



HEPATOPROTECTIVE EFFECTS OF METHANOL EXTRACT OF *ACANTHUS MONTANUS* (*Acanthaceae*) LEAVES ON ACETAMINOPHEN INDUCED LIVER INJURY IN RATS

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Abstract

Objective: The study evaluated the hepatoprotective effects of methanol extract of *Acanthus montanus* leaves on acetaminophen-induced liver injury in rats. **Materials and Methods:** The study utilized 25 male Wistar albino rats randomly distributed into 5 groups of 5 rats each and groups 1 had the normal control that received normal saline (2 ml/kg/day) for 14 days. Group 2 was the negative control; acetaminophen (2500 mg/kg) induced untreated while group 3 was the positive control that received 100 mg/kg/day of silymarin for 7 days before acetaminophen induction and treatment with silymarin continued for the subsequent 7 days. Groups 4 and 5 served as the hepatoprotective groups that received 200 and 600 mg/kg of the methanol extract of *A. montanus* leaves respectively, for days before acetaminophen induction (2500 mg/kg) and treatment with the extract continued for the subsequent 7 days. All the treatments were administered orally and standard methods were used for the biochemical analyses and histological examinations. **Results:** The acetaminophen induction caused significant ($P < 0.05$) elevation of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) activities in the negative control when compared with the normal control suggesting liver injury. The negative control group had significantly ($P < 0.05$) reduced levels of total protein and albumin, and significantly ($P < 0.05$) elevated levels of total bilirubin and direct bilirubin relative to the normal control. However, extract-treated groups showed significant ($P < 0.05$) reductions in the AST, ALT, and ALP activities relative to the negative control but comparable to the positive control. The extract groups further showed significant ($P < 0.05$) increase in the total protein and albumin concentrations, and reduced levels of total bilirubin and albumin when compared with the negative control indicative of improved liver functions. The hepatoprotective groups showed mild hepatocytes degeneration contrary to the severe wild spread hepatocyte degeneration observed in the negative control. **Conclusion:** These study findings illustrate that methanol extract of *A. montanus* leaves possesses hepatoprotective activities comparable to silymarin. Further researches are required to identify and characterize the lead bioactive phytoconstituents in *A. montanus* leaves to improve its hepatoprotective activities against various hepatic disorders.

Keywords: *Acanthus montanus* leaves, hepatic injury, liver marker enzymes, liver functions, acetaminophen

Introduction

The liver is a unique organ in the body involved in many biotransformation processes like drug metabolism, anabolic and catabolic processes, detoxification, and maintenance of internal homeostasis. These reactions taking place in the liver expose it to various hepatotoxicants, toxins, and possible liver failure which could have detrimental adverse health effects and pose a threat to the survival of the entire organism [1, 2]. Various medical conditions, drug abuse, alcoholism, excessive smoking, poor nutrition, chemical and environmental agents, genetic factor, and unhealthy lifestyle predisposes one to an increased risk of liver diseases or disorders. Prevention of hepatotoxicity by avoiding hepatotoxic agents, the use of hepatoprotective drugs, and maintaining a healthy lifestyle remains the key to preventing liver diseases and disorders. Overdose of some drugs have been known to induce liver injury and this is exploited in the experimental researches on hepatotoxicity involving animal models. Acetaminophen (paracetamol) is a common antipyretic and analgesic drug that when taken in excess dose elicit hepatic injury [2, 3]. Acetaminophen, when ingested, is biotransformed to N-acetyl-P-benzoquinonimine in the liver, been a free radical, it causes lipid peroxidative, disruption of glutathione and thiol group-containing enzymes, and liver necrosis [3]. Most common liver diseases and disorders like necrosis, cirrhosis, inflammation, and hepatitis treated with available drugs like interferon, colchicines, penicillamine, and corticosteroids sometimes do not yield the desired results and usually associated with scarcity, unpleasant adverse health effects and a huge financial burden [4]. Currently, emphases are geared towards alternative complementary medicine that is readily available with high therapeutic efficacy, cost-effectiveness, and minimal adverse health effects.

Acanthus montanus (Nees) T. Anderson is a member of *Acanthaceae* family commonly found in West African countries and parts of the world with abundant rainfall [2]. *A. montanus* leaves and its young shoots have demonstrated high medicinal properties including antihypertensive, and treatment of syphilis, cough, emetic, urethra discharge, boils, anaemia, stomach-ache, menstrual cramps, arthritis, asthma, kidney problem nausea, abdominal pains, acute gastritis and hepatic disorders [2, 5]. Most of the medicinal activities exhibited by this plant extract are its rich phytochemical constituents such as tannins, saponins, cardiac glycosides, terpenoids, steroids, flavonoids, and alkaloids [6]. The leaves of this plant have shown to possess hepatocurative activities in our previous reports. To provide a guide to the therapeutic potentials of *A. montanus* and effective management of hepatic disorders, we designed a study to investigate and ascertain the hepatoprotective activities of the leaves of this plant against acetaminophen-induced liver injury in rats, hence enhancing health outcome of patients with hepatic disorders.

