

TOXICOLOGICAL AND BIOCHEMICAL EVALUATION OF ETHANOLIC CRUDE EXTRACT OF *CYPERUS ROTUNDUS*

MANSOOR AHMAD^{1*}, MAHAYROOKH², MEHJABEEN³, ASIF BIN REHMAN⁴, NOOR JAHAN⁵

¹Research Institute of Pharmaceutical Sciences, University of Karachi, ²Department of Pharmacology, Dow University of Health Sciences,

³Department of Pharmacology, Federal Urdu University of Arts, Science & Tech., ⁴Department of Pharmacology, Hamdard University,

⁵Department of Pharmacology, College of Pharmacy, Dow University of Health Sciences Karachi, Pakistan.

Email: herbalist53@yahoo.com, mehjbn1@gmail.com

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ABSTRACT

Toxicity and biochemical study of crude ethanolic extract of *C. rotundus* was carried out in mice and rats. The extract was given at the dose of 10, 100 and 1000 mg/kg. None of the group exhibited any sign of toxicity at these doses. However, at the dose of 1000 mg/kg motor activity is slightly decreased. 10% decrease was observed in weight with treated group (1000mg/kg). The extract of *C. rotundus* was also observed on different biochemical parameters (glucose, lipid profile, cardiac enzymes, liver enzymes and kidney function test). Liver enzymes were found normal and statistically non significant while there is non significant increase in serum bilirubin, gamma-GT and SGPT was observed. Over all hematological studies showed non significant changes. Histopathological examination on different organs of rats also confirmed that the drug is safe and non toxic.

Keywords: *C. rotundus*, Hematological parameters, Histopathology, Toxicity.

INTRODUCTION

Cyperus rotundus (cypereceae) locally called as nagar motha, mainly cultivated in tropical, subtropical and temperate regions [1-2]. It is a grass like herb has tuber or rhizome [3]. Literature revealed the presence of sesquiterpene hydrocarbons in the essential oil of *C. rotundus* plant (isorotundene, cypera-2,4(15)-diene, norrotundene and the oxygenated compound cyperadione), [1-2]. This plant exhibits antipyretic, anti-inflammatory, analgesic and hypotensive effect [4-5]. Volatile oil from tuber has role in menstrual irregularity [6]. It has a potential to inhibit the growth of some fungi and bacteria [3], antihyperglycemic and antioxidant properties [7]. It has a wide range of medicinal uses in different countries [8-10]. In this study, crude ethanolic extract of *C. rotundus* was used for the toxicity and biochemical evaluation.

MATERIALS AND METHODS

Plant material: Crude extract of *Cyperus rotundus* was used in the present study. Different doses of extract (10, 100 and 1000mg/kg) were prepared by dissolving it in distilled water for experimental use. Saline (0.9% NaCl) was used as control [11-12].

Animals

NMRI Mice (20-30g) and Sprague-Dawley rats weighing between 200-225g were obtained from Aga Khan Medical University, Karachi and housed in separate cages for seven days prior to experimentation with free access to food and tap water *ad libitum*.

Toxicological studies in mice

Four groups of NMRI mice (25-30g) containing ten animals in each group (5 males, 5 females) were used in this study. All animals were treated orally once daily for fourteen consecutive days.

- Group I was treated with crude extract of *C. rotundus* (10mg/kg)
- Group II was treated with crude extract of *C. rotundus* (100mg/kg)
- Group III was treated with crude extract of *C. rotundus* (1000mg/kg)
- Group IV was served as control

Animals were weighed daily before the administration dose. All the animals were kept under observation for early two hours after the administration of dose, for any change in behavior or physical activities. Numbers of expired animals were noted at the end of study period [11-13].

Toxicological studies in rats

Three groups of Sprague-Dawley rats weighing (200-225g) were housed for 5 days prior to experimentation with free access to food and water. Each group was containing 24 animals (12 males and 12 females). They were treated orally for fourteen consecutive days and body weight was recorded every day. Group I and II were served as treated and group III served as control. The dose of 100 and 1000mg/kg body weight (dissolved in distilled water) were administered orally in group I and II rats respectively once daily for 14 consecutive days. While the control received equivalent volume of distilled water through the same route [11-13].

AUTOPSY

At the end of 14th day all survived mice and rats were anaesthetized with Pentothal sodium 40mg/kg intra peritoneal and autopsied.

