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Research Paper:

Ethanolic Extract of *Syzygium cumini* Causes Toxic Effects on Ethanol-induced Liver and Kidney Damage in Albino Wistar Rats: A Biochemical and Histological Study

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ABSTRACT

Background: The therapeutic value of *Syzygium cumini* (*S. cumini*) has been documented in traditional medicine for the treatment of many diseases and ailments. Various preparations of this plant have been made and used especially for liver inflammatory conditions in livestock. Further, many liver diseases in humans are inflammatory conditions, which are caused by alcohol intake. This study sought to examine the effect of *S. cumini* on ethanol-induced hepatotoxicity in Wistar albino rats.

Methods: Twenty-five rats were divided into five groups of five rats each. The first group was control and the other four were administered ethanol at varying doses to induce liver and kidney damages. Two doses of the *S. cumini* extract were administered at a concentration of 200 mg/kg or 400 mg/kg. Silymarin was administered to the last group at 10 mg/kg. The liver and kidney tissue samples were collected and preserved for histological analyses and the rat sera were analyzed for the associated biochemical biomarkers.

Results: Histopathological analyses revealed pyknotic nuclei and distortion in the arrangement of the hepatocytes in extract-treated groups. The kidney tissue samples showed signs of interstitial bleeding and aggregation of lymphocytes in the peri-glomerular areas. The analyses of the biochemical parameters revealed that there were significant increases in the aspartate aminotransferase (AST), alanine transaminase (ALT), Urea and creatinine in the sera of the groups treated with the extract compared to those of the controls ($P<0.05$).

Conclusion: The *S. cumini* extract caused elevation of serum hepatic and renal biomarkers at 400 mg/kg and did not have a hepatoprotective effect.

Keywords: Hepatoprotective, Histology, Kidneys, Liver, Serum Biomarkers, *Syzygium cumini*

Introduction

The alcohol-related disease of the live is an inflammatory condition caused by hepatic steatosis and injuries to the liver tissue secondary to alcohol intake, which is responsible for over 10% of the cases reported globally [1]. This condition is a progressive inflammatory disease associated with long-term alcohol intake. Prolonged and excessive alcohol consumption (≥40-80 g/day, men & more than ≥20-40 g/day, women) [2] could lead to serious illnesses, including gastrointestinal ulcers, pancreatitis, alcoholic liver disease, neurologic disorders, diabetes mellitus, and cancers [3, 4]. Among the above conditions, alcoholic liver disease has attracted much attention due to its high morbidity and mortality [2]. Alcoholic liver disease can progress over time to cirrhosis and hepatic cancers [4]. Therefore,

these pathologies must be diagnosed and treated early to mitigate the long-term devastating consequences to the liver and kidneys functions.

Syzygium cumini (*S. cumini*) is a large evergreen tree, which belongs to the *Myrtaceae* family and it grows up to 30-meter high [5]. It has been valued for its practical uses in Ayurveda and umami systems of medicines presumably for a variety of therapeutic properties. The therapeutic value of *S. cumini* has been recognized in various traditional medicine systems, and used in the treatment of different diseases and ailments in humans [6]. Most parts of the tree, especially its seeds are traditionally used to treat a wide range of ailments [5], due to its contents believed to have a variety of medicinal properties. These include antioxidant, anti-inflammatory, neuropsychopharmacological, anti-microbial, anti-HIV, anti-leishmanial and anti-fungal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, anorexigenic, gastroprotective, and anti-ulcerogenic, and has been reported to have a protective effect against the injurious outcomes of radioactivity [5-10]. Other researchers have used the plant to cure liver ailments secondary to various pathologies [5, 8, 11].

The present study aimed to investigate the effect of the *S. cumini* extract on the ethanol induced hepatotoxicity, since this plant has a history of treating liver ailments in livestock and cattle. Specifically, we conducted this experimental study to find out whether it would be effective for the treatment of alcohol-related liver damage in Wistar rats, as an available and affordable plant product in areas where this plant grows widely.

Matrials and Methods

Authentication of plant and preparation of extract: Fresh and ripe *S. cumini* fruits were acquired from a private garden and authenticated by a Botanist in the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria. The fruits were washed to remove dirt, the pulp separated from the seeds, sun dried and finally powdered. The powder was soaked in ethanol and allowed to stand with minimal agitation for 24 hours. The liquid obtained was then strained and evaporated under vacuum, using a lyophilizer. The ethanolic extraction to obtain a powdery constituent was carried out according to the method described by a previous study [12]. Ultimately, 4000 mg of the powdered extract was added to 10ml of distilled water to obtain a stock solution which contained 400mg of the extract/ml.

