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Phytochemical Investigation and Hepatoprotective Activity of *Cissampelos pareira* Against Carbon-tetrachloride Induced Hepatotoxicity

[1]Mahendra Singh Yadav*, [2]Anurag Kumar, [2]Ajeet Singh, [3]Uma Shankar Sharma, [3]Niranjan Sutar,

[1]Advance Institute of Biotech & Paramedical Sciences, Kanpur -209217, U.P.
[2]Dayanand Dinanath College, Institute of Pharmacy, Kanpur - 209214 U.P.
[3]Department of Pharmacy, Sir Madanlal Group of Institution, Etawah, U.P.-206001.

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***Corresponding author:**

E-mail: niranjansutar77@rediffmail.com

ABSTRACT

In Indian traditional system of medicine, herbal remedies are prescribed for the treatment of various diseases including liver diseases. The present study was aimed to investigate the hapatoprotective activity of the ethanolic extract of *Cissampelos pareira* against carbon tetra chloride (CCl4) induced hepatotoxicity in rats. Liver functions were assessed by the determination of SGOT, SGPT, ALP and bilirubin. Histopathological studies were carried out. The serum biochemical analysis results suggest that the use of ethanolic extract of *Cissampelos pareira* exhibited significant protective effect from hepatic damage in CCl4 induced hepatotoxicity model. Histopathological studies revealed that concurrent administration of the extract with CCl4 exhibited protective effect on the liver, which further evidenced its hepatoprotective activity.

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. Liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there is not much drug available for the treatment of liver disorders [2]. Therefore, many folk remedies from plant origin are tested for its potential hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl4) induced hepatotoxicity model is widely used for the study of hepatoprotective effect of drugs and plants [3].

Cissampelos pareira Linn. is significant plant of family Menispermaceae. There are 37 plant species summarized under this botanical name. Their taxonomic position is not clear. In most cases, *C. pareira* or Pareira is used. It is found in subtropical parts of India, Asia, East Africa and America [4]. The plant is a climbing shrub, 2 - 5m high with a thickened root. Leaves have an orbicular shape 7-14 cm in diameter. They are membranous or leathery, veined, glabrous to densely pilose. Flowers are green, male ones in short umbels, 10 - 12cm long, females in pendulous spikes, 7 - 10cm long, with a little round leaflet at the base of every flower [5].

No systematic studies have been reported for its hepatoprotective activity. Hence an effort has been made to establish the diuretic activity of alcoholic extracts of *Cissampelos pareira* root.

MATERIALS AND METHOD

Collection and preparation of Plant Extract

The roots of plant were collected from Botanical Garden of N.B.R.I. (National Botanical Research Institute), Lucknow, Uttar Pradesh state, India in the month of June 2009 and authenticated by Dr.Harish K. Sharma, Ayurvedic Medical College, Davangere, Karnataka, India. A voucher specimen was submitted at Institute's herbarium department for future reference (AN 104). Dried roots were ground to coarse powder. Powder was first defatted with pet. Ether and then extracted with ethanol which is further evaporated to dryness to obtain alcoholic extract.

Extraction and phytochemical screening of plant

The freshly collected roots (5 gm) 0f *Cissampelos pareira* were distilled with water and shade-dried. Then dried in tray drier under controlled condition and powdered the drug in fine particles. The powdered roots of *Cissampelos pareira* (1000 g) were extracted with 50% aqueous Ethanol at room temperature and the combined extracts from three successive extractions,

were filtered and concentrated to a sticky gum in a rotary vacuum evaporator (Rotavopour, Buchi, USA) and dried in lyophilizer (Labcono,USA) under reduced pressure. The yield was 92 g (9.2% (w/w).Phyto chemical screening was performed using standard procedures [6, 7].

Experimental animals

Rats (Sprague dawley) and swiss albino mice with body weight 150-185 gm and 20-25 gm respectively of either sex and of approximately the same age was procured from animal house of Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at 26±2^{0}C temperature and relative humidity 44-56% with a dark and light cycle of 12±1 h in polypropylene cages (38×23×10) with not more than six animal in one cage. Animals were provided with standard rodent pellet diet (Dayal, India) and Water ad libidum. All the procedures were reviewed and approved by the institution committee for ethical use of animals [8].All animal experiments conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC) and followed the guidelines of IAEC.

