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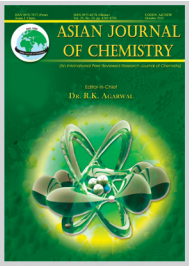

Chemical Constituents, Proximate Composition and Hepatoprotective Activity of *Abutilon muticum*

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Phytochemical studies of the aerial parts of *Abutilon muticum* resulted in the isolation of eight new source compounds, which had not been isolated so far from this investigated source. The compounds isolated were: 3,4',5,6,7-pentahydroxy flavones; 3,3',4',5,7-penta hydroxy flavone-8-O-β-D-glucopyranoside, 3,3',4',5,7-pentahydroxy flavones; stigmasterol, benzoic acid, 1-tricosanol, cholesterol and triacontyl palmitate. Proximate composition of *A. muticum* revealed that the protein content was appreciable (23.5 %) and similar to that of cotton (28.72 %). As far as human nutritional aspects are concerned *A. muticum* had significant mineral contents. Potassium is most abundant (443.01 mg/100 g), followed by calcium (395.23 mg/100 g), phosphorus (327.13 mg/100 g) and magnesium (193.42 mg/100 g). In addition hepatoprotective activity of *A. muticum* was evaluated against hepatic damage in rabbits. The substantially elevated enzyme levels were restored towards normalization significantly by the extracts. The biochemical observations were supplemented with histopathological examination of rabbit liver sections. These findings reveal *A. muticum*, indigenous to Pakistan to be potentially valuable herb for minerals, delivery of drugs and liver diseases.

Key Words: *Abutilon muticum*, Chemical constituent, Minerals, Hepatoprotective, Histopathology.

INTRODUCTION

The genus *Abutilon* of the family Malvaceae consists of 150 species, of broad leaf evergreen plants, mainly distributed in the tropics and subtropics[1]. The specie *Abutilon muticum* Del ex DC (local name; chakrabenda) occurs in plains throughout Pakistan especially more common in Sindh and abundantly in the deserts of Cholistan, Bahawalpur[1]. *A. muticum* is a tomentose under shrub, bears spherical fruit having about 25 carpals, each of which contains 3 tasteless seeds. All parts of the plant are used medicinally[2]. Seeds are used in the treatment of cold, cough bronchial infection, inflammation of urinary tract, gonorrhea, diarrhea and ulcers. The seeds cakes are also used for dairy cattle and as fertilizer[3].

Literature survey revealed that little work had done on seeds of *A. muticum* and no phytochemical work had so far been carried out on other parts of this plant species. This prompted us to carry out investigations on this plant source. The present study describes the isolation and characterization of eight constituents from different fractions of methanolic extracts of the aerial parts of the plant. These compounds were identified by comparison of their physical and spectral data with those reported in the literature. Proximate and mineral analysis plays a crucial role in assessing the nutritional significance of plants/herbs[4]. As various medicinal plant species are also used as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plant species. This paper describes the proximate composition and mineral contents of the aerial parts of *A. muticum*. The isolation of constituents has been correlated with the biological activities.

The liver because of its strategic anatomical location and its large metabolic conversions is exposed to many kinds of xenobiotics and therapeutic agents. Liver injury can be caused by toxic chemicals, drugs and virus infiltration from ingestion of infection. Efforts to find suitable curative agents for the treatment of liver diseases in natural products of plants and minerals origin are being made[5]. Paracetamol a widely used analgesic-antipyretic drug is known to cause hepatotoxicity in man and laboratory animals[6]. Carbon tetrachloride has also been widely used in animal models to investigate chemical toxin induced liver damage[7]. The modern medicines have little to offer for alleviation of hepatic ailments, whereas most important representatives are of phytoconstituents[8]. The survey of literature revealed that some species of the genus Abutilon

found uses in the traditional system of medicine as a liver tonic[9,10]. However hepatoprotective activity of *A. muticum* has not been significantly investigated. Therefore in the present study, the hepatoprotective effect of the extracts of aerial parts of *A. muticum* has been evaluated in paracetamol and carbon tetrachloride induced liver damage in rabbits.