Animal husbandry: A total of 25 albino Wistar rats of both genders were obtained from the Faculty of Pharmacy, University of Maiduguri, Borno State, Nigeria. They were housed in the vivarium of the Department of Human Anatomy. The rats were divided randomly into five groups of five rats each as shown in Table 1. They were kept in stainless steel cages and allowed to acclimatize for a period of two weeks before the experiments began. They were fed pellet rat chow and water during the study period ad libitum. They were kept under a 12hr light and dark cycle at 22±0.5°C temperature and 40%–60% ambient relative humidity. The experiments were performed according to the approved guidelines of the Institutional Animal Ethics Committee of the University of Maiduguri, Borno State, Nigeria.

The *S. cumini* extract and silymarin were administered orally through a gastric tubing for a period of seven days. At the end of this period, the animals were sacrificed and the liver and kidneys were dissected from each rat carefully and preserved in 10% formalin for the subsequent histological processing. Micrographs were taken via a microscope to examine the histological structures. Blood samples were collected from the animals by cardiac puncture through the ventricular chamber, using a syringe. The collected blood samples were centrifuged and the sera separated and analyzed to assess the marker enzymes representing liver and kidneys functions.

Histological analyses: Micrographs were taken using a light microscope (MBJX-ISCOPE, Los Angeles, USA), which was equipped with a digital camera (M500, X64, version 3.7) at several magnifications. Photomicrographs of the histological sections were obtained by a 10X objective lens, and analyzed on an Amscope image application.

Statistical analyses: The statistical analyses were performed with GraphPad Prism 9 software. Ordinary one-way ANOVA was performed to compare the means of the controls versus other groups followed by Dunnett's multiple comparisons test. $P<0.05$ was considered as significant.

Results

Analysis of serum enzymes: The enzymes representing liver and kidney functions were assessed by their concentrations in the rats' sera.

Serum ALT (Alanine aminotransferas) and AST (Aspartate transaminase): In Group D, the serum Alanine Aminotransferase (ALT) level was significantly increased ($P<0.001$) compared to that of the control group. In Group C, the AST level was also significant-

Table 1. Animal groups and the treatments

Groups	Treatments
A: Control	Distilled water
B: Negative control	0.5 ml Ethanol
C: Low dose extract	200 mg/kg *S. cumini*+0.5 ml Ethanol
D: High dose extract	400 mg/kg *S. cumini*+0.5 ml Ethanol
E: Positive control	140 mg/kg Silymarin+0.5 ml Ethanol

ly increased (P<0.001) compared to that of Group A. In Groups B and E, the ALT levels were significantly increased compared to that of the controls (P<0.01 & P<0.05, respectively); see details in Figure 1. Figure 2 illustrates the serum Aspartate transaminase (AST) levels for all groups. The AST levels in group B were significantly decreased (P<0.01) compared to that of the control groups. The levels of this enzyme were also significantly (P<0.001) increased in groups C and D compared to that of the controls. The rats that had been treated with silymarin showed a significant increase in the AST levels compared to that of other control groups.

Serum albumin, urea and creatinine: There was a significant (P<0.001) reduction in the serum albumin levels in Groups D and E; however, no significant changes were noted in the serum albumin levels in groups B and C (Figure 3). The serum urea concentrations were significantly (P<0.001) increased in Groups B and D compared to that of other control groups. In groups C and E; however, there were significant decreases (P<0.001) in these parameters compared to that of the controls (Figure 4). The serum creatinine levels were significantly (P<0.001) decreased in groups B, C, D and E compared to that of the control group A (Figure 5).

