

Original Research Article

Toxicity Evaluation of Fruit Rind of *Cucumis metuliferus* on the Biochemical, Hematological and Histological parameters of Albino Rats.

Comment [ON1]: Italicize the botanical name

Abstract

Background: Fruit rinds/peels form major part of food waste, yet they possess nutrients and biologically-active constituents which have been found useful in nutrition and therapy. Nevertheless, the safety of their usage is a drawback and therefore requires a complete evaluation of their safety profile. This study evaluated the biochemical, hematological and histological toxic effects of the ethanol extract of the fruit rind of *Cucumis metuliferus* in albino rats.

Comment [ON2]: Summarize this to include essential ingredients and aim

Methods: The acute oral toxicity test (LD₅₀) was done using Lorke's method and then 16 male albino rats in four (4) groups (A, B, C and D) of four rats (4) with mean weight of 173.8 g were daily administered orally with 100, 200, 400 mg/kg body weight of the extract respectively with group D serving as the control. Treatment lasted for 14 days after which blood samples were collected for biochemical and hematological assays. Kidney, liver and heart were removed and fixed in 10% formalin solution for histological studies.

Comment [ON3]: This is good with slight modifications

Results: The LD₅₀ result showed no death and toxic sign up to 5000 mg/kg bodyweight. The result of biochemical assays in comparison between the treated and control groups showed no significant difference ($p>0.05$) in the levels of liver enzymes, albumin, total protein and the antioxidant enzyme activities. However, creatinine, direct and total bilirubin of group A indicated a significant difference ($p<0.05$) when compared with the other groups. Hematology as well as biochemical parameters of the kidney and lipid of the treated groups showed significant difference ($p<0.05$) in the electrolytes, cholesterol and high-density lipoprotein (HDL) levels while there was no significant difference ($p>0.05$) in the urea, triglyceride and low-density lipoprotein (LDL) levels when compared with the control. Some degenerative histological changes were found in the kidney, liver and heart tissues.

Comment [ON4]: This is understandable

Conclusion: The ethanol extract of the fruit rind of *Cucumis metuliferus* caused some biochemical and histological alterations and may not be safe. Therefore, caution should be exercised when in use.

Comment [ON5]: This is the first-time ethanol extract is mentioned; Consider including in the Introduction or title to be whole reflective.

Keywords: *Cucumis metuliferus*, Kiwano, Biochemical, Hematology, Histology, Liver, Kidney, Heart.

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Introduction

Different parts of plant have been used to meet numerous nutritional, therapeutic and medicinal needs of man and animals. Fruits serve important nutritional and therapeutic purpose in providing the body with vitamins, minerals and various biologically active compounds. There has been increase in awareness in the consumption of fruits following the

increase in knowledge of the benefits from them. This increase in consumption is reflected in the amount of solid food waste which the rind of fruits are part of in the environment. In order to minimize the production of food waste associated with fruit rinds, studies have shown that these fruit rinds are packed with essential nutrients and important bioactive components [1]. Studies have equally suggested the potential usage of these fruit rinds for diets and therapeutic purposes [2]. For this reason, investigation on the toxicity or otherwise safety of these non-edible parts of fruits should be established following scientifically established methods. Generally, there is a belief that all natural medicinal products are free and devoid of toxicity. This leads to indiscriminate use which could affect various organs of the body of which the liver and kidney are most prone to damage as they are directly involved in metabolism and detoxification.

Comment [ON7]: No citation, Please include citations

Cucumis metuliferus commonly called horned melon, kiwano or jelly melon belongs to Cucurbitaceae family. It is a monoecious herb that grows wild annually [3]. It is mostly found in the tropics including Nigeria and South Africa. The fruits are green in colour but orange-skinned when ripe with jelly-like succulent pulp which have refreshing taste and embedded seeds. The fruits are eaten raw and the various parts have been found useful for different dietary and therapeutic purposes. The seeds are roasted and eaten as well as ground into powder and taken as an anti-helminthic. Although the rinds are edible but the pulp are mostly consumed. Studies have shown that the rind contains nutrients and phytochemicals [1]. We have studied and reported previously on the anti-oxidant and anti-inflammatory properties of the ethanol extract of the fruit rind [4]. Hence, to ascertain its safety, this study was undertaken to evaluate and investigate the toxicity of the ethanol extract on the biochemical, hematology and the major organs of albino rats.

Comment [ON8]: A Paragraph should be included to elucidate the justification for the study

Materials and Methods

Plant materials

The fruits of *Cucumis metuliferus* were purchased from Gboko, Benue State, Nigeria. It was authenticated at the Department of Applied Biology, Enugu State University of Science and Technology, Agbani, Nigeria. The fruits were thoroughly washed with portable water to remove dirt, cut using kitchen knife and the pulp containing the seed was scooped out and the rinds were retained. The rinds were then cut into pieces and air-dried at room temperature (25 °C) for three weeks. The dried sample was ground into powder using an electric blender and sieved through muslin cloth.