EXPERIMENTAL

Melting points (uncorrected) were determined in glass capillaries using Gallenkemp melting point apparatus. Ultraviolet (UV) spectra were recorded in methanol on Cecil 7200 Model of 7000 series spectrophotometer. Infrared (IR) spectra were measured on Thermo Nicolet, Model: M2000 infrared spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded at 300, 400 and 500 MHz on Bruker AM-300, AM-400 or AMX-500 nuclear magnetic resonance spectrometers using TMS as an internal reference. The ^{13}C NMR spectra were performed on the same instrument at 300 MHz. Low-resolution electron impact mass spectra (EI-MS) were recorded on a Finnigan MAT 312 spectrometer. Column chromatography was performed on silica gel (Si 60, 70-230 mesh, E. Merck), Purity of the samples were checked on precoated silica gel GF-254 (20 cm × 20 cm, 0.5 μm thick) (E. Merck) thin layer chromatography plates. Glass plates of uniform thickness size (20 cm × 20 cm) were coated uniformly with silica gel GF-254 for preparative thin layer chromatography.

The aerial parts (stem, leaves and flowers) of *A. muticum* were collected from Cholistan deserts of district Bahawalpur, Pakistan, in April 2007. The plant was identified and authenticated by Dr. Zaheer-ud-Din Khan, Head, Botany Department, GC University, Lahore, Pakistan. Voucher specimen (*A. muticum*; GC Herb. Bot. 138) was deposited at Dr. Sultan Ahmad Herbarium, GC University, Lahore, Pakistan.

Extraction and isolation: Freshly collected plant materials were cleaned to remove adhering dust and then dried under shade. The dried samples were pulverized in a Willy Mill to 60 mesh size and used for solvent extraction. The shade dried ground plant (20 Kg) was exhaustively extracted with methanol at room temperature. The extract was evaporated to yield the residue (432 g). The whole residue was dissolved in water and partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol, respectively.

The hexane soluble fraction (67 g) was subjected to column chromatography over a silica gel column using *n*-hexane with gradient of $CHCl_3$ up to 100 %. Seven fractions (Fr.1-7) were collected. The fraction 4 was introduced on silica gel and eluted with acetone: *n*-hexane (1:9) to get purified [fraction 6]. The fraction 5 was introduced on silica gel and eluted with $CHCl_3$: *n*-hexane (3:7) to get purified [fraction 8]. The chloroform soluble fraction (113 g) was subjected to column chromatography over a silica gel column using *n*-hexane with gradient of $CHCl_3$ up to 100 % followed by methanol. Six fractions (Fr.1-6) were collected. The fraction 2 was introduced on silica gel and eluted with acetone: *n*-hexane (0.5:9.5) to get purified [fraction 5]. The fraction 4 was introduced on silica gel and eluted with $CHCl_3$: *n*-hexane (3:7) to get purified [fraction 4]. The fraction 5 was introduced on silica gel and eluted with MeOH:$CHCl_3$ (1:9) to get four sub fractions. The sub fraction 5.1 was subjected to preparative TLC (MeOH: $CHCl_3$; 0.5:9.5) to afford [fraction 1]. The ethyl acetate soluble fraction (94 g) was subjected to column chromatography over a silica gel column using *n*-hexane with gradient of $CHCl_3$ up to 100 % followed by methanol. All the nine fractions (Fr. 1-9) were collected. The fraction 5 was introduced on silica gel and eluted with EtOAc: *n*-hexane (3:7) to get purified [fraction 2]. The fraction 7 was introduced on silica gel and eluted with MeOH: $CHCl_3$ (1.5:8.5) to get purified [fraction 3]. The *n*-butanol soluble fraction (47g) was subjected to column chromatography over a silica gel column using $CHCl_3$ with gradient of methanol up to 100 %. Nine fractions (Fr. 1-9) were collected. The fraction 4 was introduced on silica gel and eluted with methanol: $CHCl_3$ (3:7) to afford purified [fraction 7].

Proximate analysis: Proximate analysis was conducted using AOAC methods (1990). Percentage of moisture by vacuum oven (method 934.06), total fat by Soxhlet extractive (method 920.39c), protein by Kjeldahl nitrogen (method 920.152) and ash by direct analysis (method 940.26) were determined according to the AOAC method[11]. The percentage of crude protein was estimated by multiplying the total nitrogen content by a factor[11] of 5.30. Total carbohydrates were calculated by subtracting the total percentage of other components from 100.