Acute Toxicity Study:

The adult male albino mice selected for acute toxicity study. The 50% ethanolic extract of *Cissampelos pareira* root were taken at various doses levels (100, 250, 500, 1000, 2000 mg/kg body weight) dissolved in 1 % carboxymethyl cellulose administered 10 ml/kg body weight orally to pairs of mice per dose level. The control animals received 1 % carboxymethyl cellulose in distilled water (10 ml/kg) orally. The animals were observed continuously for two hour and then occasionally for further four hours and finally any mortality. Behavior (gross behavior, general motor activity, writhing, convulsion, response to tail pinching, pupil size, fecal output, water intake, feeding behavior, sedation etc.) of the animals and any other toxic symptoms also observed for 72 hours and the animals were kept under observation up to 14 days (Ghosh, 1984) [9].

The effective dose (ED50) of 50% ethanolic extract of Cissampelos pareira root was decided 1/10 0f maximum dose (2000mg/kg). So I was used the dose of 50% ethanolic extract of *Cissampelos pareira* such as 100, 200 and 400 mg/kg body weight, p.o. for hepatoprotective activity.

Hepatoprotective study:

Carbon tetrachloride induced hepatotoxicity:

Experimental Procedure:

Rats (Sprague dawley) with body weight 150-185 gm were divided into six groups of six

Group I

Animals were administered a single daily dose of 1% Carboxy methyl cellulose (1 ml/kg body weight, p.o.) for 10 days. This group served as control group.

Group II

Received 50% carbon tetrachloride with olive oil (2 ml/kg body weight, I.p.) on first and third day of experiment with a single daily dose of 1% Carboxy methyl cellulose (1 ml/kg body weight, p.o.) for 10 days. This group served as treated group.

Group III

Received a single daily dose of 100 mg/kg body weight of root extract along with 50% carbon tetrachloride solution (2ml/kg body weight, i.p. on first and third day of experiment).

Group IV

Received a single daily dose of 200 mg/kg body weight of root extract along with 50% carbon tetrachloride solution (2ml/kg body weight, i.p. on first and third day of experiment).

Group V

Received a single daily dose of 400 mg/kg body weight of root extract along with 50% carbon tetrachloride solution (2ml/kg body weight, i.p. on first and third day of experiment).

Group VI

Received silymarin, the known hepatoprotective compound (Sigma Chemicals Company, USA), at a dose of 100 mg/kg, p.o., along with carbon tetrachloride solution (2 ml/kg body weight, i.p.). The *Cissampelos pareira* root extract was given simultaneously with carbon tetrachloride. And this is served as standard group.

The *Cissampelos pareira* root extract was given 30 min before carbon tetrachloride administration. Treatment duration was 10 days respectively. Animals were sacrificed 48 h after the last administration. Blood was collected, allowed to clot and serum separated. Liver was dissected out and used for biochemical studies. Procedure is according to Rasheeduz and Mujahid. 1998. [10]

Pentobarbitone induced sleeping time in Mice:

The effect of 50% ethanolic extract of *Cissampelos pareira* on CCl4-induced prolongation in pentobarbitone sleeping time was studied in Swiss albino mice as described by Gilani et al. The mice were divided in to six groups.

Control group:

Group received four doses of vehicle (1ml/kg of 1% Carboxy methyle cellulose in distilled water) orally at 12 hr interval and Olive oil was administered as a bolus dose (7.5ml/kg; orally), 1 hr after the last dose vehicle followed after 24 hr by Pentobarbital (75mg/kg,ip).

Toxic group:

This group was given the same treatment except the olive oil was replaced with Carbon tetra chloride (1.5ml/kg body weight p.o.).

Test groups:

The three test groups received the same treatment as that of the toxic group except that the vehicle was replaced with 50% ethanolic extract of Cissampelos pareira at three dose levels 100mg/kg, 200mg/kg and 400mg/kg body weight p.o. respectively.

Standard group:

This group received the same treatment as that of toxic group except that the vehicle was replaced with 100mg/kg silymarin.

The time between loss of righting reflux and its recovery were recorded in all the groups.

Biochemical estimation

Biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) were determined by Reitman and Frankel method [11].

Serum alkaline phosphatase was determined by King and Kings Method [12]. Malby and Evelyn method [13] was followed to estimate total bilirubin content.

Statistical analysis

All the values are expressing as mean ± SEM (standard error of mean) for six rats. Statistical analysis was carried out by usinsg PRISM software package (version 3.0). Statistical significance of differences between the control and experimental groups was assessed by One way ANOVA followed by Newman-Keuls Multiple Comparision test. The value of probability less than 5% (P<0.05) was considered statically significant.

RESULTS AND DISCUSSION

Phytochemical analysis:

The 50% ethanolic extract of roots of *Cissampelos pareira* Linn were subjected to qualitative test to identified the phytoconstituents which revealed the presence of carbohydrates, alkaloids, sterols, phenolic compounds, saponins, fats and oils, tannins and flavonoids, resins.