Renal histological findings: The histopathological examinations of the micrographs revealed normal cellular architecture of the kidney tissue samples in the control group A, consisting of normal renal corpuscles surrounded with Bowman's spaces. The renal corpuscles were lined with simple squamous epithelia. The renal tubules in the parenchyma and distal convoluted tubules

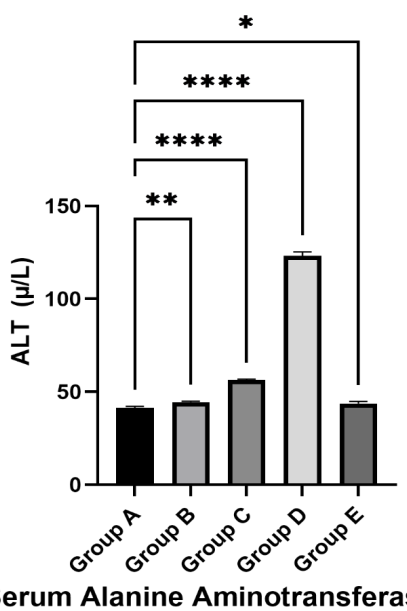

Figure 1. The level of serum Alanine Aminotransferase (ALT) in rat sera from all groups

Values are expressed as Mean±SD. *P<0.05, **P<0.01, ***P<0.001, ****P<0.001.

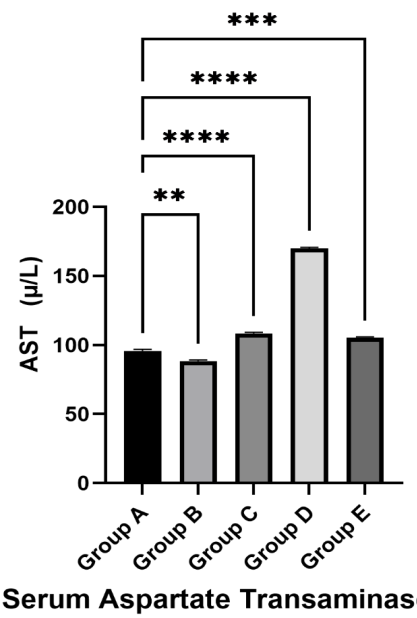

Figure 2. The level of serum Aspartate Transaminase (AST) in the serum of rats in all groups.

Values are expressed as Mean±SD. *P<0.05, **P<0.01, ***P<0.001, ****P<0.001.

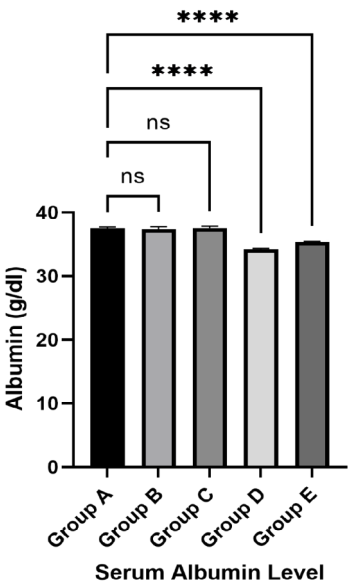

Figure 3. The level of albumin in the rat sera from all groups.

Values are expressed as Mean±SD. Ns: Not Significant, *P<0.05, **P<0.01, ***P<0.001, ****P< 0.001.

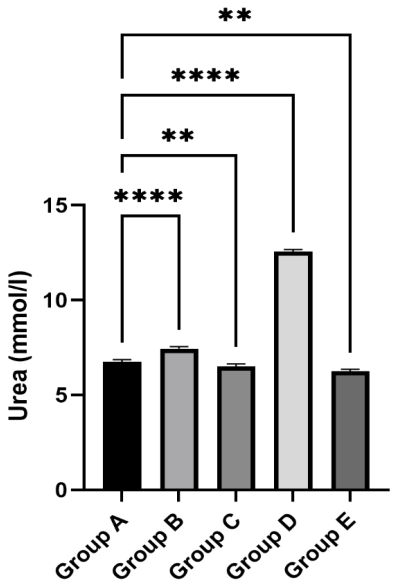

Figure 4. Serum urea levels for all rat groups

Values are expressed as Mean±SD. Ns: Not significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.001.

were distinct from the proximal convoluted tubules by their clear and wide lumens (Figure 6-A).

Figure 6-B shows the kidney tissue for the groups that was administered ethanol. There was considerable shrinkage in the renal corpuscles with the Bowman capsules widened. Also, there were marked bleeding spots in the renal parenchyma. The renal tubules remained unaffected with the tubular cells remained as simple cuboids in shape. There was also an aggregation of lymphocytes in the spaces among the renal tubules, denoting inflammatory reactions. In the rats that were treated with both alcohol and 200 mg/kg of *S. cumini* extract, the micrograph revealed distorted glomeruli and numerous lymphoid aggregations in the renal parenchyma. There was also bleeding spots in spaces among the renal tubules which remained intact in this group (Figure 6-C).