Estimation of Different Biochemical Parameters

At the end of 14th day all survived rats (Group II and III were anaesthetized with Pentothal Sodium 40mg/kg i.p. and the blood samples approximately (4 to 8ml) were withdrawn from cardiac puncture (before dissecting the animals) with sterile disposable syringe and were left at room temperature for 20 minutes. Then incubated at 37°C for 30 minutes and centrifuged separately in (BHG) Hermel 7230 (Germany) at the speed of 3000 rpm for 20 minutes. Supernatant (Serum) were separated out and the residue was discarded.

Serum obtained (1-5ml) was subjected for the study of estimation of Liver Enzymes Bilirubin, SGPT, Gamma Glutamyl Transferase (γ GT), Alkaline Phosphatase (AP), estimation of Cardiac Enzyme (Lactate Dehydrogenase; LDH, Creatinine Kinase; CK), Aspartate Amino Transferase; ASAT), estimation of Kidney Enzymes: Total Protein, Albumin, Urea, Uric Acid, Blood Urea Nitrogen (BUN), Creatinine and estimation of lipid Profile (High density lipoprotein; HDL, Cholesterol, Triglycerides; TG). Estimation of Glucose and Hemoglobin were also carried out. All tests were performed by using commercial assay kits. All these kits were purchased from Diagnostica Merck (Germany), Spectrophotometer U-2000 (Hitachi) was used to measure the absorbance of light [11-13].

Histology

At the end of toxicological studies all vital organs (Heart, liver, spleen and kidneys) were subjected to microscopic examination and compared with control animals. They were fixed in 10% formalin. After usual processes of dehydration, clearing and infiltration, tissues were embedded in paraffin wax and sectioned into 7- μ m slices through Leica RM 2145-Rotation Microtome. The tissues were stained with hematoxylin and

eosin. The slides were studied and photographed through Nikon advance trinocular research microscope OPTIPHOT model X2T-21E equipped with Nikon microphotography system; model UFX-DX-35 and phase contrast N plan [11-14].

Statistical Analysis

Data obtained from results regarding serum biochemical enzymes were compared using student's t test.

RESULTS

Toxicological studies in mice

Toxicology of *C. rotundus* was carried out in mice. None of the group examined showed significant change in body weights and any mortality (Table1).

In mice administration of higher dose of *C. rotundus* (1000mg/kg) caused decreased motor activity, corner sitting, tail erection and palpebral ptosis in early two hours after dosing but all animals return to normal within two hours.

Toxicological studies in rats

Male and female rats treated with *C. rotundus* (100 and 1000mg/kg body weight) did not show any mortality (Table 2).

None of these animals showed any type of toxicity except diarrhea but some physical behavioral changes were observed in first two hours after dosing like uncoordinated motor activity, corner sitting and palpebral ptosis. Non-significant decrease was observed in the weight of control and group I treated rats while 19% decrease was observed in group II rats treated with 1000mg/kg and this decrease is statistically significant (p<0.05) as shown in Figure 1.

Table 1: Toxicological study of *C. rotundus* after 14 day treatment in mice

Dose	Route of Administration	Mortality Symptoms
Control (Saline)	PO	-
Treated 10mg/kg	PO	-
Treated 100mg/kg	PO	-
Treated 1000mg/kg	PO	Decreased motor activity, corner sitting, palpebral ptosis, tail erection

Table 2: Toxicological study of *C. rotundus* (1000mg/kg) after 14 days treatment in rats

Dose	Days	% Change in Weight	Mortality	Toxic effects
Control (Saline)	14	1.78±1.35	-	-
Treated (100mg/kg)	14	1.23±0.28	-	-
Treated (1000mg/kg)	14	19.23±5.28*	-	Decreased motor activity, corner sitting