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Extraction procedure

The powdered sample (155.79 g) was extracted with 80% ethanol by soaking with intermittent stirring for 24 h. The mixture was then filtered using Whatman no 4 filter paper and the filtrate concentrated by evaporation in a water bath at 40 °C and a semi solid extract (9.8g) was obtained. The extract was stored in an air-tight container at 4 °C in a refrigerator prior to use.

Acute Oral Toxicity Test (LD₅₀)

The LD₅₀ of the extract was evaluated using Lorke's method [5]. Twelve (12) healthy male albino rats with mean weight of 173.8 g were randomized into six (6) groups in two phases.

In the first phase, three (3) groups of three rats each were given single oral dose of 10, 100 and 1000 mg/kg bodyweight of the extract respectively. They were monitored for 24 h. In the second phase, three groups of one rat each were given single oral dose of 1600, 2900 and 5000 mg/kg bodyweight of the extract. They were also monitored for 24 h for any sign of toxicity and mortality.

Experimental Animal Model

A total of 16 healthy male albino rats with mean weight of 173.8g were randomly distributed into four (4) groups of four (4) rats each. They were housed separately in a well-ventilated cage and fed with water and grower's mash. They were left to acclimatize to their new environment and maintained under standard laboratory condition ($27^{\circ}\text{C}\pm 3^{\circ}\text{C}$, 12-hour light/dark cycle) in the Applied Biochemistry Laboratory, Enugu State University of Science and Technology for 7 days.

Experimental Procedure

Group A: 100mg per kg bodyweight of ethanol extract of fruit rind of *Cucumis metuliferus*.

Group B: 200mg per kg bodyweight of ethanol extract of fruit rind of *Cucumis metuliferus*.

Group C: 400mg per kg bodyweight of ethanol extract of fruit rind of *Cucumis metuliferus*.

Group D: Control; received normal feed and water only

The extract was administered orally for 14 days.

Collection of blood sample and Histological Examination.

After the experimental period, the animals were fasted overnight and sacrificed under chloroform anesthesia. Blood samples were collected through cardiac puncture using 5ml syringe into a well-labelled non-heparinized tubes and allowed to clot for about 2 hours and thereafter centrifuged at 4000 rpm for 10 min to remove cells and recover serum, which was used for the biochemical assay. Part of the blood samples were also put into heparinized tubes for the hematology assay. The kidney, liver and heart were removed and fixed in 10% formalin solution for histological studies.

Analytical Methods

Liver Function Test

The liver function parameters analyzed were the liver enzymes [Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)], Bilirubin (direct and total bilirubin), Albumin and Total protein. The liver enzymes, Bilirubin and Albumin were determined according to the method of Limdi and Hyde [6] using Randox diagnostic kits while total Protein was determined using Biuret method as described by Gonall et al [7].

Kidney Function Test

Serum chloride and sodium content was determined using the method of Skeggs and Hochestrasser [8], Bicarbonate was determined using the method of Roger et al. [9] and potassium content was determined using the method of Kabiru et al. [10]. Serum urea and

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creatinine level were determined using Randox diagnostic kit according to the manufacturers instruction.

Lipid Profile Assay

Lipid profile (Cholesterol, Triglyceride, Low density lipoprotein, High density lipoprotein and Very low density lipoprotein) was determined according to the method of Sidhu and Naugler [11] using Randox diagnostic kit and measured at 546nm using Autochemistry Analyser (Mindray BA-88). LDL (low density lipoprotein) was determined by measuring the amount of cholesterol remaining in the serum after precipitation with polyvinyl sulphate. The LDL content was calculated using the standard formula of Friedewald et al [12]. VLDL (Very low density lipoprotein) was estimated as 20% of total Triglyceride (TG) concentration [12]. HDL (High density lipoprotein) was determined by measuring the amount of cholesterol remaining in the serum after precipitation of LDL, VLDL and Chylomicron by the addition of phosphotungstic acid and magnesium chloride. The HDL content was measured as the remaining cholesterol in the sample solution after precipitation.

Antioxidant Enzyme Assay

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Sun and Zigma [13]. Catalase activity was determined according to method of Aebi [14]. It was assayed colorimetrically at 620nm and expressed as μ moles of H_2O_2 consumed/min/mg protein at 25°C. Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust [15].

Hematological Assay

The hematological assay for Red Blood Cell count (RBC) and White Blood Cell count (WBC) were carried out using Olympus Binocular microscope and Neuberg's hemocytometer, Packed Cell Volume (PCV) was measured using the Micro hematocrit as described by Thomas [16], and the hemoglobin (Hb) content was assayed using the method of National Committee for Clinical Standards [17].