The group that was administered 400 mg/kg of *S. cumini* also showed the same features as observed in Group C. The bleeding spots; however, were not as abundant as those in the low *S. cumini* dose group. The renal corpuscles were continuous and undistorted, and Bowman's capsules were present, and the renal corpuscles and tubules were intact (Figure 6-D). In Group E, rats that had been treated with 140 mg/kg of silymarin, the histological architecture of the kidney micrographs closely resembled those of the normal control group. There was no observed interstitial bleeding and the glomeruli were not shrunk. The Bowman's capsules were present and lined with simple squamous epithelia, and the renal tubules were intact and the lining epithelial layers were not interrupted (Figure 6-E).

Liver histological findings: The liver slides in the control group showed normal hepatocytes in a spoke-like radiations toward the central vein. The sinusoidal spaces were clear and separated adjourning hepatocyte cords. The nuclei of the hepatocytes appeared granular (Figure 7-A). The groups that were administered ethanol showed

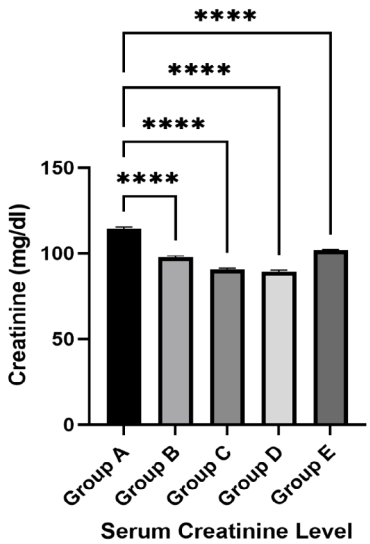

Figure 5. The level of creatinine in the sera of all rat groups

Values are expressed as Mean±SD. Ns: Not Significant, *P<0.05, **P<0.01, ***P<0.001, ****P<0.001