The value indicate Mean±SEM; *p<0.05

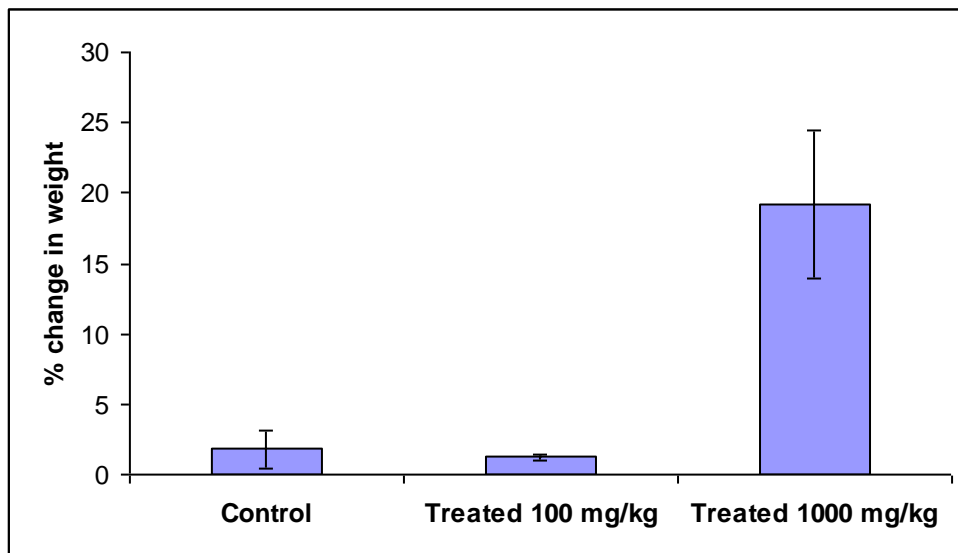


Fig. 1: Shows the effect of *C. rotundus* on body weight

Autopsy

Autopsy revealed that no gross changes were observed in organs like liver, spleen, heart and Kidney. Non significant decrease was observed in weight of heart, kidney, spleen and liver. No gross change was observed in heart and vessels. Drug did not cause any internal body hemorrhage. The shape, color, position and weight of the *C. rotundus* treated rat kidneys are comparable with controls. Kidneys, ureter and urinary bladder are comparable with its control. The shape, color, position and weight of the *C. rotundus* treated rat lungs are comparable with controls. The diameter of trachea is also comparable with its control. The stomach is filled with food. The treated animals were producing too much smell due to severe diarrhea.

Effect of *C. rotundus* on different biochemical parameters

The effect of *C. rotundus* (1000mg/kg) on different parameters was also observed. The alterations in the Glucose, Cholesterol, Alkaline Phosphatase, Bilirubin, Total protein, Triglycerides, SGOT, SGPT, γGT, Creatinine, CK, Urea, BUN, Uric acid, Albumin, HDL, HB and LDH are shown in Table 3.

Effect on liver enzyme

Oral administration of 1000mg/kg dose of *C. rotundus* for 14 consecutive days was not found to alter the liver enzymes significantly. *C. rotundus* produced slight but not significant increase in the serum bilirubin and γGT and SGPT with respect to its control, although this increase was statistically nonsignificant (p>0.05). *C.*

rotundus. Produced slight but not significant decrease as shown in

Table 3 and Figure 2-6.