Statistical Analysis

Data analysis was performed using SPSS version 23 statistical package. Values were presented as mean \pm standard deviation (SD). Statistical significance of the results between groups was determined using one way analysis of variance (ANOVA). Differences between means were considered statistically significant at $P < 0.05$.

Results

Acute Oral Toxicity Test (LD_{50})

The toxicity testing for 24 h on rats showed that the extract elicited no significant change in the behavior of the experimental rats and no death was recorded up to the dose of 5000 mg/kg bodyweight.

Effect of the extract on the Biochemical parameters

Comment [ON14]: It is important to mention the company names, location and address of the manufacturers of all commercially available kits.

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Liver function Analysis

The result of the liver function test at different doses is presented in Table 1. The Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) concentrations at dosages of 100 and 200 mg/kg increased and decreased at 400 mg/kg with no significant difference ($p > 0.05$) when compared with the control. Aspartate Aminotransferase (AST), Albumin concentrations and Total protein at the three doses increased with no significant difference ($P > 0.05$) when compared with the control. The Direct and Total Bilirubin concentrations at 100 mg/kg increased significantly ($P < 0.05$) when compared with the control and other groups.

Table 1: Serum Concentrations of Liver Function parameters of the treated rats in comparison with the control.

PARAMETER	Group A (100 mg/kg)	Group B (200 mg/kg)	Group C (400 mg/kg)	Control
ALT (U/L)	18.51±6.00	18.51±2.42	9.97±3.87	12.79±3.23
AST (U/L)	36.38±6.13	28.35±4.06	26.35±7.61	20.49±2.17
ALP (U/L)	6.00±1.98	5.52±2.60	3.22±0.65	5.06±1.95
Albumin (mg/dl)	3.27±0.69	3.02±0.48	3.62±0.29	3.36±0.94
Direct Bilirubin (mg/dl)	1.34±0.14 ^a	0.27±0.10 ^a	0.86±0.23 ^a	0.05±0.01
Total Bilirubin (mg/dl)	1.48±0.46 ^a	0.32±0.06 ^a	0.79±0.22 ^a	0.09±0.02
Protein (mg/dl)	75.95±8.99	77.73±0.75	73.37±11.09	63.97±0.97

^asignificant increase in comparison with the control ($P < 0.05$)

Comment [ON17]: Include the F values in the table for each parameter

Kidney Function Analysis

The result of the analysis of serum kidney function parameters are presented in Table 2. In comparison with the control, the levels of all the electrolytes (Na^+ , K^+ , Cl^- and HCO_3^{2-}) were significantly increased ($p < 0.05$) at all the three doses. However, sodium (Na^+) was reduced significantly ($p < 0.05$) at 100 mg/kg. The levels of urea at all doses and creatinine at 200 and 400 mg/kg were not significantly ($p > 0.05$) altered while creatinine at 100 mg/kg was significantly reduced ($p < 0.05$).

Table 2: Serum Concentrations of Kidney Function parameters of the treated rats in comparison with the control

	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^{2-} (mmol/L)	Urea (mg/dl)	Creat (mg/dl)
A 100mg/kg	99.00±7.26 ^b	3.65±0.75 ^a	74.25±7.27 ^a	12.50±2.38 ^a	3.20±0.43	2.03±0.47 ^b
B 200 mg/kg	132.00±19.31 ^a	4.50±0.57 ^a	86.00±9.83 ^a	19.25±2.22 ^a	3.15±0.49	3.09±0.24
C 400 mg/kg	160.25±20.21 ^a	5.08±0.90 ^a	100.50±8.23 ^a	24.75±2.99 ^a	2.85±0.25	3.15±0.21
Control	107.00±7.53	2.83±0.29	63.50±7.85	11.75±1.71	3.45±0.31	2.65±0.66

^asignificant increase in comparison with the control ($P < 0.05$). ^bsignificant decrease in comparison with the control ($P < 0.05$)

Comment [ON18]: Include F values

Lipid Profile Analysis

The result of the analysis of the serum lipid profile are presented in Table 3. The Total Cholesterol (TC) and High-density lipoprotein (HDL) levels were significantly ($p<0.05$) decreased at 200 and 400 mg/kg when compared with the control. The levels of Triglyceride (TG), Low-density lipoprotein (LDL) and Very low-density lipoprotein (VLDL) were not significantly ($p>0.05$) altered.

