Original Article

Hepatoprotective Effects of Aqueous Extract of Watermelon (*Citrullus lanatus*) Seeds on Ethanol-Induced Oxidative Damage in Wister Rats

ABSTRACT

Background: Watermelon (*Citrullus lanatus*) is a roundish or ovoid fruit rich in antioxidant supplements that prevent oxidative stress in hepatic tissues due to generation of reactive oxygen species following exposure to alcohol. The present study aimed to investigate the protective effects of watermelon seed on ethanol-induced oxidative damage in liver of Wister rats. **Methods:** Twenty-five adult Wister rats of both sexes were randomly divided into five groups of five rats each. Rats in group I were administered distilled water for 8 days. Group II were administered distilled water for 7 days followed by 20 mL/kg body weight of 40% ethanol per body weight on day 8. Groups III and IV were administered 200 and 400 mg/kg, respectively, of the extract for 7 days followed by 20 mL/kg of 40% ethanol on day 8. Group V received 100 mg/kg body weight of silymarin for 7 days followed by 20 mg/kg of 40% ethanol on day 8. Animals were sacrificed on day 8. Liver was excised and then processed for histopathological examination. Biochemical assay for the following hepatic marker enzymes was carried out: alanine amino-transferase (ALT), aspartate amino-transferase (AST), alkaline phosphates (ALP), and protein concentration. **Results:** The results showed that watermelon treatment leads to increase in body weight. Furthermore, pretreatment with watermelon seed extract lead to significant decrease ($P < 0.05$) in plasma AST, ALT, ALP, and protein concentration, compared to ethanol treatment group. **Conclusion:** Watermelon seed extract demonstrates hepatoprotective effect in ethanol-induced oxidation in the liver of Wister rats, which might be linked to various antioxidant phytochemical content present in the extract.

Keywords: Ethanol-induced, hepatoprotective effects, oxidative damage, watermelon seeds

Introduction

Liver is a large vascular glandular organ that secretes bile and causes significant changes in many substances present in the blood (such as conversion of excess sugar into glycogen). It has a regenerative potential, making it possible for an individual with part of his/her liver removed to survive. However, some of its roles are so delicate that anomalies start manifesting based on the nature and severity of its initial damage.[1]

Substances such as H_2O_2, O_2^{2-}, and OH^- collectively known as reactive oxygen species (ROS) are double-edged sword in that their roles can be beneficial or nocuous.[1] Cellular functions in a biological system relied on balance of oxidation–reduction reactions, often seen as prooxidants and antioxidants.[1,2] Shift from equilibrium may be in favor of oxidative stress leading to pathological conditions.[3]

Cells are equipped with effective molecular mechanisms to tightly regulate the intracellular ROS level and to control the balance between oxidant and antioxidant molecules. Oxidative stress arising from disruption in the level of ROS and antioxidant defense potential of the cell[2] give

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rise to major cellular metabolites such as DNA, lipids, and proteins. This phenomenon has been implicated in a number of human disorders including, but not limited to, cardiovascular disorders[3,4] and liver pathologies.[5,6] Acute exposure to high levels of ROS appears to be responsible for the development of various injuries including ischemic and reperfusion in the liver.[7,8] Ethanol is an acute hepatotoxic agent that causes distortion of cytoarchitecture and hepatocyte degeneration.

Chronic or heavy alcohol consumption has been reported to pose a deleterious health hazard and can lead to battery of metabolic disorder in hepatic and extrahepatic diseases.[9] Ethanol has commonly been used as hepatotoxin in experimental hepatopathy. Albeit, the pathogenesis of ethanol-induced liver disease has not been well elucidated, and available evidence suggests that ethanol-induced hepatotoxicity may be due to oxidative stress that leads to impaired liver functions and fibrosis.[10,11]

Alcoholism commonly causes hepatic, gastrointestinal, nervous, and cardiovascular injuries leading to pathophysiology.[12] Cytopathic conditions as a result of alcoholism lead to decrease in activities of antioxidant enzymes.[13,14]