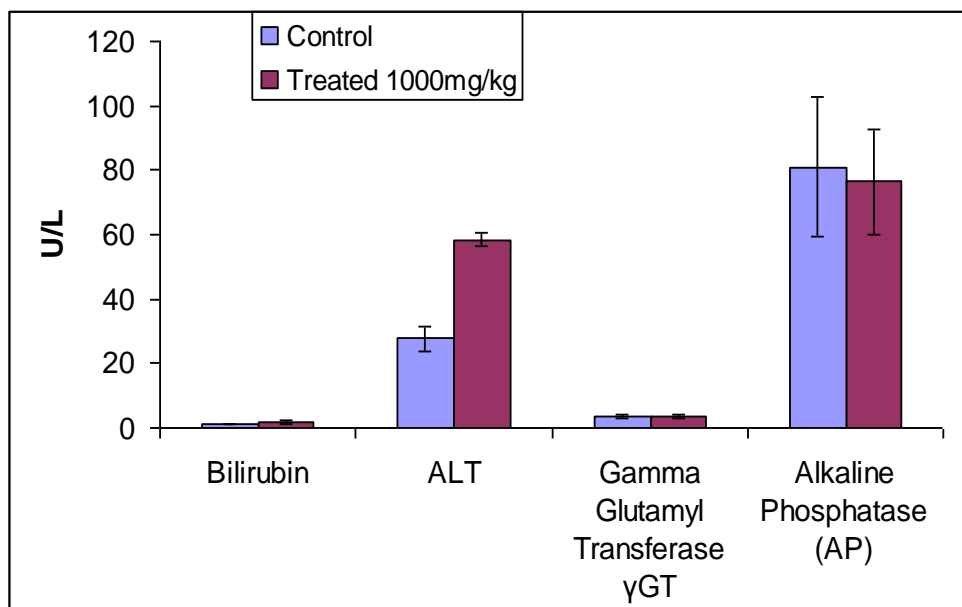


Fig. 2: Effect of *C. rotundus* (1000mg/kg) on liver enzymes

Effect on cardiac enzyme

Oral administration 1000mg/kg *C. rotundus* for 14 consecutive days was not found to alter the cardiac enzyme significantly. It reduces the serum ASAT in comparison to control rats, although this reduction was statistically non-significant ($p < 0.05$). While an increase in serum LDL and CK was observed after the administration of *C. rotundus* for 14 consecutive days. But this increase was also statistically non-significant ($p > 0.05$) in LDH and significant ($p < 0.05$) in CK as shown in Table 3 and Figure 3.

Effect on Kidney

Oral administration of 1000mg/kg dose of *C. rotundus* for 14 consecutive days was not found to alter the kidney function significantly. Statistically non-significant changes in the serum total protein and albumin, Urea and BUN (Figure 4; $p > 0.05$) levels were observed in the *C. rotundus* and treated rats in comparison to control rats. Minor reductions were found statistically non significant ($p > 0.05$). No change was observed in uric acid and

creatinine after the administration of *C. rotundus* for 14 consecutive days as shown in Table 3.

Effect on Lipid Profile

Oral administration of 1000mg/kg dose of *C. rotundus* for 14 consecutive days prevented the elevation of lipid profile significantly. It reduces the serum cholesterol.

SGOT and HDL levels were reduced with respect to its control, although this reduction was statistically non significant ($p > 0.05$). An increase was observed in serum TG and LDH level after the administration of *C. rotundus* for 14 consecutive days as shown in Table 3 and Figure 5. The increase in CK and TG level was statistically non significant ($p < 0.05$).

The effect of *C. rotundus* on the diabetic profile is presented in Figure 6. The results indicate that serum glucose level was decreased non significantly ($p > 0.05$) in the *C. rotundus* treated groups. There was no significant effect on hemoglobin levels. Therefore, administration of *C. rotundus* did not produce Anemia.

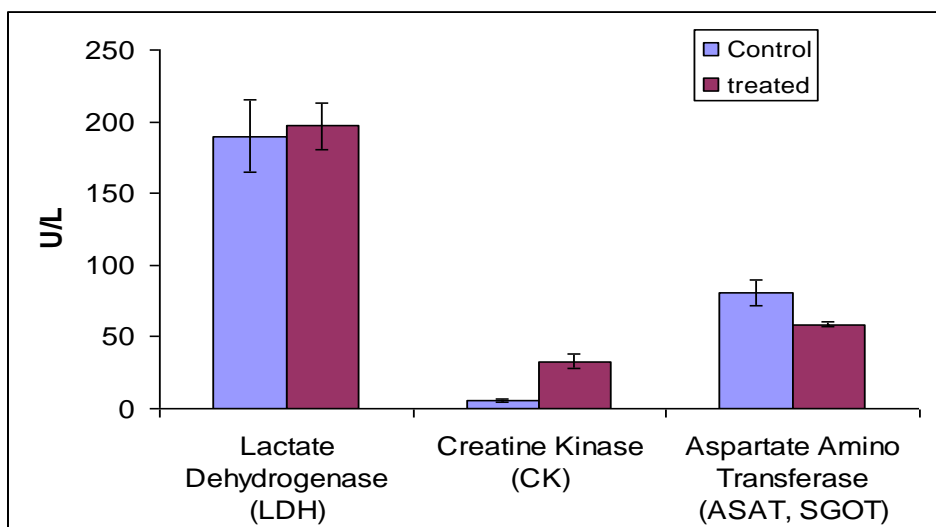


Fig. 3: Effect of *C. rotundus* on cardiac enzymes (1000mg/kg)