Mineral analysis: Ground and dried aerial parts (5 g) were ashed according to the AOAC method 985.35 to obtain ash free from carbon[11]. Minerals were determined using a Unicam 969 AA spectrometer equipped with a GF 90 furnace and FS 90 furnace auto sampler (Unicam limited Cambridge UK). Minerals were quantified on the basis of peak areas and comparison with a calibration curve obtained with corresponding standards.

Hepatoprotective activity

Paracetamol (acetaminophen, 4-hydroxy acetanilide), carbon tetrachloride, silymarin, carboxy methyl cellulose (CMC), standard kits of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate (SGOT), alkaline phosphate (ALKP) and direct bilirubin (D.Bil) were purchased from Aldrich Chemicals Co. (Gill Ingham, Dorset, UK). All other reagents and solvents were of analytical grade and purchased from either Sigma or Merck representatives.

Plant extracts and experimental animals: The fresh aerial parts of *Abutilon muticum* were extracted with 80 % aqueous methanol because at that concentration, the solvent extracts most of the constituents and also inhibit growth of the majority of the microbes[12]. Plant material was soaked thrice for 5, 3 and 2 days at room temperature and filtered. The combined extract was evaporated in vacuum, gave a greenish brown semi solid extract (yield 22.4 %).

Rabbits of either sex (weighing 600-900 g), obtained from PCSIR Laboratories Complex, Lahore Pakistan, were used for the study. The animals were housed in standard conditions with natural light and dark cycle. They were allowed standard laboratory feed and water *ad libitum*. Animals were acclimatized to their environment for one week prior to experimentation. All the experiments were performed in the morning according to current guide lines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals[13].

Acute oral toxicity studies: The extracts were administered to the test groups in graded doses ranging up to 2 g/kg body wt. and the rabbits were observed for signs of toxicity and mortality for 48 h afterwards[14].

Experimental design

Paracetamol induced experimental liver injury: In the paracetamol induced liver injury model, paracetamol (2 g/kg) was administered orally to all animals except the animal of the normal group[15]. Silymarin (100 mg/kg) was used as a reference standard[16]. The animals were segregated into five groups of six each as follows: Group-I: Control group, treated with normal diet daily for 03 days. Group-II: Treated with normal diet daily for 03 days and single dose of paracetamol on day 3. Group-III: Treated with silymarin (100 mg/kg) daily for 03 days followed by paracetamol on day 3. Group-IV: Treated with *A. muticum*, aq. MeOH extract (150 mg/kg) daily for 03 days followed by paracetamol on day 3. Group-V: Treated with *A. muticum* aq. MeOH extract (300 mg/kg) daily for 03 days followed by paracetamol on day 3.

After 48 h of paracetamol administration, blood samples of each animal were collected in sterile centrifuge tubes and allowed to clot. Serum was separated by centrifugation at 2500 rpm for 15 min and biochemical investigations were carried out.

Carbon tetrachloride induced experimental liver injury: Hepatic injury was induced in rabbits by intraperitoneal administration of a single close of 0.5 mL/kg CCl_4[17,18]. Animals were grouped as follows: Group-I: Control group, treated with normal diet three times at 12 h intervals. Group-II: Treat with normal diet three time at 12 h intervals followed by CCl_4 (0.5 mL/kg) after 48 h of last dose of vehicle. Group-III: Treated with silymarin (100 mg/kg) orally three times at 12 h interval followed by CCl_4 (0.5 mL/kg) after 48 h of last dose of silymarin. Group-IV: Treated with *A. muticum* aq. MeOH extract (150 mg/kg) three times at 12 h interval followed by CCl_4 (0.5 mL/kg) after 48 h of last does of extract. Group-V: Treated with *A. muticum* aq. MeOH extract (300 mg/kg) three times at 12 h interval followed by CCl_4 (0.5 mL/kg) after 48 h of last dose of the extract.

After 36 h of CCl_4 treatment blood was collected from all the groups of rabbits. Serum was separated by centrifugation at 2500 rpm at 37 ºC for 15 min and analyzed for various biochemical parameters.

Biochemical determinations: The bio-chemical parameters like serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase[19], alkaline phosphate[20] and direct bilirubin[21] were assayed using standard assay kits.