There is a renewed interest globally in the use of medicinal plants for prevention, treatment, and management of various pathologies.[15,16] The therapeutic effects of plants are due to the presence of phytochemicals such as tannins, polyphenols, flavonoids, alkaloids, and terpenoids.[17] Taking into account the key role played by free radicals in the etiology and progression of many disorders, the use of natural products with antioxidant properties has been suggested as an efficacious therapeutic alternative against diseases.[18]

Many studies have reported the protective effects of antioxidants such as epigallocatechin-3-gallate,[19] curcumin,[20] pumpkin oil,[21] quercetin,[22] and resveratrol[23] against alcohol-induced tissue damage. Further, other studies have shown the protective effects of antioxidant-rich natural compounds against various toxicant-mediated tissue damage.[24-26]

Watermelon (*Citrullus lanatus*) is a large oblong, ovoid, or roundish fruit with a hard green or white rind often variegated or striped with a sweet watery pink, yellowish, or red pulp with many seeds. It is a native of tropical Africa that yields about 93% water, thus the name watermelon. In recent years, *C. lanatus* has attracted scientific interest owing to its bioactivities.[27] The seeds of *C. lanatus* is a natural source of the following phytochemicals: phenols, saponins, tannins, flavonoids, and alkaloids.[28] The seeds are rich in protein, tannins, and minerals,[29] and citrulline, a potent precursor of L-arginine.[29,30] They are also rich in beta-carotene,[31] vitamin C, and lycopene.[32] There is paucity of information on the hepatoprotective effects of aqueous extract of watermelon seed on ethanol-induced oxidative stress. Here, we evaluate the antioxidant potential of watermelon seeds pretreatment on ethanol-induced oxidative stress in the liver of Wister rats.

Materials and Methods

Chemicals

Silymarin (Micro Labs Limited, 92, SIPCOT, Hosur, Tamil Nadu, India) was purchased from M.U.B. Pharmaceutical Limited (Zaria, Nigeria). Braford reagent, 2,4-dinitrophenylhydrazine, α-oxaloacetate, L-aspartate, sodium hydroxide, α-oxoglutarate, and L-alanine were obtained from Randox assay kit (Randox Laboratories Limited, Crumlin, UK). All other reagents were of analytical grade.

Collection of plant material

Fresh fruit of *C. lanatus* was purchased from Sabon Gari market in Zaria. The fruits were then authenticated in the herbarium at the Department of Biological Sciences, Ahmadu Bello University, Zaria. The seeds were then weighed and kept in an airtight container.

Preparation of watermelon seed extract

Aqueous extraction of the seeds was done by cold maceration process. The fruit of the *C. lanatus* was opened, after which the seeds were removed, washed, and shed dried. The dried seeds were pounded to powder form. A mass of 250 g of the pounded seeds was soaked in a maceration apparatus with 1000 mL of distilled water for 24 h. After 24 h, it was then filtered and allowed to settle for 1 h, decanted, and evaporated in water bath at 60°C, after which the dried residue was scraped out of the dish.

Acute oral toxicity studies

Acute oral toxicity study is determined according to laid down guidelines of the Organization for Economic Co-operation and Development (OECD) adopting the up-and-down approach (OECD guideline no. 423). Based on the approach, a limit test was conducted to group the toxicity class of the compound. The main test was performed on three male Wister rats to estimate the precise LD_{50}. The animals were fasted overnight with free access to water, weighed, and a single dose of the test substance was administered. Animals were observed individually during the first 30 min,

periodically during 48 h with special attention given during first 4 h (short-term toxicity), and daily, thereafter for total of 8 days (long-term toxicity). LD_{50} was found to be greater than 2000 mg/kg body weight, in limit test. The test substance could be classified in the hazard classification as Class 5–1500 mg/kg $<$ LD_{50} $<$ 4000 mg/kg in the globally harmonized system. The LD_{50} of test drug was found to be 25,000 mg/kg from the main test.[33]

Hepatotoxin

Ethanol is a known hepatotoxin in terms of severity of injury. It causes toxic necrosis leading to biochemical changes that present clinical features similar to those of acute viral hepatitis. Liver injury was induced by administration of ethanol mixed with water. Animals were administered single dose of 40% ethanol on day 8.

