding the experimental animals with *C. milleni* on the weight of organs were recorded. No significant variation was observed in the organ to body weight ratio of the animals

fed with seed, defatted seed, and pulp. Only in the spleen to body-weight ratio was lower ratio obtained in the treated groups compared with the control.

**Table 1.** Weekly food and water intake of Albino Rats fed with *C. milleni*

Groups	Weekly Food and Water Intake of Rats					
	Week 1		Week 2		Week 3	
	Food(g)	Water (ml)	Food(g)	Water (ml)	Food (g)	Water (ml)
Control	86.33±6.33	75.33±17.76	92.57±3.39	84.29±7.75	77.57±1.56	88.57±8.84
Pulp	96.67±0.61	71.17±10.56	98.29±0.64	85.71±4.68	93.43±1.29	98.57±19.33
Defatted seed	87.50±2.59	98.33±16.10	93.71±1.36	88.00±10.83	89.86±3.14	89.25±6.21
Seed	87.33±3.00	79.00±17.05	90.14±2.41	94.43±8.55	93.71±1.23	112.86±10.17

Values were expressed as mean±SEM

**Table 2.** Organ to weight ratio of Albino Rats fed with seed and pulp of *C. milleni*

Groups	Organ to weight ratio			
	Liver	Kidney	Heart	Spleen
Control	0.04	0.006	0.004	0.007
Pulp	0.03	0.004	0.003	0.004
Defatted seed	0.03	0.007	0.004	0.004
Seed	0.03	0.005	0.003	0.004

Organ weight/Weight of animals at week 3 (Sample size, 5)

**Table 3.** Liver function enzymes in Albino Rats fed with *C. milleni*

Groups	Liver Function Markers (U/L)		
	AST	ALT	GGT
Control	43.52±5.69 <sup>a</sup>	28.52±3.51 <sup>a</sup>	2.53±0.43 <sup>a</sup>
Pulp	56.78±2.46 <sup>ab</sup>	32.26±2.16 <sup>a</sup>	1.89±0.25 <sup>a</sup>
Defatted seed	74.89±3.95 <sup>b</sup>	26.15±1.61 <sup>a</sup>	1.56±0.29 <sup>a</sup>
Seed	65.83±7.73 <sup>b</sup>	27.54±2.79 <sup>a</sup>	1.66±0.17 <sup>a</sup>

Values were expressed as mean±SEM and considered significant at  $p < 0.05$ . Values with different superscript along the same row are significantly different (Sample size, 5)

### 3.1.2. Effects of *C. milleni* on the live function of Rats

The liver function markers of rats fed with *C. milleni* seed and pulp are showed in Table 3. Aspartate Amino Transferase (AST) activity increased significantly ( $p < 0.05$ ) in the groups fed with the defatted seed and seed, while no significant difference ( $p > 0.05$ ) was observed between the control and group fed with pulp. No significant difference was observed in the

Alanine Amino Transferase (ALT) and Gamma Glutamyl Transferase (GGT) activities across the groups.

### 3.1.3. Effects of *C. milleni* on the Renal function of Rats

Figure 2a and b represented the creatinine and urea concentration respectively of rats fed with *C. milleni* and the commercial diet. Highest creatinine concentration was observed in the control group. Comparison between the control

and groups fed with pulp and seed of *C. milleni* showed no significant difference ( $p < 0.05$ ). However, significant decrease was observed in the creatinine concentration of the group fed with the defatted seed. Urea on the other hand decreased significantly ( $p < 0.05$ ) in the group fed with seed of *C. milleni* while no significant difference ( $p > 0.05$ ) was observed across the other groups.

