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Effect of Alkaloidal and Aqueous Ethanol Extracts of Roots of *Boscia angustifolia* (Capparidaceae) on Hepatorenal Functions in Albino Rats

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Abstract: The alkaloidal composition, histopathological and toxicity studies of alkaloid and aqueous ethanol extracts of *Boscia angustifolia* on biochemical indices of kidney and liver functions in rats were studied. The amount of alkaloids detected in 50 g powdered root extract of the plant was 11.44% (w/v). Renal and liver indices were significantly ($p<0.05$) altered at higher doses of 703.60, 1125.70 (alkaloidal extract), 839.30 and 1342.80 mg kg^{-1} body weight (aqueous ethanol extract). The aqueous ethanol and alkaloidal root extracts produced histopathological lesions of the liver and kidney at higher doses. These lesions include perivascular cuffs, protein cast, infiltration (kidney) and slight infiltration and perivascular cuffs (liver). There was a significant ($p<0.05$) dose dependent decrease in weight in the rats given higher doses of the aqueous ethanol and alkaloidal root extracts of *Boscia angustifolia*. The use of this plant is associated with some levels of organ toxicity.

Key words: *Boscia angustifolia*, alkaloidal composition, hepatorenal function, histopathological studies

Introduction

Plants and plant extracts have been used since the dawn of civilization by man. The use of ethnobotanical preparations for various reasons justified or not, is still continued by various cultures all around the world (Massond *et al*., 2002). Many medicinal plants used traditionally have been in use by man without the actual knowledge of their toxic potential (s). In Sokoto and other parts of northern Nigeria, one of such plants used is *Boscia angustifolia* for the treatment of bacterial diseases.

Boscia angustifolia (family Capparidaceae) is commonly called a rough-leaved shepherds tree. It is a small tree of savannah to 25ft. high with glabrous branches and fragrant greenish flowers. It is widely spread in northern Nigeria and Sudan (Keay *et al*., 1960). The leaves and roots of the plant are used for the treatment of diarrhoea, pneumonia, boils, wound infection and urinary infection (Keay *et al*., 1960). In Sokoto and other parts of northern Nigeria, the roots extract of *B. angustifolia* is used in the treatment of bacterial diseases. The root extract contains alkaloids and saponins and have been reported to have antibacterial activity (Hassan *et al*., 2006).

The growing interest in herbal therapy demands information on the toxicity risk assessment of *Boscia angustifolia*, which is used tradomedically as an antibacterial plant in northern Nigeria. The main objective of the present study was to provide information on the safety/toxicity risk potential of the roots of *Boscia angustifolia* on the liver and kidney of albino rats. This was determined by assaying the biochemical indicators of liver and kidney functions.

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Materials and Methods

Chemicals

All chemicals used were of analytical grade.

Collection of Plant and Authentication

The roots of *Boscia angustifolia* were obtained from Kara market, Sokoto, Nigeria. It is preferred than other parts of the plant by traditional healers in northern Nigeria for treatment of bacterial diseases. The plant was botanically authenticated at the Herbarium, Botany unit, Usmanu Danfodiyo University, Sokoto, Nigeria. Voucher specimen was deposited in the Herbarium of the same institution. The root obtained was open-air-dried under the shade, pulverized in to a moderately coarse powder (using a wooden pestle and mortar) and stored until required for use (Onoruvwe and Olorunfemi, 1998).

Animals

Male albino rats (Wister strains) weighing 223-263 g were purchased from animal house, Department of Biological science, Usmanu Danfodiyo University, Sokoto, Nigeria. The animals were kept at the animal house of the same department in wire mesh cages and were fed with pellet diet, seasonal vegetables and tap water *Ad libitum* for one week to acclimatize them before starting the experiment.

Preparation of Plant Extract

The dried powdered (40 g) root was extracted with 50% ethanol at room temperature over night and filtered through Whatman No. 1 filter paper. The filtrate was concentrated to dryness in an oven at 40°C and the yield was 9.0% (w/w). The extract was stored in the refrigerator until required for reconstitution in distilled water (for oral administration). This procedure enabled us obliterate the possible contributory effect of the organic solvents.

