Original Article

Toxicological and Biochemical Studies of Ethanolic Fruit Extract of *Adenopus breviflorus* (*Lagenaria breviflora* Roberty) in Male Albino Wistar Rats

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ABSTRACT

The present study was undertaken to evaluate the toxicological and biochemical effects of administration of ethanolic fruit extract of *Adenopus breviflorus* (EEAB) on some liver and kidney parameters of albino rats. Phytochemical screening was carried out on the fruit of *A. breviflorus* sample. The effect of the extract on the histology of the liver tissues was also investigated. Thirty (30) male albino rats were randomly divided into 5 groups (n=6) rats each. Group 1 served as the control and was administered 2mL distilled water. The remaining groups received 500, 1000, 2000 and 4000 mg/kg/ b.w.t/day of EEAB respectively orally over a period of 56 days. The animals were sacrificed twenty four hours after administration on the fifty sixth day. Liver and blood samples were collected and biochemical indices such as alanine transaminase-ALT, aspartate transaminase-AST, alkaline phosphatase-ALP, urea, creatinine, total bilirubin, total protein, albumin and globulin assays were determined in the serum using standard techniques. Data were subjected to one-way ANOVA and the level of significance was at P=0.05. The extract significantly increased (P=0.05) the serum levels of urea, creatinine, and total bilirubin in all the treatment groups when compared to the control in dose-dependent manner except in animals administered 500mg/kg/ b.w.t of EEAB. Similar results were observed for serum activity of ALP, ALT, and AST. However, a significant decreased was observed in the serum level of albumin, globulin, and total protein in all treated groups except in group 2 animals. Phytochemical studies revealed the presence of saponins (triterpenoids). The LD_{50} of the ethanolic

extract was determined to be above 5000mg/kg BW. Routine H and E histological study revealed features of hepatotoxicity. The study suggests that ethanolic extract of *A. breviflorus* predisposes the animals to adverse effects and it was therefore concluded that the extract could be safe at the tested dosage of 500mg/kg body weight but hepatotoxic at higher doses.

Keywords: *Adenopus breviflorus*, Fruit extracts, Phytochemical screening, Serum biochemical parameters, Histology, Rats.

INTRODUCTION

Adenopus breviflorus Benth is a tree plant, commonly known as *"lagenaria breviflora* Roberty", belongs to the family Cucurbitaceae[1,2]. It is a perennial climber ascending to the forest canopy, occurring from Senegal to the West Cameroons, and generally widespread in tropical Africa. The family is a diverse family of plants in the temperate zones but also thrives in hot arid regions of the world[3]. In Nigeria, different tribal groups have their indigenous names as: "Ogbenwa" in Igbo, "Tagiri" in Yoruba) and so on[4]. The leaves are scabrid and sand papery. The stem when crushed has an unpleasant smell and a decoction from it is said to be used in Africa for headache and as a vermifuge[5]. Its seeds and fruits have been used in folk medicine since antiquity. The fruit of *Lagenaria breviflora* Roberty is widely used in folklore medicine in West Africa as herbal remedy for the treatment of measles, digestive disorders, and as wound antiseptics (e.g. umbilical incision wound) in man, while the livestock farmers use it for the treatment of Newcastle disease and coccidiosis in various animal species, especially poultry[6]. Laboratory investigations have shown evidences in support of its anti-implantation activity[7], miracicidal and cercaricidal activities[5] and antibacterial activity[8]. In addition to its medicinal application, so much has been reported on the taxonomy[9] and chemical constituents of the plant[10-13]. No attempt till date has been made to evaluate the safety of the administration of the extract of the whole fruit of *A. breviflorus*. Such an investigation would provide the necessary data on the safety of therapeutic application of the plant used in folkloric medicine. These are the objectives which the present investigation sought to achieve.

MATERIALS AND METHODS

Experimental animals

Thirty male albino rats weighing between 180 to 200 g were obtained from the Central Animal House, Faculty of Basic Medical Sciences, Ebonyi State University, Abakaliki, Nigeria. They were housed in netted cages under standard laboratory conditions and were fed with standard rat's pellets (Pfizer Feeds PLC, Enugu, Nigeria) and water allowed *ad-libitum*. Excess feeds and water were removed and replaced daily. The rats were allowed to stabilize for 2 weeks before commencement of the experiment. The experimental procedures and techniques used in the study were in accordance with accepted principles for laboratory animal use and care by National Institute of Health (NIH, 1985) all protocol and procedure were approved by Animal Ethics Committee of the University with reference number (EBSU/REC/BM14/011).