### **3.1.4. Effects of *C. milleni* on the histology of liver and kidney of rats**

#### *3.1.4.1. Effects of C. milleni on the liver histology of rats*

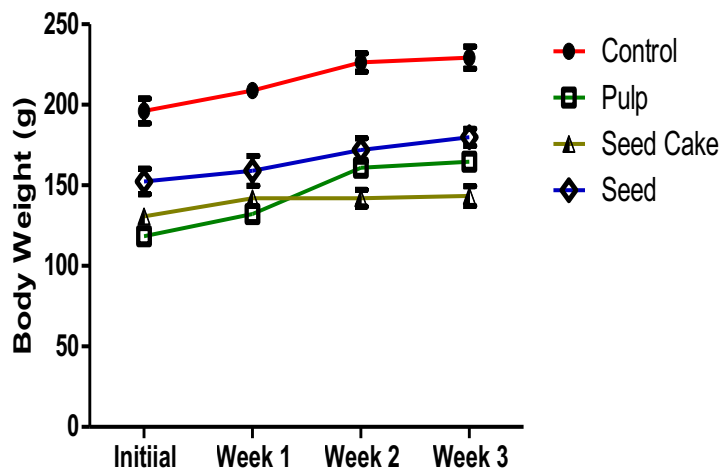
Plate 3a and b represent the photomicrograph of haematoxylin and eosin (H and E) stained liver section of rats in the control and treatment groups at 100um and 50um magnifications respectively. The representative photomicrograph showed the portal triad (PT), hepatic vein (HV), hepatic artery (HA), hepatic duct (HD), bile duct (BD) and the well distributed hepatocytes (H). Observed in the photomicrograph included mild degenerative changes that can be termed as necrosis, some mild fibrosis and hemorrhage characterized by the presences of infiltrated red hemorrhagic and inflammatory cells (Group B-D). Also observed is a distortion (well defined dilation in the portal triad) in the walls of the blood vessels (red arrow), pyknotic hepatocytes and signs of inflammation (red arrow) is seen more especially in the group fed with *C. milleni* seed powder (group D). Group A on the other hand showed normal morphological presentation with

an array of well outlined hepatocytes as well as the portal triad system.

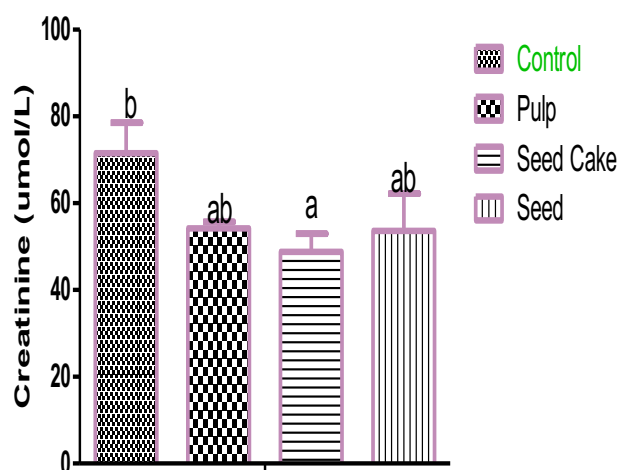
#### *3.1.4.2 Effects of C. milleni on the kidney histology of rats*

The representative photomicrograph of H and E stained section of the kidney of rats across the treatment and the control groups are presented in plate 4a and b. Demonstrated across the study groups is the Renal Corpuscles (RC), Renal glomeruli (G), Macula densa (MD), Distal and Proximal (DCT & PCT) convoluted tubules (renal tubules RT) and the bowman's capsule (BC). The red arrows indicate areas with marked pathomorphological changes.

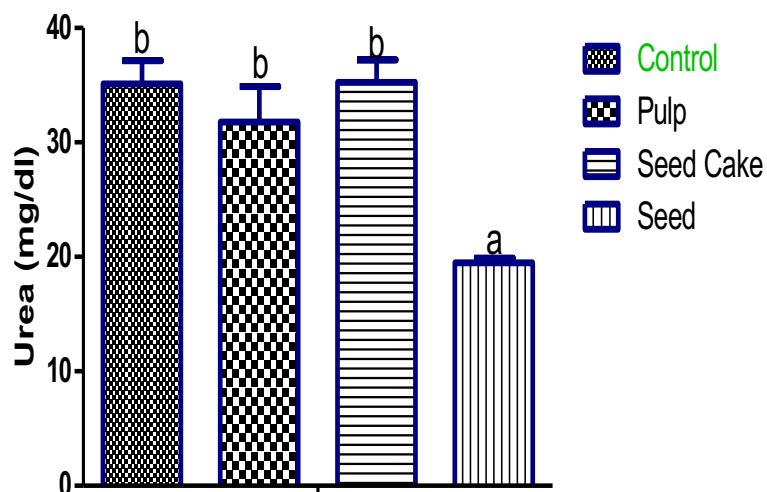
The collagen (type IV) of the basement membrane outlines the glomerular capillaries. The collagen of the parietal layer (PL) of Bowman's capsule (BC) and the basal membrane (BM) of a distal tubule are observable from the photomicrographs. Marked degenerative changes characterized with fibrosis and hemorrhage (red arrows) showing varying degrees of renal injury evidenced by focal sclerosis of the glomerulus, widening of the Bowman's space and hyper-cellularity and complete collapse of the glomerulus. There is hyaline arteriosclerosis, interstitial fibrosis, interstitial inflammation, as well as acute tubular necrosis are all observed in a mild to severe degree in treated group B-D relative to group A that appears normal. Features are consistent with chronic glomerulonephritis and or glomerulosclerosis. (Red arrows).



