Research Article

Hepatocurative effects of methanol extract of *Acanthus montanus* leaves on acetaminophen-induced liver failure in rats

Uroko Robert Ikechukwu[1]*, Chinedu Paulinus Nwuke[1], Nancy Oluomachi Uchenna[1], Chidimma Lilian Asadu[2], Emeka John Dingwoke[3], Fatima Amin Adamude[4], Nwobodo Ndubuisi Nwobodo[5]

ABSTRACT

Objective: This study was designed to investigate hepatocurative activities of the methanol extract of *Acanthus montanus* leaves on acetaminophen-induced liver failure in rats. **Materials and Methods:** The acute toxicity and hepatocurative activities of the extract were studied using mice and Wistar albino rats, respectively. The rats were distributed into five groups of five rats each. Group 1 served as normal control while Goups 2–5 were acetaminophen-induced orally. Groups 2 and 3 served as negative and positive controls, respectively, while Groups 4 and 5 served as the hepatocurative groups. Liver failure in rats was induced with 2500 mg/kg body weight of acetaminophen administered orally. Group 3 was treated with silymarin, 100 mg/kg body weight whereas Groups 4 and 5 were treated with the extract, 200 and 500 mg/kg body weight of the methanol extract of *A. montanus* leaves, respectively. **Results:** The acute toxicity results on methanol extract of *A. montanus* leaves in mice showed that the extract caused no adverse reactions or death in the mice. Treatment of acetaminophen-induced liver failure in rats with the extract caused significantly ($P < 0.05$) reduction of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities, and total bilirubin concentrations and significantly ($P < 0.05$) increased total protein, albumin, direct bilirubin concentrations, and improved liver histomorphology that were negatively altered by acetaminophen toxicity. **Conclusion:** The findings of this study suggest that methanol extract of *A. montanus leaves* possesses hepatocurative activities capable of restoring liver failure and ameliorating adverse effects associated with hepatotoxicity.

KEY WORDS: *Acanthus montanus,* Acetaminophen, Hepatotoxicity, Histomorphology, liver functions, Liver marker enzymes

INTRODUCTION

Liver is a vital organ in the body that plays a central role in the metabolic processes including the metabolism of nutrients, biotransformation of drugs and xenobiotics, biosynthesis of important biomolecules, detoxification, and maintenance of internal homeostasis. Liver failure may be caused by xenobiotics, alcohol consumption, malnutrition, infection, anemia, and medications.[1,2] The role of liver in the detoxification and metabolism of drugs and xenobiotics makes it vulnerable to injury due to oxidative stress resulting from excess reactive oxygen species generated during these processes that deplete antioxidants in the body.[2,3] Hepatotoxic chemicals including acetaminophen overdose, hemochromatosis, alcoholic liver injury, toxin exposure, and viral hepatitis damage liver cells through lipid peroxidation.[3] Many plants possess hepatoprotective properties due to their richness in antioxidants phytochemicals such as phenols, flavonoids, alkaloids, carotenoids, coumarins, essential oil, alkaloids and organic acids capable of inhibiting lipid peroxidation, healing, and rapid regeneration of damaged liver cells.[4]

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

[1]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, [2]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria, [3]Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, [4]Department of Biochemistry, Federal University Lafia, Nasarawa State, Nigeria, [5]Department of Pharmacology and Therapeutics, College of Medicine, Parklane Campus, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria

***Corresponding author:** Uroko Robert Ikechukwu, Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Phone: +2348065914471. E-mail: greaturoko@gmail.com

Received on: 13-03-2019; Revised on: 18-05-2019; Accepted on: 24-05-2019

Acetaminophen-induced hepatotoxicity is caused by its intermediate metabolic product, N-acetyl-p-benzoquinone mine that causes oxidative stress and glutathione depletion.[5] Under normal conditions and therapeutic doses of acetaminophen, benzoquinone integrates with intracellular glutathione to become a non-toxic mercapturic derivative which is excreted in the urine. Ingestion of high dose of acetaminophen leads to the generation of excess benzoquinone that overwhelms the capacity of liver for detoxification. The excess benzoquinone generated bind to cellular components such as to sulfhydryl groups in proteins causing cell necrosis and lipid peroxidation, impairment of cellular membrane stability, acute liver injury and eventually the death of the hepatocyte in the absence of immediate interventions to ameliorate its toxic effects.[6,7]