Plant materials

The fresh fruit of *Adenopus breviflorus* was collected at the back of Food Processing Laboratory, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The fruit sample was identified and authenticated as *A. breviflorus* by Dr. A.T.J. Ogunkunle in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. Authenticated voucher specimen (LHO 227) was deposited in the Herbarium of the same department.

Extraction and preparation of extract

The whole fruit of *Adenopus breviflorus* was rinsed with distilled water and reduced into smaller sizes. The smaller sizes were air dried in the shade for 12 to 14 days and then pulverized to coarse powder, and stored in an air tight container until required. 1000g of the powdered fruit was weighed and suspended in 2.5liters of 96% ethanol and shaken vigorously at intervals, this was allowed to stand for 48 hours in a dark room environment. Thereafter it was filtered by a mesh cloth and the filtered was concentrated into a greenish-brown syrupy mass in a rotary evaporator with a temperature of about 40^0C under reduced pressure for 3h. The extract syrup formed was left over a water bath for final concentration into solid paste which gave a percentage yield of 21.8%. The concentrate was later reconstituted in sterile distilled water to give the required doses of 500, 1000, 2000, 4000 mg/kg/ body weight in 2ml of the vehicle respectively using tween-80 as suspending agent. The solutions were prepared fresh on the day of experiment prior to the administration.

Preliminary phytochemical screening

The ethanolic fruit extract of the plant was subjected to various qualitative phytochemical tests, to identify the secondary metabolites; saponins, tannins, terpenes, steroids, alkaloids, flavonoids and cardiac glycosides present in the fruit. The methods of analysis employed were those described by Trease and Evans[14] and Sofowora[15].

Acute oral toxicity study

The lethal dose (LD_{50}) of the ethanolic fruit extract of *A. breviflorus* was determined by the method of Lorke[16,17] using thirteen (13) rats. In the first phase rats were divided into three groups of three (3) rats each and were treated with the ethanolic fruit extract of *A. breviflorus* at doses of 10, 100 and 1000mg/kg body weight intraperitoneally. They were observed for 24 hours for signs of toxicity. In the second phase four rats were divided into four (4) groups of one rat each and were also treated with the ethanolic fruit extract *A. breviflorus* at doses of 1000, 1600, 2900 and 5000mg/kg body weight (i.p). The median lethal dose (LD_{50}) was calculated using the second phase.

Experimental design and animal treatment

A total of thirty (30) Wistar rats were used for the study. The rats were randomly divided into five (5) groups consisting of six (6) animals each and coded to prevent observer bias. The rats in group 1 received 2mL of distilled water daily while the experimental groups 2, 3, 4 and 5 received 2mL graded doses of 500, 1000, 2000 and 4000mg/kg body weight of the ethanolic fruit extract of *A. breviflorus* respectively orally via an orogastric syringe for fifty- six days. Twenty-four hours after the last administration, the animals were anesthetized with sodium pentobarbital (30mg/kg, intraperitoneally). When the rats became unconscious, the neck area was quickly cleared of fur and skin to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with

interstitial fluid) were then sharply cut with a sharp sterile blade. Blood samples for sera preparation were collected into sterile plain tubes and allowed to clot at room temperature for 1h. It was then refrigerated for another 45 minutes[18]. The clear serum was collected with Pasteur pipette into clean, dry sample bottles and stored in refrigerator until required 12 h later. All analyses were completed within 24h of sample collection.

Determination of serum biochemical parameters

Serum urea and creatinine levels were determined using spectrophotometric methods described by Coles[19]. The total bilirubin concentration was determined as described by Balistreri and Shaw[20]. The activities of hepatic marker enzymes were determined in the serum. Alkaline phosphatase (ALP) activity was determined using the method described by Wright *et al*.[21]. Alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined using the method described by Reitman and Frankel[22]. Total protein was measured using Biuret reaction as modified by Donninger *et al*.[23], while albumin level was measured by spectrophotometric estimation using the Sigma Diagnostic Kit (Sigma Diagnostics, UK). Globulin was obtained from the difference of total protein and albumin.