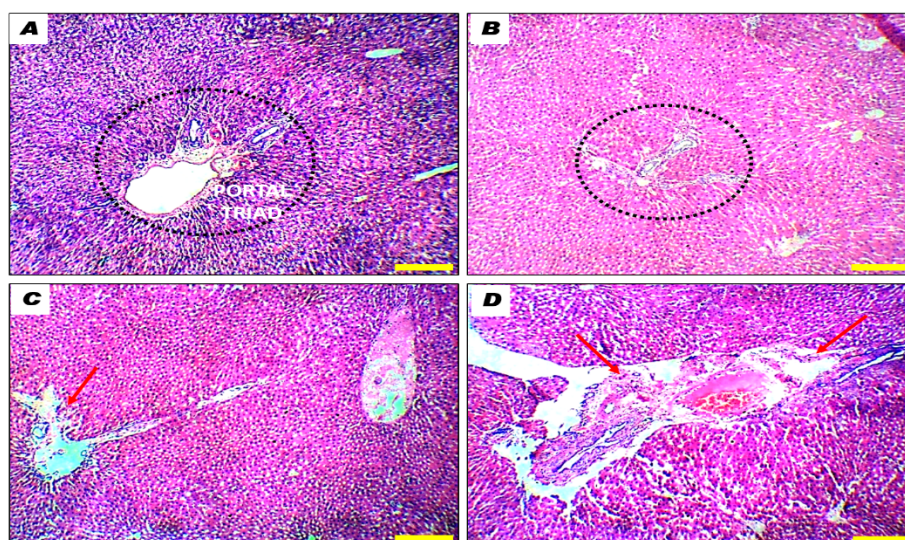
**Figure 1.** Weekly total body weight of Rats fed with *C. milleni*  
Values were expressed as mean±SEM



**Figure 2 a,** Serum creatinine concentration in Rats fed with *C. milleni*  
Values were expressed as mean±SEM and considered significant at  $p < 0.05$ . Values with different superscript are significantly different

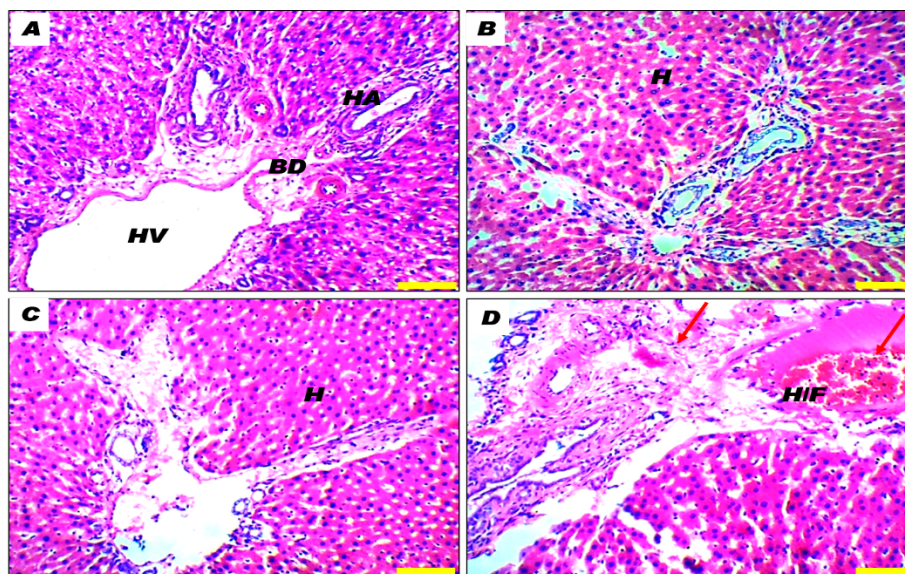


**Figure 2b,** Serum Urea concentration in Rats fed with *C. milleni*  
Values were expressed as mean $\pm$ SEM and considered significant at  $p < 0.05$ . Values with different superscript are significantly different



**Plate 3a,** Photomicrographs of the panoramic views of liver general micromorphological presentations in Adult Wistar rats across the study groups. Hematoxylin and Eosin stain (scale bar 100um).





