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Assessment of the Effect of *Citrullus lanatus* Pulp and Seed Aqueous Extract on the Liver of Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The purpose of this study was to assess the impact of an aqueous extract of watermelon (*Citrullus Lanatus*) seeds and pulp extract on the Albino rat's liver tissues.
Study Design: A pre-clinical experimental trial using rat models.
Study Area: This study was carried out at the Department of Medical Laboratory Sciences, University of Nigeria Enugu Campus, and the Department of Morbid Anatomy, Enugu State University Teaching Hospital.

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Methods: Eighteen albino rats were grouped from A to F. Group A and B served as the baseline and positive control respectively and were provided distilled water and 100mg/kg body weight of Vitamin C for 21 days, respectively. Group C-F received watermelon extract in the following concentration 25mg/kg, 50mg/kg, 100mg/kg, 150mg/kg respectively for 21 days. All dosages were administered orally using gavage once a day. Following the experiment, samples of blood were taken in order to measure the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Total bilirubin (TB) using QCA (Quinica Clinical applicator assay kits). **Results:** The results showed that a statistical significant decrease in the Aspartate transaminase (AST) level was observed in the rats that received 25mg/kg *Citrullus lanatus* extract (CLE) [61.67±2.67] when compared with the baseline control [106.67±4.98]. A notable statistical increase in the C-reactive protein value was observed in rats in group E and F that received 100mg/kg and 150mg/kg of *Citrullus lanatus* extract respectively when compared with the baseline and positive control. Histological findings on the liver also showed normal histomorphology across all the groups. In conclusion, the pulp and seed extract of *Citrullus lunatus* did not significantly alter the albino rat's liver's relative organ weight, but they did exhibit an increase that is dose-dependent in antioxidant activity.

Keywords: Watermelon; liver tissue; C-reactive protein; aqueous extracts; Citrullus lanatus.

1. INTRODUCTION

Water melon, or *Citrullus lanatus*, is a flowering plant that resembles a vine and belongs to the Cucurbitaceae family. Since the fruit is the edible portion of the plant, it is mostly produced for its flavor [1]. Due to its large edible fruit—a rare berry variety with a hard skin and no internal division-globally, the plant is cultivated in both tropical and subtropical climates. It is usually huge, round or oval in shape, with juicy, sweet, soft flesh that is either pink or deep red coloured [2].

Typically, the fruit's unripened portion is green or greenish-white colored. It is easiest to identify ripe, full fruit when shattered since both ripe and unripe fruit often have smooth, greenish exterior surfaces [1]. The fruit's flesh, which has several pips dispersed within, may be pink or red (often) orange, white, yellow or green [3]. The fruit's rind is mid to dark green and typically blotched or striated. It is one of these therapeutic plants that have generated interest among scientists because of its bioactivities. Antioxidants such citrulline, vitamin C and beta carotene are naturally found in *Citrullus lanatus*. Red-fleshed watermelons are also a fantastic source of lycopene [4].

As an essential organ in the human body, the liver controls a number of functions, including metabolism, detoxification, digestion, immunity, and vitamin storage. It makes up around 2 percent of the body weight of a healthy adult [5]. The liver is a unique organ because it receives blood from both the portal vein, that supplies about 75% of its flow of blood, and the hepatic artery provides about 25% [5]. The tight upper abdominal quadrant contains the diaphragm in the human body system. It also performs other metabolic functions, such as regulating the amount of glycogen stored, metabolizing red blood cells, and generating hormones [6]. Additionally, it facilitates the elimination of the harmful chemical, bilirubin that is produced during the lysis of red blood cells and which, when accumulated, can be extremely toxic and cause jaundice [6,7].

Phytochemicals, alternatively referred to as secondary metabolites of plants, are constituents that have biologic function and are present in plants [8]. Plants produce compounds called phytochemicals for a number of uses that are advantageous to the plants themselves. They are present in many parts of plants, including as the pulp, bark, roots, leaves, flowers, and seeds [8]. Humans have used their ability to prevent disease by employing them as medicines since plants first utilized them to defend themselves against environmental factors [9]. Phytochemicals' biological attributes include antibacterial and antioxidant action, a variety of detoxifying enzymes, immune system activation, decreased platelet aggregation, hormone metabolism modification, and anticancer capabilities [8,9].