Hepatotoxicity study

After the blood samples were taken, the rats abdomen were opened and the liver excised , rinsed with saline and fixed in 10% buffered formalin solution. The liver was routinely processed; paraffin sections of 10 um thick were obtained and stained with hematoxylin and eosin for histopathological signs of toxicity[24]. Thereafter microscopic slides were photographed and interpreted.

Statistical analysis

The data were statistically evaluated by one way ANOVA. Comparison between treatment and control group were made by Student's t- test then followed with Fisher's exact. Differences between groups were considered significant at $P = 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical analysis indicate that the extract contains saponins (triterpenoids).

Acute oral toxicity test

The oral administration of the ethanolic fruit extract of *A. breviflorus* to albino rats up to the dose of 5000mg/kg BW did not record any mortality. However, a mild clinical sign of writhing, increase in motor activity and tremors in rats treated with the dosage of 5000mg/kg BW was observed. Thus, the LD_{50} was considered to be above 5000mg/kg BW.

Biochemical studies

Total protein (TP) concentration

The mean serum total protein decreased significantly (*P=0.05*) for all the treatment group when compared with the control group (73.93 ± 2.41 g/dl) but showed no significantly increased in group 2 animals (77.33±3.42 g/dl).

Albumin and globulin levels

The serum level of albumin decreased significantly for the entire treatment group when compared with the control (20.35±.93g/dl) except in group 2 animals (21.78±.93g/dl) which increased non-significantly. The serum level of globulin decreased significantly across all the treated group when compared with the control (55.58±0.94g/dl) except in group 2

animals (55.55±0.57g/dl) which increased non-significantly (*P*>0.05).

Alkaline phosphatase (ALP) activity

The mean value of serum ALP activity increased significantly (*P*=0.05) across the entire treated group when compared with the control (130.7±1.09 IU/L) except in group 2 animals (128.8±.66 IU/L) which decreased non-significantly.

Alanine aminotransaminase (ALT) activity

The serum ALT activity increased significantly (P=0.05) across all the treatment groups when compared with the control (90.23±1.05 IU/L) but decreased non-significantly (*P*>0.05) in group 2 animals (88.28±.60 IU/L).

Aspartate aminotransferase (AST) activity

The serum AST activity increased significantly (*P*=0.05) across all the treated groups when compared with the control except in group 2 animals (80.02±1.11 IU/L) which increased non-significantly *(P*>0.05).

Urea level

The urea concentration increased significantly (*P*=0.05) across all the treated groups when compared with control (16.73±.21 g/dl) but decreased non-significantly in group 2 animals (15.68±.46g/dl).

Creatinine (CRT) level

The serum creatinine levels increased significantly *(P*=0.05) across all the treated groups when compared with control (434.9±1.26g/dl) but decreased non-significantly in group 2 animals (432.1±1.65g/dl).

Total bilirubin level

The total bilirubin concentration increased significantly (*P*=0.05) across all the treated groups when compared with control (101.9±.78 g/dl) but decreased non-significantly *(P>0.05)* in group 2 animals (100.2±.48g/dl).

Hepatotoxicity findings

The liver section from group 1 (control, 2ml distilled water) showed normal architecture of the liver (Figure 1). The liver section from group 2 given 500 mg/kg body weight of EEAB orally showed relatively normal hepatocytes with nuclei, central vein, sinusoid, portal triad and some inflammatory cells scattered all over (Figure 2). The liver section from group 3 given 1000 mg/kg body weight of EEAB showed hepatic injury due to slight loss of normal liver architecture with evidence of shrunken hepatocytes, with pale pykinotic nuclei and slightly congested central vein, the portal triad was sinuded by inflammatory cells and there is loss of intervening sinusoids (Figure 3). But the liver section from groups 4 and 5 (EEAB 2000 mg/kg body weight orally and EEAB 4000mg/kg body weight orally) shows shrunken hepatocytes, congested central vein, the portal triad was sinuded by inflammatory cells, intervening sinusoids and dilation of the central vein (Figure 4 and 5).