Table 3: Serum Lipid profile Concentrations of the treated rats in comparison with the control

	TC(mg/dl)	TG(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
A 100 mg/kg	20.03±1.12	17.79±7.72	11.49±3.78	3.79±0.6	3.56±1.54
B 200 mg/kg	15.37±2.84 ^b	26.64±0.04	7.39±1.42 ^b	3.98±1.82	5.33±0.01
C 400 mg/kg	10.41±3.14 ^b	18.33±6.39	4.37±2.61 ^b	2.38±1.41	3.67±1.28
Control	21.92±0.05	23.33±0.98	11.93±0.23	5.32±0.18	4.67±0.20

^b significant reduction in comparison with the control ($p<0.05$)

Comment [ON19]: Include the F values

Hematology Analysis

The result of the analysis of the hematology parameters are presented in Table 4. The packed cell volume (PCV), Red blood cell (RBC) and Hemoglobin levels were significantly reduced ($p<0.05$) while the White Blood Cell (WBC) count was significantly increased in all the treated groups when compared with the control.

Table 4: Hematological indices of the treated rats in comparison with the Control.

	PCV (%)	RBC x 10 ¹¹	Hb (mg/dl)	WBC x 103/ μ L
A 100 mg/kg	26.96±3.84 ^b	2.36±0.12 ^b	7.65±1.44 ^b	10.93±1.09 ^a
B 200 mg/kg	23.18±2.31 ^b	2.11±0.22 ^b	7.30±1.24 ^b	11.51±0.94 ^a
C 400 mg/kg	21.15±1.25 ^b	1.4±0.46 ^b	7.12±1.14 ^b	13.71±2.36 ^a
Control	34.31±4.87	2.44±0.34	9.66±1.2	10.74±0.71

^asignificant increase ($p<0.05$) in comparison with the control. ^bsignificant decrease ($p<0.05$) in comparison with the control.

Antioxidant Enzymes

The result of the analysis of antioxidant enzyme activity is presented in Table 5. In comparison with the control, the activity of Superoxide Dismutase (SOD) was significantly increased ($p<0.05$) at 400 mg/kg, Catalase (CAT) was not significantly altered at all the doses while the levels of Malondialdehyde (MDA) were significantly increased ($p>0.05$) at all the doses.

Table 5: Serum concentrations of antioxidant enzymes and MDA in comparison with the control

SOD	CAT (x10 ⁻³ U/L)	MDA (x10 ⁻²)
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	(x10 ³ U/L)		μM/L)
A 100 mg/kg	3.84±0.38	5.74±0.85	8.92±0.71 ^a
B 200 mg/kg	3.04±0.72	4.6±0.18	4.71±0.75 ^a
C 400 mg/kg	5.08±0.29 ^a	6.25±0.13	5.11±0.11 ^a
Control	3.64±0.49	5.47±0.87	2.46±0.13

^asignificant increase in comparison with the control ($p<0.05$)

Histopathological Analysis

The micrograph of the liver section is presented in Fig 1. The histology of liver section of the control group (a) shows that the hepatocytes are normal in comparison with the liver section in the extract treated groups of 100 (b), 200 (c) and 400 mg/kg (d). There was mixed cell focus amidst normal hepatocytes in the group treated with 100 mg/kg, marked hepatic vacuolar degeneration was observed in 200 mg/kg extract treated group while in 400 mg/kg treated group, moderate periportal inflammation and mild sinusoidal infiltration were observed but the hepatocytes were normal.