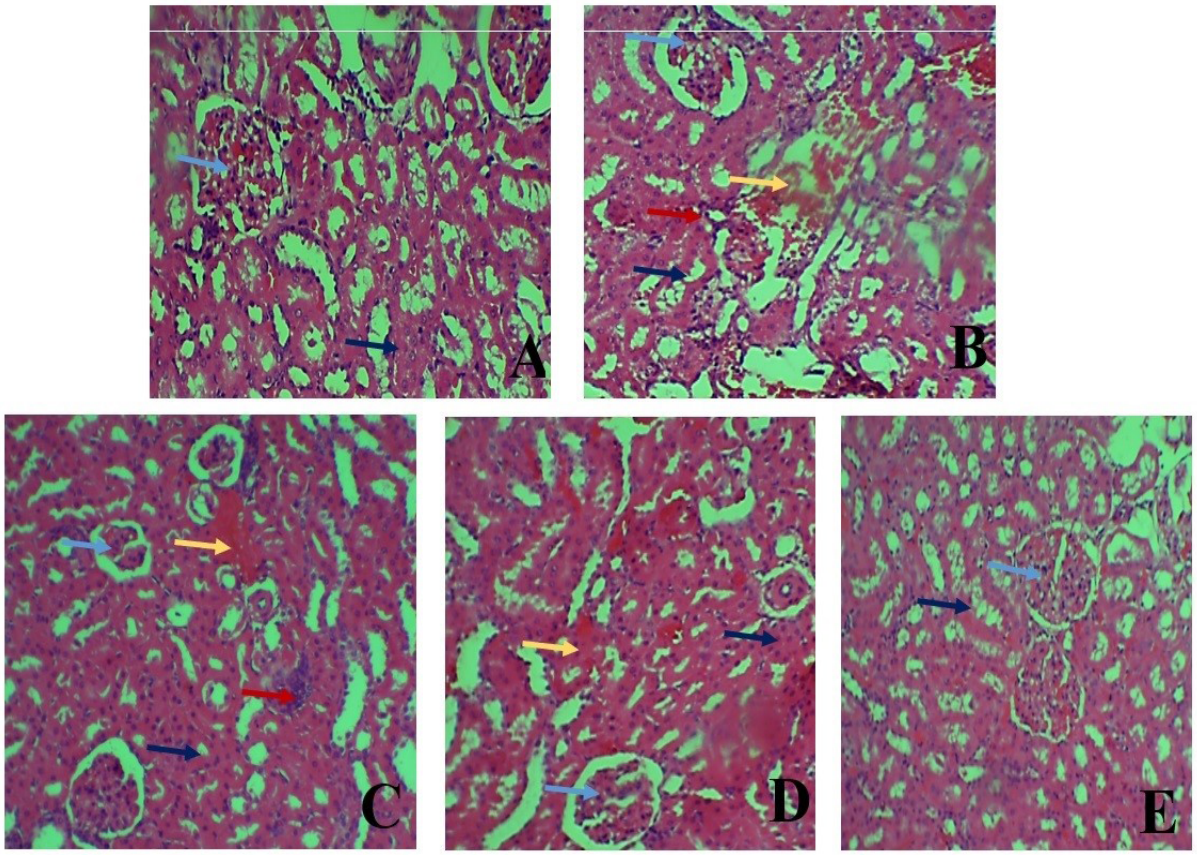

Figure 6. The kidney tissue in Groups A–E showing renal corpuscles (pale blue arrows) which were atrophied in Groups B, C and D, renal tubules (dark blue arrows)

There were bleedings (yellow arrows) in the renal parenchyma of Groups B, C and D, and infiltration of lymphocytes (red arrows) in the renal parenchyma. H&E stained; X100.

distorted central vein and widening of the hepatic sinusoids, with exaggerated sinusoidal spaces (Figure 7-B).

The liver of the rats that were administered with the low dose of the extract showed distorted hepatic tissue. The hepatocytes were less eosinophilic and had pyknotic nuclei, the sinusoids were collapsed, and the cells were clumped together. The central veins were dilated and the hepatocyte cords were aligned side by side as they were oriented towards the central veins (Figure 7-C).

The rats in the group that were administered a higher dose of the extract showed better restorations in the hepatocytes' arrangements, which radiated towards the central veins. The sinusoidal spaces were reestablished and clear, demarcating among hepatocyte cords with pyknotic nuclei (Figure 7-D). Figure 7-E reveals liver parenchyma with features that resemble normal liver tissue samples. The hepatocyte arrangements, the central veins and sinusoids had normal appearances.

Discussion

Excessive alcohol consumption causes many diseases, with the liver and kidneys being the seriously affected organs. This is of immense clinical importance, since these organs play vitals roles in the body, including detoxification, protein synthesis and filtration of toxins and metabolic wastes in the human body. The *S. cumini* extract is used traditionally to treat liver in many pathological conditions. In the present study, the ethanolic extract of the *S. cumini* fruits was administered at two doses, 200 mg/kg and 400 mg/kg, for its potential protective effects on the liver and kidney functions against repeated doses of ethanol.

Several agents are usually used to induce renal and hepatic stress in experimental animals in order to assess the hepato-protective ability of certain substances. In the present study, ethanol was used to induce toxic alterations in the liver and kidneys of rats by causing structural membranes damage and necrosis. Silymarin was also used