**Plate 3b,** Photomicrographs of liver general micromorphological presentations in Adult Wistar rats across the study groups. Hematoxylin and Eosin stain (scale bar 50um).

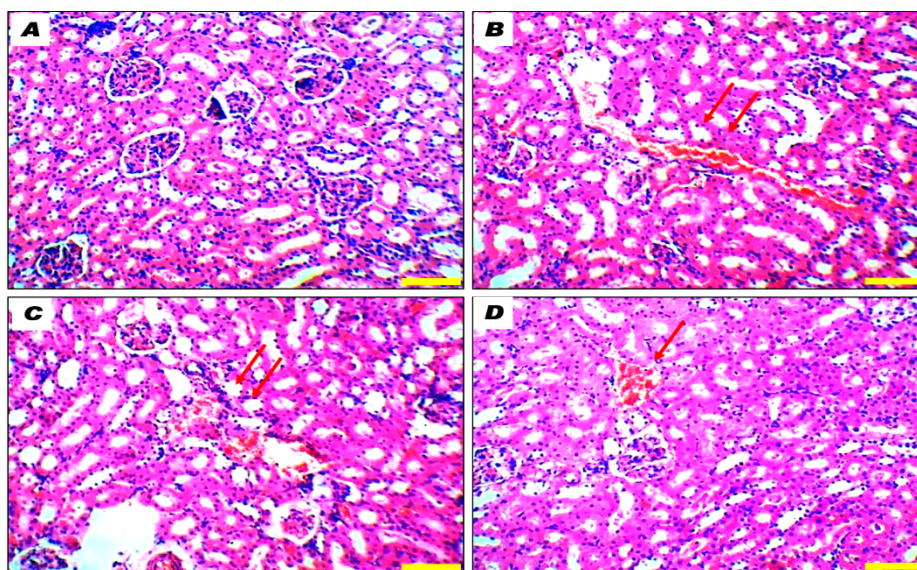
Key

A, Control group

B, Group fed with the pulp of *C. milleni*

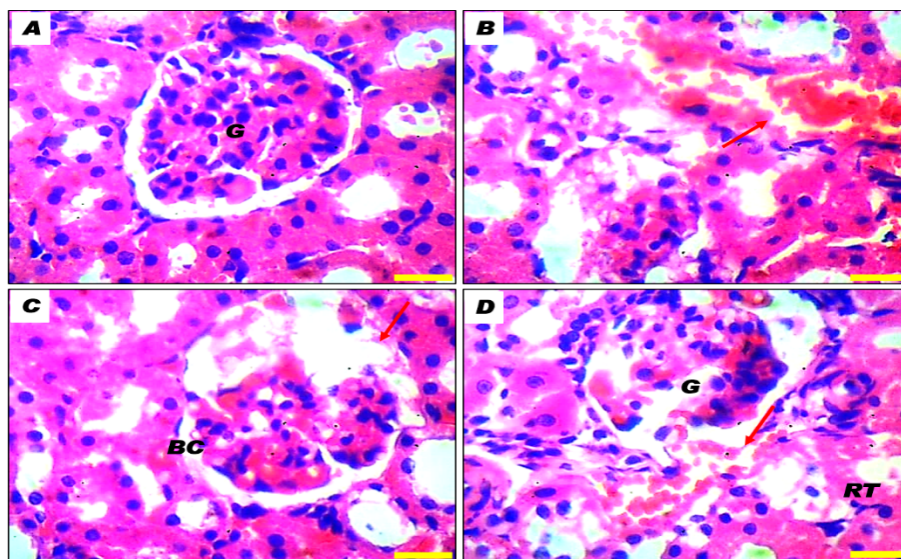
C, Group fed with the defatted seed of *C. milleni*

D, Group fed with the seed of *C. milleni*



**Plate 4a,** Photomicrographs of the renal cortex showing panoramic views of Kidney general micromorphological presentations in Adult Wistar rats across the study groups. Hematoxylin and Eosin stain (scale bar 100um).