Extraction of Alkaloids

Fifty gram powdered plant sample (root) was extracted with a liter of methanol: water (1:1 V: V) mixture. It was filtered through Whatman No. 1 filter paper and the filtrate evaporated. The resultant residue was mixed with 200 mL of 0.0025M H_2SO_4 and partitioned with ether to remove unwanted materials. The aqueous fraction was basified with strong NH_3 solution and then extracted with excess chloroform to obtain the alkaloidal fraction or separated by filtration. The chloroform extraction was repeated several times and the bulk extract was concentrated to dryness. The final alkaloid was weighed and the percentage was calculated with reference to the initial weight of the powder (Trease and Evans, 1978; Nuhu *et al*., 2000).

$$\% \text{ Alkaloids} = \frac{\text{Weight of alkaloid residue} \times 100}{\text{Volume taken}}$$

Administration of Extracts

The crude alkaloidal and aqueous ethanol extracts of *Boscia angustifolia* were used for the toxicity test. The rats were divided into two sets of 4 groups of 4 each. In first set, Groups 2, 3 and 4 were orally administered with 1 mL of alkaloidal extract (281.43, 703.60 and 1125.70 mg kg^{-1} body weight, respectively) and the second set, with aqueous-ethanol residue (335.71, 839.30 and 1342.80 mg kg^{-1} body weight, respectively) daily for a period of 28 days. Group 1 in each set served as controls and received only drinking water by the same route. The weight of the animals was taken prior to the administration of the plant extract and after 28 days of the experiment.

Body Weight

The body weights of all the animals before and after 28 days of the extracts administration were recorded.

Blood Samples and Clinical Chemistry

Animals were sacrificed and blood samples were collected, allowed to clot and centrifuged to obtain sera. The biochemical parameters, serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined using Randox assay kit by standard methods of Reitman and Frankel (1957). Alkaline phosphatase (ALP) was estimated by the Randox (colorimetric) method of Rec (1972). The 5^{1}-nucleotidase (5^{1}-NLT) was determined by the methods of Campbell (1962); Harold *et al*. (1980). Total bilirubin (TBL), conjugated bilirubin (CBL) and unconjugated bilirubin (UCBL) were analyzed (Randox kit) using the methods of Jendrassik and Grof (1938); Sherlock (1951). Albumin (Bromocresol green) and urea (Diacetylmonoxime) were done by the methods of Cheesbrough (1991) and Wybenga *et al*. (1971), respectively. Electrolytes, creatinine (colorimetric with deproteinization) were estimated by the methods of Uriyo and Singh (1974) and Henry (1974), respectively. Uric acid was by the method of Collins and Diehl (1959) and Morin and Prox (1973).

Histopathological Assessment

Histopathological examinations were carried out on the liver and kidney of the rats. These were fixed in 10% formalin, dehydrated in gradual ethanol concentrations (50-100%), cleared in xylene and embedded in paraffin. Sections (4-6 μM thick) were prepared and then stained with hematoxylin and eosin (H-E) dye for photomicroscopic observation under light microscope at high (x 400 objective) power magnifications (Wasfi *et al*., 1994; Guntupalli *et al*., 2006) .

Statistics

The data collected in the study was subjected to one way analysis of variance (ANOVA), Benferoni compare all columns using Graph Pad Prism. Values were expressed as mean±standard error.

Results

Alkaloidal Composition

The amount of alkaloids detected in 50 g powdered extract was 11.44 g% (w/v).

Body Weight

There was noticeable significant ($p<0.05$) changes in the body weight of the animals that received higher doses of the alkaloidal and aqueous ethanol extracts of *Boscia angustifolia* compared with control group (Table 1).

Table 1: Mean body weight changes in rats administered alkaloidal and aqueous ethanol root extracts of *Boscia angustifolia*

Concentration (mg kg^{-1})	WBT (g)	WAT (g)
Control (ALK)	231.50±2.38*	238.25±22.0
281.43 (ALK)	225.00±1.83	223.00±1.73
703.57 (ALK)	259.75±2.50*	249.63±1.38
1125.71 (ALK)	235.75±1.20*	226.50±2.08
Control (AQE)	231.50±2.38	238.23±2.22
335.71 (AQE)	238.75±2.63	237.50±3.11
839.29 (AQE)	250.00±2.83*	245.75±2.36
1342.86 (AQE)	262.25±0.96*	253.50±1.12

WBT = Weight of rats before administration of extract, WAT = Weight of rats after administration of extract. ALK = Alkaloidal, AQE = Aqueous ethanol extract. Values are mean±standard deviation, *significantly different ($p<0.05$) compared with weight of rats after (28 days) administration of extract by using the student t-test (n = 4)

Subacute Toxicity (Hepatorenal function)

Some hepatorenal Indices (Table 2 and 3) are significantly different at concentrations of 703.60 and 1125.70 (alkaloidal extract) and 839.30 and 1342.80 mg kg^{-1} body weight (aqueous ethanol extract).