A. montanus a member of A*canthaceae* family is a small perennial shrub with few branches found in many regions of the world and largely in West African. It is called "Elele-nyijuo," "Agamsoso," "Agameebu," and "Agameru" in the Southeast of Nigeria where it is used in traditional medicine.[8] Extracts of the leaves are used to treat urinary diseases, boils, urethral pain, endometritis, urinary disease, cystitis, leukorrhea, cardiovascular diseases, and respiratory and gastrointestinal diseases and have shown to possess analgesic, anti-inflammatory, and antipyretic properties.[9,10] It has been used by many traditional medicine practitioners to treat liver enlargement, hepatitis, and various hepatic disorders with claims that the leaves extracts possess hepatoprotective properties, although there is no scientific or experimental evidence to substantiate these claims. In this study, the effects of the methanol extract of *A. montanus* leaves on liver function indices of acetaminophen-induced liver failure in rats were investigated.

MATERIALS AND METHODS

Collection and Identification Plant Leaves

Fresh leaves of *A. montanus* were collected from Forestry Research Institute of Nigeria, Eastern Station, Ahia Eke Ndume, Umuahia, Abia State and identified with voucher number FHI23965 at the Department of Forestry, College of Natural Resources and Environmental Management Michael Okpara University, Umudike, Abia State, Nigeria.

Preparation and Extraction of Plant Leaves

The *A. montanus* leaves were properly hand-picked from their stems, washed with clean running water, dried under shade at room temperature for 2 weeks. The dry leaves were pulverized using pestle and mortar, sieved and stored in a dry clean, sterile container for subsequent extraction. A quantity, 500 g of the coarsely ground *A. montanus* leaves was weighed into a dry clean container and 1.5 L of absolute methanol, covered properly to prevent vaporization of the methanol solvent and allowed to stand for 72 h with occasional agitation by manual shaking. After 72 h, it was filtered with Whatman filter paper No. 1. The filtrate was concentrated in a water bath at 50°C temperature and allowed to evaporate completely. The evaporated extract was then weighed, and the percentage yield calculated. The extract was covered with an aluminum paper foil and stored in a refrigerator for the experiment.

Experimental Animals

Thirty male Wistar albino rats and 18 male albino mice were used in this study for investigation of hepatocurative effects of the methanol extract of *A. montanus* leaves on acetaminophen-induced liver failure and acute toxicity study, respectively. The rats were purchased from the Animal House of the Department of Zoology, University of Nigeria, Nsukka, and acclimatized for 7 days under 12 h dark and light cycle at the Animal House of the College of Natural Sciences, Michael Okpara University of Agriculture, Umudike with free access to standard feed and drinking water.

Experimental Design

The 18 male albino mice were distributed into six groups of three mice each and three groups each were used for Phase I and Phase II of acute toxicity study of the extract, respectively. The 30 male Wistar albino rats were randomly distributed into five groups of six rats each and used for the evaluation of hepatocurative effects of the methanol extract of *A. montanus* leaves. Group 1 (normal control) received 1 ml/kg body weight of distilled water every 24 h for 14 days. Group 2 (negative control) received acetaminophen (2500 mg/kg body weight) on day 7 and day 14 of the experiment without any treatment. Group 3 (positive control) received acetaminophen, 2500 mg/kg body weight and treated with silymarin (100 mg/kg body weight) for 14 days. Group 4 received acetaminophen (2500 mg/kg body weight) on days 1 and 7 of the experiment and treated with 200 mg/kg body weight of the methanol extract of *A. montanus* leaves for 14 days. Group 5 received acetaminophen (2500 mg/kg body weight) on days 1 and 7 of the experiment and treated with (500 mg/kg body weight) methanol extract of *A. montanus* leaves for 14 days. Groups 4 and 5 were hepatocurative groups investigated for hepatocurative effects of the methanol extract of *A. montanus* leaves. Blood samples and livers were collected on the 15th day of the experiment for biochemical and histological analyses, respectively.

Induction of Liver Failure

Liver failure was induced by oral administration of acetaminophen dissolved in normal saline (2500 mg/kg body weight) to the rats and starved for 24 h before the commencement of treatment with free access to drinking water only.