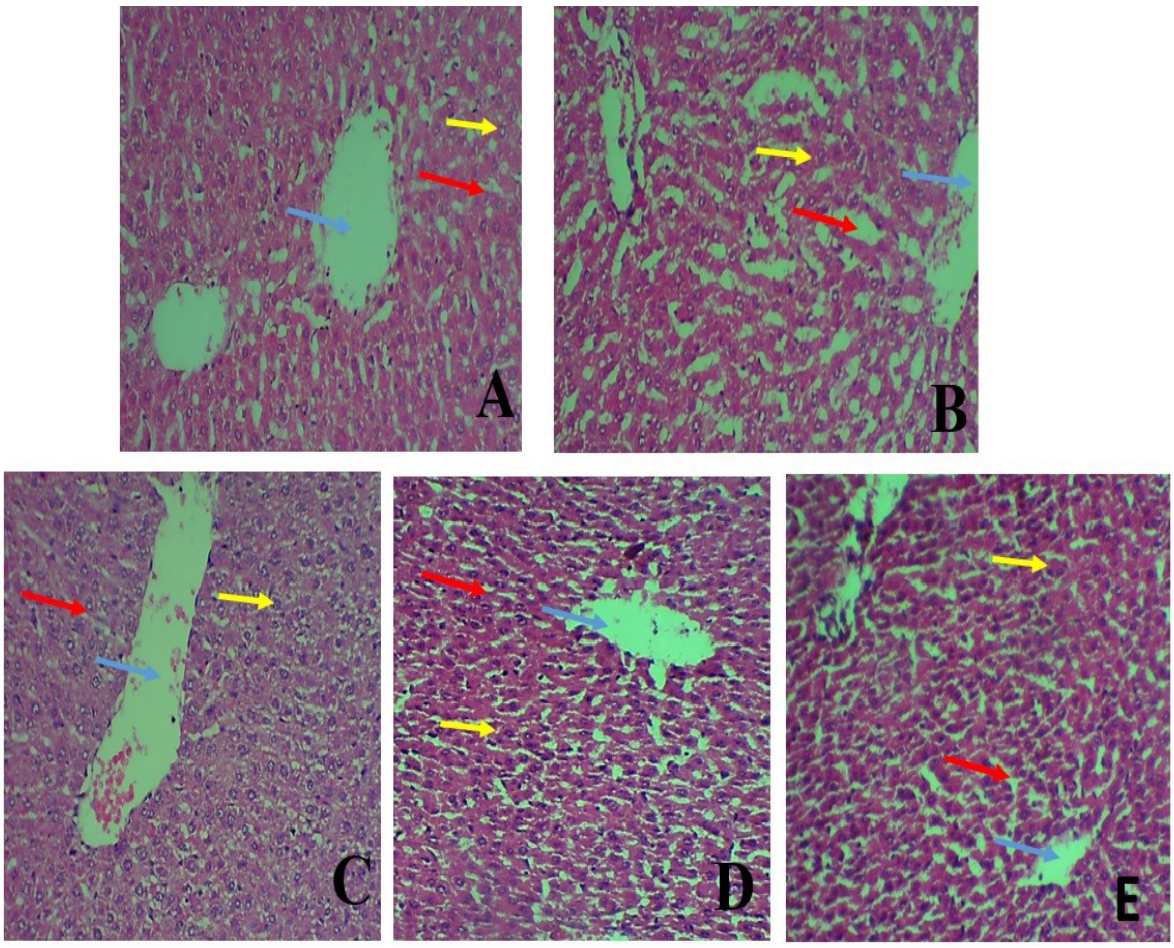

Figure 7. The histology of the liver in the Groups A–E with the blue arrow on the central vein which is dilated in group C

The red arrows point to the sinusoids which are obliterated in group C. The hepatocytes (yellow arrows) are pyknotic in Groups C and D. H&E stained. Magnification: X100.

as a standard drug because of its proven activity, safety and well-established role in inhibiting lipid peroxidation, stimulating protein synthesis along with its antioxidant, anti-inflammatory and anti-fibrotic activities [13].

Biochemical profiles which are monitored in the liver and kidney tissues are useful markers for tissue injury assessment [14]. Measurement of enzyme activities in various bodily tissues and fluids plays a significant role in pathological investigations and assessing the toxicity of drugs [14, 15]. Tissue enzymes can also specify the cellular damages caused by chemical compounds before showing structural alterations observable by histological techniques [14]. These indices have had important roles in establishing the effects of many substances on the liver, kidneys and other bodily organ tissues [16, 17].

Serum ALT and AST: The serum ALT and AST levels are among the current indicators of liver damage and toxicity. When the liver is damaged, especially by toxins, ALT and AST are released into the blood stream and their serum levels rise. The concentrations of ALT and AST in the serum are correlated with the extent of liver tissue damage [17, 18]. Conversely, the reoccurrence of the aminotransferases' activities in the serum close to the normal levels indicates the hepatocytes regeneration and the recovery of the liver parenchyma [3, 18, 19]. Based on the results of the current study, there were indications of the liver damage in the group that received a high dose of the extract, as evident by the elevated serum AST and ALT levels. The findings indicate that *S. cumini* did not provide a protective effect on the liver at the given concentrations. In contrast, previous reports have claimed that there were no significant increases in the serum AST and ALT levels in rats who received varying doses of ethanol for 30, 90 or even 180 days [20].

An earlier study [11] has demonstrated that the mean AST and ALT levels in all treated groups were significantly lower than that of the tetracycline-treated group.

The reported findings suggest that the *S. cumini* extract at either doses and in those animals treated with silymarin protected the liver from the damage induced by tetracycline. However, contrary to the results obtained in the current study, the reported AST and ALT levels were significantly lower in the group treated with the higher dose of the extract (500 mg/kg). In the current study, the lower dose of the extract at 200 mg/kg caused a significantly lower AST and ALT levels compared to the group that received the higher dose at 400 mg/kg.