Materials

Reagents

All reagents used in this study were of analytical grade or equivalent and obtained from Sigma Company, USA, and other chemical stores at Onitsha, Anambra State, Nigeria.

Equipment

All apparatus and equipment used in this study are of international laboratory analytical standards and are found at the Department of Biochemistry Laboratory, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, and Shalom Laboratory, Nsukka, Enugu State, Nigeria.

Collection and identification of plant material

The *A. montanus* leaves used in this study were sourced from the Forestry Research Institute of Nigeria, Eastern Station, Ahia Eke, Ndume, Umuahia, Abia State, Nigeria. The collected plant leaves were identified and authenticated (voucher number; FHI 23965) as *A. montanus* leaves at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia, State Nigeria

Experimental animals

Twenty-five (25) adult Wistar albino rats of both sexes were used for this study. The animals were obtained from Animal House of the Department of Zoology and Environmental Sciences at the University of Nigeria, Nsukka, Enugu State, Nigeria. The animals were acclimatized at the Animal House of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, for 7 days and were fed *ad libitum* before the commencement of study.

Preparation and extraction of plant material

The freshly collected *Acanthus montanus* leaves were handpicked and washed under running clean water and air-dried under shade (include the number of days) at room temperature. The dried-leaves sample was then pulverized into coarse powder and stored in a dry clean sterile container for subsequent extraction. 500 g of the pulverized *Acanthus montanus* leaves were soaked in 1.5 L of methanol for 72 h. It was then filtered firstly, with a mesh cloth and finally with a Whatman No. 1 filter paper. The filtrate was then concentrated in a water bath at 50°C until all the methanol was evaporated and concentrated extract was weighed and stored in a refrigerator until analysis.

Experimental design

The 25 rats were randomly grouped after acclimatization into 5 groups containing 5 rats

each for the evaluation of hepatoprotective properties of methanol extract of *A. montanus* leaves. Group 1 was the normal control where the rat models received 2 ml/kg/day body weight (b. wt.) of normal saline orally for 14 days only. Group 2 was the negative control where models received 2500 mg/kg b. wt. of acetaminophen orally on the day 7 and 14 respectively without any treatment. Group 3 served as the positive control where models received 100 mg/kg/day b. wt. of silymarin for 7 days and after 30 min of silymarin administration on the 7th day, 2500 mg/kg b. wt. of acetaminophen was orally administered to the group and treatment with 100 mg/kg silymarin continued till the 14th day. Groups 4 and 5 were the hepatoprotective groups where models were administered 200 and 600 mg/kg/day b. wt. orally of methanol extract of *A. montanus* leaves, respectively for 7 days. After 30 min post administration of the extract to groups 4 and 5 on the 7th day, 2500 mg/kg b. wt. of acetaminophen was orally administered to the rats in each of the groups and treatment with the respective dose of the extract continued till the 14th day. Blood samples and liver were collected from the animals on day 15th for biochemical analyses and histological examination respectively.

Biochemical analyses

The liver marker enzymes: alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activities were assayed using the methods described by Reitman and Frankel as outlined in their respective Randox commercial kits [7]. Total protein concentration was determined using Peterson's modifications of the Lowry method *et al.*, using a protein assay kit (Biuret method) while serum albumin level was determined using the method of Rodkey [8, 9]. The total bilirubin and direct bilirubin were determined using the methods described by Jendrassik and Grof as contained in Randox assay kits [10].

Histopathological analysis of the liver

The experimental animals were euthanized on day 15th of the study period. Tissue sections of the livers were collected for histopathological studies. The samples were fixed in 10% phosphate-buffered formalin for a minimum of 48 hours before tissue preparation. Tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70%, 80%, 90%, and absolute alcohol); cleared in 3 grades of xylene and embedded in molten wax. Upon solidification, the tissue-containing wax blocks were cut into 5µm thick sections with a rotary microtome, floated in a water bath and incubated at 60°C for 30 minutes. The 5µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90%, 80%, and 70%). The sections were then stained with Hematoxylin for 15 minutes. The Blueing of sections was performed with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX.

Slide examination

The prepared slides were examined under a MoticTM compound light microscope using x4, x10, and x40 objective lenses. The photomicrographs were taken using a MoticTM 5.0 megapixels microscope camera at x400 magnifications.

Statistical analysis

The data obtained were analysed using a one-way analysis of variance (ANOVA) with Statistical Products and Service Solutions (SPSS) version 22. Means were subjected to Duncan's Multiple Comparison post hoc test (LSD) and results were presented as mean \pm standard deviation ($n = 5$). Statistical significance was established at 95 % confidence level ($P < 0.05$).