Biochemical Assays

The acute toxicity study of the methanol extract of *A. montanus* leaves was determined as described by Lorke.[11] ALT, AST, and ALP activities were assayed according to the methods of Reitman and Frankel as outlined in Randoxkit.[12] Total serum protein and albumin concentrations were determined according to the methods of Lowry *et al.* and Rodkey, respectively.[13,14] Furthermore, total serum bilirubin and direct bilirubin concentrations were determined as described by Jendrassik and Grof using Randox assay kits.[15]

Histopathological Examination of Liver Morphology

The experimental animals were euthanized at the end of the study period with chloroform and tissue sections of the liver were collected for histopathological studies. The livers were fixed in 10% phosphate-buffered formalin for a minimum of 48 h before tissue preparation. The tissues were subsequently trimmed, dehydrated in four grades of alcohol (70%, 80%, 90%, and absolute alcohol); cleared in three grades of xylene and embedded in molten wax. On solidifying, the tissue-containing wax blocks were cut into 5 µm thick sections with a rotary microtome, floated in water bath and incubated at 60°C for 30 min. The 5 µm thick sectioned tissues were subsequently cleared in three grades of xylene and rehydrated in three grades of alcohol (90%, 80%, and 70%). The sections were then stained with Hematoxylin for 15 min. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a DPX as mountant. The prepared slides were examined with a Motic™ compound light microscope using x4, x10, and x40 objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at x160 and ×400.

Statistical Analysis

The data obtained were analyzed using Statistical Package for the Social Sciences version 21, and the results were presented as mean ± standard deviation while the means were compared for statistical significance using Duncan's multiple comparison *post hoc* test. Significant differences in the results were established by one-way analysis of variance with acceptable significance at $P < 0.05$.

RESULTS

The percentage yield of the extract obtained from the extraction of 500 g of finely ground *A. montanus* leaves with 1.5 L methanol solvent was found to be 6.8% equivalent to 34 g which indicated that much of the polar constituents of the leaves were relatively extractable with methanol.

The results of acute toxicity study of the methanol extract of *A. montanus* leaves showed that none of the mice died or showed any sign of adverse reactions within 24 h after they were administered graded doses of the methanol extract in Phases I and II of the acute toxicity studies, respectively.

The data in Figure 1a represent the AST activities of acetaminophen-induced rats treated with the methanol extract of *A. montanus* leaves. The acetaminophen induction of live failure caused significant ($P < 0.05$) increase in the AST activities in the rats when compared with the normal control (Group 1) that was not acetaminophen induced. The AST activities of the negative control (Group 2) that was acetaminophen-induced untreated and the positive control acetaminophen-induced treated with silymarin, respectively, showed significant ($P < 0.05$) increase when compared with the normal control. However, there was significant ($P < 0.05$) decrease in AST activities of the positive control when compared with negative control (Group 3). The hepatocurative groups (Group 4 and 5) showed significant ($P < 0.05$) decrease in their AST activities when compared with the AST activities of the negative and positive controls, respectively, and no significant ($P > 0.05$) increase in their AST activities when compared with normal control.

It was evidenced in the ALT activities of acetaminophen-induced liver failure in rats treated with methanol extract of *A. montanus* leaves in Figure 1b that there was significant ($P < 0.05$) increase in ALT activities of each of the acetaminophen-induced rat groups when compared with the normal control with that of the negative being the highest (Group 2). Treatment of the acetaminophen-induced liver failure in rats with silymarin (positive control) and the methanol extract caused significant ($P < 0.05$) decrease in the ALT activities of the positive control and hepatocurative groups, respectively, when compared with the negative control. However, there were significant ($P < 0.05$) increase in the ALT activities of positive control treated with silymarin and Group 4 treated with the extract (200 mg/kg body weight) when compared with the normal control. The Group 5 that received the methanol extract (500 mg/kg body weight) showed no significant ($P > 0.05$) increase in their ALT activities when compared with the normal control.

From the ALP activities of acetaminophen-induced rats treated with methanol extract of *A. montanus* leaves in Figure 1c, it was observed that the acetaminophen induction of liver failure caused significant ($P < 0.05$) increase in ALP activities of all the induced rats when compared with the normal control. Acetaminophen-induced rats treated with silymarin (positive control), and the methanol extract (Group 4 and 5) showed significant ($P < 0.05$) decrease in their ALP activities when compared with the negative control. There were no significant ($P > 0.05$) differences observed between ALP activities of the positive control treated with silymarin and hepatocurative groups treated with graded doses of the methanol extract. However, Group 5 rats treated with the methanol extract (500 mg/kg body weight) showed significant ($P < 0.05$) decrease in ALP activities when compared with the group treated with (200 mg/kg body weight) the methanol extract while the ALP activities of Group 5 rats were no significantly ($P > 0.05$) higher than the normal control rats.