Serum albumin, urea and creatinine: The serum albumin concentration is an indication of liver function [19, 20]. In the present study, the serum albumin level in the group that received the low dose of the extract was similar to that of the control group. Interestingly, the group that received the high dose of the extract and those treated with silymarin, had significantly lower serum albumin levels. The findings suggest that in these groups, the liver function had been markedly impaired.

Urea and creatinine are the most frequently ordered blood analyses to assess the status of the kidney function. Urea is produced by protein breakdown and excreted in urine whereas creatinine is a non-protein nitrogenous compound that is produced by the breakdown of creatine in muscles and excreted by filtration through the kidney at a constant rate [18]. In the current study, the serum urea levels were elevated in the rat group that received the extract at a high dose. This was in agreement with the previously reported results in the study conducted by Nahid et al. [9]. The damage to the rats' kidneys might be due to lipid peroxidation or formation of free radicals secondary to the ethanol administration. In a previous study [21], there was no significant difference in the levels of serum urea compared to that of the control groups.

The serum creatinine levels were significantly reduced in all of the experimental groups compared to those in the controls. The administration of the extract might have reduced the serum creatinine levels through mechanisms which is not currently clear. Silymarin is a well-known drug with protective property toward the liver. It is commonly used as a reference drug in liver-related studies as a test compound [13]. The results of the biochemical assays in this study suggest that silymarin was more effective than the ethanolic extract of *S. cumini* in protecting both the kidneys and liver by effectively reducing the serum levels of ALT, AST, urea and creatinine in the experimental groups.

Histopathological findings: The biochemical findings of this study corroborate the histopathological results observed for the liver and kidneys. The kidneys of the rats in the extract-treated groups showed interstitial bleeding in the renal parenchyma but the kidney tissue in the group treated with silymarin showed fairly normal histological features. The observations confirm the efficacy of silymarin in treating hepatotoxicity and renal damage induced by ethanol. The liver tissue in the treated groups also showed evidence of hepatotoxicity as observed by the collapse of sinusoids and marked damages to the hepatocytes. The group treated with silymarin showed features that were similar to those of the control group. Contrary to our findings; however, another study has reported on the protective effect of *S. cumini* extract on the liver tissue in alloxan induced diabetic mellitus [17]. Finally, two earlier studies [9] have reported that *S. cumini* extract protected the liver cellular architecture from tetracycline-induced damages. These studies also reported that *S. cumini* extract at high doses caused some changes in biochemical parameters but did not produce morphological changes in the cellular architecture of the liver, kidneys, heart, lungs, stomach, intestine and pancreas in animal models [9, 17].

Conclusions

Based on the results of the present study, the ethanolic extract of *S. cumini* caused elevation of serum hepatic and renal biomarkers at the high concentration administered. The histological assessment of the hepatic and renal tissues corroborated the results obtained by the biochemical assays. The tissue samples exhibited signs of toxicity as evident by the lymphoid aggregations and distortion of tissue in the kidneys and disorganized arrangement of hepatocytes in the liver. These effects; however, were not observed in rats treated with silymarin.

The current study considered only the light microscopic images and biochemical assays of the liver and kidneys after the rats were administered *S. cumini* extract. We did not have resources for sophisticated cytologic examinations of changes due to the effects of *S. cumini*.

We recommend that lower concentrations of *S. cumini* extract be studied to see if the adverse effects could be minimized on the animals' tissues. The biomarkers of oxidative stress may also be investigated to uncover the effect of the extract on these parameters.

Ethical Considerations

Compliance with ethical guidelines

This study was conducted following the University of Maiduguri Research and Ethical Committee guidelines,

the ARRIVE guidelines specific to reporting in vivo experiments, and the National Institutes of Health (NIH) guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The study protocol was approved by the Ethics Committee of the Department of Human Anatomy, University of Maiduguri (Code: UM/HA/UGP 18.19 009).

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Authors' contributions

Conceptualization, methodology, and data collection: Martha Orendu Oche Attah, Abba Aji Manu, and Muhammad Bello Musa; Supervision: Martha Orendu Oche Attah, and Helga Ishaya Bedan; Resources: Abba Aji Manu and Muhammad Bello Musa; Funding acquisition: Abba Aji Manu and Muhammad Bello Musa; Writing - reviewing, editing and approval, data analysis: All authors.

Conflict of interest

The authors declared no conflict of interests.

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