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Evaluation of Hepatoprotective and Hepatotoxic Activities of Root Extracts of *Cyperus pertenuis* in Rats

Aamir Mushtaq[1*], Shehla Rehmat[2], Mehwish Jamil[2], Asifa Bashir[2], Qurat-Ul-Ain[2], Habib Anwar[2] and Sarah Rashid[2]

[1]Gulab Devi Institute of Pharmacy, Gulab Devi Educational Complex, Lahore, Pakistan.
[2]Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore, Pakistan.

Authors' contributions

This research article was designed by the combined efforts of all authors. Author AM supervised the project. Authors Shehla Rehmat, MJ, HA, AB and Sarah Rashid did all the experimental work including statistical work. Authors MJ and QUA compiled this research article. All the authors read and approved the final draft of manuscript.

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Original Research Article

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ABSTRACT

Aim of Study: The present study was conducted to assess the role of root extracts of *Cyperus pertenuis* (CP) in its ability to protect liver cells alongside the evaluation of its hepatotoxic effects if any.

Methodology: Dried powdered roots (1kg) of CP were macerated in ethanol (5 L) for five days. The residue obtained after filtration was then macerated in n-hexane (2 L) for 5 days. Both filtrates were concentrated by rotavapor to semi-solid pastes and in-vitro anti-oxidant activity was found by 2,2–Diphenyl–1–Picrylhydrazyl (DPPH) method. 42 rats were placed in seven groups (n=6). Group-I (vehicle control) was administered with 5% Carboxymethyl cellulose (CMC), Group-II (disease control) was given paracetamol (1 g/kg/p.o) for seven days. Group-III (standard) was

**Corresponding author: E-mail: aamir_mushtaq@hotmail.com;*

administered with silymarin (25 mg/kg/p.o) for seven days. Groups IV–VII were given plant extracts in different doses for seven days prior to administration of paracetamol. Blood was collected 18 hours after administration of paracetamol and serum levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) and Total Bilirubin (TB) were assessed. Moreover, toxicity and histopathological studies were also performed.

Results: In disease control group, the levels of ALT, AST, ALP and TB were observed as 174.5±1.8 u/l, 215.7±5.4 u/l, 689.8±9.1 u/l and 1±.06 mg/dl, respectively which were reduced significantly to 146.7±3.3 u/l, 139.5±6.5 u/l, 508.3±3.2 u/l and .81±.04 mg/dl in plant treated group (400 mg/kg/p.o). Necrotic lesions were observed in histopathological slides of disease control group while extract treated liver were free of necrosis and other structural damages. LD_{50} calculated for plant extract was 3162 mg/kg/p.o.

Conclusion: It is thus concluded that *C. pertenuis* root extract is a potent hepatoprotective agent and shows hepatotoxicity only at very high doses.

Keywords: Hepatocytes; Cyperus pertenuis; alkaline phosphatase; total bilirubin; aspartate aminotransferase; histopathology.

1. INTRODUCTION

Hepatic disorders are increasing day by day and it has become a challenge to cope with liver injuries caused by the uninterrupted use of drugs, beverages and particularly alcohol [1,2]. Paracetamol has become the most common drug of everyday use for the management of pain and hyperpyrexia. Although, this is considered the safest drug at normal therapeutic dose but its overdose may lead to severe hepatic necrosis, extrahepatic lesions, nephrotoxicity and even death in human beings [3]. Its overdose produces severe oxidative stress of hepatocytes by generation of NAPQI (n-acetyl-p-benzoquinone imine) [4], lipid peroxidation and overall depletion of glutathiones which consequently, produces irreversible membrane damage and ultimately cell death [5]. Trend is shifting towards the use of natural herbs for the management of hepatic cell damage caused by oxidant substances. The plants having antioxidant activities are usually focused for this purpose. A lot of work has been done on plants such as *Picrorhiza kurroa, Allium sativum, Curcuma longa*, *Tinospora cardiofolia*, *Cichorium intybus*, *Bauhinia racemosa* and *Silybum marianum* [6], for evaluation of hepatoprotective activity in management of drug induced liver damage. Moreover, conventional preparations available in India may contain hundreds of plants which are widely used for antioxidant activities [7]. *Capparis spinosa* [8], *Daccus carota* [9], *Euphorbia antisyphilitica* [10], *Hygrophyla auriculata* [11], *Lycium chinensis* [12], *Rubia cordifolia* [13], *Silybum marianum* and *Zingiber officinale* [9,14], have been extensively studied for their hepatoprotective activities. *Cyperus pertenuis* is a herb that belongs to family Cyperaceae and it has been traditionally used with the combination of others plants in gastrointestinal and liver disorder. The family of Cyperaceae is rich in essential oil containing a mixture of cyperone, cyperene, flavoniods, alkaloids and sesquiterpenes which constitute the bulk of the examined oil. *Cyperus rodundus, Cyperus alopecuroides* and *Cyperus scariosus* are the others plants that have been studied for their antibacterial activity and hepatoprotective actions. Major constituents of these plants are same. In the past, it has been reported that phenolic and flavonoid contents have anti bacterial, antioxidant, anticancer, anti inflammatory and anti diabetic activities [15,16]. This plant is first time evaluated for its hepatoprotectivity. The objective of present study was to evaluate hepatoprotective action of *Cyperus pertenuis* dried roots in albino rats along with its marked effect on hepatocytes in higher doses.