The total protein concentrations of acetaminophen-induced rats treated with the methanol extract of

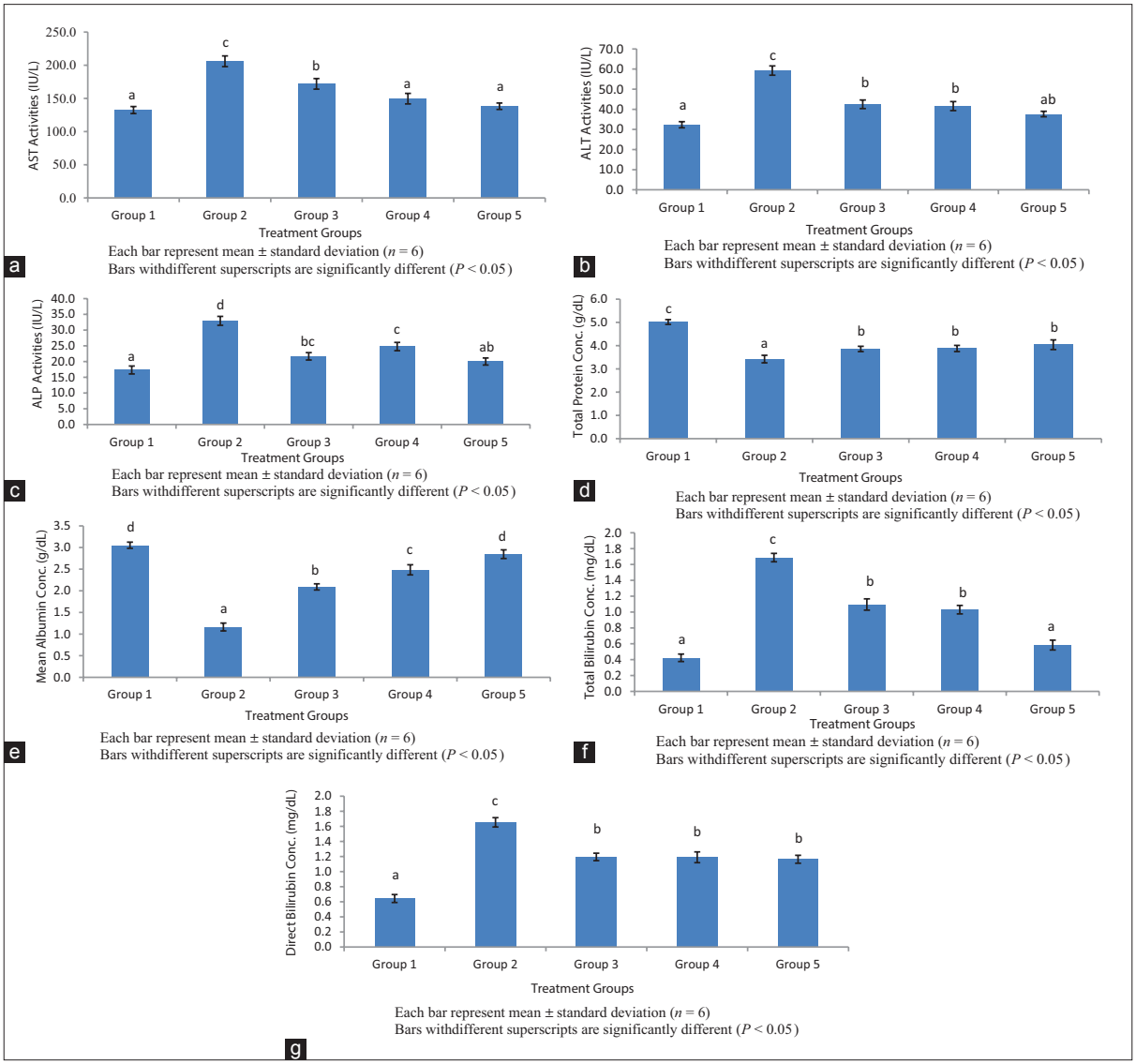

Figure 1: (a) Aspartate aminotransferase activities of Wistar albino rats that suffered liver failure from acetaminophen induction and treated with the methanol extract of *Acanthus montanus* leaves. (b) Alanine aminotransferase activities of Wistar albino rats that suffered liver failure from acetaminophen induction and treated with the methanol extract of *A. montanus* leaves. (c) Alkaline phosphatase activities of Wistar albino rats that suffered liver failure from acetaminophen induction and treated with methanol extract of *A. montanus* leaves. (d) Total protein concentrations of Wistar albino rats that suffered liver failure from acetaminophen induction and treated with methanol extract of *A. montanus* leaves. (e) Albumin concentrations of Wistar albino rats that suffered liver failure from acetaminophen induction and treated with the methanol extract of *A. montanus* leaves. (f) Total bilirubin concentrations of Wistar albino rats that suffered liver failure from acetaminophen induction and treated with methanol extract of *A. montanus* leaves. (g) Direct bilirubin concentrations of Wistar albino rats that suffered liver failure from acetaminophen induction and treated with methanol extract of *A. montanus* leaves

A. montanus leaves indicated that normal control rats had the highest total protein concentrations among all the rat groups [Figure 1d]. It was further observed that all the acetaminophen-induced rats had significant ($P < 0.05$) decrease in their total protein concentrations relative to the normal control rats. However, treatment with silymarin (Group 3) and the extract (Groups 4 and 5) caused significant ($P < 0.05$) increase in their total protein concentrations when compared with the negative control (Group 2) while there was no significant ($P > 0.05$) difference between the total protein concentrations of the positive control and hepatocurative groups (Groups 4 and 5).

The albumin concentrations of acetaminophen-induced rats treated methanol extract of *A. montanus* leaves in Figure 1e showed that the acetaminophen induction caused significant ($P < 0.05$) reduction of the albumin concentrations in the rats when compared with the normal control. However, positive control (Group 3) rats and hepatocurative groups (Groups 4 and 5) treated with silymarin and the methanol extract, respectively, showed significant ($P < 0.05$) increase in their albumin concentrations with that of hepatocurative groups been dose-dependent and significantly ($P < 0.05$) higher than the positive control.