Experimental animals

Twenty-five adult Wister rats of both sexes were purchased from the animal house in Pharmacology Department, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The rats were housed in the Department of Human Anatomy animal house and maintained in standard laboratory condition at ambient temperature (35°C), humidity, and under 12 h photoperiod. The animals were allowed to acclimatize to their new environment for 2 weeks prior to commencement of administration. The rats had free access to standard pellet chow (vital feeds from Nigeria Ltd.) and water *ad libitum* throughout the experiment with the exception of 12 h fasting on the last day of the experiment before they were sacrificed. All experimental protocols were reviewed and approved by the Ahmadu Bello University Teaching Hospital Research and Ethics committee.

Experimental design

The rats were divided into five groups of five animals each. Watermelon seed extract was administered orally for 8 days with daily treatment with both the extract and drugs as follows:

Group I (C): Control rats administered distilled water (2 mL/kg body weight) only for 8 days.

Group II (C + E): Rats treated with distilled water for 7 days followed by 20 mL/kg body weight of 40% ethanol per body weight on day 8.

Group III (W_1 + E): Rats treated with 200 mg/kg of the extract for 7 days followed by 20 mL/kg of 40% ethanol per body weight on day 8.

Group IV (W_2 + E): Rats treated with 400 mg/kg of the extract for 7 days followed by 20 mL/kg of 40% ethanol per body weight on day 8.

Group V (S + E): Rats treated with 100 mg/kg of silymarin for 7 days followed by 20 mL/kg of 40% ethanol on day 8.

The 20 mL/kg of 40% ethanol was administered after 6 h of fasting. The 40% ethanol administered at a total accumulative dosage of 20 mL/kg body weight by four equally divided gavages in 20-min intervals intragastrically. All administrations were carried out at 9.00 a.m. It has been well documented that the dose of ethanol used in the present study can elicit tissue toxicity and oxidative stress in Wister rats (Han *et al.,* 2015).[34] For the morphological study, all animals in each group were weighed prior and after the experiment. On the last day of the treatment, all animals were fasted for 12 h and then humanely sacrificed. Liver was excised, weighed, and homogenized in 50 mmol/lTris-HCl buffer (pH 7.4), and then centrifuged at 5000 *g* for 15 min for biochemical studies. The resultant supernatants were immediately kept frozen until when needed.

Further, the dose of the extract at 200 and 400 mg/kg of *C. lanatus* seed were adopted according to Okunrobo *et al.* (2012).[35] Hepatotoxicity induction was done according to a modification of binge-drinking model designed by Carson and Pruett (1996).[36] The dose of silymarin (100 mg/kg body weight) was adopted according to Madhavi *et al.* (2012).[60]

Histological studies

Sequel to administration, the experimental animals were humanely sacrificed following chloroform anesthesia. Blood samples were collected from the apex of the heart via cardiac puncture technique from each animal by the use of syringe and needle for biochemical analysis. The liver tissue was harvested and fixed in neutral buffered formalin for histological analysis.

Statistical analysis

The statistical analysis was conducted by one-way analysis of variance. All mean ± standard error of mean values were used to compare mean difference between and within groups and a two-tailed P value <0.05 was considered statistically significant. Tukey's multiple comparison test was applied for comparison of mean values of different groups treated with different dose levels of extracts and positive controls. Statistical analyses were carried out using STATA 13 software (StataCorp LP, College Station, TX, USA).

Results

Morphological studies

Rats in the C + E group have the least weight gain (2.00 ± 0.45) g compared to rats in other groups [Table 1]. Rats in the silymarin (S + E) group have the highest weight gain followed by those administered with 400 mg/kg body weight of watermelon seed extract. The