Histopathological Studies

Aqueous ethanol and alkaloidal root extracts produced histopathological lesions of the kidney and liver (Table 4). The lesions are perivascular cuffs and protein cast, infiltration (kidney) and slight infiltration with perivascular cuffs (liver).

Table 2: Serum liver function indices in rats administered alkaloidal and aqueous ethanol root extracts of *Boscia angustifolia*

Conc. (Mg kg^{-1})	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})	5^{1}-NLT (U L^{-1})
Control	5.20±0.40	10.75±1.44	89.70±6.90	22.50±4.75
281.43 (ALK)	6.80±0.77	13.75±0.75	97.35±7.550	27.50±2.50
703.57 (ALK)	15.10±0.87*	30.00±1.00*	524.40±15.95*	65.00±3.54*
1125.71 (ALK)	25.85±0.85*	36.00±5.01*	745.20±27.66*	94.00±8.50*
335.71 (AQE)	6.40±0.66	11.50±0.86	96.60±7.95	25.00±2.89
839.29 (AQE)	13.03±0.84*	29.25±2.78*	379.50±13.21*	60.00±2.60*
1342.86 (AQE)	21.10±0.70*	33.50±2.51*	579.60±27.68*	80.00±5.67*

Conc. (mg kg^{-1})	ALB (g L^{-1})	TB (µmol L^{-1})	CB (µmol L^{-1})	UCB (µmol L^{-1})
Control	1.70±0.10	7.86±0.47	6.20±0.71	2.33±0.16
281.43 (ALK)	1.62±0.03	8.79±0.49	5.54±0.62	3.25±0.64
703.57 (ALK)	0.73±0.03*	12.49±0.47	4.31±0.62	8.18±0.89
1125.71 (ALK	0.56±0.11*	16.65±1.86*	3.69±1.23	12.96±3.09*
335.71 (AQE)	1.44±0.05	8.30±0.54	6.15±0.71	2.18±0.18
839.29 (AQE)	0.86±0.06*	11.10±0.75	3.08±0.62	8.03±0.44
1342.86 (AQE)	0.84±0.06*	15.73±0.93*	3.69±1.23	12.04±0.30*

Values are mean±standard error of the mean, *Significantly different from the control ($p<0.05$) by using the analysis of variance (ANOVA) (n = 4), ALT = Alanine transaminase, AST = aspartate transaminase ALP = alkaline phosphatase, ALB = albumin, 5^{1}-NLT= 5^{1}-nucleotidase, TB = total bilirubin, CB = conjugated bilirubin, UCB = unconjugated bilirubin, ALK = Alkaloidal and AQE = Aqueous Ethanol Extracts

Table 3: Serum kidney function indices in rats administered alkaloidal and aqueous ethanol root extracts of *Boscia angustifolia*

Concentration (mg kg^{-1})	Urea (mmol L^{-1})	Creatinine (µmol L^{-1})	Sodium (ppm)	Potassium (ppm)	Uric acid (µmol L^{-1})
Control	1.43±0.09	132.75±22.55	30.75±0.86	1.58±0.13	235.57±23.78
281.43 (ALK)	1.84±0.17	154.88±22.15	29.00±0.41	1.80±0.04	285.10±12.40
703.57 (ALK)	6.04±0.09*	1991.30±25.55*	17.50±0.29*	4.03±0.03*	768.60±14.30*
1125.71 (ALK)	8.39±0.46*	2168.25±44.38*	15.50±1.50*	5.65±0.15*	1313.98±24.85*
335.71 (AQE)	1.66±0.08	154.90±42.37	30.00±0.71	1.60±0.07	247.96±20.28
839.29 (AQE)	3.97±0.12*	1239.05±11.51*	18.50±0.65*	3.78±0.07*	669.58±14.32*
1342.86 (AQE)	5.07±0.09*	1371.75±44.38*	16.50±0.50*	4.85±0.05*	1115.63±24.87*

Values are mean±standard error of the mean. *Significantly different from the control ($p<0.05$) by using the analysis of variance (ANOVA) (n = 4), ALK = Alkaloidal and AQE = Aqueous Ethanol Extracts

Table 4: Histopathological features of liver and kidney of rats administered alkaloidal and aqueous ethanol root extracts of *Boscia angustifolia*