Results

The result in Fig. 1 shows the aspartate transaminase (AST) activities of rats pre-treated with methanol extract of *A. montanus* leaves before acetaminophen induction. The result showed that a significant ($P < 0.05$) increase in the AST activities of the acetaminophen-induced rats when compared to normal control. The positive control (group 3) and extract-treated groups (4 and 5) respectively, showed a significant ($P < 0.05$) decrease in AST activities relative to the negative control rats (group 2). Similarly, group 5 that received 600 mg/kg of the extract before acetaminophen induction showed significant ($P < 0.05$) decrease in AST activities whereas group 4 that received 200 mg/kg of the extract before acetaminophen induction showed no significant ($P > 0.05$) increase in AST activities when compared to the positive control that received 100 mg/kg of silymarin.

The alanine transaminase activities of rats pre-treated with methanol extract of *A. montanus* leaves before acetaminophen induction demonstrated that ALT activities significantly ($P < 0.05$) increased in all the acetaminophen-induced rats when compared to the normal control. Positive control (group 3) pre-treated with 100 mg/kg b. wt. of silymarin, and extract pre-treated groups (4 and 5) respectively, showed significant ($P < 0.05$) decrease in ALT activities when compared to the negative control (group 2). Furthermore, group 4 received 200 mg/kg b. wt. of the extract showed a significant increase in ALT activities whereas group 5 that received 600 mg/kg of the extract showed no significant ($P < 0.05$) increase in ALT activities when compared to the positive control (group 3) that received silymarin drug.

The result of the alkaline phosphatase (ALP) activities of rats pre-treated with methanol extract of *A. montanus* leaves before induction of liver injury with a high dose of acetaminophen (Fig. 3) indicates that the acetaminophen induction caused significant ($P < 0.05$) increase in ALP activities of the rats.

However, the groups of rats pre-treated with the methanol extract (groups 4 and 5) and silymarin (group 3), respectively showed significant ($P<0.05$) decrease in ALP activities as compared to the negative control (group 2) that received no treatment before and after acetaminophen induction. Also, the ALP activities of the extract-treated groups showed no significant ($P>0.05$) difference as compared to the positive control pre-treated with silymarin.

The result in Fig. 4 shows the total protein concentrations of rats pre-treated with methanol extract of *A. montanus* leaves before induction of liver injury with a high dose of acetaminophen. The result reveals a significant ($P<0.05$) reduction in the total protein concentrations in all the rats that received a high dose of acetaminophen (groups 2 – 5) as compared to the normal control that received only normal saline. The total protein concentrations observed in the positive control (group 3), and groups 4 – 5 pre-treated with silymarin and the methanol extract respectively, showed significant ($P<0.05$) increase when compared to the negative control (group 2). However, there was no significant ($P>0.05$) difference observed between the total protein concentrations of the positive control that received silymarin and extract-treated groups (groups 4 and 5), respectively.

The data in Fig. 5 shows the albumin concentrations in rats pre-treated with methanol extract of *A. montanus* leaves before induction of liver injury with high amounts of acetaminophen. The result showed a significant ($P<0.05$) decrease in albumin concentrations of groups 2 – 5 rats induced acetaminophen as compared to the normal control. The positive control (group 3) pre-treated with silymarin and groups 4 – 5 pre-treated with graded doses of the methanol extract before acetaminophen induction had significantly ($P<0.05$) elevated albumin concentrations when compared to the negative control. The albumin

concentrations in groups 4 and 5 rats treated with 200 and 600 mg/kg b. wt. of the methanol extract respectively, were significantly ($P<0.05$) high when compared with the positive control pre-treated with 100 mg/kg b. wt. of silymarin.

The total bilirubin concentrations of rats pre-treated with silymarin and methanol extract of *A. montanus* leaves before induction of liver injury with a high dose of acetaminophen showed significant ($P<0.05$) increase in all the acetaminophen-induced rats when compared to the normal control rats (Fig. 6). Relative to the negative control, group 3 pre-treated with silymarin, and groups 4 and 5 rats pre-treated with varying doses of methanol extract showed significant ($P<0.05$) decrease in total protein concentrations. Group 5 pre-treated with 600 mg/kg of the methanol extract showed a significant ($P<0.05$) decrease in the total protein concentration when compared with the positive control pre-treated with 100 mg/kg of silymarin.

The result in Fig.7 shows the direct bilirubin concentrations of rats pre-treated with silymarin, and methanol extract of *A. montanus* leaves subsequently induced liver injury with a high dose of acetaminophen. It showed that the rats induced with a high dose of acetaminophen had a significant ($P<0.05$) increase in direct bilirubin concentrations when compared with the normal control. The positive control (group 3) pre-treated with 100 mg/kg of silymarin showed a significant ($P<0.05$) decrease indirect bilirubin concentration relative to the normal control rats. However, groups 4 and 5 rats pre-treated with graded doses of the methanol extract showed no significant ($P>0.05$) decrease indirect bilirubin concentrations when compared to the negative control rats that received no pre-treatment before induction of liver injury with a high dose of acetaminophen.