DISCUSSION

Phytochemical studies revealed the presence of saponins without tannins, therefore is described as triterpenoids. The acute toxicity LD_{50} of ethanolic fruit extract of *A. breviflorus* in albino rats was determined to be above 5000mg/kg body weight according to the method of Lorke[16,17]. The elimination of toxic substances is just one of the many functions of the liver and kidneys. The kidney plays a very important role in removal of metabolic wastes from the blood stream. Its functionality therefore can be assessed among many others by determining the serum level of excretory constituents[25]. The

liver converts nutrients into energy, forms proteins and stores carbohydrates. However, these organs can be remarkably resilient in the elimination of toxins and their other functions can be damaged in the process[26]. The significant increase in serum urea and creatinine levels in all the treatment groups except in group 2 animals for fifty-six days period of treatment may be suggestive of an alteration in the secretory and excretory functions of the kidney[27] caused by the ethanolic fruit extract of *A. breviflorus*. The increase in serum total bilirubin concentration by the extract may be suggestive of liver dysfunction rather than kidney dysfunction. This may result from the interference of the components of the extract with the process of getting rid of the ammonia produced from amino acid catabolism in the liver[28]. Another possible explanation is that urea may be converted to other products undetectable by the direct method of urea determination used in this study. Pre-treatment with ethanolic fruit extract of *A. breviflorus* produced a significant decrease in the albumin, globulin and total protein serum levels which suggest that the extract will inhibit protein biosynthesis[29]. Increase in total protein is said to augment the buffering capacity of the blood since plasma proteins have been reported to be responsible for 15% of the buffering capacity of blood[30]. The three most important and common liver enzymes in liver profile were AST, ALT and ALP[31,32] and that of the kidney are urea and creatinine. Kidney and liver are the sensitive organs, whose functions are known to be affected by a number of factors such as drugs including phytochemical of plant origin that ultimately lead to renal and liver failure[33]. The significant increase in serum alkaline phosphatase (ALP) activity for all the treatment groups except in group 2 animals for fifty-six days is suggestive of a possible damage to tissue cell plasma membrane by the ethanolic fruit extract of *Adenopus breviflorus*, thus leading to leakage of membrane component into extracellular fluid[34]. This is further supported by the increase in alkaline phosphatase (ALP) activity in the liver. This may imply loss of enzyme to extracellular fluids or the inactivation of the enzyme in situ by the components of the extract and their metabolites. The membrane damage done to the liver by the extract had started manifesting through the serum elevation of cellular enzymes (AST and ALT). Alanine transaminase (ALT) activities in the blood are increased in the conditions in which liver cells are damaged or dead[35]. Elevation of Alanine transaminase and aspartate transaminase (ALT and AST) activities in the serum following fifty-six days administration may indicate derangement of hepatic cytosolic content and interference with kidney amino acid metabolism[36]. This coupled with increase in serum urea, creatinine and total bilirubin concentration, still point to the fact that the extract may adversely interfere with amino acid metabolism of the liver in a concentration dependent manner. Urea and creatinine are the major indicators of kidney functionality. Elevation of urea is an indication that kidney functions have been compromised as may be seen in diabetes mellitus. Serum electrolytes, urea and creatinine are used to assess the renal functional capacity[37]. The significant changes observed in urea showed that the plant extract did alter the functional capacity of the kidney and may have effect on the urea cycle[38]. The slight changes observed in creatinine at specific doses could be attributed to physiological response by the animals to counteract the effect of the extract or to recover from the assault of the extract[39]. The extract is characterized by a very high degree of toxicity. The hepatotoxicity finding in liver sections of the extract groups was characterized by

morphological changes such as loss of hepatic sinusoidal arrangement or loss of radial arrangement of hepatocytes, vascular derangements (affecting the central vein and portal triad), pykinotic nuclei, degeneration of the hepatocytes in the groups administered 1000, 2000 and 4000mg/kg/ BW EEAB. These effects were observed to be dose dependent as feature of hepatic damage were progressive as the doses increases. In consistent with previous reports hepatic cytoplasmic and nuclei changes: cytoplasmic vacoulations, pykinotic nuclei, vascular derangements and loss of hepatocytes are signs of toxicity and degenerative liver change impact on the liver by toxin substances including drugs[40]. Consequently this translates into change in liver function and depending on the degree of impact either an increase or decrease in liver function, but most commonly these features are also liver adaptive changes[40].