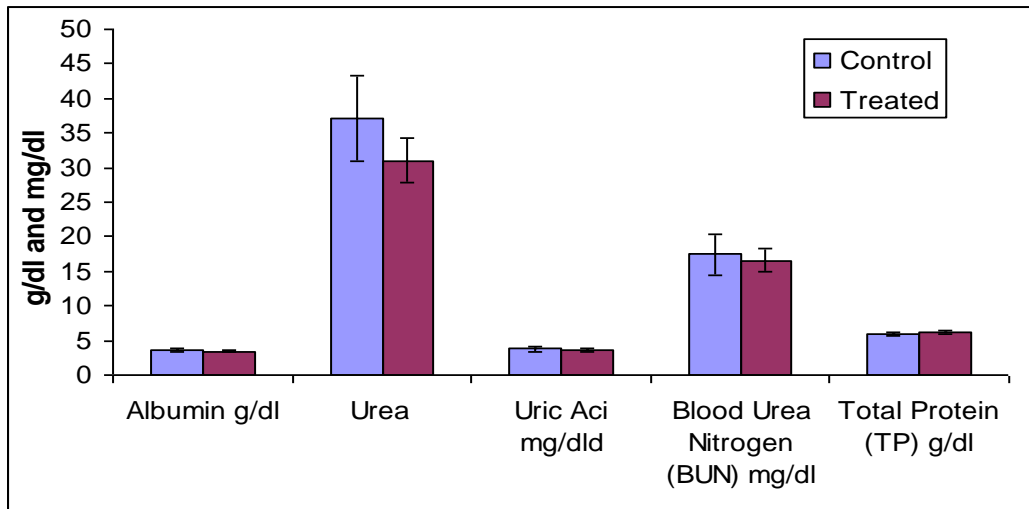


Fig. 4: Effect of *C. rotundus* on kidney functions (1000mg/kg)