2. MATERIALS AND METHODS

2.1 Equipments

Digital electronic balance (FA2004B,Yoke Galvano), Centrifuge machine (Pro-economic), Vortex Mixer (VM-300), Grinder (Waves, Pakistan), Merck Microlab (Merck, Germany), Microwave oven (DHG-9053A), Microscope, Spectrophotometer (UV1900, Yoke Galvano), Dissection kit, syringes, Measuring cylinder and Beakers.

2.2 Drugs and Chemicals

Diagnostic kits of ALT, AST, ALP and TB (Human-Germany), ethanol, formalin, n-hexane, methanol, sodium bicarbonate, xylene, Paraffin, hematoxylin and eosin dye are of analytical grade (Merck, Germany) and purchased from licensed supplier. Paracetamol was donated by Consolidated Chemical Laboratories Pvt Ltd. Silymarin was purchased from Abbott Laboratories, Pakistan.

2.3 Collection of Plant

The roots of *Cyperus pertenuis* was purchased from local market of Lahore and identified by Department of Botany, University of the Punjab, Lahore. A specimen of plant was preserved in herbarium of Riphah Institute of Pharmaceutical Sciences Lahore which was given voucher no RIU-02-07-33.

2.4 Extracts Preparation

Dried roots were ground into fine powder and accurately weighed. The dried roots powder (1000 g) was extracted using maceration method in ethanol (97% w/v, 5 L) for five days. Mixture was filtered by muslin cloth and then by Whatman filters paper no. 1. The filtrate was subjected to rotary evaporator to recover the solvent. The residue was macerated in n-hexane (2 L) for five days and semi solid extract was obtained after evaporating the solvent by rotary evaporator. The percentage yield (w/w) of n-hexane and ethanolic extracts of *Cyperus pertenuis* was calculated by using following formula. Percentage yield = weight of extract / total weight of powdered material × 100.

2.5 Assay of Free Radical Scavenging Activity by DPPH Method

The free radical scavenging activity of different concentrations of standard ascorbic acid and crude extracts of *Cyperus pertenuis* roots were assayed for the free radical scavenging activity by using DPPH radical scavenging method. The various crude extracts or standard ascorbic acid solutions (1 ml each) at different concentrations (20, 40, 80, 160, 320 and 640 µg/ml) were taken in separate test tubes. Two milliliters of 1.0 mmol/L DPPH radical solution, prepared in methanol, was added to each test tube. The solution was mixed rapidly on vortex and allowed to stand in dark at 37 ℃ for 30 min. In the same way blank was prepared without extract. By using UV-Vis spectrophotometer, the decrease in absorbance of each solution was measured at 517 nm. The percentage of radical scavenging activity of tested extracts was calculated by using the following formula: [17]

$$\text{Free radical scavenging activity (\%)} = [Ac-As]/A_c \times 100$$

Where Ac=Absorbance of control at 517 nm and As=Absorbance of the sample.

2.6 Experimental Animals

Albino rats weighing about 150–180 g were used in this study, which was sourced by the University of Veterinary and Animal Sciences (UVAS) Lahore. The animals were kept in animal house of Riphah Institute of pharmaceutical Sciences (RIPS) where they were provided with standard condition of temperature (25 ^{o}C), humidity (50%) and 12/12 h of light and dark cycles and fed with standard pellet diet and water. They were housed individually in cages containing sterile paddy husk throughout the experiment and had free access to sterile food and water. The study was started after getting approval for the usage of animals from departmental research and ethical committee with protocol no RIU–ACE–117.

2.7 Evaluation of Hepatoprotective Activity

Animals used in this study were divided randomly into seven groups. Each group comprised of six rats. Blood samples (2 ml each) of all rats were collected by cardiac puncture for baseline analysis of liver biomarkers (ALT, AST, ALP & total bilirubin) in serum. Carboxymethyl cellulose (0.5 g) was dissolved in 1000 ml of distilled water. Suspensions of both extracts i.e (n–hexane and ethanolic extract) and paracetamol were prepared in 0.5% CMC. One way ANOVA was applied to reveal the significant difference in baseline mean serum level of ALT, AST, ALP & total bilirubin among all groups. Group-I was marked as vehicle control group and orally administered with 0.5% CMC (1 ml/100 g) once daily for 7 days. Group-II was marked as diseased group and was administered only with paracetamol suspension (1 g/kg/p.o) at 7th day. Group-III was standard control group administered with silymarin (25 mg/kg/p.o.) once daily for seven days. Groups-IV and V were administered with ethanolic extract suspension of

C. pertenuis (200 and 400 mg/kg/orally, respectively, once daily for 7 days. Groups-VI and VII were administered with n-hexane extract suspension of *C. pertenuis* (200 and 400 mg/kg/orally, respectively, once daily for 7 days. Paracetamol suspension was administered orally at the dose of 1 g/kg/rat on the seventh day to all above groups except group-1. After 18th hours of last dose animals were anesthetized by chloroform. Blood was collected by cardiac puncture and Serum was separated by centrifugation at 2500 rpm at 30℃ for 15 min and utilized for the estimation of various biochemical parameters [18].