Histopathological analysis:

Pieces of liver from each liver lobe were fixed in Bouin's fluid for 24 hrs and washed in running tap water to remove the colour of Bouin's fluid and dehydrated in alcohol ascending and descending order, embedded in paraffin and cut at 5µm (Automatic tissue processor) in a rotary microtome. These sections were deparaffinised in xylene, stained with hematoxylin-eosin dye and mounted with Canada balsams. The histopathological slides were examined and photographs were taken with a digital stereomicroscope (Olympus).

Acute Toxicity Study

The aim of performing acute toxicity studies is for establishing the therapeutic index of a particular drug and to ensure the safety in-vivo. Acute toxicity study in generally carried out for the determination of LD50 and ED50 value in experimental animals.

The 50% ethanolic extract of roots of *Cissampelos pareira* has shown 0% mortality at a dose corresponds to 250, 500, 1000, 2000 mg/kg body weight after observing continuously for 2 hours and then occasionally for further 4 hours and finally overnight mortality recorded. Behaviour of animals and any other toxic symptoms also observed for 72 hours and the animals were kept under observation up to 14 days. On the bases of above study the effective dose was found 1/10 of maximum dose (200mg/kg). Hence three random doses 100. 200, 400 mg/kg body weight p.o. were selected for study.

Hepatoprotective activity:

Carbon tetra-chloride induces hepatotoxicity:

It is clearly evident that the CCl4 caused significant elevation liver serum markers. In the CCl4 treated rats, the level of SGPT (24.95± 0.46 - 79.41± 2.01, p<0.001), SGOT (169.87± 2.87 - 352.24± 14.31, p<0.001), ALP (231.27± 3.68 - 388.20 ± 4.06. p<0.001), Total protein (6.86± 0.10 - 3.47± 0.04, p<0.001), Albumin (1.34± 0.02 - 0.69± 0.01, p<0.001) and Total billirubin (0.55± 0.02 - 1.73± 0.03, p<0.001).

In contrast the groups treated with 50% ethanolic extract of *C. pareira* at a dose of (100-400 mg/kg) once daily for 10 days prevented the hepatotoxicity in dose dependent manner. The ranges of protection in the serum marker were found to be SGPT (79.41± 2.01 - 53.06± 0.91, p<0.05 to p<0.001), SGOT (352.24± 14.31 - 191.68± 2.72, p<0.05 to p<0.001), ALP (388.20± 4.06 - 246.61± 3.43, p<0.05 to p<0.001), Total protein (3.47± 0.04 - 7.06± 0.04, p<0.05 to p<0.001), Albumin (0.69± 0.01 - 1.18± 0.01, p<0.05 to p<0.001), and Total billirubin (1.73± 0.03 - 0.62± 0.01, p<0.05 to p<0.001).

The protection of silymarin ranged for SGPT (79.41± 2.01 - 46.60± 0.62, p<0.001), SGOT (352.24± 14.31- 167.18± 2.13. p<0.001), ALP (388.20± 4.06 - 237.88± 1.12, p<0.001), Total protein (3.47± 0.04 - 7.18± 0.01, p<0.001), Albumin (0.69± 0.01 - 1.27± 0.01, p<0.001), and Total billirubin (1.73± 0.03 - 0.99± 0.01, p<0.001) respectively as shown in Table No.3. The histological observation also basically support the results obtained from the serum enzyme assay (Table No.3).

Pentobarbitone induced sleeping time in mice:

The results of this study were given in Table No.1. Pentobarbitone at a dose of 75 mg/kg (i.p.) caused sedation in mice of control group for a period of 73±10.71 min., where as treatment of animals with Carbon tetrachloride (Toxic group) prolonged the petobarbitone sleeping time to 234.50± 21.21 min, the value that was significantly higher (P<0.001) than that of control. Prior treatment with animals with 50% ethanolic extract of *Cissampelos pereira* (100mg/kg, 200mg/kg and 400mg/kg body weight p.o.) and silymarin (100mg/kg) significantly shortened the pentobarbitone sleeping time as compared to the toxic group. Though all the extract exhibited activity the maximum reduction in sleeping time were observed in *Cissampelos pareira* at dose of 400mg/kg groups, which was closed to the sleeping time observed in the reference drug, silymarin group (P>0.05).