The data in Figure 1f showed the total bilirubin concentrations of acetaminophen-induced rats treated with methanol extract of *A. montanus* leaves which indicated that acetaminophen induction caused significant ($P < 0.05$) increase in their total bilirubin concentrations when compared with normal control with negative control having the highest total bilirubin concentrations. Acetaminophen-induced rats treated with silymarin (Group 3) and extract-treated groups (Groups 4 and 5), respectively, showed significant ($P < 0.05$) decrease in total bilirubin concentrations when compared with the negative control. It was observed that Group 5 treated with the extract (500 mg/kg body weight) had no significantly ($P > 0.05$) higher total bilirubin concentration than the normal control while that of the positive control and Group 4, respectively, were significantly higher than the normal control.

The direct bilirubin concentrations in acetaminophen-induced rats treated with methanol extract of *A. montanus* leaves showed that normal control rats had the lowest direct bilirubin concentrations among all the rats [Figure 1g]. The acetaminophen-induced rats had significantly ($P < 0.05$) high direct bilirubin concentrations when compared with the normal control; however, treatment with silymarin and the methanol extract, respectively, caused significant ($P < 0.05$) decrease in direct bilirubin concentrations of the positive control and hepatocurative groups, respectively, when compared with the negative control though significantly ($P < 0.05$) higher than the normal control.

Sections of the liver collected from the normal control rats showed normal hepatic histomorphology for laboratory rodents [Figure 2a]. The tissue sections showed normal hepatic lobules made up of normal hepatocytes arranged in interconnecting cords (hepatic cords) around the central veins. The hepatic cords are separated by the hepatic sinusoids and radiate toward the periphery of the hepatic lobules (portal areas), where they meet with the components of the portal triads (branches of the hepatic artery, hepatic vein, and bile duct) which are suspended in the loose connective tissue matrix.

The liver sections from rats in this group showed severe degeneration of the hepatocytes in the periportal and mid-zonal areas of the hepatic lobules while the centrilobular hepatocytes (black arrow) were normal [Figure 2b]. The affected hepatocytes appear swollen; containing multiple coalescent intracytoplasmic clear vacuoles (white arrow) and partially occludes the adjacent hepatic sinusoids (blue arrow). This type of degeneration is classified as microvesicular steatosis.