2.8 Effect of Extracts on Body Weight

The effect of *C. pertenuis* extract on mean body weight of experimental animals of acute model was determined by finding weights of animals at the start of study and at day seventh.

2.9 Toxicity Study (LD_{50})

Study of acute toxicity of plant extract was performed according to guidelines of Organization for Economic Cooperation and Development (OECD 423, 2001). The study was conducted on 24 rats each weighing 250 g approximately. Rats were deprived of food and only water was supplied to them. Vehicle group was only administered with CMC 0.5% suspension. Rats in experimental groups 1-3 were treated with single oral dose of ethanolic extracts (1000, 2000 and 5000 mg/Kg, respectively). Experimental animals were also observed for behavioral changes and signs of toxicity like convulsions, twitching, rigidity, sedation, jumping, loss of lightening reflex, ataxia and mortality on daily basis. The LD_{50} of *Cyperus pertenuis* was calculated by using following formula.

$$LD_{50} = \sqrt{Do \times D100}$$

Where D_0 = Dosage of 0% mortality
D100 = Dosage of 100% mortality

The animals which survived were sacrificed for examination of signs of toxicity and sera were collected for evaluation of ALT, AST, ALP and TB values after observing their behavior for 12 days.

2.10 Histopathological Slides Preparation and Grading

The process for slide preparation was as followed;

- Accessing of specimens
- Fixing the tissues
- Dehydration
- Clearing from fixing solution and water
- Impregnation
- Embedding of tissues
- Sectioning of tissues
- Staining of slide

Liver specimens were generally graded into four categories [19,20] which were periportal necrosis, intralobular necrosis, portal inflammation and fibrosis. Scoring is described in Table as follow:

Table 1. HAI (Histology activity index) for grading of slides

Indication	Stage	Score	Omitted number
Normal	0	0	0
Periportal ±Bridging necrosis	Mild	1	2,7,8,9
	Moderate	3	
	Marked	4,5,6,10	
Portal inflammation	Mild	1	2
	Moderate	3	
	Marked	4	
Fibrosis	Mild	1	2
	Moderate	3	
	Marked	4	
Focal necrosis and	Mild	1	2
Intralobular degeneration	Moderate	3	
	Marked	4	

2.11 Statistical Analysis

All results were expressed by Mean ± S.D and were statistically analyzed by using one way/two way analysis of variance (ANOVA) test. Value of $P < .05$ was considered significant.

3. RESULTS

3.1 Percentage Yield, Solubility and Antioxidant Activity

The percentage yield of ethanolic extract of dried roots of *Cyperus pertenuis* was 2.4% while for n-hexane extract it was 0.45%. Results of solubility and antioxidant activity by DPPH methods are shown in Tables 2 and 3 respectively.

3.2 Evaluation of Hepatoprotective Activity

The present study was based on evaluation of the hepatoprotective activity of ethanolic and n-hexane extracts of *Cyperus pertenuis* roots against paracetamol induced hepatotoxicity in albino rats (n=6) at two dose levels 200 mg/kg and 400 mg/kg body weight. The sera of all rats were assayed for the level of biological markers of liver such as ALT, AST, ALP and total bilirubin. The results have been illustrated in Table 4. It was found that serum level of ALT, AST, ALP and total bilirubin of Vehicle group was 109.3±2.8 u/l, 110.5±3.0 u/l, 226.7±4.6 u/l and 0.60±0.04 mg/dl, respectively. Disease group (n=6) showed significant increase ($p < .05$) in serum level of ALT up to 174.5±1.8 u/l, AST up to 215.7±5.4 u/l, ALP up to 689.8±9.1 u/l and total bilirubin up to 1.0±0.06 mg/dl as compared to vehicle and standard groups. The Standard group (n=6) showed significant decrease ($p < .05$) in serum level of ALT up to 136.7±1.8 u/l, AST up to132±3.1 u/l, ALP up to 346.5±12.4 u/l and total bilirubin up to 0.61±0.01 mg/dl as compared to the disease group. Significant decrease in serum level of ALT, AST, ALP and total bilirubin was also observed in rats treated with extract in 200 mg/kg as compared to 400 mg/kg of extracts dose. Treatment of rats with ethanolic extract at 400 mg/kg/p.o. an hour prior to paracetamol resulted in significant decrease ($p < .001$) in serum level of ALT (139.5±4.2) u/l, AST (160±3.7) u/l, ALP (534.3±3.9) u/l and total bilirubin (0.80±0.03) mg/dl as compared to disease group. The dose of 200 mg/kg administered an hour prior to paracetamol decreased serum level of ALT up to 152.7±4.7 an AST up to 144.7±3.4 u/l, ALP up to 541.7±3.8 u/l and total bilirubin level up to 0.71±0.04 mg/dl respectively. In experimental groups C & D ALT, AST, ALP and TB were 154.2±2.5 u/l, 156.2±4.5 u/l, 528.3±5.5 u/l, 0.70±0.05 mg/dl and 146.7±3.3 u/l, 139.5±6.5 u/l, 508.3±3.2 u/l, 0.81±0.04 mg/dl, respectively.