Histopathological studies: One animal from the treated groups showing maximal activity as indicated by improved bio-chemical parameters from each group were utilized for this purpose. The animals were sacrificed and the abdomen was cut open to removal the liver. The livers were quickly preserved in neutral buffered formalin. Histological liver sections were prepared as described previously by Luna *et al.*[22] 5 mm thick pieces of the liver were fixed in different concentrations of EtOH, then embedded in paraffin and stained, using haematoxylin eosin dye and finally observed under microscope for histopathological changes in liver architecture and their photographs were taken.

Data analysis: Results were expressed as mean ± SEM. Different statistical techniques such as; analysis of variance (ANOVA), Duncan's multiple range method and regression analysis were carried out for analyzing the data obtained from different samples Differences at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Chemical constituents: In the family Malvaceae genus Abutilon occupies key position as it contains plants with diverse applications. To the best of our knowledge (through extensive literature survey) no work has been reported on the chemical constituents of *A. muticum*. Only few researchers have reported the characterization of seeds of this plant species[23]. Eight new source compounds including; 3,4',5,6,7-pentahydroxy flavone [**1**][24], 3,3',4',5,7-pentahydroxy flavone-8-O-β-D-glucopyranoside [**2**][25], 3,3',4',5,7-pentahydroxy flavone [**3**][26], stigmasterol [**4**][27], benzoic acid [**5**][28], 1-tricosanol [**6**][29], cholesterol [**7**][30] and triacontyl palmitate [**8**][31] were isolated for the first time. These compounds were identified by comparison of their spectral data from the corresponding values in the literature or with authentic samples.

Proximate composition: The results of proximate analysis showed variant concentrations/proportions of biochemicals and other content (Table-1). The moisture content of the aerial parts of *A. muticum* was comparatively less as this specie wildly grows in the desserts and dry lands along with temperate regions. Dietary fiber plays an important role in human health. Numerous studies had shown that vegetarian or individuals with high fiber intakes have blood pressure measure very lower than those with low fiber intake. In the present study the fiber content was suggestive that this plant is a good source of dietary fiber. The fats in plant leaves are important as membrane constituents in the chloroplasts and mitochondria. The high amount of crude fat detected may be due to the fact that fats are more extractable with alcohol. There are very large number of different nitrogen containing substances exists in plants as alkaloids, amines, cyanogenic glycosides, purines, pyrimidines and cytokines[32]. *A. muticum* showed average nitrogen percentage (16.13). Therefore, Abutilon species could be recommended as protein supplement. However, their use as food supplement depends on interplay of factors like the digestibility of their nutrients and the presence of anti nutritional factors[33]. These contents still need to be investigated with aerial parts of *A. muticum.*

TABLE-1
PROXIMATE COMPOSITION OF AERIAL PARTS OF *Abutilon muticum*

Component	*A. muticum* (aerial parts)
	Proximate composition (%)
Moisture	8.23
Fat/oil	4.21
Protein	23.46
Fiber	16.13
Ash	17.29
Carbohydrate	32.04

Minerals: Although each mineral has its own health benefits, minerals are generally important as constituents of bones,

teeth, soft tissues, haemoglobin, muscle, blood and nerve cells. Minerals are also vital to overall mental and physical well being[34]. The mineral analysis of the medicinal plant, *A. muticum* showed significant variation among different minerals (Table-2). Potassium was most abundant (443.01 mg/100 g), followed by calcium (395.23 mg/100 g), phosphorus (327.13 mg/100 g) and magnesium (193.42 mg/100 g). The concentration of Zn, which affects human health ranges[35] from 100-500 mg/kg. The Zn level in *A. muticum* was in good agreement with required standard. In case of Pb, the suggested concentration in plant species[36] is 2-6 mg/kg, However, *A. muticum* carries negligible level of Pb, which further clarifies their need as food supplement. With regard to human nutritional aspects; *Abutilon* specie has significant mineral contents.

TABLE-2
MINERAL CONTENTS OF AERIAL PARTS OF *Abutilon muticum*

Minerals	*A. muticum* (aerial parts)
	Concentration (mg/100 g)
Aluminium	5.16
Cadmium	0.02
Calcium	395.23
Iron	3.21
Lead	0.02
Magnesium	193.42
Phosphorous	327.13
Potassium	443.01
Zinc	21.05

Hepatoprotective activity

Biochemical observations: The effects of aqueous methanolic extract of *A. muticum* on serum enzymes were studied and the results are given in Tables 3 and 4. Paracetamol an analgesic and antipyretic is assumed to be safe in low doses. Small doses are eliminated by conjugation followed by excretion but increased doses produce liver necrosis[37]. Paracetamol intoxication in normal rabbits elevated the levels of serum glutamate pyruvate transminase, serum glutamate oxaloacetate, alkaline phosphate and direct bilirubim significantly, indicating acute centrilobular necrosis. The rabbits treated with aqueous methanolic extract of *A. muticum* showed (Table-3) significant reduction in all the biochemical parameters elevated by paracetamol. This reduction in biochemical parameters is similar when compared with that of silymarin.