The liver sections from rats in this group showed a moderate widespread degeneration of the hepatocytes in the periportal and mid-zonal areas [Figure 2c]. The hepatocytes in the centrilobular areas (white arrow) of the hepatic lobules appear normal. The affected hepatocytes (black arrow) appear swollen, partially occluding the hepatic sinusoids and contain numerous coalescent clear vacuoles in their cytoplasm.

The liver sections from rats in this group showed a mild to moderate vacuolar degeneration of the hepatocytes in the periportal and mid-zonal areas of the hepatic lobules only. The hepatocytes in the centrilobular areas appear normal [Figure 2d]. The affected hepatocytes (arrow) appear slightly swollen and contain clear vacuoles in their cytoplasms.

Liver sections from rats in this group showed a mild to moderate vacuolar degeneration of the hepatocytes in the periportal and mid-zonal areas of the hepatic lobules only [Figure 2e]. The hepatocytes in the centrilobular areas appear normal. The affected hepatocytes appear slightly swollen and contain clear vacuoles in their cytoplasms.

DISCUSSION

A. montanus leaves extracts are used in the treatment of various diseases by local traditional medicine practitioners including hepatic disorders, but there is insufficient experimental evidence to validate the potency of its extracts in the management of hepatic disorders as claimed. This study investigated

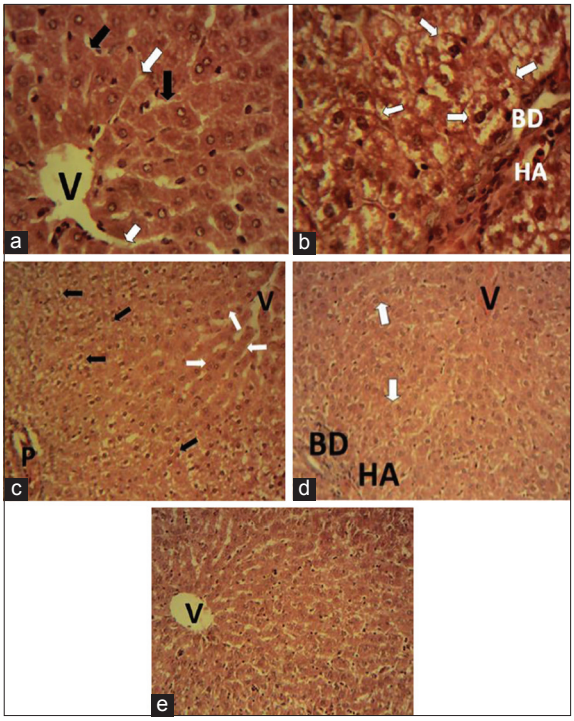

Figure 2: (a) Liver histomorphology of normal control rats (Group 1). Key: V = Central vein; Black arrow = Hepatic cords; Sinusoids (white arrow). (b) Liver histomorphology of the negative control rats (Group 2). Key: BD = Bile duct; HA = Hepatic artery. (c) Liver histomorphology of the positive control rats (Group 3). Key: V = Central vein; P = Portal area. (d) Liver histomorphology of paracetamol-induced rats treated with a low dose of methanol extract of *Acanthus montanus* leaves (Group 4). Key: V = Central vein; HA = Hepatic artery; BD = Bile duct. (e) Liver histomorphology of paracetamol-induced rats treated with a high dose of methanol extract of *A. montanus* leaves (Group 5). Key: V = Central vein

hepatocurative activities of the methanol extract of *A. montanus* leaves on the liver function indices of acetaminophen-induced rats with the view of validating its use in the management of hepatic disorders. The absence of adverse reactions such as nervousness, drowsiness, restlessness, coordination loss, and/or death in the mice administered graded doses of the methanol extract of *A. montanus* leaves after 24 h of the oral administration indicated that the extract was relatively safe for consumption. Although the extract showed no acutely toxicity adverse effects, its chronic consumption could elicit adverse effects, and thus, it should be consumed with caution to prevent any chronic toxic effects that may result from its consumption.