C. pertenuis at dose of 400 mg/kg was found to be more effective than CP extract at dose of 200 mg/kg in reducing serum level of ALT, AST, ALP and TB.

Table 2. Solubility testing of *Cyperus pertenuis* extracts

Solvents	Ethanolic extract 1:1 w/v	n-hexane extract 1:1 w/v
Water	Insoluble	Insoluble
Normal saline	Insoluble	Insoluble
Ethanol	Soluble	Soluble
n-hexane	Insoluble	Soluble
Ethyl acetate	Slightly soluble	Soluble
DMSO	Soluble	Soluble
Chloroform	Soluble	Soluble

Table 3. Percentage inhibition of DPPH free radical essay by crude extracts of *Cyperus pertenuis*

Concentration (µg/mL)	Ascorbic acid	n-hexane extract of *C. pertenuis*	Ethanolic extract of *C. pertenuis*
20	85.0±0.17	83±0.80	82±1.05
40	87.3±1.02	85±1.11	83±0.76
80	89.0±0.53	88±0.42	84±0.59
160	93.0±1.14	89±0.63	87±0.81
320	94.8±0.06	90±0.81	88±0.48
640	95.2±0.73	92±0.68	89±0.19

3.3 Histopathological Grading

Histopathological findings were expressed in the term of histology activity index (HAI) of Knodell et al. [20] which is numerical scoring system for assessment of histological activity in asymptomatic chronic active hepatitis as described in Table 5. Histopathological scoring of liver transverse sections of vehicle group showed intact liver architecture with normal hepatocytes and well defined nucleus with mean HAI=0 (Fig. 2A). In contrast, disease group treated with paracetamol showed moderate to marked periportal necrosis, moderate periportal and bridging necrosis, mild to moderate hepatocytes degeneration, mild fatty changes and moderate portal inflammation with mean HAI=8.4 (Fig. 2B). Histopathology of Standard Group, treated with silymarin (25 mg/kg) along with paracetamol, showed intact liver architect with normal hepatocytes and well defined nucleus with mean HAI=0 (Fig. 2C). On histological examination, the transverse sections of liver specimens of rats treated with *Cyperus pertenuis* extracts (ethanolic & n-hexane) at 200 mg/kg along with Paracetamol (on seventh day) showed moderate to marked periportal necrosis, mild degeneration of hepatocytes and fatty changes with mean HAI=4.4 (Fig. 2D and 2E) respectively. No pathological lesions were observed in transverse sections of liver biopsy specimens of rats treated with *Cyperus pertenuis* extracts (ethanolic & n-hexane) at dose of 400 mg/kg i.e. hepatocytes with well-defined nucleus were seen in transverse sections of liver biopsy specimens of Experimental groups B & D with mean HAI=0 (Fig. 2F and 2G) respectively.

3.4 Effect of *Cyperrus pertenuis* Extracts on Body Weight

Mean weights of animals before and after treatment periods are shown in Table 6 which indicates that weight of disease group is reduced after treatment periods while all other groups were significantly increased as shown in Table 6.

Table 4. Evaluation of hepatoprotective activity

Groups	Serum ALT (u/l)	Serum AST (u/l)	Serum ALP (u/l)	Serum TB (mg/dl)
Vehicle group	109.3±2.8	110.5±3.0	226.7±4.6	0.60±0. 04
Disease group	174.5±1.8[a]	215.7±5.4[a]	689.8±9.1[a]	1.0±0.06[a]
Standard group	136.7±1.8	132±3.1	346.5±12.4	0.61±0.01
Experimental group A	152.7±4.7[***]	144.7±3.4[***]	541.7±3.8[***]	0.71±0.04[***]
Experimental group B	139.5±4.2[***]	160±3.7[***]	534.3±3.9[***]	0.80±0.03[***]
Experimental group C	154.2±2.5[***]	156.2±4.5[***]	528.3±5.5[***]	0.70±0.05[***]
Experimental group D	146.7±3.3[***]	139.5±6.5[***]	508.3±3.2[***]	0.81±0.04[***]

*All values were expressed as Mean ± S.D. and two ways ANOVA was applied. Superscript "[a]" indicated significantly different from vehicle group with P < .05. Superscript "***" indicated significantly different from disease group with P < .001*