It is a known fact that plants make these compounds for their own safety, but new research has revealed that many phytochemicals can also protect people from illness [10]. The components of watermelon

extracts are widely known for their antioxidative properties and ability to combat free radicals. Due to its bioactivities, this medicinal plant has attracted the attention of experts [4]. *Citrullus lanatus* naturally contains antioxidants such beta-carotene, vitamin C, and citrulline. Lycopene is abundant in watermelons with red flesh [4,11]. Consequently, the claim that eating this fruit may have a defensive impact on the liver will be explored further in the current study. The study will help in assessing the effects of *Citrullus lanatus* seed and pulp extract using the liver of Albino rats as a prototype.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh *Citrullus lanatus*, or water melon, fruit was bought from a market in Enugu North Local Government Area of Enugu State, Nigeria. A botanist from the Department of the Botany University of Nigeria Nsukka, Enugu State, properly identified the fruit and assigned it the voucher number UNH/03/0316B.

2.2 Plant Material and Extraction

The purchased *Citrillus lanatus* was extracted from the pulp, the *Citrullus lanatus* seeds were left to air dry for 48 hours before being ground in a gasoline-powered grinder. The crude aqueous extract was made by dissolving 100g of finely ground *Citrullus lanatus* seed in 400ml of water. After ten minutes of stirring with a wooden stirrer, the liquid was left to stand for an hour while being periodically stirred. Muslin cloth was used to seive the homogenate. 700 ml of blended Citrullus lanatus pulp juice was then mixed with three (300 ml) of the resultant filtrate. Before administering the mixture to the experimental rat models, the mixture was thoroughly stirred, kept in an airtight container to avoid moisture absorption, and kept in the refrigerator at 2-8°C

2.3 Experimental Animals

The research made use of eighteen male Albino rats that were acquired from Nsukka, Enugu state, Nigeria, for the experiment. The animals were housed at the Animal House in the Department of Anatomy at the University of Nigeria Enugu Campus. The rats were weighed, placed in six groups of three rats each, and kept in cages made of metal. They were retained in a typical laboratory setting with a temperature of (27±2°C) and a 12-hour light and dark cycle. A typical Grower mash meal (Vital Feeds Nigeria ltd)® and water were made available to the animals *ad libitum* The experiment, which lasted 21 days, was conducted with the animals in the animal house. Before the study started, a two-week acclimatization period was given to Albino rats.

2.4 Experimental Design

Eighteen (18) male Albino rats weighing 100–135g and 3–4 months of age were used in this study. Based on body weight, the rats were split up into six groups (A-F). Rats in groups C through F were the test groups, whereas rats in groups A and B were the baseline and positive control. For 21 days, rats in Groups C–F, respectively, were given oral gavages of a watermelon and seed extract mixture with dosages of 25 mg/kg, 50 mg/kg, 100 mg/kg, and 150 mg/kg body weight. When rats in group B got 100 mg/kg of vitamin C, group 'A' rats were given water. Each animal was cared for in accordance with the organization's policies regarding the treatment and care of animals used in scientific research [12].

2.5 Biochemical Tests

The animals were weighed, blood samples were obtained from the rats' medial canthus using a retroorbital puncture, serum was extracted from each blood sample, and several biochemical parameters, such as total bilirubin, alanine transaminase (ALT) and aspartate transaminase (AST), were measured after treating the rats with *Citrillus lanatus* seed and pulp extract for twenty-one (21) days. These measurements were made using the colometric method developed by Reitman and Frankel (1957) as cited by [13]. The enzyme-linked immunosorbent assay (ELISA) kit that was bought from Elabscience® was used to determine the amount of C-reactive protein.