CONCLUSION

The study revealed that the repeated administration of ethanolic fruit extract of *A. breviflorus* at varying doses brought about increase in urea, creatinine and total bilirubin implying kidney dysfunction and compromise of secretory and excretory functions of the kidney. The significant changes in alkaline phosphatase (ALP), Alanine transaminase (ALT) and aspartate transaminase (AST) activities in various tissues studied suggested that administration of the extract at doses higher than 500mg/kg may cause damage to hepatic and renal cells as well as disrupt amino acid metabolism. It is evident from this investigation that administration of ethanolic fruit extract of *A. breviflorus* above the dose of 500mg/kg may lead to damage in the liver.

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Table 1. Effects of daily administration of ethanolic fruit extract of *Adenopus breviflorus* fruit (EEAB) for fifty six days on rat serum biochemical parameters

	Group 1 (2ml/kg B.W)	Group 2 (500mg/kg B.W) of EEAB	Group 3 (1000mg/kg B.W) of EEAB	Group 4 (2000mg/kg B.W) of EEAB	Group 5 (4000mg/kg B.W) of EEAB
Total protein (g/dl)	73.93±2.41	77.33±3.42	30.9±1.76*	25.28±0.86*	17.83±0.79*
Albumin (g/dl)	20.35±0.93	21.78±0.93	16.15±0.78*	12.18±0.67*	9.77± 0.42*
Globulin (g/dl)	55.58±0.94	55.55±0.57	14.75±0.42*	13.1±0.57*	8.07±0.31*
ALP (IU/L)	130.7±1.09	128.8±0.66	150.3±1.34*	176.4±0.87*	189.7±0.96*
ALT (IU/L)	90.23±1.05	88.28±0.60	145.6±0.63*	170.7±0.76*	178.4±1.02*
AST (IU/L)	86.32±0.66	71.02±1.11	160.4±0.77*	166.4±0.62*	180.4±0.67*
Urea (mg/dl)	16.73±0.21	15.68±0.46	50.68±0.37*	56.42±0.46*	75.33±0.48*
Creatinine (mg/dl)	434.90±1.26	432.1±1.65	800.5±0.69*	856.10±0.42*	900.7±1.46*
Total Bilirubin (mg/dl)	101.9±0.78	100.20±0.48	120.4±0.57*	138.9±0.35*	145.0±0.33*

Values represented as mean ± SEM; n = 6, (*P =.05)→Significant, DW and EEAB means Distilled water and Ethanolic Fruit Extract of *Adenopus breviflorus* respectively.

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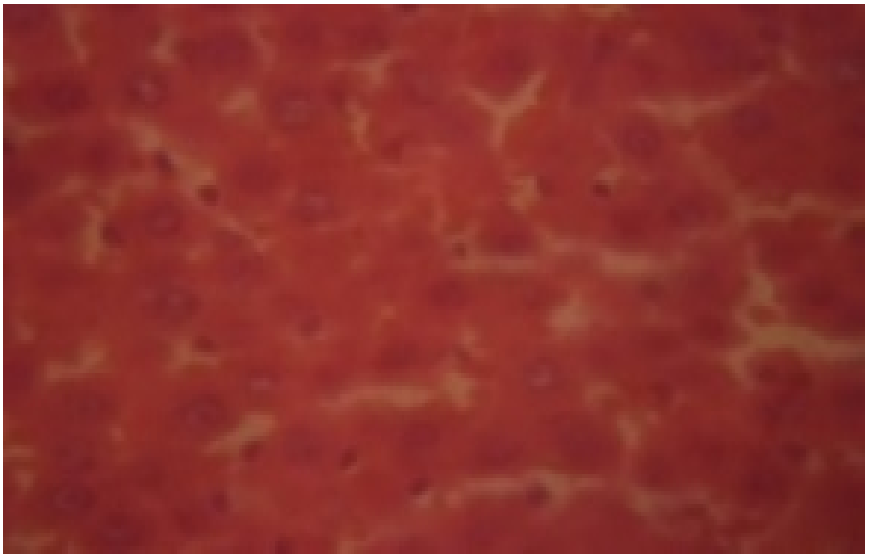