It is well known that carbon tetrachloride is used as a hepatotoxic agent on various animals[38,39]. Administration of CCl_4 to rabbits produced hepatotoxicity showed by significant increase in the serum levels of serum glutamate oxaloacetate, serum glutamate pyruvate transminase, alkaline phosphate and direct bilirubim in comparison to control group. The aqueous methanolic extract of *A. muticum* showed a significant decrease in the serum enzymes when compared to the CCl_4 compared control groups. Standard drug, silymarin (100 mg/kg) also exhibited similar results. Carbon tetrachloride elevated serum enzyme levels due to its enzymatic activation of CCl_3 free radical, which in turn alters the structure and function of liver cells[40]. Pretreatment with *A. muticum* aq. methanolic extract showed a dose dependant protection against the injurious effect of CCl_4. The reduction in biochemical parameters exhibited by aq. methanolic extracts of *A. muticum* was similar to that of silymarin.

Histopathological observations: The histopathological profile of the rabbits treated with aq methanolic extract showed no visible changes, confirming the safety of the extract at selected dose (Fig. 1). The control group (group I) also showed normal cellular architecture with well brought out central vein, well presented cytoplasm and prominent nucleus (Fig. 2). The liver section of paracetamol treated animals (group-II) showed hepatic cells with severe toxicity characterized by disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis (Fig. 3). Rabbits treated with silymarin and intoxicated with CCl_4 and paracetamol showed less disarrangement and degeneration of hepatocytes, indicating marked regeneration activity (Fig. 4).

Acute toxicity studies: Aqueous methanolic extract of *A. muticum* did not show any sign and symptoms of toxicity and mortality up to 2000 g/kg dose.

Conclusion

Due to the increasing awareness regarding health beneficial effects of plants/herbs many research groups have examined plants for exploiting novel potential sources of natural medicine. In the present study aqueous methanolic extract of *A. muticum*

TABLE-3
EFFECTS OF *A. muticum* EXTRACT ON BIO-CHEMICAL PARAMETERS OF THE RABBITS, INTOXICATED WITH PARACETAMOL

Group	Design of treatment	SGOT (U/L)	SGPT (U/L)	ALKP (U/L)	D. Bil (mg/dL)
I	Control	67.1 ± 3.10	50.46 ± 1.58	96.62 ± 3.39	0.86 ± 0.02
II	Paracetamol	109.61 ± 5.12	160.43 ± 8.60	363.50 ± 6.56	3.27 ± 0.11
III	silymarin + paracetamol	67.85 ± 3.22	46.45 ± 2.34	121.7 ± 3.06	0.53 ± 0.09
IV	*A. muticum* (150 mg/kg) + paracetamol	86.56 ± 5.99	90.56 ± 1.50	190.95 ± 5.91	1.15 ± 0.09
V	*A. muticum* (300 mg/kg) + paracetamol	73.34 ± 2.61	78.72 ± 2.24	170.25 ± 2.94	0.91 ± 0.02

TABLE-4
EFFECT OF *A. muticum* EXTRACTS ON BIOCHEMICAL PARAMETERS OF THE RABBITS, INTOXICATED WITH CCl_4

Group	Design of treatment	SGOT (U/L)	SGPT (U/L)	ALKP (U/L)	D. Bil (mg/dL)
I	Control	61.05 ± 1.85	57.55 ± 2.49	138.30 ± 4.57	0.91 ± 0.03
II	CCl_4	216.04 ± 5.74	116.70 ± 4.00	446.39 ± 6.58	1.47 ± 0.05
III	Silymarin + CCl_4	89.49 ± 3.09	56.26 ± 6.32	139.94 ± 2.35	0.49 ± 0.03
IV	*A. muticum* (150 mg/kg) + CCl_4	115.42 ± 5.46	92.83 ± 2.67	234.47 ± 3.38	1.24 ± 0.15
V	*A. muticum* (300 mg/kg) + CCl_4	97.08 ± 2.99	76.59 ± 3.88	197.34 ± 2.00	0.75 ± 0.03