Table 5. Histological scoring of transverse sections of liver specimens [20] in acute toxicity model

Groups (n=6)	Histopathological lesions				
	Periportal+/- bridging necrosis	Interlobular degeneration & focal necrosis	Portal inflammation	Fibrosis	HAI
Vehicle group	0	0	0	0	0
Disease group	4	1.4	3	0	8.4
Standard group	0	0	0	0	0
Experimental group A	3.4	1	0	0	4.4
Experimental group B	0	0	0	0	0
Experimental group C	3.4	1	0	0	4.4
Experimental group D	0	0	0	0	0

Table 6. Effect of *Cyperrus pertenuis* extracts on body weight of experimental animals of acute toxicity model

Groups	1st day weight (g)	7th day weight (g)
Vehicle group	152.2±1.384	161.7±1.563[a]
Disease group	153.3±2.789	148.2±2.247[**]
Standard group	154.3±3.007	163.5±2.754[a]
Experimental group A	159.5±2.872	167.7±2.906[a]
Experimental group B	154.8±2.949	164.2±2.798[a]
Experimental group C	157.3± 2.108	166.0±2.113[a]
Experimental group D	158.3±2.836	167.0±3.077[a]

All values were expressed as Mean ± S.D and Superscript "[a]" represents insignificant change in mean body weight compared to first day at P < .05. Superscript "[**]"shows significant change in mean body weight compared first day where P < .01

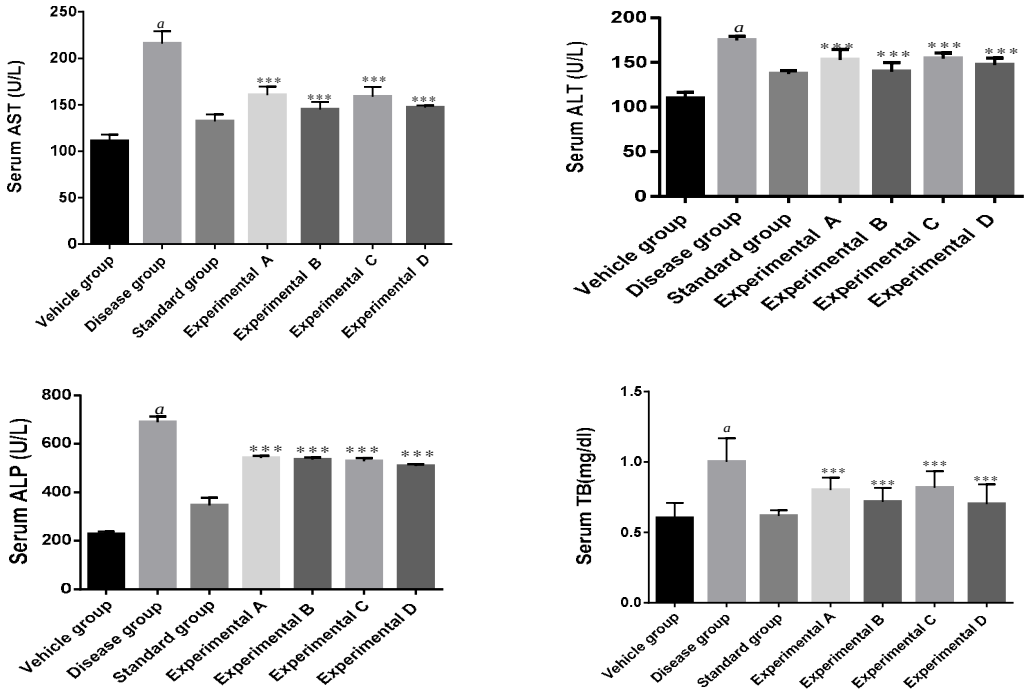

Fig. 1. Evaluation of hepatoprotective activity

*All values were expressed as Mean ± S.D. and two ways ANOVA was applied. Superscript "[a]" indicated significantly different from vehicle group with P < .05. Superscript "***" indicated significantly different from disease group with P < .001*

3.5 Evaluation of Hepatotoxicity

All animals died when extract was administered in dose 5000 mg/Kg thus LD_{50} was calculated as 3162 mg/kg. Livers of dead rats were preserved for histological analysis (Table 9). The animals who survived after administering acute high doses were observed for behavioral changes as shown in Table 8. Results of hepatic serum biomarkers are shown in Table 7 which indicated that hepatic enzymes are elevated in the rats which were administered with high acute dose (5000 mg/Kg).

3.6 Histopathological Studies for Evaluation of Hepatotoxic Effect of *Cyperrus pertenuis* Extracts

All toxicological results are expressed in Table 9 with their histological scorings of transverse sections of liver tissues usually observed at 100 x magnifications. Histopathological evaluation

indicated normal hepatocytes with dilated sinusoids in vehicle control group (Fig. 4A) as well as in experimental group-I treated with 1000 mg/kg ethanolic extract of *Cyperus pertenuis* (Fig. 4B). A mild degree of necrosis was observed in experimental group-II which was treated with ethanolic extract of *Cyperus pertenuis* in dose 2000 mg/kg as shown in Fig. 4C. However, a severe degree of necrosis was observed in experimental group-III which was treated with ethanolic extract of *Cyperus pertenuis* in dose 5000 mg/kg as shown in Fig. 4D.