Table No.1: Effect of 50% ethanolic extract of *C. pareira* on Pentobarbital induced sleeping time in mice

Groups	Sleeping Time (Min.)	Percentage decrease in sleeping time (%)
Control group	73 ± 10.71	--
Toxic group	234.50 ± 21.21	--
Test group-1 (100mg/kg)	206.16 ± 13.12	17.65
Test group-2 (200mg/kg)	171.50 ± 09.31	39.50
Test group-3 (400mg/kg)	146.33 ± 15.18	54.93
Standard group	138.00 ± 17.78	60.00

Effect of Drug extract on body weight, liver weight, and kidney weight of carbon tetrachloride induced hepatotoxicity in rats.

50% ethanolic extract of *Cissampelos pareira* at a dose of 100, 200 and 400 mg/kg once daily for 10 days and standard drug silymarin at a dose of 100mg/kg were subjected for studying the body weight, lever weight and kidney weight in hepatotoxic rats. The study showed that the body weights are significantly decreased from (195-185.5) in CCl4 treated rats. However, 50% ethanolic extract of C. *pareira* showed a dose dependent

Table No.2: Effect of 50% ethanolic extract of *Cissampelos pareira* on body weight, liver weight, and kidney weight of carbon tetrachloride induced hepatotoxicity'

Treatment/Dose	Body weight (gm)	Liver weight(gm)	Kidney weight(gm)
Control (Vehicle)	195.00 ± 4.84	6.30 ± 0.07	0.98 ± 0.03
Toxic (CCL4)	185.5 ± 3.51	7.53 ± 0.16	1.10 ± 0.08
C. pareira (100mg/kg)	186.16 ± 4.30	7.2 ± 0.05	0.99 ± 0.05
C. pareira (200mg/kg)	187.5 ± 4.97	6.60 ± 0.23^{c}	0.95 ± 0.05
C. pareira (400mg/kg)	188.5 ± 3.36	6.46 ± 0.10^{c}	0.99 ± 0.06
Silymarin (100mg/kg)	188.33 ± 3.34	6.31 ± 0.03^{c}	1.03 ± 0.06

Values expressed as are Mean ± SEM of six rats in each groups.^{Z}p<0.001when compared to respective control and ^{c}p<0.001when compared to respective CCl_4 control.

Table No.3. Effect of 50% ethanolic extract of *C. pareira* on biochemical parameters against control and carbon tetra chloride induced hepatotoxicity.

Treatment /Dose	SGPT(U/L)	SGOT(U/L)	ALP(U/L)	Total Protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)
Control group (1%CMC)	24.95 ± 0.46	169.87± 2.87	231.27± 3.68	6.86± 0.10	1.34± 0.02	0.55± 0.02
Toxic group (CCL4 +Olive oil)	79.41± 2.01^{Z}	352.24± 14.31^{Z}	388.20± 4.06^{Z}	3.47± 0.04^{Z}	0.69± 0.01^{Z}	1.73± 0.03^{Z}
Test group (C. pareira 100 mg/kg)	73.22± 1.16	251.04± 10.82^{c}	360.77± 4.13	4.88± 0.04^{a}	0.93± 0.009^{a}	1.09± 0.01^{c}
Test group (C. pareira 200 mg/kg)	65.60± 0.83	224.31± 6.50^{c}	301.62± 1.87^{c}	6.30± 0.06^{c}	1.03± 0.01^{a}	0.89± 0.01^{c}
Test group (C. pareira 400 mg/kg)	53.06± 0.91	191.68± 2.72^{c}	246.61± 3.43^{c}	7.06± 0.04^{c}	1.18± 0.01^{c}	0.62± 0.01^{c}
Standard group (Silymarin 100 mg/kg)	46.60± 0.62	167.18± 2.13^{c}	237.88± 1.12^{c}	7.18± 0.01^{c}	1.27± 0.01^{c}	0.99± 0.01^{c}

Values expressed as are Mean ± SEM of six rats in each group.
^{Z}p<0.001when compared to respective control and ^{a}p<0.05 and ^{c}p<0.001when compared to respective CCl_4 control.

protection in body weight. Result for highest dose (400mg/kg) is comperatable with the standard drug silymarin (100mg/kg).

The study showed that the liver weight was increased from 6.3 ± 0.07 to 7.53 ± 0.16 in CCL4 treated group. However, 50% ethanolic extract of *Cissampelos pareira* showed a dose dependent protection in liver weight. Silymarin (100mg/kg) showed significant reduction in liver weight compared to CCL4 treated group, The study showed that the kidney weight was slightly increased from 0.98 ± 0.03 to 1.10 ± 0.08 in animals of CCL4 treated group. However, treatment with 50% ethanolic extract of *Cissampelos pareira* revert the changes (Table No.2).

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