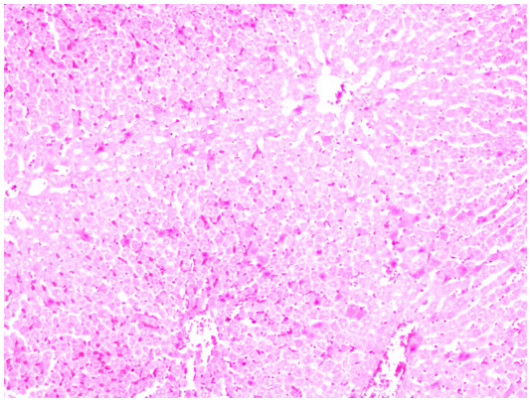

Fig. 1. Liver of rabbit treated with aq methanolic extract of *A. muticum*

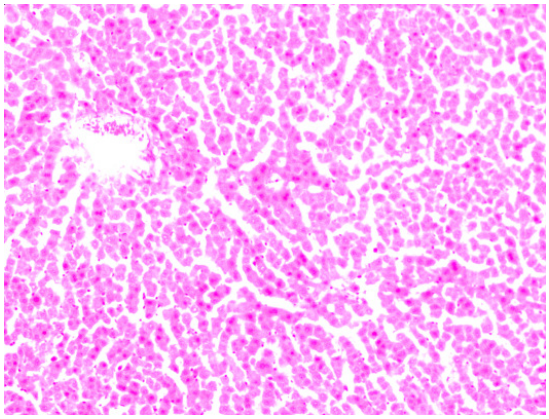

Fig. 2. Liver of rabbit treated with normal diet

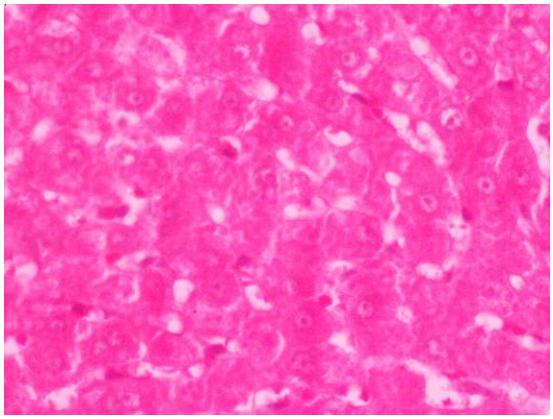

Fig. 3. Liver of rabbit treated with paracetamol

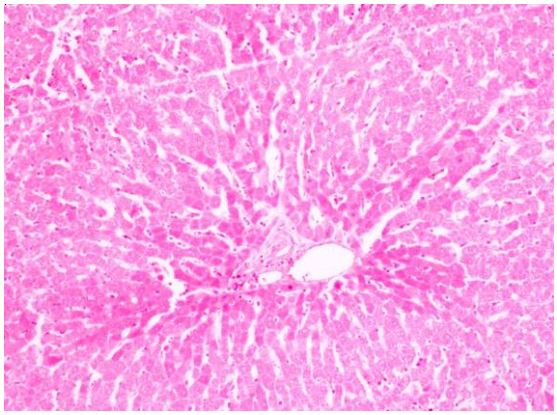

Fig. 4. Liver of rabbit treated with silymarin intoxicated with paracetamol

was evaluated for the chemical composition and hepatoprotective activity using hepatotoxicity induced by paracetamol and CCl_4 in rabbit model and find out therapeutically better efficacious extract. The results indicated that pretreatment with aqueous methanolic extracts could reduce damage produced by paracetamol and CCl_4. Since the phytochemical analysis of the extracts had shown the presence of flavonoids and phenolic compounds, which had been known for their antioxidant and hepatoprotective activities[41], thus it can be concluded that possible mechanism of hepatoprotective activity may be due to the presence of flavonoids and phenolic compounds in the extracts. The aerial parts of the plant have nutritional qualities which when used in right proportions could be of tremendous benefits to the body. The results may therefore, offer a scientific basis for use of *Abutilon* species, in human diet and other commercial products. More works need to be conducted to elucidate viable potentials of *A. muticum* as a prelude to a tangible therapeutic entity.

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