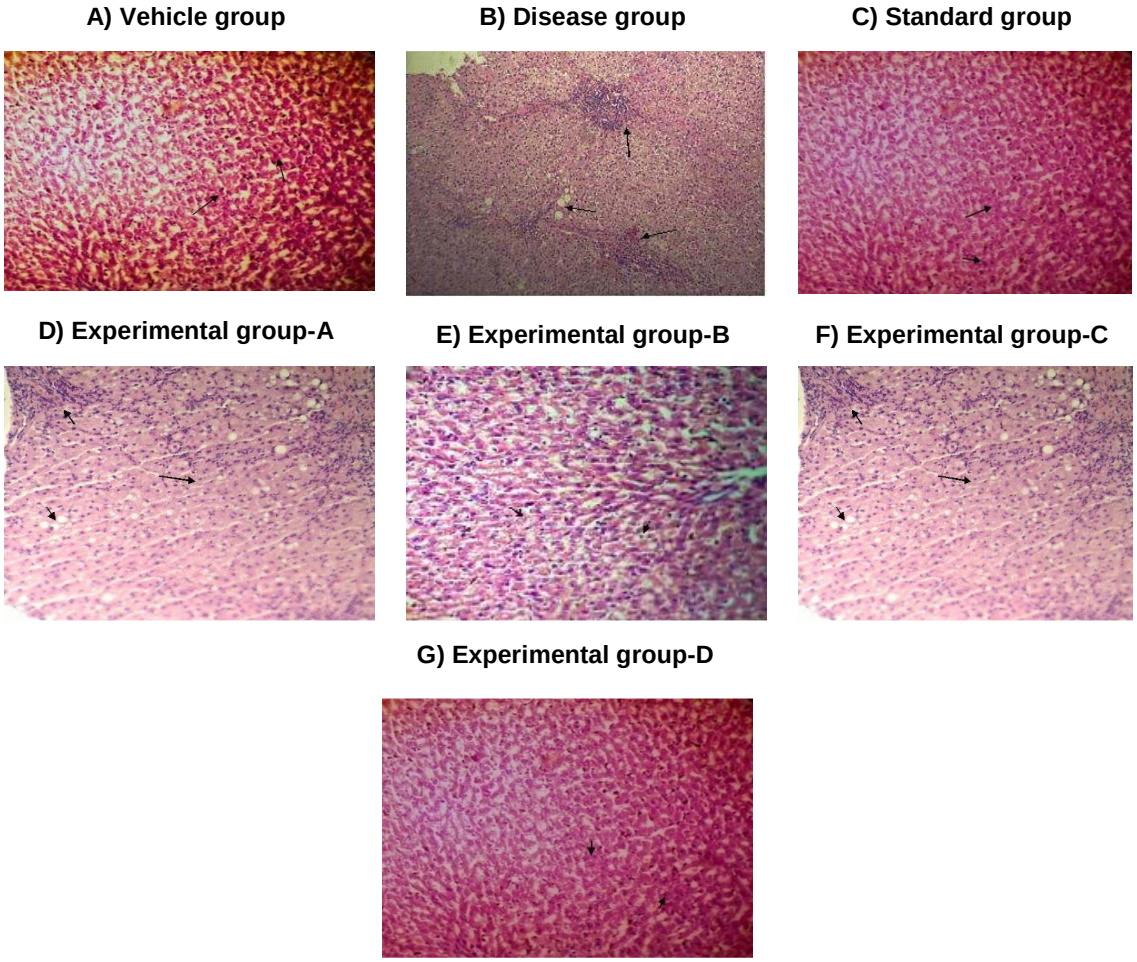

Fig. 2. Histopathological slides of hepatic tissues of different groups (100 X Magnification power)

Table 7. Evaluation of hepatotoxic effect of *Cyperrus pertenuis* extracts

Groups	ALT (u/l)	AST (u/l)	ALP (u/l)	TB (mg/dl)
Vehicle group	87.6±1.89	103.5±11	148.3±7.47	0.55±0. 05
Experimental group 1	102.3±2.97^{ns}	110.8±9.1^{ns}	162.5±9.2*	0.58±0.04^{ns}
Experimental group 2	102.5±3.05^{ns}	138.8±5.4*	174.8±16.2*	0.73±0.081*
Experimental group 3	236.2±4.02**	188.0±15.21**	351.5±53.8**	1.23±0.21**

All values were expressed as Mean ± S.D and Superscript "^{ns}" represented not significant at P < .05. Superscript "*"showed significant change at P < .01 and "**" indicated highly significant at P < .001 when compared to vehicle group