Table 1: Mean ± SEM of body weight and percent weight gain

Groups	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Liver–body weight ratio
	Mean ± SEM	Mean ± SEM	Mean ± SEM	(%)
C	182.60 ± 27.89	197.40 ± 28.58	14.80 ± 3.18	3.69 ± 0.38
C + E	186.00 ± 24.56	188.00 ± 24.89	2.00 ± 0.45[?]	5.17 ± 0.19[?]
W_1 + E	191.80 ± 24.89	197.20 ± 24.80	5.40 ± 0.25[†]	4.73 ± 0.20 [?]
W_2 + E	194.20 ± 26.38	202.60 ± 26.68	8.40 ± 1.08[†]	4.26 ± 0.08[†]
S + E	184.20 ± 19.68	194.40 ± 19.82	10.20 ± 0.86[†]	4.04 ± 0.14[†]

C: control rats treated with distilled water, C + E: control rats treated with 20 mL/kg body weight of 40% ethanol, W_1 + E: rats pretreated with 200 mg/kg body weight of *C. lanatus* followed by ethanol intoxication, W_2 + E: rats pretreated with 400 mg/kg body weight of *C. lanatus* followed by ethanol intoxication. Data are presented as mean ± standard error of mean (SEM), $n = 5$ rats per group. Weight gain indicated significant difference ($P < 0.01$) across the groups. The least weight gain was among rats in the C + E group. Those administered 400 mg/kg body weight gained more weight than those administered 200 mg/kg body weight. Consequently, liver–body weight ratio of group C + E was significantly higher compared to other groups. Means with superscripts are statistically significant at $P < 0.05$.

Table 2: Effect of aqueous extracts of *Citrullus lanatus* seeds on biochemical markers in ethanol-induced hepatotoxicity

Group	AST (μ/L)	ALT(μ/L)	ALP (μ/L)	AST/ALT ratio
	Mean ± SEM	Mean ± SEM	Mean ± SEM	
C	35.40 ± 1.47	56.00 ± 8.00	16.46 ± 1.42	0.63
C + E	75.20 ± 4.50*	103.20 ± 10.39*	26.63 ± 1.91*	0.73*
W + E_1	51.00 ± 5.97[?,†]	74.40 ± 8.35[?]	22.08 ± 1.05[†,?]	0.69[?]
W + E_2	45.20 ± 4.92[?]	69.60 ± 5.88[?]	19.11 ± 2.11[?]	0.65[?]
S + E	42.20 ± 3.72[?]	66.40 ± 5.88[?]	18.46 ± 1.23[?]	0.64[?]

ALT, alanine amino-transferase; ALP, alkaline phosphates; AST, aspartate amino-transferase; C: control rats treated with distilled water, C + E: control rats treated with 20 mL/kg body weight of 40% ethanol, W + E_1: rats treated with 200 mg/kg body weight of *C. lanatus*, W + E_2: rats treated with 400 mg/kg body weight of *C. lanatus*. Data are presented as mean ± standard error of mean (SEM), $n = 5$ rats per group. Means with superscripts are statistically significant at $P < 0.05$.

difference in weight gain is statistically significant ($F = 10.43$, $P < 0.01$). This indicates that ethanol administration impaired food consumption among rats whereas watermelon seed extract administration did not impair food consumption. Paired sample Student -*t*-test showed statistical significant difference ($P < 0.01$) between the initial and the final body weight in all groups except for the C + E group ($P > 0.05$). Further, silymarin offered a better weight gain compared to group administered higher dose of watermelon seed extract. Relative organ weight showed statistical significant difference ($F = 3.07$, $P < 0.05$), with the C + E group having higher relative organ weight (5.17 ± 0.19) g compared to other groups.

Effects of watermelon seed pretreatment on antioxidant enzyme activities in Wister rat liver induced by ethanol

Ethanol treatment significantly increased aspartate amino-transferase (AST), alanine amino-transferase (ALT), and alkaline phosphates (ALP) activity in the liver of rats compared to other groups [Table 2]. Watermelon pretreatment reversed this alteration in the liver. Aqueous extract of watermelon conferred significant ($P < 0.05$) hepatoprotection against oxidative stress by mitigating AST, ALT, and ALP activities. Serum level of AST, ALT, and ALP levels are marginally lower at higher dose (400 mg/kg body weight) than at lower dose (200 mg/kg body weight). However, the level of the enzymes is lower in the silymarin-treated group than in the high dose of the extract group. The ratio of AST to ALT remained low in all groups with the C + E group having higher AST to ALT ratio. Therefore, watermelon seed extract significantly decreased ethanol-induced oxidative stress in the liver.