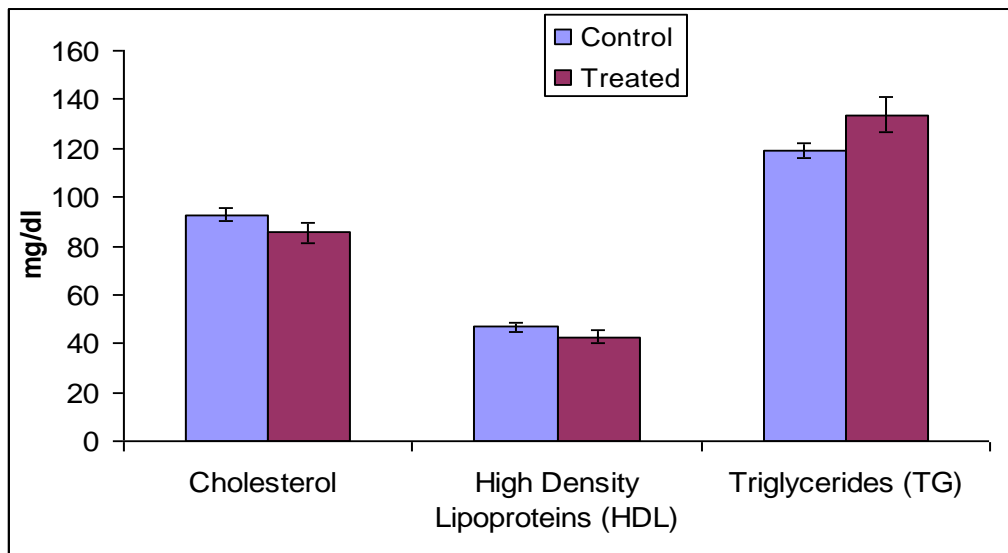


Fig. 5: Effect of *C. rotundus* on Lipid profile

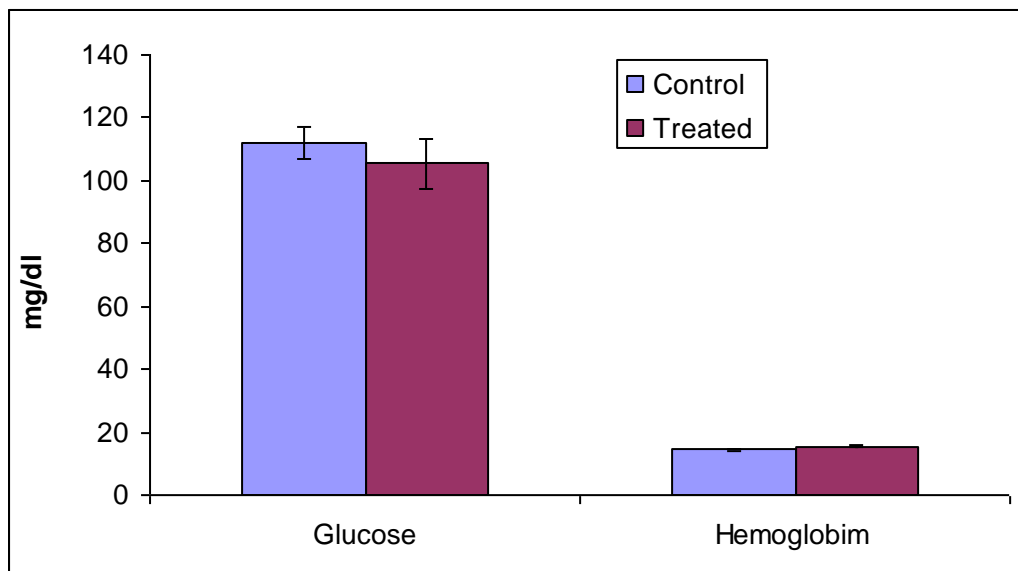


Fig. 6: Effect of *C. rotundus* (1000mg/kg) on Glucose and Hemoglobin level

Table 3: Effect of *C. rotundus* (1000mg/kg) on different biochemical parameters after 14 days of oral administration

Parameters	Control	<i>C. rotundus</i> Treated	p-value
Bilirubin	1.16±0.1912	1.74±0.4512	P>0.05
Alanine aminotransferase (ALAT, SGPT)	27.64±3.6612	32.58±3.3512	P>0.05
Gamma Glutamyl Transferase γ GT	3.35±0.6612	3.72±0.5712	P>0.05
Alkaline Phosphatase (AP)	80.96±21.6312	76.4±16.212	P<0.05
Lactate Dehydrogenase (LDH)	190.23±24.7912	196.99±16.3812	P>0.05
Creatine Kinase (CK)	5.71±1.5512	32.86±4.7612	P<0.05
Aspartate Amino Transferase (ASAT, SGOT)	80.31±9.0512	58.49±1.8512	P<0.05
Total Protein (TP)	6.025±0.2512	6.25±0.2312	P>0.05
Albumin	3.595±0.1912	3.42±0.0912	P>0.05
Urea	37.16±6.2512	30.97±3.1812	P>0.05
Uric Acid	3.815±0.3812	3.66±0.2612	P>0.05
Blood Urea Nitrogen (BUN)	17.45±2.9312	16.56±1.6312	P>0.05
Creatinine	0.875±0.0812	0.77±0.0712	P>0.05
Cholesterol	92.72±2.7612	85.4±3.912	P>0.05
High Density Lipoproteins (HDL)	46.67±1.7412	42.71±2.5912	P>0.05
Triglycerides (TG)	119±2.7912	133.7±7.0912	P<0.01
Glucose	112.04±5.2512	105.38±8.1812	P>0.05
Hemoglobin	14.496±0.4312	15.44±0.20612	P>0.05

Histopathological Examination of Heart

Histopathological examination suggested that cardiac muscle in both control and treated (*C. rotundus*, 1000mg/kg) are normal (Figure 1a and b). They are comparable with each other and they did not show any significant histological abnormality.

Histopathological Examination of Kidney

There was no significant abnormality observed after the administration of *C. rotundus* (1000mg/kg) in kidney (Figure 2a and

b). Epithelial cells of renal corpuscles are arranged normally after the administration of *C. rotundus* (1000mg/kg).

Histopathological Examination of Liver

The liver cells are arranged in to lobules in both control and treated slide. Hepatic sinusoids appear to radiate from central vein. Necrotic lesions were not seen in *C. rotundus* (1000mg/kg) treated animals and hepatocytes were comparable with the control as shown in figure 3a and b.