The liver section obtained from the normal control rats showed the normal hepatic histomorphology of laboratory rodents (Fig.

8). The tissue sections showed normal hepatocytes in the hepatic lobules arranged in interconnecting cords (hepatic cords) around the central veins. The hepatic cords are separated by the hepatic sinusoids and radiate towards 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. Central vein (V); Hepatic cords (Black arrow); Sinusoids (white arrow).

The liver section from the negative control rats that were acetaminophen-induced and untreated showed severe degeneration of the hepatocytes in the periportal and mid-zonal areas of the hepatic lobules while the centrilobular hepatocytes (black arrow) appeared normal (Fig. 9). The affected hepatocytes were swollen with multiple coalescent intracytoplasmic clear vacuoles (white arrow) and partially occludes the adjacent hepatic sinusoids (blue arrow). This demonstrates a typical example of microvesicular steatosis. Central vein (V); Bile duct (BD); Hepatic artery (HA).

The histomorphology of the liver obtained from positive control rats (group 3) that were pre-treated with silymarin before acetaminophen induction showed a moderate widespread degeneration of the hepatocytes in the periportal and mid-zonal areas (Fig.10). The hepatocytes in the centrilobular areas (white arrow) of the hepatic lobules appeared normal. However, the affected hepatocytes (black arrow) appeared swollen, partially occluding the hepatic sinusoids containing numerous coalescent clear vacuoles in their cytoplasm suggestive of microvesicular steatosis.

Sections of the liver obtained from the rats in this group portrayed moderate degeneration of the hepatocytes in the periportal, mid-zonal, and outer parts of the centrilobular areas of the hepatic lobules (Fig. 11). Though, the affected hepatocytes appeared swollen;

containing single, large, or multiple coalescent intracytoplasmic clear vacuoles (black arrow), they partially occluded the adjacent hepatic sinusoids. This type of degeneration is classified as micro-vesicular steatosis. Randomly distributed areas of coagulative necrosis of the hepatocytes with nuclear pyknosis were also observed (blue arrow).

The liver pre-treated with 600 mg/kg of methanol extract of *A. montanus* leaves before and after acetaminophen induction collected from rats in group 5 showed moderate widespread degeneration and necrosis of the hepatocytes (Fig. 12). The observed hepatocyte degenerative changes involved all the cells in the centrilobular areas of the hepatic lobules. Central vein (V); Portal area (P).

Discussion

Acetaminophen (paracetamol) is one of the most used and abused over the counter analgesic and antipyretic drug that inhibit prostaglandin production in the central nervous system as a major mechanism of its action [11]. It is metabolized in the liver to N-acetyl-p-benzoquinone imine (NAPQI) which is detoxified by glutathione and excreted in the urine. When acetaminophen is ingested in excess above its therapeutic dose, the glucuronidation and sulfation processes responsible for its detoxification become saturated, due to excess production of NAPQI which interacts with glutathione and thereby depleting it [12]. The excess NAPQI accumulates and binds covalently to the cysteinyl sulfhydryl groups of cellular proteins. The resulting NAPQI-protein adducts can lead to oxidative stress, hepatocyte injury, and possibly kidney damage [13, 14]. This study investigated hepatoprotective effects of methanol extract of *A. montanus* leaves on rats to validate its hepatoprotective claims by many traditional medicine practitioners and to enable its optimal use as a prophylactic and/or therapeutic target in enhancing human health.

The significant increase in the liver marker enzyme activities of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) in the rats induced liver injury with high doses of acetaminophen is indicative of liver damage and leakage of these liver enzymes into the extra-hepatic tissues. The high-dose acetaminophen-induced liver injury resulting to increased marker enzymes activities is in tandem with our earlier report that acetaminophen-induced liver injury results in elevated levels of ALT, AST and ALP activities due to leakage of these enzymes to extra-hepatocellular tissues attributable to possible disruption of liver integrity and architecture [2]. This denote the toxic effects of high doses of acetaminophen on the liver cells. Despite the insignificant activities of these marker enzymes due to low concentration the serum, the presence of a liver injury or damage resulting from a compromised liver architecture and increased permeability enhances the leakage of these marker enzymes into the extrahepatic environment ensuing an inevitable increase in serum concentrations with a consequent rise in activities of these enzymes. The increased AST and ALP activities are a less specific indicator of liver injury, as kidney and heart injuries among other tissue damage could contribute much of these enzymes to the circulating blood and increase their serum activities aside liver injury. An increase in serum ALT activity is a better indicator of liver injury than AST and ALP though, in combination, the increasing activities of the enzymes (AST, ALT, and ALP) could confidently serve as a better indicator of liver injury. The significant reduction in the AST, ALT, and ALP activities of the positive control and extract pre-treated groups (4 – 5) respectively relative to the negative control is suggestive of the hepatoprotective properties of *A. montanus* against acetaminophen toxicity. Although the rats pre-treated with the extract experienced some degree of liver injuries due to acetaminophen toxicity, it was relatively negligible as compared to the

negative control rats. These findings are in synergy with findings of Uroko et al., and Al-Snafi et al., who had independently reported that reductions in the activities of liver marker enzymes of hepatoprotective groups relative to negative control suggest improvement in the integrity and permeability of plasma membrane of hepatocytes and recovery liver injury [15, 16].