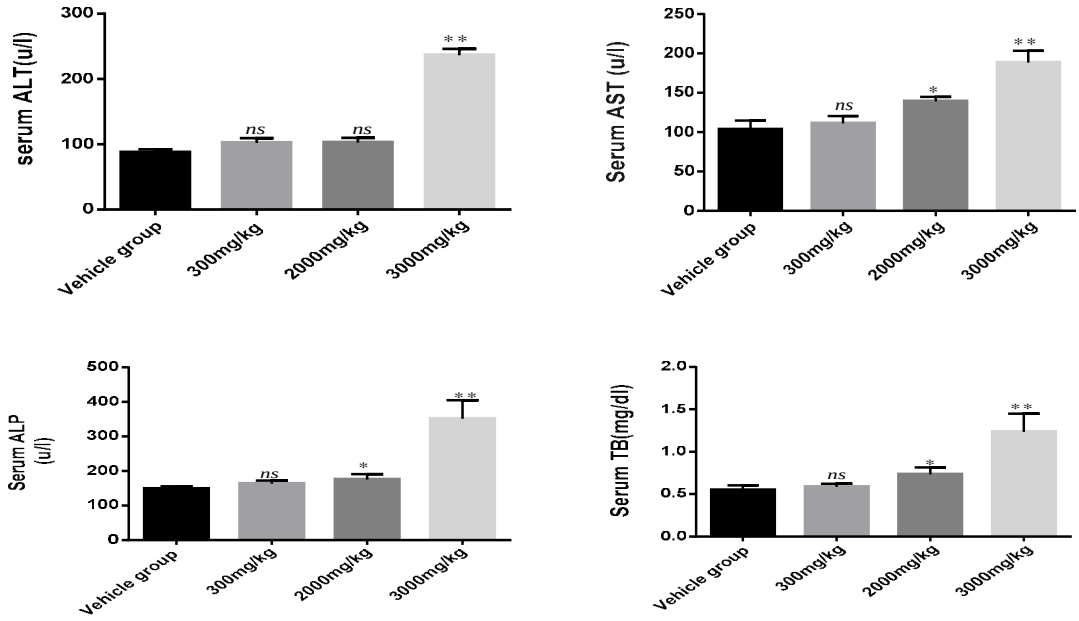

Fig. 3. Evaluation of hepatotoxic effect of Cyperrus pertenuis extracts

All values were expressed as Mean ± S.D and Superscript "ns" represented not significant at P < .05. Superscript ""showed significant change at P < .01 and "**" indicated highly significant at P < .001 when compared to vehicle group*

Table 8. Evaluation of behavioral changes in experimental rats at dose 5000 mg/kg

Behavior changes	Days											
	1	2	3	4	5	6	7	8	9	10	11	12
Hyperactivity	-	-	+	+	+	-	-	-	-	-	-	-
Rigidity	-	-	-	-	-	+	+	+	+	+	+	+
Twitching	-	-	-	-	-	-	-	-	+	+	+	+
Irritability	-	-	-	-	-	+	+	+	+	+	+	+
Jumping	-	-	+	+	+	-	-	-	-	-	-	-
Tonic /Clonic convulsions	-	-	-	-	-	-	+	+	-	-	-	-
Sedation	-	-	-	-	-	+	+	+	+	+	+	+
Ataxia	-	-	-	-	-	-	+	+	+	+	+	+
Loss of traction	-	-	-	-	+	+	+	+	+	+	+	+
Strabo Tail	-	-	-	-	+	+	+	+	+	+	-	-
Blanching	-	+	-	+	+	+	+	+	-	-	-	-
Abnormal secretion	-	-	-	-	-	-	+	+	+	+	+	+
Cyanosis	-	-	-	-	-	-	+	+	+	+	+	+
Reddeness	-	-	-	-	+	+	+	+	+	+	+	+

-: Indicates no change in observing parameter
+: Indicates change in observing parameter

4. DISCUSSION

Paracetamol is a renowned drug for its analgesic and antipyretic activity but potential damage of hepatocytes by its overdose is another fact which cannot be denied. Actually, it depletes the glutathione levels in body when used in overdose by covalently binding its metabolite N-acetyl-p-benzoquinone imine (NAPQI) [21] to cysteine residues [22]. The protection mechanism fails due to depletion of glutathione levels which prevents covalent binding of NAPQI to hepatic proteins [23]. In human body, liver is quite sensitive to the toxicities of certain chemical substances entering in portal circulation. So, once the liver cells are damaged then level of hepatic enzymes serum glutamic oxaloacetic transaminase (SGOT), serum

glutamic-pyruvic transaminase (SGPT), ALP and TB is increased which can be used as marker of hepatic cell damage [24]. In the present study paracetamol was used to induce hepatotoxicity in albino rats. As the results indicated that significant hepatic cell damage occurred which is clear from the elevated levels of serum biochemical markers i.e. AST, ALT, ALP and TB. Elevated level of SGOT also called AST is often accompanied with rise in level of SGPT also known as ALT which converts amino acids to keto-acids [25]. Administration of *Cyperus pertenuis* in all doses significantly reduced the levels of both AST and ALT which suggests that plant extract is capable of maintaining the membrane integrity of hepatocytes. But on the other hand elevated levels of serum ALP and TB indicates structural damage of hepatocytes [26]. Serum ALP and TB are also significantly reduced by plant extracts which indicates its marked potential in prevention of hepatic cell injury.