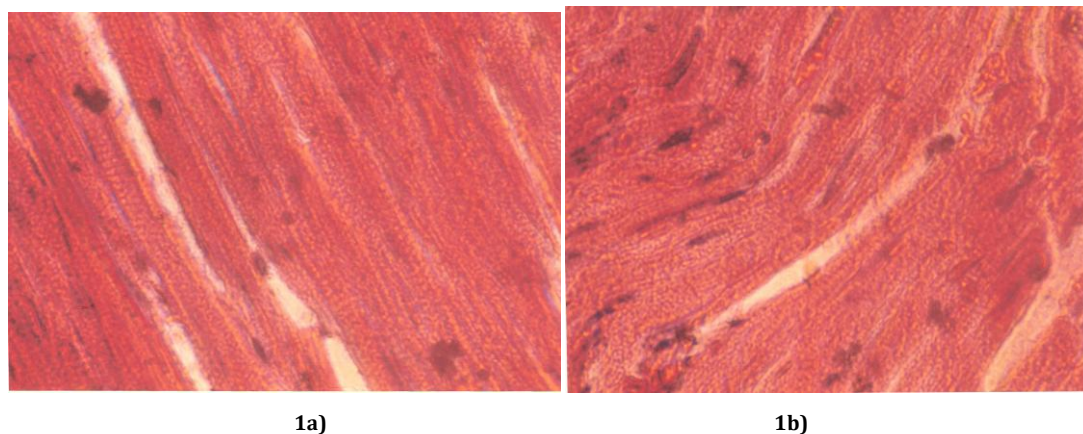


Fig. 1: a) Control Heart and 1b) treated heart (20X) of Rat treated with *C. rotundus*

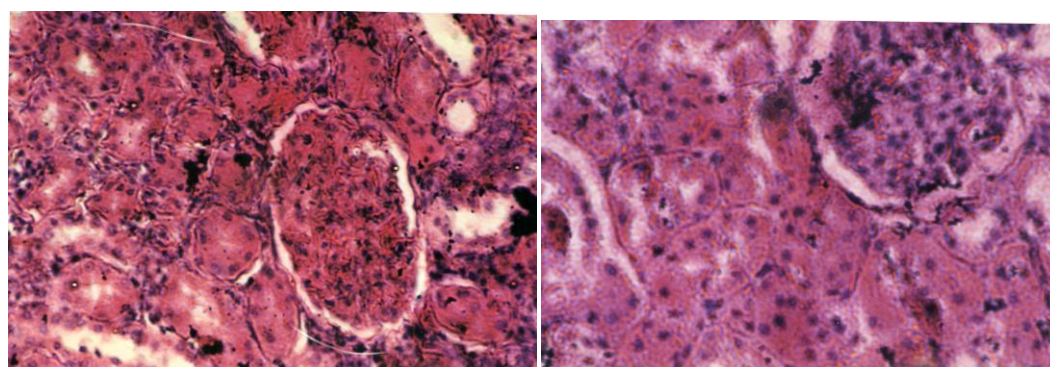


Fig. 2: a) Control kidney and 2b) treated kidney (10X) of Rat treated with *C. rotundus*

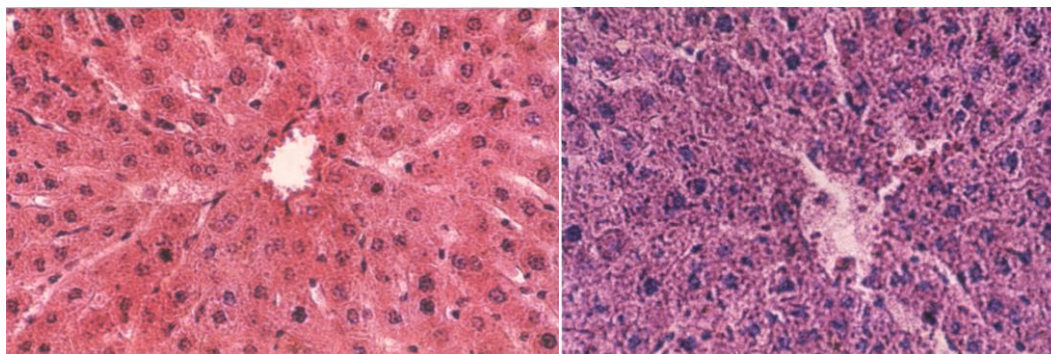


Fig. 3: a) Control liver and 3b) treated liver (20X) of Rat treated with *C. rotundus*