The high total protein, albumin, and low total bilirubin and direct bilirubin concentrations in the normal control rats indicate normal liver functions whereas, the reverse cases observed in the negative control rats suggest impaired liver functions due to liver injury from acetaminophen toxicity. Elevated blood total bilirubin level is a major indicator of liver and bile duct disease but it can also occur in haemolytic anaemia. The normal control rats' livers were functioning properly and were able to synthesize enough proteins including albumins needed to maintain normal biochemical functions. These sufficient albumins produced by the normal rats were able to transport much of the circulating bilirubin in the blood to the livers for detoxification and excretion in bile. The low direct bilirubin concentrations in the normal rats revealed that the rats' livers were able to perform their normal bilirubin detoxification effectively, which increased its solubility and accretion in bile. Similarly, the low total protein and albumin concentration observed in the negative control rats (group 2) bespeak that their liver cells suffered injury or damage which resulted in their impaired protein synthesis, similar to the finding of Al-Snafi et al. [16]. However, reduced serum albumin levels can also occur in malnutrition and chronic medical conditions like diabetes and kidney diseases. These findings are consistent with Kanchana and Sadi that hypoproteinaemia is an indication of liver injury and compromised liver functions because the healthy liver is required to synthesize sufficient serum proteins [17]. The high total bilirubin concentration in the

negative control rats could be attributed to the inability of their low albumin level to transport a significant amount of circulating bilirubin from blood to the liver for conjugation into a more water-soluble form. However, the significantly elevated levels of total proteins and albumin concentrations in the hepatoprotective groups relative to the negative control suggest that pre-treatment of the rats with the methanol extract protected the rats from massive liver damage from acetaminophen toxicity. The hepatoprotective rats' groups had increased protein synthesis including albumin required for various biochemical functions and transport of blood circulating bilirubin to the liver for detoxifications and further showed *et al* that rats had uncompromised liver functions similar to the findings of Rajandra *et al.* [18]. The lower direct bilirubin concentrations in hepatoprotective groups showed that the methanol extract pre-treated rats were able to carry out conjugation of bilirubin efficiently than the negative control rats which also suggest that acetaminophen induction was unable to their liver functions largely. An excessive blood level of bilirubin can adversely affect DNA synthesis, and uncouple oxidative phosphorylation leading to a low ATP level in the body that could impair vital biochemical and metabolic functions. Whereas, the presence of a moderately raised level of bilirubin in the blood could confer some antioxidants to protect against free radicals and improved health status [19].

The normal hepatic histomorphology observed in the normal control rats showed that the rats were healthy and free from any hepatic injury before and after the study. Thus, any alteration observed in the rats treated with a high dose of acetaminophen could be attributed to the hepatotoxic effects associated with the ingestion of an overdose of acetaminophen. The severe widespread degeneration of the hepatocytes of the negative control rats showed that the rats suffered a severe liver injury due to

acetaminophen toxicity, as there was no treatment given to ameliorate acetaminophen toxicity. However, the moderate degeneration of the hepatocytes in the silymarin treated positive control and methanol extract of *A. montanus* leaves treated hepatoprotective groups showed that silymarin and the extract respectively possess hepatoprotective activities but could not confer the rats with total hepatoprotection against acetaminophen toxicity.

Conclusions

The findings of this study unveiled that the methanol extract of *A. montanus* leaves possesses hepatoprotective activities comparable to silymarin and could be used in the prevention of hepatotoxicity. However, the methanol extract of *A. montanus* leaves showed limited hepatoprotective activities as the rats administered the extract demonstrated some levels of hepatotoxicity relative to normal control rats but mild relative to the severe hepatotoxicity experienced by the untreated rats that received high doses of acetaminophen drug.

Conflict of interest

The authors have declared no conflict of interest.