Figure 1. Photomicrograph of the liver of control rat given 2ml distilled water showing normal arrangement of hepatocytes with nuclei, sinusoid, portal triad and central vein (H&E stain, 400x)

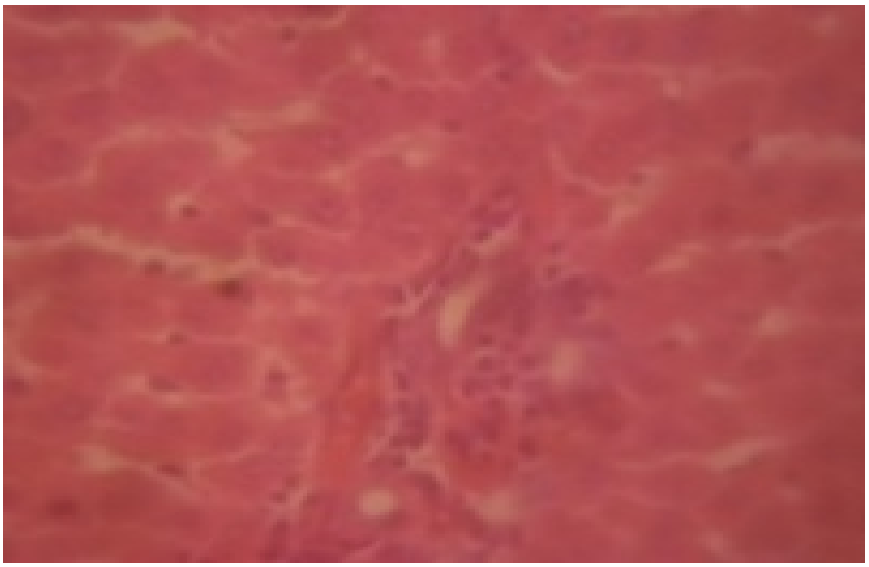

Figure 2. Photomicrograph of the liver of rats administered 500mg/kg body weight of EEAB, showing relatively normal hepatocytes with nuclei, central vein, sinusoid, portal triad and some inflammatory cells scattered all over (H&E stain, 400x)

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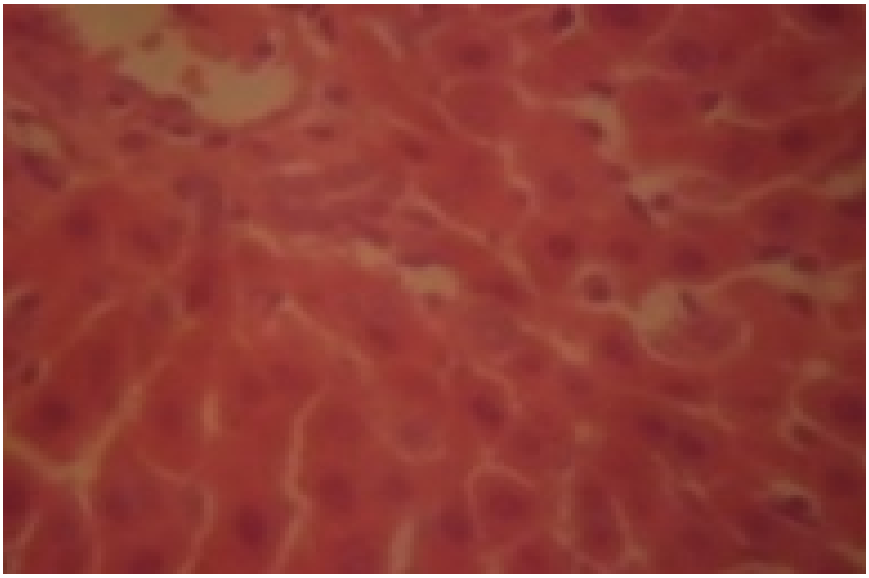