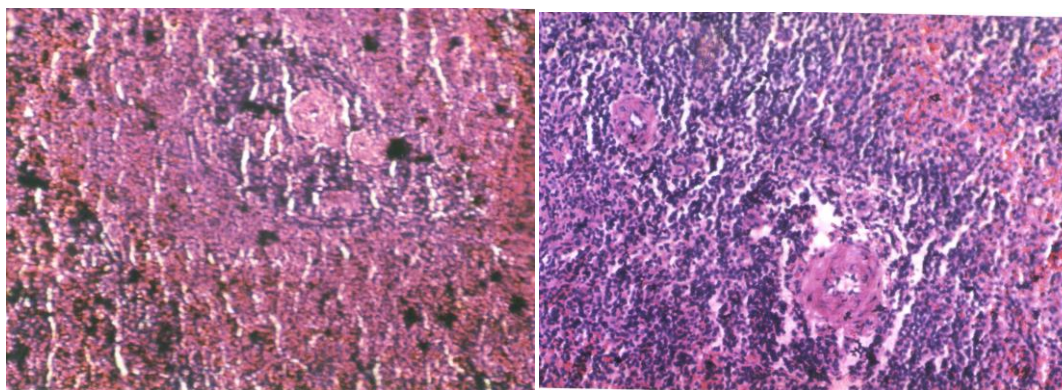


Fig. 4: a) Control spleen and 3b) treated spleen (20X) of Rat treated with *C. rotundus*

Histopathological Examination of Spleen

Spleen is subdivided into red and white pulp. White pulp is arranged as a cylindrical sheath of lymphocytes around a blood vessel known as a central artery as shown in both control and *C. rotundus* treated spleen (Figure 4a and b). Red pulp consists of sinusoids. In control spleen connective tissue septa derived from the capsule penetrate the substance of spleen, conveying blood vessels into the interior of the organ while treated spleen contains an area of germinal center. The marginal zone also present around lymphatic nodules present in *C. rotundus* treated spleen (Figure 4a and b).

DISCUSSION

Toxicology of *C. rotundus* was also carried out in mice. The doses used were 10 mg/kg, 100 mg/kg and 1000 mg/kg. None of the groups examined showed significant change in body weights and mortality. At higher dose 1000 mg/kg, a decreased motor activity, corner sitting, tail erection and palpebral ptosis in early two hours after dosing but animals return to normal within two hours. Toxicity test in male and female rats with 100 and 1000 mg/kg body weight crude extract of *C. rotundus* exhibited no mortality and none of these animals showed any sign of toxicity except diarrhea but physical behavioral changes were observed in the first two hours. After dosing like uncoordinated motor activity, corner sitting and palpebral ptosis. Non-significant decrease was observed in weight of control group I rats while 10% decrease was observed in group II rats treated with 1000 mg/kg and this decrease is statistically significant ($p < 0.05$) as shown in table 1 and 2.

Autopsy revealed that no gross changes were observed in organs like liver, spleen, heart and kidney. No gross changes were observed in heart and vessels. Drug did not cause any internal body hemorrhage. In urinary system that is kidney, ureters and urinary bladder were found comparable with its control. The shape, color, position were found similar to control.

Autopsy of respiratory system in shape, color and position were found comparable with control. The diameter of trachea was also

found same in comparison to control. The stomach was filled with food. The treated animals were producing too much smell due to severe diarrhea. Intestinal movement of treated rats was accelerated.

Histopathological examination of different organs (heart, kidney, liver and spleen) was carried out. After the administration of *C. rotundus* (1000 mg/kg) in animals, no significant results were obtained because histopathological examination showed normal histology and pathology in both control and treated animals.

Akperbekova & Abdullaev (1966) reported the LD_{50} 90.0 gm/kg in mice when ethanol extract of root was administered intraperitoneally [15-16]. Woo et al., (1977) reported that when ethanol extract (defatted with petroleum ether) of dried roots was administered i.p. to mice of both sexes produced $LD_{50} > 0.5$ mg/kg [17]. Dhawan et al., (1910) reported that on administration of ethanol-water (1:1) extract of rhizome i.p. to mice of both sexes produced LD_{50} 681.0 mg/kg [18]. Our toxicological results showed nontoxic effects in general on body, autopsy and histopathology of organs of treated animals (mice). These results confirm the claims that it is a safe and nontoxic drug.

The effects of extract of *C. rotundus* on different biochemical parameters (glucose, cholesterol, alkaline phosphate, bilirubin, total protein, triglycerides, SGOT, SGPT, γ GT, creatinine, CK, urea, BUN, uric acid, albumin, HDL, LDL and Hb) were observed when 14 consecutive days 1000 mg/kg sample was orally administered in rats. Liver enzymes were found normal and statistically non-significant. A non-significant ($p < 0.05$) increase in serum bilirubin, γ -GT and SGPT was also observed with respect to its control. Extract of *C. rotundus* produced slight but non-significant decrease in the serum alkaline phosphate (AP) but this increase was also non-significant.

CONCLUSION

There were no major changes found in biochemical parameters and histological studies, therefore, it may be assumed that this drug is a safe drug and can be used for a prolonged period.

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