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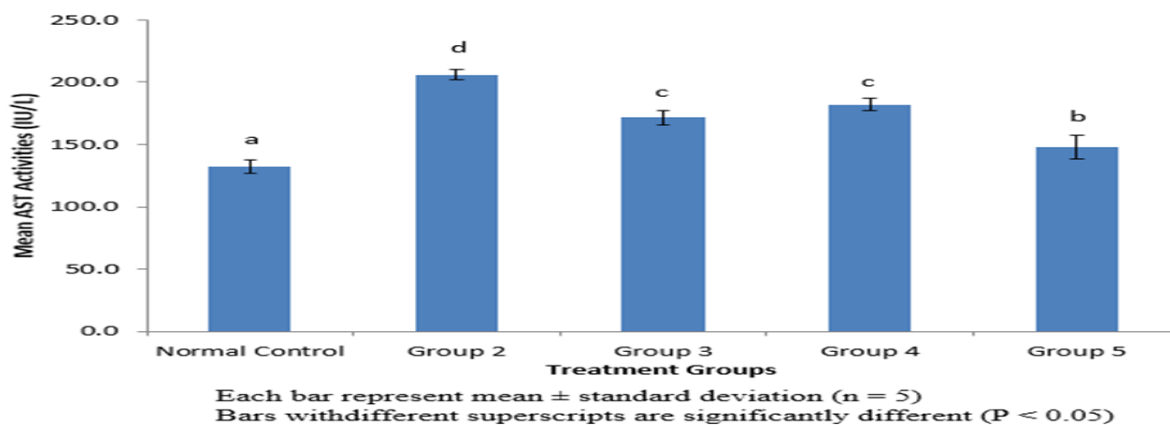


Fig. 1: Aspartate transaminase (AST) activities of acetaminophen (2500 mg/kg b. wt.) induced rats treated with methanol extract of *Acanthus montanus* leaves

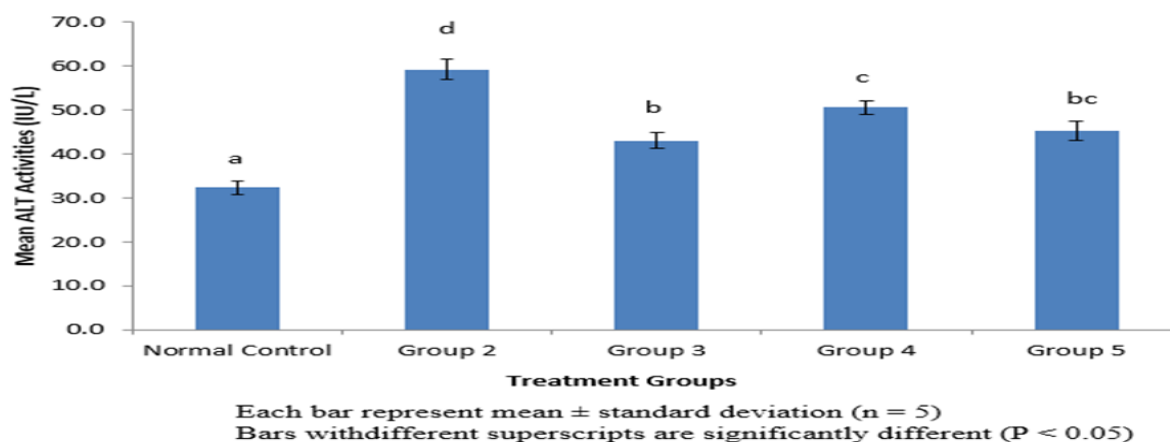


Fig. 2: Alanine transaminase (ALT) activities of acetaminophen (2500 mg/kg b. wt.) induced rats treated with methanol extract of *Acanthus montanus* leaves

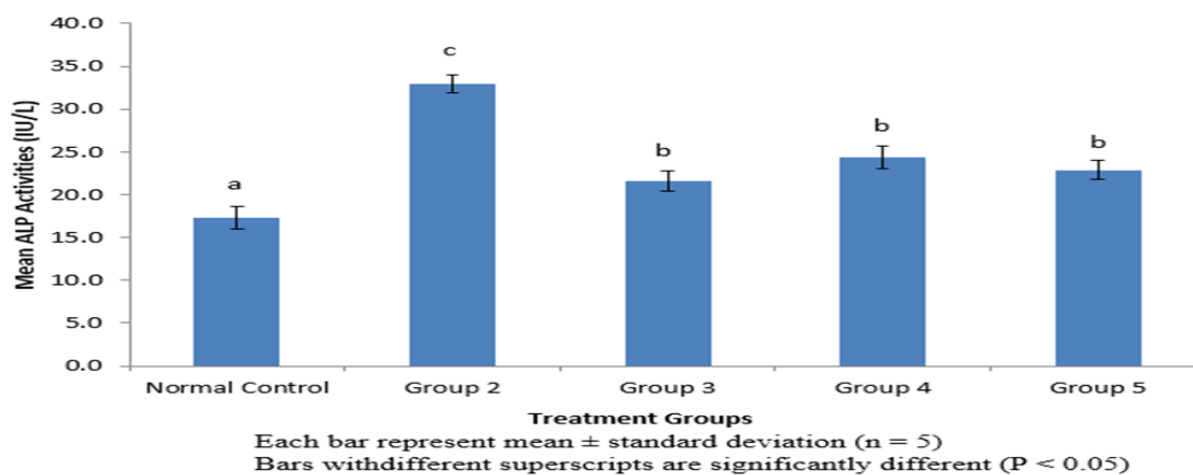


Fig. 3: Alkaline phosphatase (ALP) activities of acetaminophen (2500 mg/kg b. wt.) induced rats treated with methanol extract of *Acanthus montanus* leaves