Concentration (mg kg^{-1})	Kidney	Liver
Control	No visible lesion (normal)	No visible lesion (normal)
281.43 (ALK)	No visible lesion (normal)	No visible lesion (normal)
703.57 (ALK)	Protein cast	Slight infiltration and perivascular cuffs
1125.71 (ALK)	Infiltration	Slight infiltration and perivascular cuffs
335.71 AQE	No visible lesion (normal)	No visible lesion (normal)
839.29 (AQE)	Perivascular cuffs	Slight infiltration and perivascular cuffs
1342.86 (AQE)	Protein cast	Slight infiltration and perivascular cuffs

ALK = Alkaloidal and AQE = Aqueous Ethanol Extracts

Discussion

Many medicinal plants have been used to treat bacterial diseases without risk assessments. Sub-acute toxicity studies of the alkaloidal and crude aqueous ethanol root extracts of *B. angustifolia* were elucidated in small laboratory animals. The alkaloidal composition in the study presents an easy method that can be used for analyzing the composition of alkaloids. The detection of some amount of alkaloids in the plant extract could possibly contribute to the antibacterial activity of *Boscia angustifolia*, since alkaloids and other phenolic compounds have been reported to have antimicrobial activity (Hostettman and Nakanishi, 1979; Okwute and Mann, 1999). However, in the present investigation the doses of the extracts were calculated according to the body weight of the animals, which shows appreciable results in the rats studied in dose dependent manner.

In this study, the alkaloidal and aqueous ethanol root extracts of *Boscia angustifolia* caused significant changes in hepatorenal indices at only concentrations of 703 to 1343 mg kg^{-1} body weight (Table 2 and 3). Thus, this finding did not provide evidence of clinical safety of the plant at higher concentrations as indicated by the results. The significant ($p<0.05$) increases of ALT and AST resulted from possible necrotic injury of the liver and cholestasis (Speech and Liehr 1983; Panteghini *et al*., 1984; Lott and Wolf, 1986). Elevations of ALP and 5'-NLT are indicative of cholestasis (Birkett *et al*., 1986; Van Hoof and De Broe, 1994). The significant ($p<0.05$) increase of serum TBL and UCBL with decrease of albumin indicate defective liver excretory function (Weiss *et al*., 1983; Cheesbrough, 1991) and impaired synthetic function of the liver (Harold *et al*., 1980; Cheesbrough, 1991). The significant ($p<0.05$) marked increase in serum urea, creatinine and uric acid at higher doses of the extracts are indication of damaged renal function (Harold *et al*., 1980; Cheesbrough, 1991) and the significant ($p<0.05$) decrease of sodium and increase of potassium are also signs of renal failure (Cheesbrough, 1991).

Histopathological examinations of the tissue (Fig. 1 to 10) indicated presence of lesions in the kidney and liver of rats treated with higher doses of *Boscia angustifolia* and have justified the significant alterations of the hepatorenal indices. This may not be a surprise since these organs are sites for biotransformation and excretion, respectively. Thus, the 2 organs may have been exposed to the toxic principle present in the extracts. This finding in the present study is in agreement with the observation of Muyibi *et al*. (2000) who noted tubular necrosis and cast in animals fed with *Cassia occidentalis*. On the basis of this and at higher doses of the extracts, it is clear that the plant has no clinical safety. Alkaloids and saponins present in the root extracts may highly be responsible for the altered hepatorenal indices. Some alkaloids have cytotoxic effect on organs; they damage the cells of the liver, lungs, heart and kidney (Harbone, 1972) and saponins cause haemolysis of the red cells (Clark and Myra, 1975). The significant ($p<0.05$) decreases in weight loss after 28 days of extracts

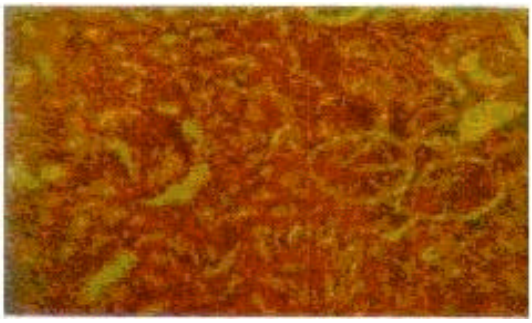

Fig. 1: Kidney photomicrograph section of normal rat (control). Hematoxylin and Eosin (H and E) X 400