2.6 Relative Organ Weight

Under chloroform anesthesia, the rats were sacrificed and dissected. The liver was grossly examined. The rats' excised liver tissues were cleansed, and any lesions or other abnormalities were checked for. Each rat's liver was weighed to estimate the relative organ weight (ROW), which was computed as follows:

$$ROW = \frac{Absolute\ organ\ weight\ (g)}{Body\ weight\ of\ rats\ on\ sacrifice\ (g)} \times 100$$

2.7 Histological Processing

The excised liver tissues were chopped into slabs that were about 0.5µm thick and preserved in 10% formalin. The liver tissues were processed using the automatic tissue processing utilizing the paraffin wax embedding technique. Using a Rotary Microtome (Heitz 150 Rotary Microtome, Cambridge type), sections of 5µm thick was produced. The Hematoxylin and Eosin staining method was used.

2.8 Microscopy and Photomicrography

Olympus binocular microscope with integrated illumination system was used to examine the sections. Consequently, the sectioned tissues was photographed under a microscope with an Olympus trinocular microscope and a Samsung Model SS850 digital camera.

2.9 Statistical Analysis

Where appropriate, the results were expressed using the mean and standard error mean (SEM). The study was conducted using the Statistical Package for Social Sciences Software Program (SPSS, Chicago, IL, version 23.0). A one-way analysis of variance (ANOVA) was performed on the data. The tukey highest significant difference (HSD) post-hoc test and group-to-group parameter differences came next. A significance threshold of $p<0.05$ was taken into account.

3. RESULTS

3.1 Morphological Studies

Prior to the animals being sacrificed, weights were taken both before and after the administration process. For every group of rats, the rodents' body weight increased after the experiment when compared with the initial weights of the rats. The group that received distilled water treatment had the largest increase in mean weight gain. The group that received the greatest dosage of *Citrullus lanatus* extract-150 mg/kg body weight—came next.

Table 2shows the relative organ (liver) weights (ROW) after the administration of *Citrullus lanatus* extract which was examined across different experimental groups. There was no significant difference in the ROW of rats treated when the groups were compared with the two controls. The hepatic index (Liver-body weight ratio %) showed no significance when also compared the groups with the positive and baseline controls after the administration of the CLE.

3.2 Biochemical Analysis of Liver Enzymes (AST, ALT, TB)

Table 3 shows the liver enzyme serum level enzymes in each group. Group C and D expressed decreased level of AST, at ($p\leq0.002$) and ($p<0.025$) respectively in contrast to the positive control (84.67 ± 4.33). Group C (61.67 ± 2.67), group D (74.67 ± 8.59). C. *lanatus* pulp and extract from the seed produced a dose-dependent and also statistically remarkable decrease in the level of the liver enzymes at 25mg/kg AST ($p\leq0.002$) and also at 50mg/kg ($p<0.025$), in contrast to group B which is the positive control that took 100mg/kg of Viamin C.

3.3 Analysis of the C-reactive Protein

Table 4 shows the serum level of c-reactive protein in each group C. *lanatus* pulp and extract from the seed produced a dose-dependent also statistically notable increase in the level of the c-reactive protein at 100mg/kg ($p\leq0.002$) and also at 150mg/kg ($p<0.025$), in contrast to group A, the baseline control and group B which is the positive control that took 100mg/kg 0f Vitamin C.

Table 1. Body Weight Changes Before and After Administration of *Citrullus lanatus* Extract

Groups	Number of rats	Body weight Before (MEAN±SEM)		Body Weight After (MEAN±SEM)	
Group A [Baseline control]	3	100.00±0.00		171.77±17.43	
Group B [100mg/kg Vitamin C]	3	101.00±1.00		153.53±7.62	
Group C [25mg/kg CLE]	3	101.00±4.67		153.20±2.08	
Group D [50mg/kg CLE]	3	109.00±4.93		153.20±4.43	
Group E [100mg/kg CLE]	3	118.67±1.33		153.20±8.54	
Group F [150mg/kg CLE]	3	126.00±4.58		169.70±9.67	
		F-ratio	**Sig**.	**F-ratio**	**Sig**.
		8.921	0.001	0.680	0.674