Protein concentration in the liver

There was decrease in the protein concentration in hepatocytes of Wister rats in the control group compared to other groups and an increase in the protein concentration of the liver in the ethanol group, which can be seen in Figure 1.

Histopathological studies

The histopathology of liver for various groups using H&E and Masson's trichrome stains were shown in Figures 2 and 3.

Discussion

The aim of this study was to evaluate the hepatoprotective effects of *C. lanatus* seeds on hepatic damage induced by ethanol. Increase in body weight was observed in all the groups except the water control group (positive control). The 400 mg/kg extract group had the highest gain in body weight when compared to the ethanol control group (negative control). This suggests that *C. lanatus* seed aqueous extract did not affect food intake of the rats. Consequentially, the increase

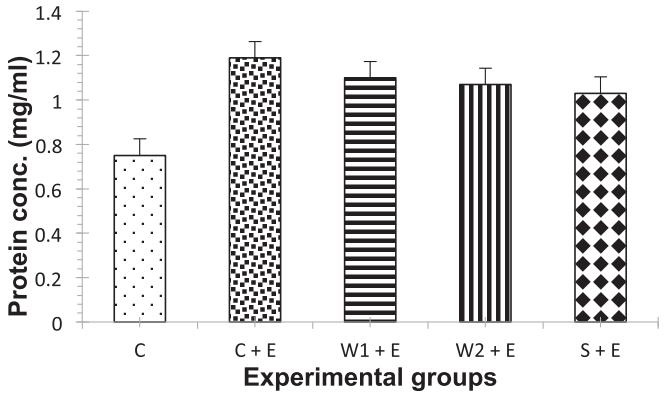

Figure 1: Biochemical analysis of protein concentration in the liver tissue. Mean protein concentration (mg/mL) in hepatocytes of Wister rats in different experimental groups.