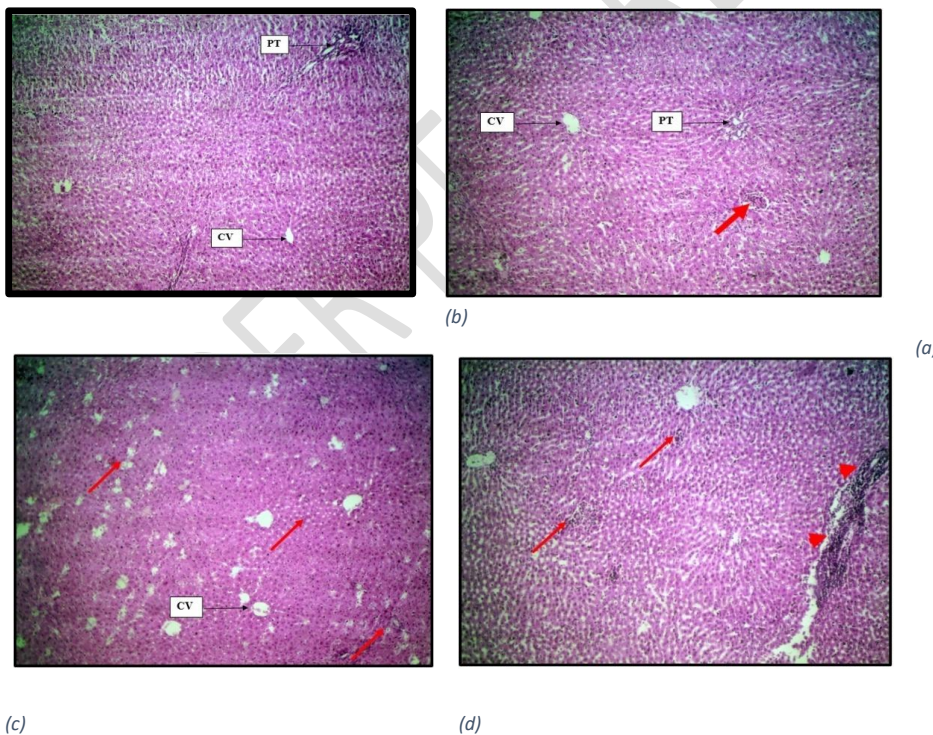


Fig. 1: The liver micrograph of the extract treated groups in comparison with the control. CV – central vein; PT – portal triad. Haematoxylin and Eosin. Magnification: X100.

(a) : control showing normal hepatocytes; (b) extract dose of 100 mg/kg showing mixed cell focus (arrow) amidst normal hepatocytes.; (c) : extract dose of 200 mg/kg showing marked

hepatic vacuolar degeneration (arrows); (d): extract dose of 400 mg/kg showing normal hepatocytes with moderate periportal inflammation (arrow head) and mild sinusoidal infiltration (arrows).

The micrograph of the kidney section is presented in Fig 2. The histology of the kidney section of the control group (a) shows normal histological features in comparison with that of 100 (b), 200 (c) and 400 (d) mg/kg treated groups. In the 100 mg/kg treated group there appear to be eosinophilic deposits in the tubules as well as infiltration by neutrophils. The tubules have sloughed off epithelium and the glomeruli are normal in 200 mg/kg treated group while in the 400 mg/kg treated group, the renal tubules are dilated with increased lumen and normal glomeruli.

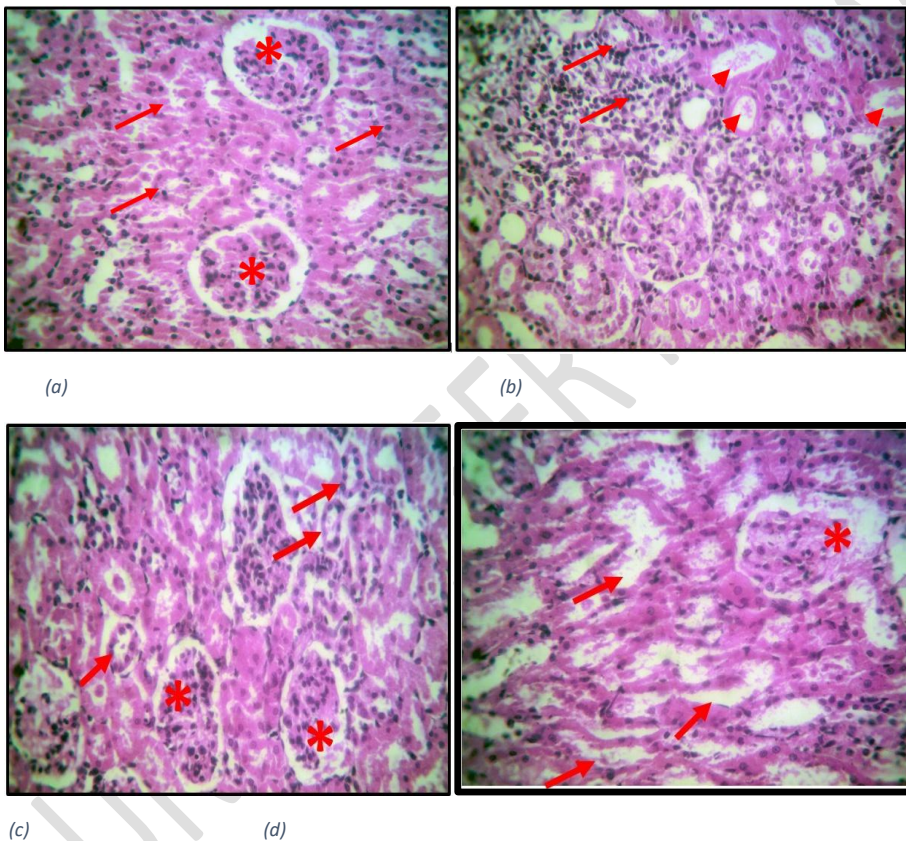


Fig. 2:The kidney micrograph of the extract treated groups in comparison with the control. Haematoxylin and Eosin. Magnification: X100.

(a): Control kidney with normal histological features. The glomeruli (*) and tubules (arrows) are normal; (b): 100 mg/kg treated group showing eosinophilic deposits in the tubules (arrows) and infiltration by neutrophils (arrow head); (c): 200 mg/kg treated group showing that tubules (arrows) have sloughed off epithelium and the glomeruli are normal (*); (d): 400 mg/kg treated group showing dilated renal tubules with increased lumen (arrows) while the glomeruli (*) are normal.