The data was expressed as mean ± SEM. P < 0.05 is considered significant. Statistical analysis by one-way Anova

Table 2. Relative Organ Weights and Liver index After Administration of *Citrullus lanatus* Extracts

Groups	Number of rats	Relative Organ Weight (MEAN±SEM)		Liver index (MEAN ±SEM)	
Group A [Baseline control]	3	5.87±0.35		3.46±0.21	
Group B [100mg/kg Vitamin C]	3	6.13±1.13		3.97±0.60	
Group C [25mg/kg CLE]	3	6.13±0.35		4.00±0.22	
Group D [50mg/kg CLE]	3	6.27±0.63		3.83±0.28	
GroupE [100mg/kg CLE]	3	6.50±0.58		3.95±0.17	
Group F [150mg/kg CLE]	3	6.30±0.15		3.73±0.19	
		F-ratio	**Sig.**	**F-ratio**	**Sig.**
		0.14	0.98	0.43	0.82

P< 0.05 is considered significant

Table 3. Comparison of ALT, AST, and Total bilirubin level of Test Groups against Control groups (A and B)

Groups	Number of Rats	ALT Mean±SEM	AST Mean±SEM	Total bilirubin Mean±SEM
Group A [Baseline control]	3	32.33±3.33	106.67±4.98	0.53±0.26
Group B [100mg/kg Vitamin C]	3	22.33±1.33	84.67±4.33	0.74±0.32
Group C [25mg/kg CLE]	3	24.00±5.13	61.67±2.67*^{b}	0.85±0.09
Group D [50mg/kg CLE]	3	30.00±9.00	74.67±8.59*^{b}	0.81±0.15
Group E [100mg/kg CLE]	3	18.00±5.13	89.00±0.00	0.53±0.27
Group F [150mg/kg CLE]	3	33.67±4.67	111.33±9.49	0.95±0.30

*Data are expressed as mean ± SEM of five rats in each group. Stastistical analysis by one way ANOVA followed by Turkey 's posthoc test. Where * represent significance at (p≤0.05), ** represent highly significance at (p≤0.01), b= All group compared with group B*

Table 4. Comparison of C-reactive protein level of Test groups against Control groups (Group A and B)

Groups	Number of Rats	C-reactive protein Mean ± SEM
Group A [Baseline control]	3	2.43±0.07
Group B [100mg/kg Vitamin C]	3	2.40±0.10
Group C [25mg/kg CLE]	3	2.53±0.15
Group D [50mg/kg CLE]	3	2.90±0.58
Group E [100mg/kg CLE]	3	4.13±0.19**^{ab}
Group F [150mg/kg CLE]	3	3.10±0.21**^{ab}

The mean ± SEM of five rats per group is used to express the data. Statistical analysis using Turkey's post hoc test after one-way ANOVA.

*Where * represent significance at (p≤0.05), ** represent highly significance at (p≤0.01)*

a= All group compared to baseline control

b= All group compared with group B

3.4 Histopathological Studies

The histoarchitecture of the liver parenchyma was normal in the water control group's liver segment (Plate 1). Liver section of the ethanol control group (Plate 2) showed a normal histo-architecture showing no obvious histomorphological alteration. Plates 3,4,5,6, the liver sections of the rats that took 25mg/kg, 50mg/kg, 100mg/kg, 150mg/kg respectively showed no sign of tissue damage, the histo-architecture were intact.