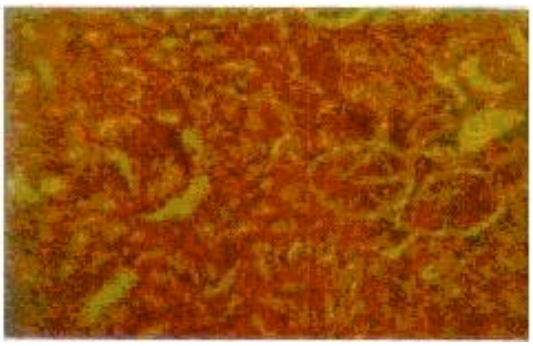

Fig. 2: Kidney photomicrograph section of rat administered alkaloidal (281.43 mg kg^{-1}) and aqueous ethanol (335.71 mg kg^{-1}) root extracts of *Boscia angustifolia*: Showing no visible lesion. Hematoxylin and Eosin (H and E) X 400

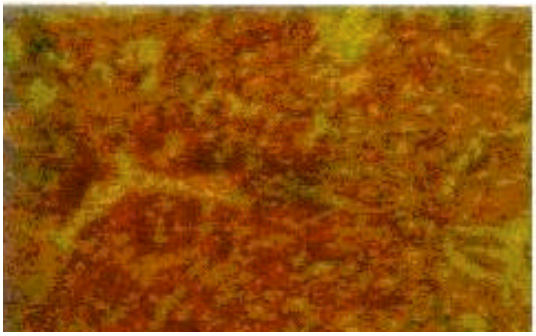

Fig. 3: Kidney photomicrograph section of rat administered (839.29 mg kg^{-1}) aqueous ethanol root extract of *Boscia angustifolia*: Showing perivascular cuffs. Hematoxylin and Eosin (H and E) X 400

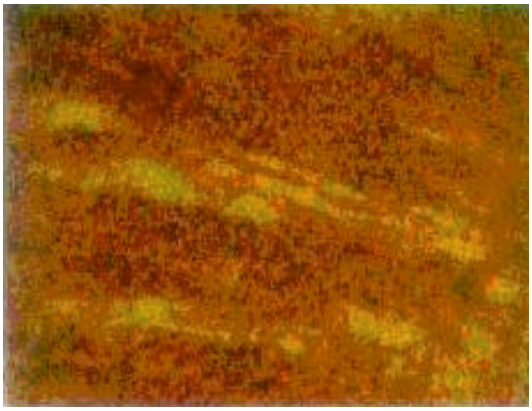

Fig. 4: Kidney photomicrograph section of rat administered (1342.86 mg kg^{-1}) aqueous ethanol root extract of *Boscia angustifolia*: Showing protein cast. Hematoxylin and Eosin (H and E) X 400

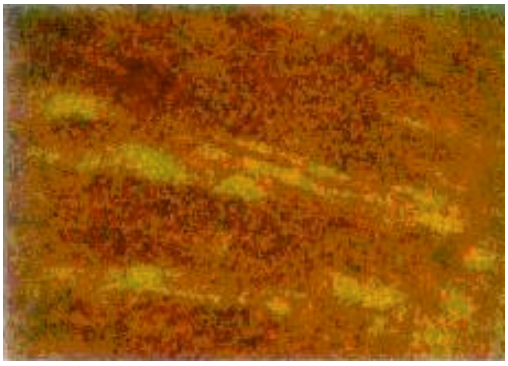

Fig. 5: Kidney photomicrograph section of rat administered (703.57 mg kg^{-1}) alkaloidal root extract of *Boscia angustifolia*: Showing protein cast. Hematoxylin and Eosin (H and E) X 400

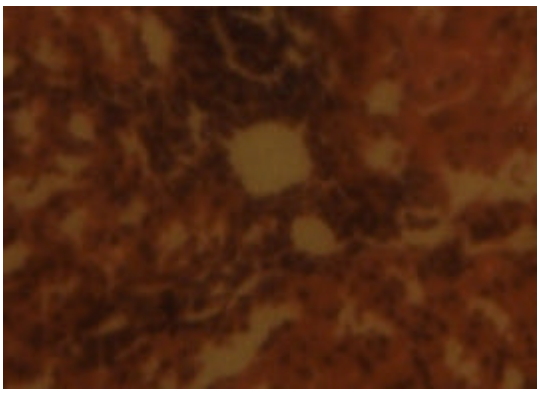

Fig. 6: Kidney photomicrograph section of rat administered (1125.71 mg kg^{-1}) alkaloidal root extract of *Boscia angustifolia*: Showing infiltration. Hematoxylin and Eosin (H and E) X 400