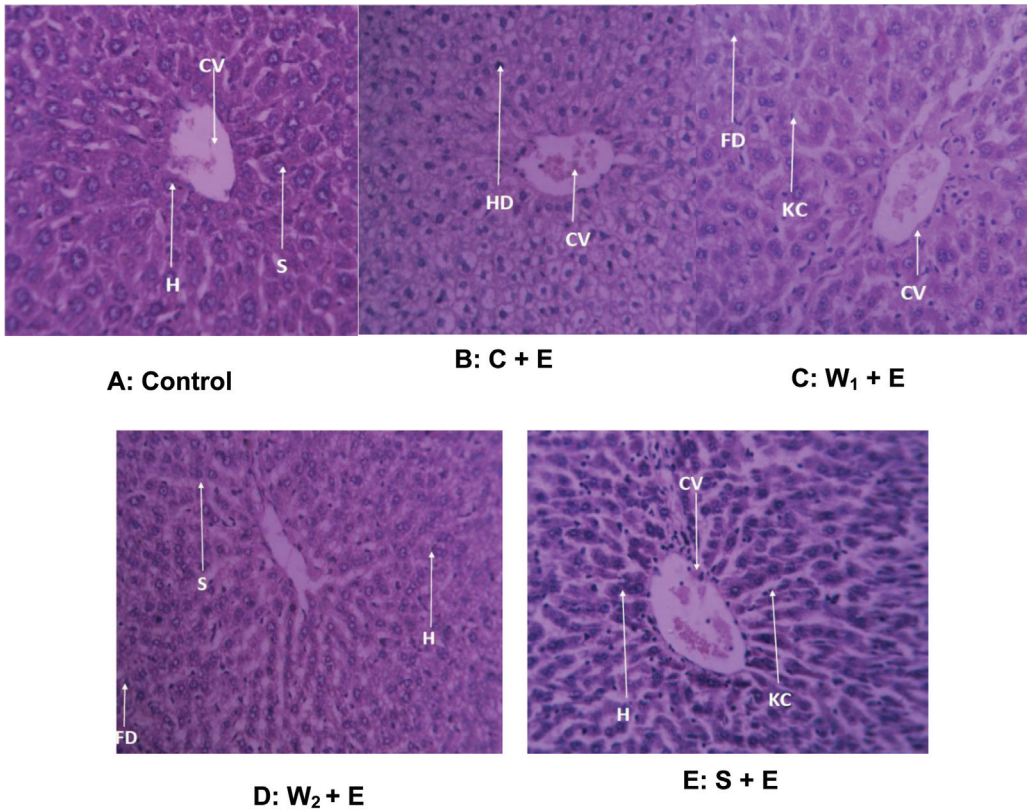

Figure 2: Histopathology of rat liver (H&E x250). (A) Control animals showing normal cytoarchitecture of the liver parenchyma. CV: central vein, H: Hepatocyte, S: sinusoid. (B) Negative control; 20 mL/kg ethanol was administered to animals in this group. The micrograph shows distorted cytoarchitecture as evidence by hepatocyte degeneration. HD: hepatocyte degeneration. (C) Liver of watermelon seed treated with 200 mg/kg body weight of the extract then ethanol. The photograph shows a relatively preserved cytoarchitecture. However, there was evidence of focal area showing degeneration of hepatocytes. Kupffer cells have become more numerous and conspicuous. H: hepatocyte, KC: Kupffer cell, FD: focal degeneration. (D) Liver of watermelon seed treated with 400 mg/kg body weight of the extract then ethanol. The micrograph showed an improved liver cytoarchitecture except for areas of focal degeneration of hepatocytes. S: sinusoid. (E) Liver of rats treated with standard drug silymarin then ethanol (positive control). The photograph shows normal histology and preserved liver cytoarchitecture as evidenced by the numerous viable hepatocyte and Kupffer cells.

in body weight might be due to hepatomegaly or hepatotrophy. Rats exposed to ethanol for 1 day in the negative control group have the least final body weight (188.00 ± 24.89) g. This observation is in keeping with earlier reports on the effects of alcohol on digestion, absorption, storage, utilization, and excretion of essential nutrients such as vitamins, minerals, and proteins.[37,38] Alcohol impairs nutrient absorption by damaging cells lining the stomach and intestines, and disabling transport of some nutrients into the blood.[39] Alcohol also inhibits the breakdown of nutrients into usable substances, by decreasing the secretion of digestive enzymes from the pancreas.[40]