The micrograph of the heart section is presented in Fig. 3. The histology of the heart section of the control group (a) shows normal cardiac fibres in comparison with the 100 mg/kg treated group (b) also with normal cardiac fibres, 200 mg/kg treated group (c) showing normal cardiac fibres with a mild infiltration by mononuclear cells and 400 mg/kg treated group (d) showing wavy cardiac fibres.

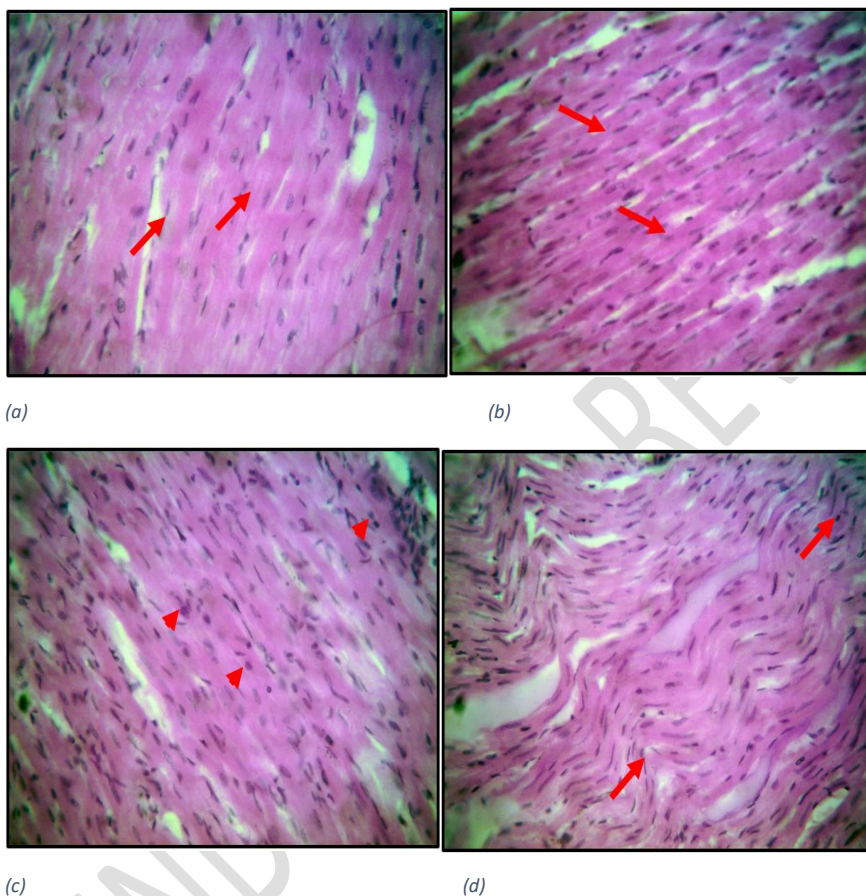


Fig 3: The Heart micrograph of the extract treated groups in comparison with the control. Haematoxylin and Eosin. Magnification: X100.

(a): Control heart with normal cardiac fibres (arrows); (b): 100 mg/kg treated group showing normal cardiac fibres (arrows); (c): 200 mg/kg treated group showing normal cardiac fibres (arrows) with mild infiltration by mononuclear cells (arrow heads); (d): 400 mg/kg treated group showing wavy cardiac fibres (arrows).

Discussion

Acute Oral Toxicity test

The result from the 24 h acute oral toxicity test showed that the extract did not cause any change in behavior of the rat and no mortality was recorded up to 5000 mg/kg. This shows

that the extract has a wide safety margin and potentially safe when used with in a short period. Despite the assumed safety of the extract due to a wide safety margin and the reported benefits from studies, the fact that the rind of the fruit is not commonly consumed and with available evidences of involvement of plant materials in pathology of different toxicity [18], it is important that further investigation be carried out on their possible toxicity. To fully establish the safety of the extract, we evaluated its effects on the body organ histology as well as the biochemical and hematological parameters which are indices of toxicity. This will be a guide for appropriate safe use of the extract for medicinal and nutritional purposes.

Comment [ON20]: More literature should be compared and stating possible reasons for agreement or disagreement.