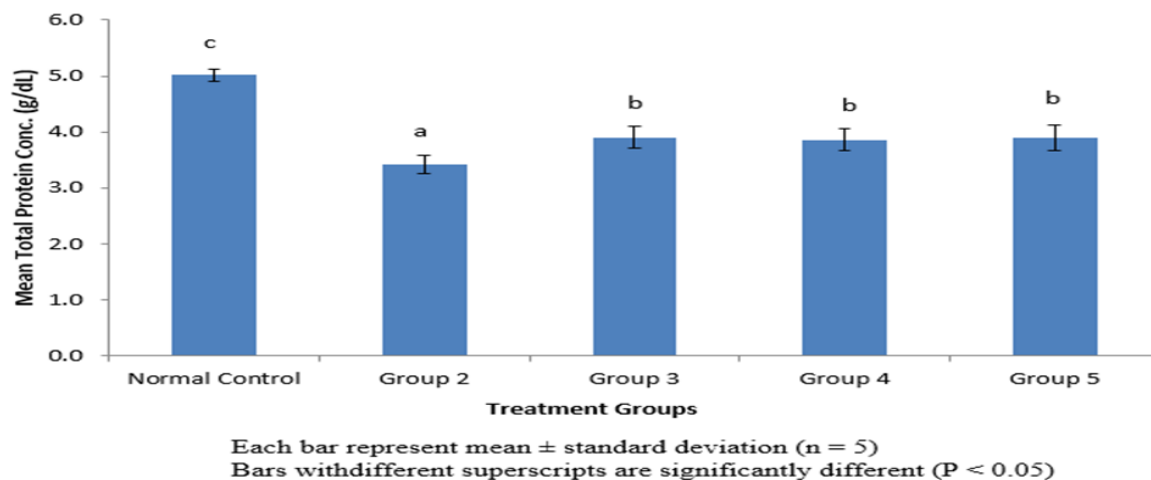


Fig. 4: Total protein concentrations of acetaminophen (2500 mg/kg b. wt.) induced rats treated with methanol extract of *Acanthus montanus* leaves

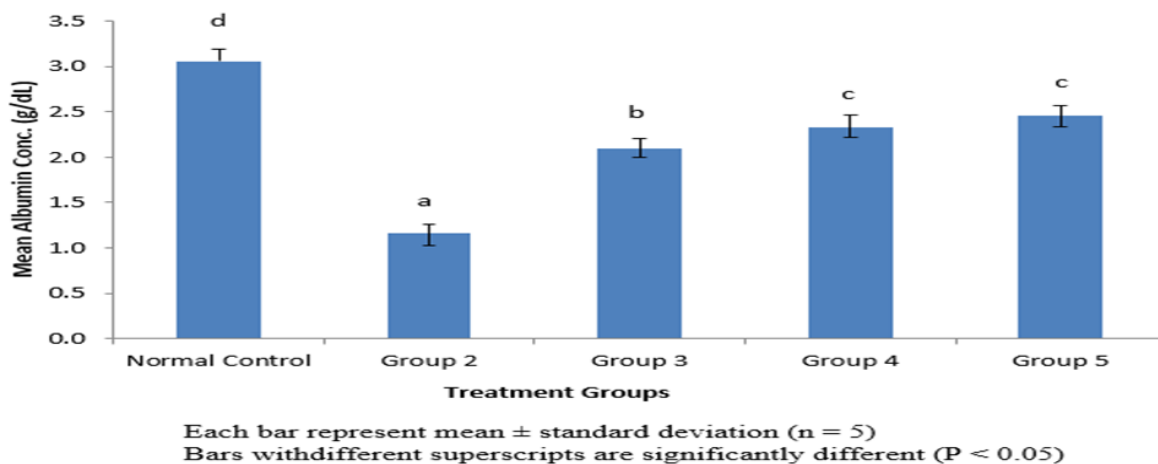


Fig. 5: Albumin concentrations of acetaminophen (2500 mg/kg b. wt.) induced rats treated with methanol extract of *Acanthus montanus* leaves