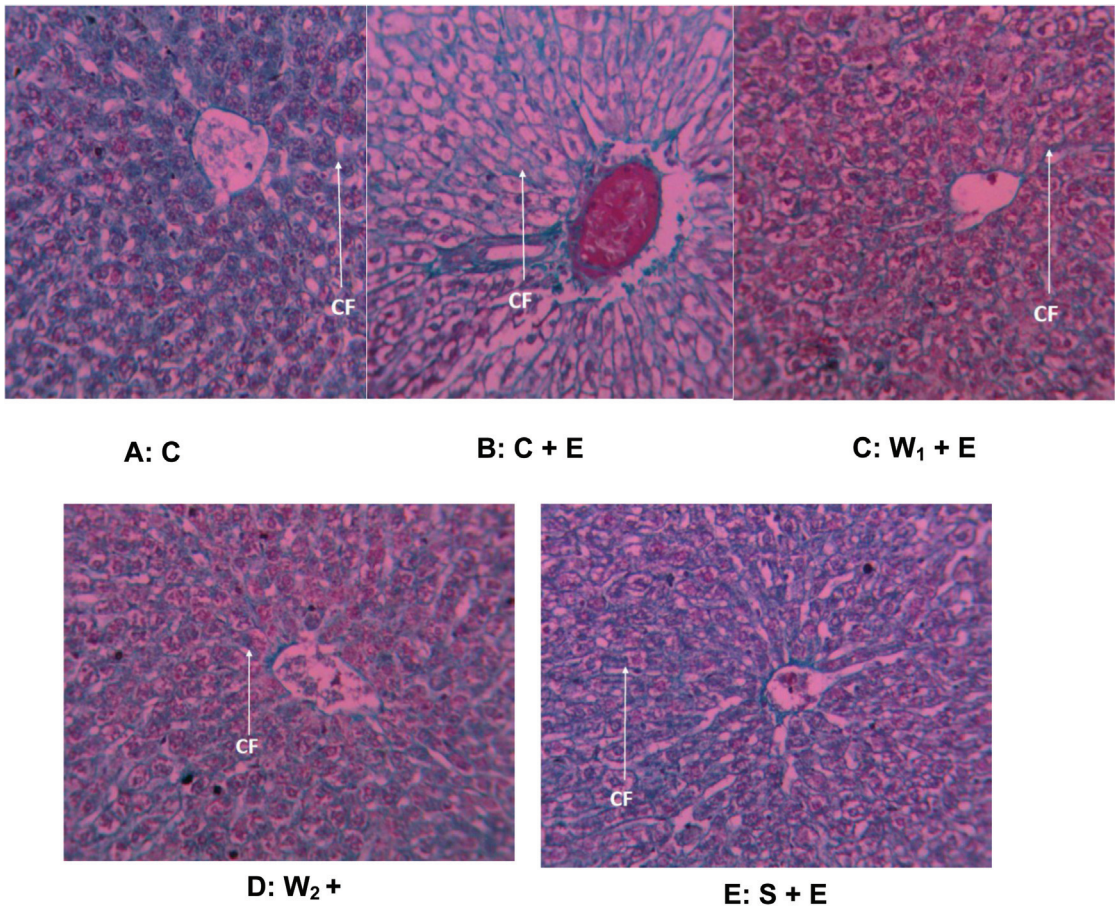

Figure 3: Histopathology of liver of various groups (Masson's trichrome x250). (A) Liver of control animals showing normal distribution of collagen fibers in the liver parenchyma. CF: collagen fibers. (B) Microscopic section of liver of rats treated with ethanol (negative control). The micrograph shows decrease in the distribution of collagen fibers in liver parenchyma. (C) Liver of rats treated with ethanol extract of *C. lanatus* (200 mg/kg) showing slight improvement in the distribution of collagen fibers in liver parenchyma. (D) Liver of rats treated with ethanol extract of *C. lanatus* (400 mg/kg) showing significant improvement in the distribution of collagen fibers in liver parenchyma. (E) Liver of rats treated with standard drug (positive control), and silymarin showing normal histology of collagen fibers in liver parenchyma.

Both ethanol and acetaldehyde have been implicated in cell injury and necrosis by increasing the production of ROS through the process of lipid peroxidation Kurose *et al.*[41] Administration of ethanol to normal rats increased plasma levels of AST, ALT, and ALP. The increased level of these serum enzymes among hepatotoxic rats is a cardinal sign of oxidative stress due to liver damage, enhanced permeability, and necrosis of the hepatocytes.[42] Plasma level of AST, ALT, and ALP among the ethanol treatment group was statistically significant ($P < 0.05$) higher than any other group. This could be due to hepatocellular damage as these enzymes reside in the cytoplasm of a normal cell but released into circulation only after cellular damage.[43] The statistically significant decrease ($P < 0.05$) in the activities of ALT, AST, and ALP at 200 and 400 mg/kg doses of the extract is itself an evidence of protective activity demonstrated by the seed extract.

The increase in the hepatic index (liver to body weight ratio) in the ethanol control group, as shown in Table 1, may be attributed to the fact that the first metabolite acetaldehyde (an unstable and toxic substance) produced as the body processes ethanol in the liver, through the enzyme alcohol dehydrogenase, and the hydrogen produced in this same reaction displaces fatty acids as fuel leading to an increase in the quantity of fatty acids and triglycerides in the liver thereby resulting in alcoholic fatty liver disease.[44,45] Alcohol decreases fatty acid oxidation in the liver[46] resulting in lipids, proteins, and hepatic triglycerides accumulation in hepatocytes, [47] thereby leading to hepatomegaly. Water retention in the cytoplasm of hepatocyte leads to increased total liver mass and volume whereas steatosis and cholestasis increases weight of the liver.[48]