The significant increase in the liver marker enzymes (AST, ALT, and ALP) activities of acetaminophen-induced rats indicated that rats suffered liver injury, which caused leakage of liver enzymes to the extrahepatic tissues. The increased liver marker enzymes activities in the acetaminophen-induced rats could be attributed to hepatic failures such as acute hepatic necrosis and disruption of hepatic cell membrane that compromised liver architecture, integrity, and permeability. This hepatic failure could have occurred from lipid peroxidation by reactive N-acetyl-p-benzoquinone, which is produced from acetaminophen breakdown by cytochrome P_{450} enzymes. Significant increase in the individual hepatic enzymes activities is less specific indicator of hepatic failure though; it is generally acceptable that increase in serum ALT activities is more specific indicator of hepatic failure than the increase in the serum activities of AST and ALP activities, respectively. However, when there are significant increases in the serum activities of ALT, AST, and ALP activities, it becomes more likely that there is liver failure. This is an agreement with the findings of other researcher that increase in the activities of liver marker enzymes in the serum was an indication of liver injuries or failure.[16-18] Hepatic enzymes concentrations and activities in the serum are very low, but when there is liver failure, these enzymes leak out of hepatocytes into the extrahepatic tissues causing an increase in their serum concentrations and activities, respectively. This is in line with the findings of Marghoob *et al.*, who reported increased concentrations and activities of serum liver marker enzymes inpatients with liver failure due to viral hepatitis, alcoholic liver disease, and cirrhosis, respectively.[19] Damage to kidney and heart can also lead to increase in the concentrations and activities of these enzymes and thus, make changes in the activities of these enzymes less specific to liver failure except for ALT that is relatively confined to liver cells. The significant reduction of these liver marker enzymes activities in acetaminophen-induced rats, treated with methanol extract of *A. montanus* leaves indicated recovery of the rats from hepatic injury caused by acetaminophen toxicity. The bioactive constituents of the extract could have stimulated regeneration of damaged hepatic cell membrane that prevented continued leakage of the hepatic enzymes to the extrahepatic tissues and caused a decrease in their activities. The extract possibly exhibited antioxidant activities that scavenged free radical generated from acetaminophen breakdown and prevented further lipid peroxidation either through non-enzymatic antioxidant activities of the phytoconstituents of the extract or through induction of synthesis of antioxidant enzymes and stabilization of the available antioxidant enzymes. The dose-dependent decrease in the serum liver marker enzymes activities of the rats treated with the methanol extract showed that the extract possesses better hepatocurative activities than silymarin.

Similarly, the significant reduction in the total protein, albumin, direct bilirubin, and increase in the total bilirubin concentrations in acetaminophen-induced rats further showed that the rats suffered liver injury

resulting in compromised liver functions. These indicated that the rats were unable to carry out their normal metabolic activities and detoxification of toxicants, which greatly endangered their lives. The increased total bilirubin concentrations showed that rats suffered decreased hepatic clearance, whereas the decreased total protein and albumin concentrations depicted their decreased synthesis by the hepatocytes. Treatment with the methanol extract reversed the trends of the total protein, albumin, direct bilirubin, and total bilirubin concentrations observed in the acetaminophen-induced rats in dose-dependent manner. This showed that the extract-treated rats had improved liver function indices relative to the acetaminophen-induced untreated rats which were further reflection of recovery of the extract-treated rats from liver injury caused by acetaminophen toxicity. The extract caused increased protein synthesis, detoxification and hepatic clearance of bilirubin as well as the promotion of biliary functions in the rats which are in agreement with the findings of Jain *et al*.[20,21]

The liver section from acetaminophen induced untreated rats showed swollen and severe degeneration of the hepatocytes in the periportal and mid-zonal of hepatic lobules. This is a typical example of microvesicular steatosis associated with liver injury mostly due to acetaminophen toxicity and indicated that the rats induced with acetaminophen suffered various degree of liver injury. Treatment with silymarin ameliorated the liver injury as depicted by a shift from severe degeneration of hepatocytes to the moderate degeneration of the hepatocytes in the periportal and mid-zonal areas of the liver of the positive control rats. This indicated that the rats were recovering from the injury due to hepatoprotective activity of silymarin. In like manner, the mild to moderatevacuolar degeneration of the hepatocytes in the periportal and mid-zonal areas of the hepatic lobules only, in the extract-treated rats indicated that the rats were recovering from the liver injury due to the hepatocurative activities of the methanol extract of *A. montanus* leaves relatively faster than the groups treated with silymarin. The improved liver histomorphologies of the rats treated with the methanol extract also indicated dose-dependent hepatocurative activities of the extract and agreed with the findings of Sivakumar *et al*.[18]

CONCLUSION

The findings of this study show that the methanol extract of *A. montanus* leaves possessed dose-dependent hepatocurative effects against acetaminophen-induced liver failure and could be used in the treatment of hepatic disorders as claimed by local traditional medicine practitioners. Further research on the hepatocurative activity of methanol extract of *A. montanus* leaves on acetaminophen-induced hepatic injury may lead to the formulation of potent hepatoprotective and curative herbal medicine for the treatment of patients with hepatic disorders.