Glutathione is non-enzymatic tripeptide most abundant in liver which protects it from oxidative stress of free radicals like peroxide, superoxide, nascent oxygen and alkoxy radicals [27]. DPPH assay of present study indicates that *Cyperus pertenuis* is a strong antioxidant which prevented the hepatic injury by capturing oxidizing agents. In fact, paracetamol toxicity depleted all the glutathione levels from body. Thus plant extracts played their hepatoprotective role by scavenging reactive species in liver. Furthermore, phytoconstituents like presence of phenolic compounds and flavoniods show antioxidant activity in many plants [28].

Table 9. Histological scoring of transverse sections of liver specimens in hepatotoxicity study [20]

Groups (n=6)	Histopathological Lesions				
	Periportal +/- bridging necrosis	Interlobular degeneration & focal necrosis	Portal inflammation	Fibrosis	HAI
Vehicle Group	0	0	0	0	0
Experimental Group-I (100 mg/kg)	0	0	0	0	0
Experimental Group-II (2000 mg/kg)	3	1.4	0	0	4.4
Experimental Group-III (5000 mg/kg)	5	1	3	0	9

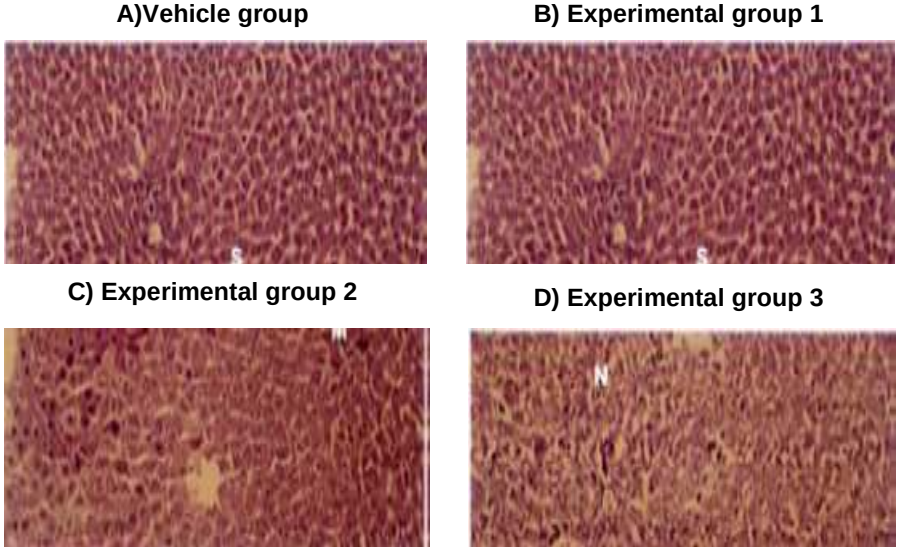

Fig. 4. Histological studies of transverse sections of liver specimens for toxicological evaluation (100 X magnification power)

Another possible mechanism of hepatoprotection by *Cyperus pertenuis* is the elevation of catalases [26]. Catalase is an enzyme that act as an antioxidant and is widely distributed in different body tissues, most abundant in liver and RBCs [29]. Catalase causes decomposition of H_2O_2 and prevents the generation of free hydroxyl radicals [30]. Thus reduction in level of catalase causes damage to cells.

Histopathological evaluation of slides indicated the severe necrosis of hepatocytes with complete disappearance of nucleus in diseased group treated only with paracetamol as shown in Fig. 2B. This clears that paracetamol is toxic in high doses for liver cells. Hepatic damage by paracetamol is due to oxidative stress of free reaction oxygen species [30]. The animals treated with *Cyperus pertenuis* extracts indicated less structural hepatic damage, necrosis and degenerations as cleared from histopathological slides (Fig. 2D-2G). The plant extracts might have role in regeneration of hepatocytes along with the prevention of fibrosis and nodules [31].

Study was also aimed to conduct acute toxicity study and consequently liver were analyzed for hepatotoxic effects of *Cyperus pertenuis* at $LD_{50.}$ Mortality and other behavioral changes were observed in rats which concluded that LD_{50} value was 3162 mg/kg in rats. 200 mg/kg ($1/15^{th}$) and 400 mg /kg ($1/7.5^{th}$) doses are effective and safe for chronic use of this plant for hepatoprotectivity.

5. CONCLUSION

From the analysis and discussions made on the results of the evaluation of biochemical and histopathological parameters, it is concluded that *Cyperus pertenuis* has strong ability to protect the liver against paracetamol induced acute hepatic damage in rats. Moreover, its hepatoprotective effects are comparable with silymarin.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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