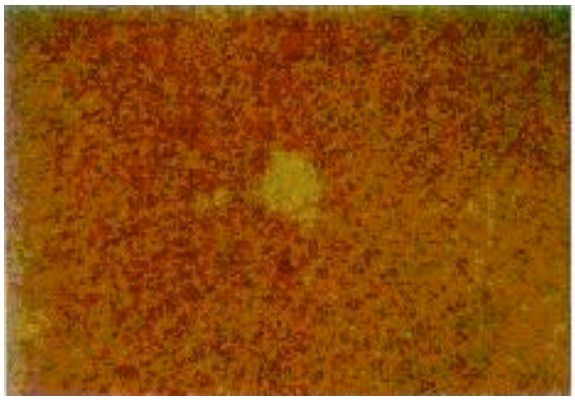

Fig. 7: Liver Photomicrograph section of normal rat (control). Hematoxylin and Eosin (H and E) X 400

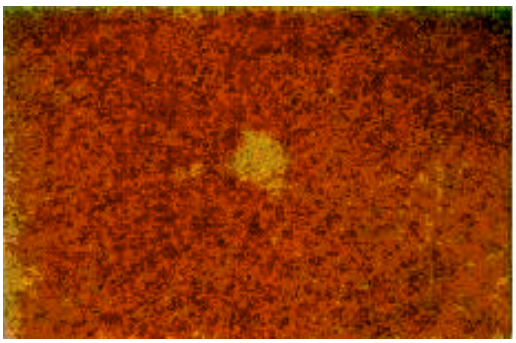

Fig. 8: Liver photomicrograph of section of rat administered alkaloidal (281.43 mg kg^{-1}) and aqueous ethanol (335.71 mg kg^{-1}) root extracts of *Boscia angustifolia*: Showing no visible lesion. Hematoxylin and Eosin (H and E) X400

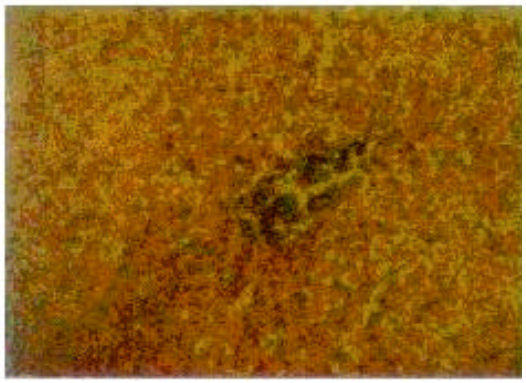

Fig. 9: Liver photomicrograph section of rat administered alkaloidal (703.57 mg kg^{-1}) and aqueous ethanol (839.29 mg kg^{-1}) root extracts of *Boscia angustifolia*: Showing slight infiltration and perivascular cuffs. Hematoxylin and Eosin (H and E) X 400

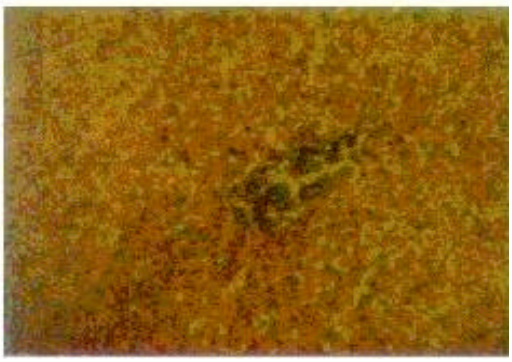

Fig. 10: Liver photomicrograph section of rat administered alkaloidal (1125.71 mg kg^{-1}) and aqueous ethanol (1342.86 mg kg^{-1}) root extracts of *Boscia angustifolia*: Showing slight infiltration and perivascular cuffs. Hematoxylin and Eosin (H and E) X 400

administration compared with control could be due to reduced feed and water intake observed from the animals. The presence of alkaloids and saponins may have caused loss of appetite resulting in decreased weight gain as do extracts of *Cassia occidentalis* containing these compounds (Muyibi *et al*., 2000).

The present research has shown the effect of roots extracts of *Boscia angustifolia* on kidney and liver tissues. The plant must therefore, be used cautiously and in small therapeutic doses, since herbs that have higher toxicity can be used safely and effectively if taken in small doses (Klein, 1996).

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