Ethical Approval

In adherence to the International and University standards for conducting research with animals, written ethical approval was obtained and preserved by the authors.

REFERENCES

1. Mroueh M, Saab Y, Rizkallah R. Hepatoprotective activity of *Centaurium erythraea* on acetaminophen-induced hepatotoxicity in rats. Phytother Res 2004;18:431-3.
2. Hiraganahalli BD, Chinampudur VC, Dethe S, Mundkinajeddu D, Pandre MK, Balachandran J. Hepatoprotective and antioxidant activity of standardized herbal extracts. Pharmacogn Mag 2012;8:116-23.
3. Bruck R, Aeed H, Avni Y, Shirin H, Matas Z, Shahmurov M, *et al.* Melatonin inhibits nuclear factor kappa B activation and oxidative stress and protects against thioacetamide induced liver damage in rats. J Hepatol 2004;40:86-93.
4. Reddy GV, Kumar RV, Rama V, Reddy MK, Reddy YN. Preliminary hepatoprotective activity of medicinal plant extracts against carbon tetrachloride induced hepatotoxicity in albino rats. Int J Rec Sci Res 2015;6:4946-51.
5. Boyd EH, Bereczky GM. Liver necrosis from acetaminophen. Br J Pharmacol 1966;26:606-14.
6. Basu S, Haldar N, Bhattacharya S, Biswas S, Biswas M. Hepatoprotective activity of *Litchi chinensis* leaves against acetaminophen induced liver damage in rats. Middle East J Sci Res 2014;7:292-6.
7. Köksal E, Gülçin I, Beyza S, Sarikaya O, Bursal E. *In vitro* antioxidant activity of silymarin. J Enzyme Inhib Med Chem 2009;24:395-405.
8. Igoli JO, Tor-Anyiin TA, Usman SS, Oluma HO, Igoli PN. Folk medicines of the lower Benue Valley in Nigeria. In: Singh VK, Govil SH, Singh S, editors. Recent Progress in Medicinal Plants. Ethnomedicine and Pharmacognosy II. Vol. 7. USA: Science Tech Publishers; 2004. p. 327-38.
9. Asongalem EA, Foyet HS, Ekobo S, Dimo T, Kamtchouing P. Antiinflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. J Ethnopharmacol 2004;95:63-8.
10. Adeyemi OO, Okpo SO, Okpaka O. The analgesic effect of the methanolic extract of *Acanthus montanus*. J Ethnopharmacol 2004;90:45-8.
11. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983;54:275-87.
12. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
14. Rodkey FL. Direct spectrophotometric determination of albumin in human serum. Clin Chem 1965;11:478-87.
15. Jendrassik L, Grof P. Determination of total bilirubin. Biochemistry1938;279:81-9.
16. Uroko RI, Sangodare RS, Muhammad KH, Asadu CL. Effect of methanol extract of *Abrus precatorius* leaves on male Wistar albino rats induced liver damage using carbon tetrachloride. J Biol Sci 2015;15:116-23.
17. Ochulor OC, Njoku OU, Uroko RI, Egba SI. Nutritional composition of *Jatropha tanjorensis* leaves and effects of its aqueous extract on carbon tetrachloride induced oxidative

stress in male Wistar albino rats. Biomed Res 2018;29:3569-76.
18. Sivakumar V, Sadiq AM, Bharathi SD. Hepatoprotective activity of *Centella asiatica* linn against acetaminophen induced liver damage in experimental animals. Emergent Life Sci Res 2018;4:19-26.
19. Marghoob H, Mohd AH, Abdelmarouf HM. Comparative levels of ALT, AST, ALP and GGT in liver associated diseases. Eur J Exp Biol 2013;3:380-4.
20. Jain S, Dixit VK, Malviya N, Ambawatia V. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Amorphophallus campanulatus* roxb. Tubers. Acta Pol Pharm 2009;66:423-8.
21. Ansari RA, Tripathi SC, Patnaik GK, Dhawan BN. Antihepatotoxic properties of picroliv: An active fraction from rhizomes of *Picrorhiza kurrooa*. J Ethnopharmacol 1991;34:61-8.

Source of support: Nil; Conflict of interest: None Declared