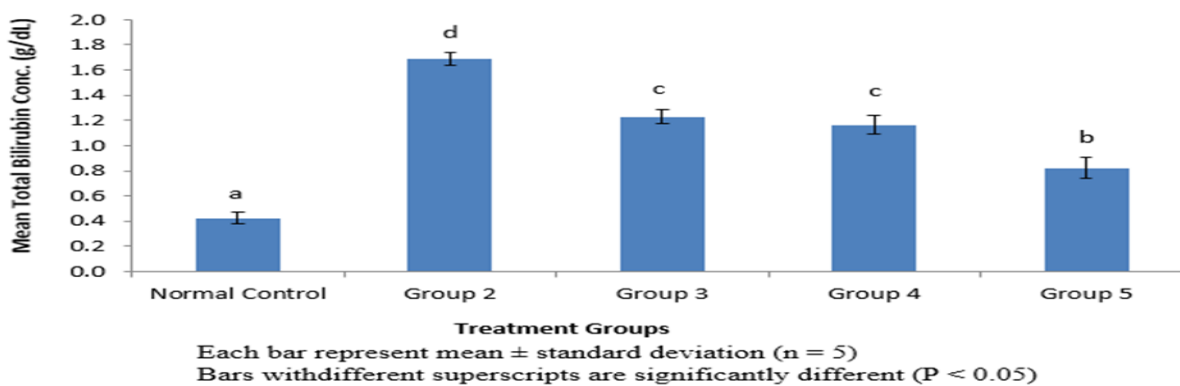


Fig. 6: Total bilirubin concentrations of acetaminophen (2500 mg/kg b. wt.) induced rats treated with methanol extract of *Acanthus montanus* leaves

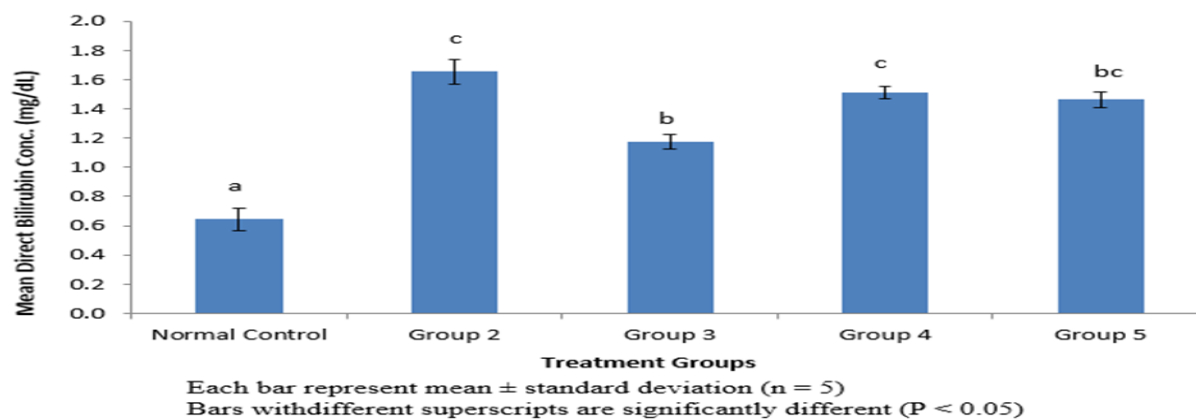


Fig. 7: Direct bilirubin concentration of acetaminophen (2500 mg/kg b. wt.) induced rats treated with methanol extract of *Acanthus montanus* leaves

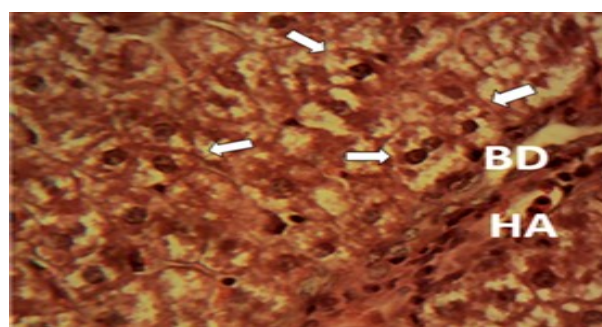


Fig. 9 Histomorphology of liver from untreated acetaminophen Induced rat (group 2).

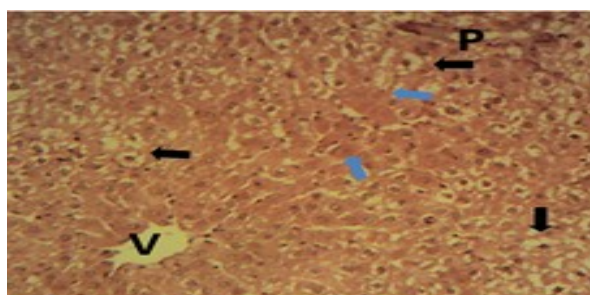


Fig. 11 Histomorphology of liver from rats treated with 200 mg/kg b. wt. methanol extract of *Acanthus montanus* leaves before and after acetaminophen induction (group 4).

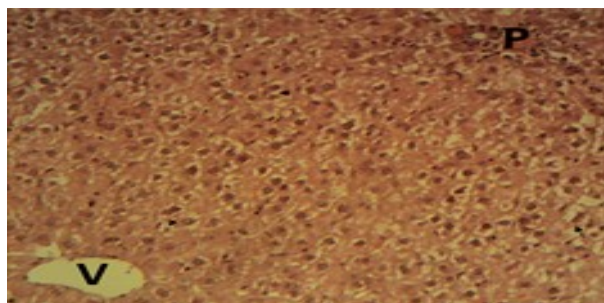


Fig. 12 Histomorphology of liver from rats treated with 600 mg/kg b. wt. methanol extract of *Acanthus montanus* leaves before and after acetaminophen induction (group 5).