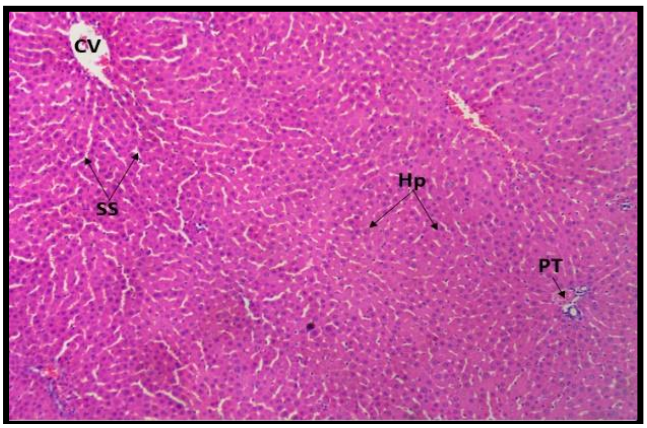

Plate 1. Liver section photomicrographs from normal control rat (Group A) showing normal histoarchitecture of the hepatic tissue. Normal features including central veins (CV), hepatocytes (Hp), portal tracts (PT) and sinusoidal spaces (SS) appear normal. (Stain: H&E; Mag: x100)

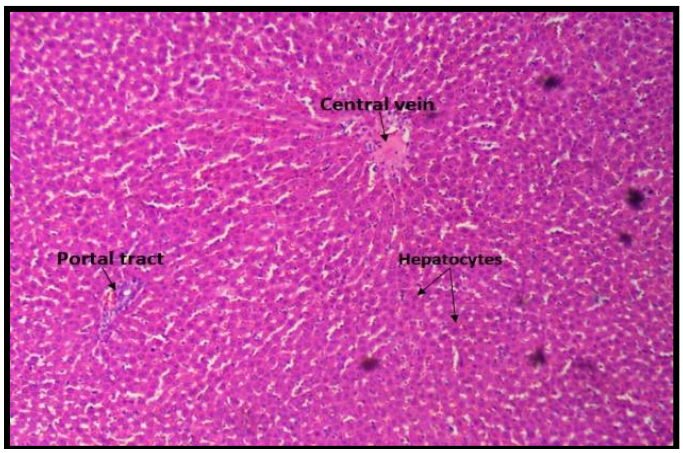

Plate 2. Liver section photomicrograph from rat treated with 100mg/kg body weight (b.wt.) of Vitamin C (Group B) showing no obvious histomorphological alteration. (Stain: H&E; Mag: x100)

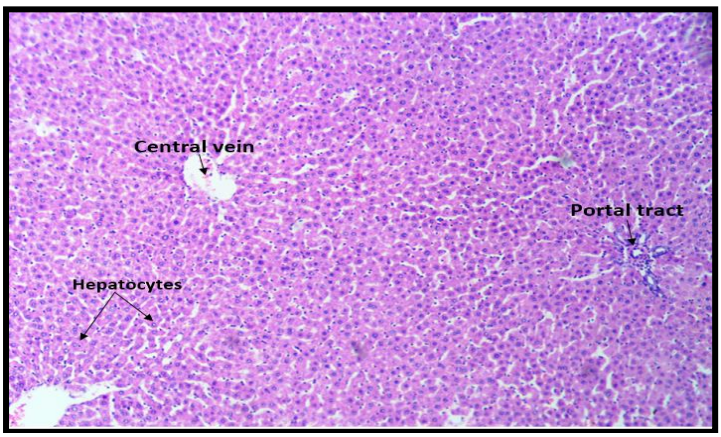

Plate 3. Liver section photomicrograph from rat treated with 25mg/kg of CLE (Group C) revealing intact histomorphology of the hepatic tissue. No obvious tissue alteration is seen. (Stain: H&E; Mag: 100)