**Plate 4b,** Photomicrographs of the renal cortex showing magnified views of Kidney general micromorphological presentations in Adult Wistar rats across the study groups. Hematoxylin and Eosin stain (scale bar 50um).

#### Key

A, Control group

B, Group fed with the pulp of *C. milleni*

C, Group fed with the defatted seed of *C. milleni*

D, Group fed with the seed of *C. milleni*

### 3.2. Discussions

*Cola millenii* is an important plant of the transitional forest of Southern Nigeria (Ubon *et al.*, 2008). The current interest of various experiment on the plant is owned to its many nutritional potentials (Borokini *et al.*, 2014; Ibrinke *et al.*, 2013; Odugbemi, 2006). Data in the literature presents conflicting evidences as regards the nutritional risk of consuming *C. millenii* as food. Therefore, this present study assessed the health risk of consuming *C. millenii* seed and pulp in Albino rats. In this study, no death was observed among the experimental groups and the animals gained weight normally throughout the period of study. Therefore, feeding of the animals with *C. millenii* had no lethal effects as well as negative impact on the growth, food and water intake of the experimental animals.

Liver, which is the organ responsible for metabolism of most xenobiotics, is susceptible to chemical injury (Hodgson *et al.*, 1988). Kidney on the other hand functions in getting rid

of the waste materials, which are either ingested or produced by detoxification process of the liver (Olorunnisola *et al.*, 2019). Therefore, accumulation of these toxic metabolites or chemicals predisposes them to damage (Arthur and John, 2000). In this study, the toxic effect of *C. milleni* seed and pulp was assessed in the liver and kidney of rats.

Hence, the biochemical parameters monitored are useful indices for the assessment of liver and renal toxicity. It included the Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Gamma Glutamyl Transferase (GGT), creatinine, and urea.

AST, ALT and GGT enzymes are mainly localized in the liver and are released into circulation upon damage to the hepatic cells. Therefore, increase in their serum activity is usually an indication of damage to the liver cells and their concentration could reflect the extent of hepatotoxicity (Olorunnisola *et al.*, 2019). Observation in this study i.e. increased observed in the AST activity of the group fed with the

defatted seed and whole seed might reflect the toxic nature of *C. millenii* seed.

The histopathological studies on the liver further substantiate the result of the biochemical studies. The mild degenerative changes observed in the group fed with the seed of *C. millenii* are a reflection that the oil of *C. mileni* is toxic to the liver. Ogbeh *et al.* (2018) previously recorded the high acidity of *C. millenii* oil. According to the study, *C. millenii* had acid value far above the recommended for cooking oil and the value established by the Ministry of Public Health for various types of edible fats and oils. The results in this study also support the report of Itoandon *et al.* (2016) who reported potential toxicity of leaf extracts of *C. milleni*.

In the monitoring of renal function, urea and creatinine concentration can give insight to the effects of the plant sample on the tubular and glomerular functions of the kidney (Ukwuani *et al.*, 2012). Therefore, high serum urea and creatinine level may be a pointer of renal failure (Hodgson *et al.*, 1988). Furthermore, removal of creatinine from plasma is achieved during glomerular filtration process and excreted in the urine. However, when the plasma level increases above normal, creatinine could also be excreted through the tubules. The implication is that the serum creatinine level in renal disease generally does not increase until the renal function is substantially impaired (Faulkner *et al.*, 1982).

Observations in this study substantiate the claim as no distortion was observed in the renal excretion of creatinine and urea whereas, histological studies revealed mild damage in the renal architecture.

Anti-nutritional factors in plants have been reported to cause modification in biochemical processes of the body. For instance, tannin was reported to reduce protein digestibility consequently reduce amino acid bioavailability (Aletor, 1993; Carnovale *et al.*, 1991; Chai and Liebman, 2004). In this study, decreased serum urea concentration, which is an excretory product of protein digestion observed in the group fed with the seed, relative to the control

group might be a reflection of decreased amino acid bioavailability rather than renal protection.

#### 4. Conclusions

The result of this study showed that *C. millenii* seed might be slightly toxic to the liver and kidney. The study also revealed that the oil of the seed might have contributed to this toxicity in rats.

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