Damage of hepatocytes or their plasma membrane in hepatocytes leads to leakage of high concentration of plasma ALT, AST, and ALP.[14] In our study, there is significant increase in ALT, AST, and ALP in the ethanol-treated group compared to the control group indicating liver damage due to ethanol. The high level of ALT and AST

in the serum is an indication of the degree of damage to the liver caused by ethanol.[22,49-52] Administration of rats with aqueous extract of *C. lanatus* seed remarkably prevented ethanol-induced elevation of serum levels of ALT, ALP, and AST. The results obtained indicated a high degree of protection against the hepatotoxic effect of ethanol. These results are in keeping with the results of Chaturvedi *et al.* (2011) who demonstrated that *Genista quadriflora* extract significantly ($P < 0.05$) decreased the activities of serum ALT and AST, which are biochemical markers of liver damage.[52] This suggested a hepatoprotective activity of the extract against acute toxicity of ethanol.[42]

ALT are abundant in the cytosol of hepatic parenchymal cells, whereas AST are found in cytosol and mitochondria of hepatocytes.[53] AST are also distributed in cardiac muscle, skeletal muscle, pancreas, and kidney. Hence, ALT measurement is more specific to determine liver damage.[54] Increased ALT enzymes in ethanol-induced liver toxicity were due to loss of structural integrity of the liver. As the enzyme is localized in the cytoplasm, it will be released into the blood circulation after cellular damage resulting in their elevation.[55] Increase of AST enzymes indicated that alcohol administration to rats causes both plasma membrane and mitochondrial membrane damage.[56]

Furthermore, the AST/ALT ratio is the ratio between the concentration of AST and ALT in the blood. It is useful during diagnosis to differentiate between the causes of liver damage or hepatotoxicity.[57] Briefly, in acute liver damage, AST to ALT ratio is ≤1.0, whereas in alcoholic hepatitis, AST to ALT ratio is always >1.0. The AST to ALT ratio was highest (0.73) in the ethanol control group compared to other groups, which is suggestive of alcohol-related injury.

Regarding protein concentration, ethanol exposure increased protein concentration in liver tissue of ethanol-treated group (negative control). This is in agreement with the results of Shalan[58] who reported that hepatic protein content markedly increase after alcohol intoxication.[58] Cunnane *et al.*[59] showed that increased liver weights resulted from hepatic triglyceride accumulation after chronic alcohol abuse.[59] Aqueous extract of *C. lanatus seed* causes a decrease in the level of protein concentration in the liver.Liver section of control rats showed normal hepatocytes and normal architecture [Figures 2 and 3]. Liver sections from ethanol-treated rats (C + E) demonstrated destruction of architectural pattern [Figures 2B and 3B). Liver sections from watermelon seeds extract-treated rats showed regeneration of normal hepatocytes and lobular architecture. Consequently, liver section from the watermelon seeds extract-treated rats showed normal lobular architecture with no necrosis or fatty changes or any inflammatory reaction [Figures 2D and 3D). These histopathological findings demonstrate a hepatoprotective effect of the extracts against ethanol-mediated liver damage. The findings obtained in the histopathological studies showed that aqueous extract of *C. lanatus* seed given to rats ameliorate the histopathological changes associated with ethanol toxicity and restores normal architecture of the liver. It also restored the glycogen pattern, DNA presentation, and connective tissue distribution in the liver parenchyma that showed distortion in the ethanol treatment group. This is in line with the previous studies showing that *C. lanatus* seed oil extract was hepatoprotective based on histology of the liver.[60]

Conclusion

The results of this study showed that ethanol can induce oxidative stress in rats. Seeds extract of watermelon has an antioxidant phytoconstituents that were able to proffer some hepatoprotection against ethanol-induced oxidative damage in rats especially at 400 mg/kg body weight. We strongly recommend consumption of the seeds along with the fruit than being spitted out as customary. We equally reject addressing the seeds as the "spitted one." Future work should focus on unravelling the molecular mechanism of the antioxidant effects of seed extract of watermelon in averting ethanol-induced damage.

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Conflicts of interest

There are no conflicts of interest.

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