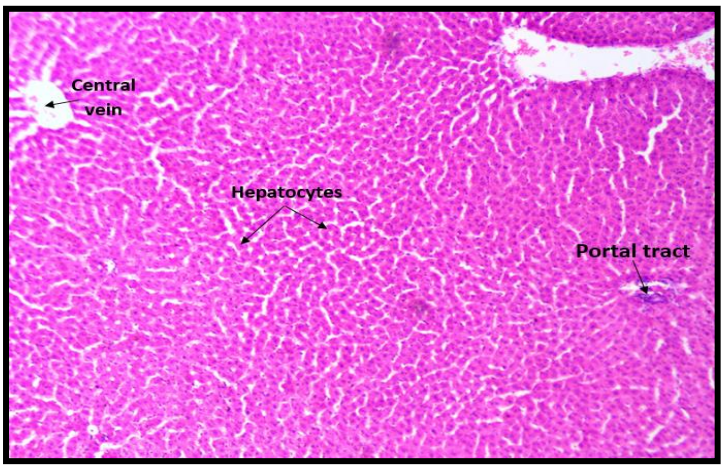

Plate 4. Liver section photomicrograph from rat treated with 50mg/kg of CLE (Group D) revealing intact hepatic histomorphology. No alteration is observed. (Stain: H&E; Mag: 100)

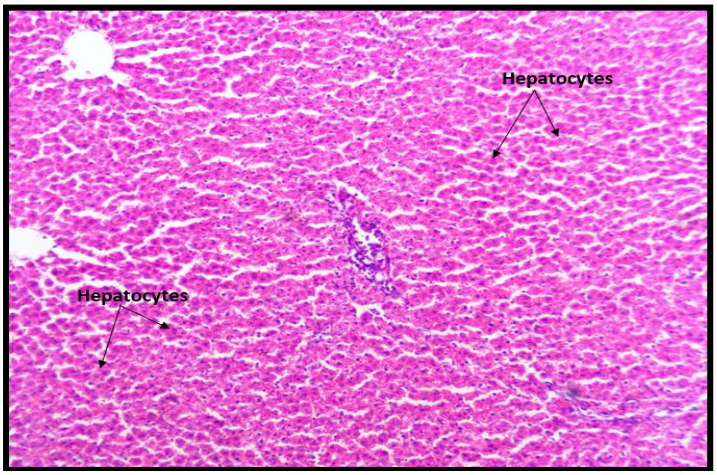

Plate 5. Liver section photomicrograph from rat treated with 100mg/kg of CLE (Group E) showing no histomorphology alteration. (Stain: H&E; Mag: 100)

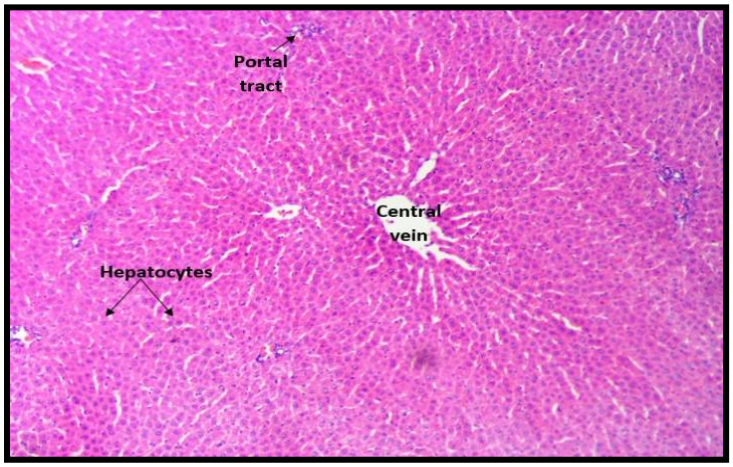

Plate 6. Liver section photomicrograph from rat treated with 150mg/kg of CLE (Group F). Features show intact tissue morphology with no obvious alteration seen. (Stain: H&E; Mag: 100)

4. DISCUSSION

This present study was aimed at evaluating the impact of *Citrullus lanatus* seed aqueous extract and pulp extract on liver of albino rats. All of the groups showed a rise in body weight, including the control groups, suggesting that *Citrullus lanatus* pulp and seed aqueous extract did not affect nutrient uptake by the body.