Effect of the extract on the liver function

Liver function parameters serve as markers in detection of liver damage caused by the presence of a substance which might be toxic to the liver. The liver is an important organ involved in the detoxification and metabolism. Any damage in the liver is evidenced in a change in the histology as well as in the level of the liver function parameters which include the liver enzymes (AST, ALT and ALP), albumin, protein, total and direct bilirubin. Liver enzymes are marker enzymes for liver function and integrity [19, 20]. Studies have shown that liver enzymes are released into the blood following damage of liver cells thereby increasing the activity of these enzymes in the plasma [21, 22]. This increase in the concentration of the enzymes can cause tissue necrosis of which the liver is specifically affected [23]. From our study, the levels of the liver enzymes of the extract treated rats were not significantly affected when compared with that of the control (Table 1). This shows that the extract did not cause any significant necrotic damage on the liver of the treated rats. Nevertheless, the increase found in the concentrations of the three enzymes at 100 and 200 mg/kg although not significant indicates a disturbance in the cell membrane integrity and damage of the hepatocytes [24, 25], which may be more pronounced following chronic use. This is also evidenced from the histological studies as the extract at 200 and 400 mg/kg induced some cellular lesions like hepatic vacuolar degeneration and periportal inflammation with mild sinusoidal infiltration. This indicates that the toxicity effect of the extract on the liver was more structurally than functionally.

The level of the serum protein in all the treated rats were observed to increase insignificantly ($p > 0.05$) when compared to the control rats. Serum total protein is a marker of the synthetic function of the liver and an essential guide in assessment of the severity of liver damage [26]. A reduction usually indicates damage to hepatocytes and increased rate of conversion of amino acid to insulin or reduced ribosomal protein synthesis. From the result, it can be deduced that the extract did not affect protein synthesis rather it increased protein synthesis. It could be said that the extract has a protective effect as it has been reported that an increase in serum protein level has a hepatoprotective effect [27]. The total and direct bilirubin levels were also found to increase in all the treated rats and significantly ($P < 0.05$) at 100 mg/kg when compared with the control group. The level of total bilirubin is also an important marker of liver damage as it is conjugated in the liver for removal through the bile or kidney. An increase in bilirubin level indicates that liver function has been compromised. This occurs because of reduction in the liver conjugation and uptake or increase in the formation of bilirubin [28] due to liver damage.

Effect of the extract on the kidney function

The kidney as a major excretory organ of the body is crucial for the proper functioning of the body. It plays a vital role in good health maintenance through its ability to maintain the homeostasis of the body, excrete waste products of metabolism, chemicals and drugs. Urea and creatinine are among the waste products of metabolism excreted by the kidney while the electrolytes are re-absorbed in the tubules in order to maintain the homeostasis of the body [30]. Creatinine and urea are cleared by the body through glomerular filtration, hence, evaluation of serum urea, creatinine and electrolytes (Na^+ , K^+ , Cl^- , HCO_3^{2-}) are important and sensitive bio-markers commonly used in the diagnosis of damage and renal failure [31]. Electrolytes regulate the osmotic pressure in cells and aid in maintaining the function of muscle and nerve cells. Increased levels of serum urea and creatinine indicate kidney damage and also change in levels of serum electrolytes are indicative of tubular dysfunction. In this study, we observed an insignificant reduction ($p>0.05$) in the level of urea in the extract treated rats and an insignificant increase in the creatinine level in 200 and 400 mg/kg group when compared with the control. Nevertheless, there was a significant reduction in creatinine level of the 100 mg/kg treated rats when compared with the control. Amount of creatinine is always constant and so increased levels indicate reduced renal function since it is easily excreted by the kidneys [32]. Increased level of creatinine shows that more creatinine was retained in the blood. From the result, it means that higher dose of the extract led to the elevated level of creatinine observed although not significantly but it is a sign of renal impairment. We equally observed a significant increase ($p<0.05$) in the levels of the electrolytes (Na^+ , K^+ , Cl^- , HCO_3^{2-}) when compared with the control. Electrolyte imbalance is a hallmark of altered electrolytes distribution [33]. This commonly occurs due to dehydration, fever and kidney failure. Sodium and potassium are the main cation of extracellular and intracellular fluids respectively. Sodium plays functions centrally in maintaining the normal distribution of the osmotic pressure and water in the various fluid compartments. High sodium (hypernatremia) or low sodium (hyponatremia) can lead to malfunction of cells, and extremes in the level of blood sodium (higher or lower than normal) can be deleterious. Potassium is also a vital component of the extracellular fluid for its influence on the activity of the muscle. Increase in potassium levels (hyperkalemia) are often linked to dehydration, shock, renal failure or adrenal insufficiency while reduced potassium levels (hypokalemia) are linked to negative nitrogen balance, malnutrition, gastrointestinal fluid losses and hyperactivity of the adrenal cortex [34]. Chloride is vital in maintaining the cation/anion balance between intra and extracellular fluids. It is also important in the control of proper hydration, osmotic pressure, and acid/base equilibrium. Increased serum chloride values may be seen in dehydration, hyperventilation, congestive heart valve, and prostatic or other types of urinary obstruction [34]. The level of serum bicarbonate is a significant indicator of dispersion of electrolyte and anion deficit. A change in the level of bicarbonate and CO_2 dissolved in plasma are features of imbalance of acid-base, which may be as a result of renal tubular acidosis, hyperkalemic acidosis, renal failure or keto acidosis [35]. From the result of this study, the significant increase ($p<0.05$) observed in the concentration of all the electrolytes in all the treated rats when compared with the normal shows that the extract altered the functioning of the kidney. This was also evident in the histology result of the kidney in which there was some histological distortions in the kidney of all the extract treated rats showing eosinophilic deposits and dilated tubules with infiltration by neutrophils.