Figure 3. Photomicrograph of the liver of rats administered 1000mg/kg body weight of EEAB, showing shrunken hepatocytes, with pale pykinotic nuclei and slightly congested central vein, the portal triad is sinuded by inflammatory cells. There is loss of intervening sinusoids (H&E stain, 400x)

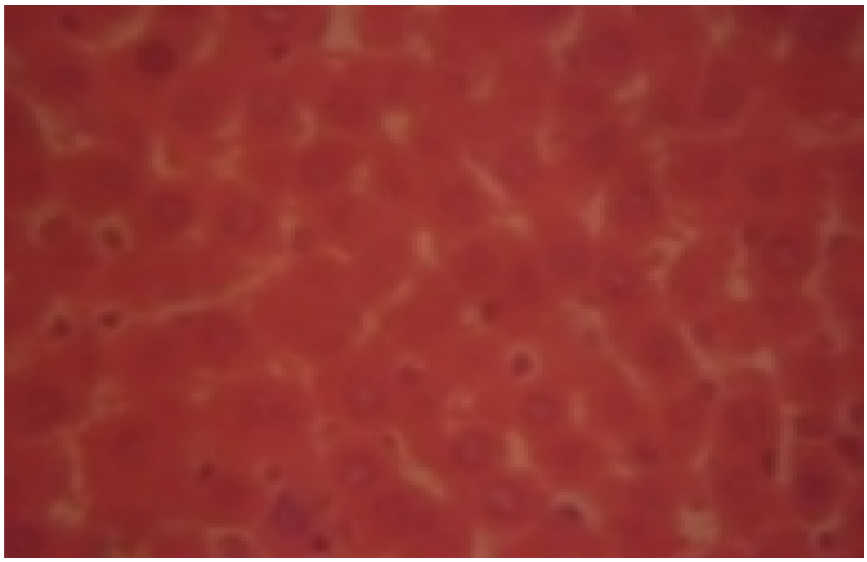

Figure 4. Photomicrograph of the liver of a rat administered 2000mg/kg body weight of EEAB, showing shrunken hepatocytes, congested central vein, the portal triad is sinuded by inflammatory cells, intervening sinusoids and dilation of the central vein (H&E stain, 400x)

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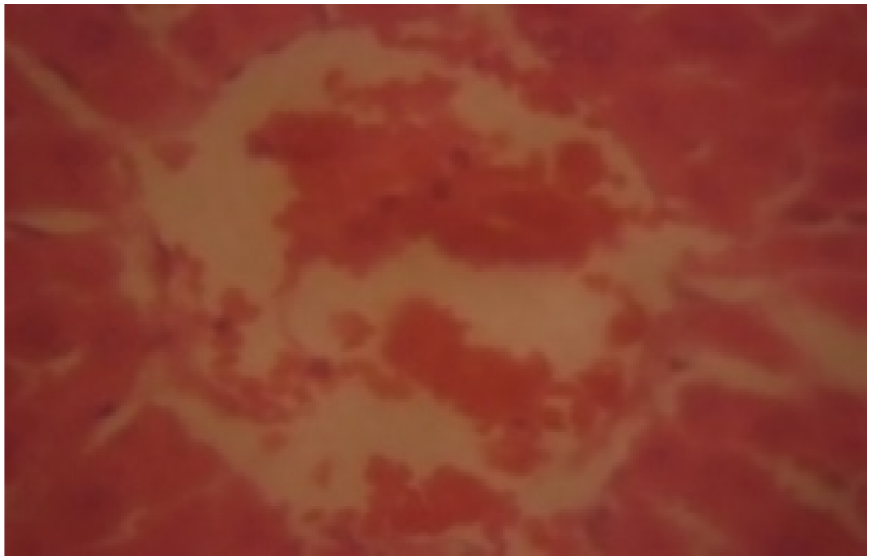

Figure 5. Photomicrograph of the liver of a rat administered 4000mg/kg body weight of EEAB, showing gross dilation of the central vein, loss of normal liver architecture. There is also loss of radial arrangement of hepatocytes, pale pykinotic nuclei, degeneration of the hepatocytes (H&E stain, 400x)