Similarly, there was no significant changes in the liver index across all groups which suggest that the extract doesn't damage the hepatocytes. This is as opposed to when compared with the hepatic index of rats that were induced with alcohol which showed a rise in the ratio to of liver body weight to hepatic index [14]. According to [15,16], this might be due to effect of the acetaldehyde, the first metabolite, which is a poisonous and unstable chemical that is created when the body breaks down ethanol in the liver using the enzymes alcohol dehydrogenase. The amount of fatty acids and triglycerides in the liver increases as a result of this metabolite and the hydrogen created in the same reaction dislodging fatty acids as fuel, raising the relative organ weight [14, 15, 16].

It is significant to remember that relative organ weight measurements offer valuable insights on the toxicity of substances to these organs [17, 18]. In the study conducted by [19, 20, 21] using various plant extracts including *Benincasa hispida*, and *Pterocarpus santalinus,* it was observed that there was no rise in the relative stomach, heart and lung weights of a few of the animals receiving treatment with further outcome of no toxicity in these groups across the studies. In corroboration with the outcome of this study, it strongly suggests that the less effect on the organ weight, the less toxic these extracts are to the liver, and indeed the whole body [19, 20]. But it's also plausible that the experimental animals' lower body weights contributed to the increased relative organ weight [22]. This is as opposed to the present study where there was no notable (p=0.98) difference in the body weight of the rats across all groups, which suggests that the extract is not toxic. Table 2 illustrates this by comparing the relative organ weights of the animals in the different treatment groups to the control group. Additionally, a physical inspection of the excised tissues revealed no alterations that would indicate organ toxicity.

AST and ALT are members of Transaminase Family of enzymes. ALT and AST are found in large amount in the liver and also small amount are found in the heart, kidney and muscles [5]. When the liver is injured or inflamed as the case may be via its exposure to various forms of toxic substances, the level of these enzymes in the blood is usually elevated [23]. The result obtained from this study revealed a notable drop in levels of AST at reduced dosages of the aqueous extract 25mg/kg and 50mg/kg. This shows that there was no injury in the liver due to the ingestion of the aqueous extract, and also a strong signifier of potential protective property of the extract. When compared to some studies that focused on the effect of this extract on already diseased liver using acetaminophen, outcomes showed that the mesocarp and seed extracts are imbued with lots of alkaloids, phenolics, tannins, and terpenoids, producing protective and/or therapeutic effect on an inflamed liver at 200mg/kg of body weight [24].

It is important to also add that in this present study, there is a significant decrease in Aspartate Transaminase (AST) in Group C and D that took 25mg/kg CLE and 50mg/kg CLE respectively in contrast to the baseline control Group A that took only water. This suggests that the extract provided more hepatoprotective effect compared to the group that were exposed to nothing. Conversely, according to [25] studying the impact of an aqueous *Citrullus lanatus* seed extract on Liver and Kidney showed a marked increase in the kidney parameters at very high doses, suggesting that this extract maybe toxic when over-consumed.

The liver produces a chemical called C-reactive protein (CRP) in reaction to inflammation. It is part of the body's immune response and serves as a marker for inflammation in the body [26]. In this study the groups that took the higher doses of the extract which is the group E and F that took 100mg/kg and 150mg/kg respectively revealed a notable rise in the C-reactive protein when compared to both the baseline that took only water and the positive control that took 100mg/kg of Vitamin C, this could indicate a high antioxidant activity, but it is dose- dependent. This means that the extract may have a greater effect on CRP levels at higher doses than at lower doses because according to the result in Table 4, there was no significance in the groups that took the low dose of the extracts. This dose dependent antioxidant activity may be attributed to the high level of phenolics present in the peels of *Citrillus lanatus,* as described by [27] in their study on the antioxidant and antimicrobial effect

of the peel, pulp, rind, and seeds of *Citrillus lanatus.*

5. CONCLUSION

This research indicates that the watermelon extracts have no discernible impact on the liver's relative organ weight of the albino rats. It also shows that when the extract is taken in higher concentration, the antioxidant activity is increased therefore signifying a hepatoprotective potential at such doses.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The Department of Animal Science at the University of Nigeria Nsukka provided ethical approval for this work.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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