From the analysis of the effects of the extract on the lipid profile of the rats, we observed a dose-dependent significant decrease ($p < 0.05$) in the total cholesterol (TC) and high-density lipoprotein (HDL) levels when compared with the control. This is a good report as the blood level of TC should be low to avoid the risk of heart disease. The levels of triglycerides (TG) and very low-density lipoprotein (VLDL) at 100 and 400 mg/kg were reduced but increased insignificantly ($p > 0.05$) at 200 mg/kg when compared with the control. However, there was an insignificant reduction of low-density lipoprotein levels in all the extract treated groups when compared with the control. High levels of TC, TG and LDL have been reported to be the main cause of coronary heart diseases. From our result, it shows that the extract may not cause any heart issues as the levels of these lipids were kept low. However, at 200 mg/kg, the increase in the TG and VLDL is an indication of potential to cause arteriosclerosis as high levels of TG in the blood may lead to the thickening or hardening of the artery walls, increasing the risk of heart attack, stroke and heart diseases. This result correlates with the result of the histology of the heart in which at 200mg/kg there was histological changes in the heart of rats evidenced by mild infiltration by mononuclear cells. Mononuclear cell infiltration is a feature of chronic inflammatory reactions [36]. Mononuclear cell infiltrates in the heart can be of particular concern since they can be more difficult to distinguish from inflammation and can be associated with myocyte injury [37]. This shows that the extract at the dose of 200mg/kg may have caused some degenerative changes in the heart of the rat which was accompanied by cell infiltrate.

The result of the effect of the extract on the antioxidant enzymes (CAT and SOD) activities and the level of Malondialdehyde (MDA) showed that the extract had an effect on the serum enzymatic antioxidants. The significant increase in activity of SOD in all the treated groups and in CAT at 400 mg/kg is an indication that the enzymes were induced by the extracts in the rats. Both enzymes (SOD and CAT) are said to be inducible enzymes whose production could be activated. Previous findings have shown that these two enzymes can be induced in animals as seen with the extract of black rice [38], and fraction of ethyl acetate leaf extract of *Globimetulabranuii* [39]. Increase in activity of these enzymes indicates increased antioxidant ability of the animals [40]. SOD acts first in defense by converting free radicals to H_2O_2 which is further detoxified to form water and molecular oxygen by CAT. We also observed significant increase ($p < 0.05$) in MDA level which was less at 200 and 400 mg/kg dose in the treated rat groups when compared with the control. The increase in the MDA level is an indication of lipid peroxidation as MDA is the product of lipid peroxidation. The observed reduction at higher doses suggest that increase in dose could be more effective in inhibiting lipid peroxidation processes.

The effect of the extract on the hematological parameters was also determined. In toxicity studies, evaluation of hematological parameters is very essential and sensitive index [41]. Studies have shown that there are changes and alterations in hematological parameters following consumption of toxic agents [42, 43]. From the result, the packed cell volume (PCV), HB and RBC levels were reduced while the white blood cell (WBC) level was increased significantly when compared with the control. This reduction could be that the extract had direct toxic effect on the haemopoietic tissues or an indirect action on haemopoiesis by reducing erythropoietin synthesis [44]. The significant increase in the white blood cells count observed in all the doses of the extract compared with the control shows that the extract had leucopoietic effect which led to increase in WBC production [45]. In this

case, the rat's ability to generate antibodies during phagocytosis will increase leading to increase in resistance to diseases and its ability to adapt in disease conditions which may be prevalent in an environment [46].

Limitation of the study

The limitation of this study was lack of adequate funding to carry out long term/chronic toxicity studies which would be helpful to ascertain safety in long term usage.

Conclusion

This study has shown that the fruit rind of *Cucumis metuliferus* has a wide safety margin from the acute oral toxicity study. Although the rind is apparently safe in acute use but the results from the biochemical and hematological indices showed its potential to cause toxic effects to the organs which was more obvious in the histological studies. The toxic effect may be more pronounced following chronic use. Therefore care must be taken when in use.

Ethical Considerations

Compliance with ethical guidelines

"Principles of laboratory animal care" were followed, the protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and the study was approved by the University's Committee on Research Ethics and Standards.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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