Vol. 3/2 (2003) 148 - 154

JOURNAL OF NATURAL REMEDIES

Hepatoprotective activity of leaves of *Feronia elephantum* Correa (Rutaceae) against carbontetrachloride-induced liver damage in rats

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Received 12 August 2002 ; Revised and accepted 3 April 2003

Abstract

Objective: The objective of the present investigation was to study hepatoprotective activity of aqueous extract of leaves of *Feronia elephantum* Correa., Fam. Rutaceae using carbon tetrachloride-induced liver damage model in rats. Methods: Liver damage in rats was produced by carbon tetrachloride (1.25 mg/kg, i.p.) in olive oil. Aqueous extract of leaves of the plant was administered to rats daily for seven or fifteen days. The physical, functional and biochemical parameters were investigated. Histopathological changes in liver were studied. Concurrently Liv 52® was used as standard hepatoprotective agent. Results: The result indicated that physical, functional and biochemical changes produced by carbon tetrachloride were restored to normal by aqueous extract of the leaves of *Feronia elephantum* Liv 52®. The hepatoprotective action of aqueous extract of leaves and Liv 52® was confirmed by histopathological examination. Conclusion: The aqueous extract of leaves of *Feronia elephantum* showed hepatoprotective effect in carbon tetrachloride induced liver damage model in rats.

Key words: Hepatoprotective, *Feronia elephantum* Correa.

1. Introduction

A number of indigenous plants have been described in the literature to possess hepatoprotective activity [1, 2]. The leaves of the plant *Feronia elephantum* Correa, (Rutaceae) commonly known as 'Kavath', have been claimed to be useful in the treatment of jaundice [3]. A decoction (*kadha*) administered orally before breakfast has been advocated by local traditional medicine practitioners [4]. The evaluation of the leaves of *Feronia elephantum* in the treatment of liver diseases has not been reported in the laboratory animals. The paucity of scientific report prompted us to undertake the present study.

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The objectives of the present investigation were to study effect of cold macerated aqueous extract of the leaves of the plant *Feronia elephantum* on various functional, physical, histo-pathological and biochemical parameters using carbon tetrachloride induced hepatotoxicity as an experimental model of liver damage [5].

2. Materials and methods

2.1 Collection of plant materials

The leaves of the plant *Feronia elephantum* Correa were collected from the medicinal plant garden at Bharati Vidyapeeth's Poona College of Pharmacy, Pune. The plant was authenticated by Agharkar Research Institute, Pune (The voucher herbarium specimen number of plant was AHMA-16126). The leaves were dried in shade for one week. The dried leaves were powdered and passed through sieve No.22.

2.2 Extraction

The fine powder (1 kg) obtained was macerated with sufficient amount of distilled water in a closed percolator for 24 h shaking frequently for 6 h and then was allowed to stand for 18 h. Sufficient overhead volume of water was kept initially. Then it was allowed to elute and filtered. Filtrate was evaporated under reduced pressure. The practical yield after extraction was 310.8 g (31.08% w/w).

The residue obtained was collected and stored in amber colored bottle in a cool and dry place. The residue thus obtained was reconstituted by suspending in 4% gum acacia and used for the biological study.

2.3 Pharmacological experimentation

Albino rats (Sprague Dawley) of either sex, obtained from National Toxicology Centre, Pune, weighing between 125-175g were used. Animals were fed with standard rat diet (Chakan oil mills, Pune) and drinking water was supplied freely. The animals were divided into ten groups with six animals in each group. Group 1 received 4% Gum acacia suspension alone (5ml/kg p.o.) for 16 days. Group 2 received carbon tetrachloride on 16th day along with other groups, otherwise it received the same treatment as that of control group i.e. Group 1.

Group 3 and 8 received 400mg/kg of extract and Liv 52® respectively. Group 4 (prophylactic) and 6 (therapeutic) received extract 400 mg/kg for seven days. Group 5 (prophylactic) and 7 (therapeutic) received extract 400 mg/kg for 15 days. Group 9 (prophylactic) and 10 (therapeutic) treated with standard drug (Liv 52®) for 15 days. Carbon tetrachloride (1.25 mg/kg) was administered 24 h before extract or standard drug treatment in therapeutic groups and on last day of extract or standard drug treatment in prophylactic groups.

2.4 Collection of blood sample

On the 17th day animals were anaesthetized with anaesthetic ether. Blood was withdrawn directly from heart with sterile syringe and collected in sterilized vial for serum separation and analysis. After collecting the blood, vials were kept at room temperature for 2 h for blood coagulation and serum was separated out by centrifugation at 2,500 rpm.

The biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT) or asparate aminotransferase (AST), serum glutamate pyruvate transminase (SGPT) or alanine aminotransferase (ALT), serum alkaline phosphatase (ALP), total proteins (TP) and cholesterol were determined using ERBA CHEM-5 Semi-autoanalyser.

2.5 Hepatoprotective study

The results of preliminary study (unpublished data) indicated that the extract showed a dose

dependent effect (Doses of 200mg, 400mg, 800mg, 1600mg/kg p.o.). A dose of 400 mg/kg was used for further study. The experimental plan was designed to study prophylactic as well as therapeutic effect of the drug on carbon tetrachloride-induced hepatic damage.

It was also thought worthwhile to plan the treatment days with the extract and the standard drug (Liv 52®) in such a way to study hepatoprotective effect of short-term (7 days) and long term (15 days) drug administration.

Liv 52®, a polyherbal hepatoprotective formulation of Himalaya Drug Company was purchased locally. Main constituents of the formulation are powders of Kasani (*Cichorium intydus*), Mandur bhasma, Kakamachi (*Solanum nigrum*), Arjuna (*Terminalia arjuna*), Kasamarda (*Cassia occidentalis*), Biranjasipha (*Achelliea millefolium*), Jhavuka (*Tamarix gallica*) etc.

2.6 Physical Parameter

Abdomen of the anaesthetized animal was opened. Liver was carefully dissected out, dried by keeping on a filter paper. Dry weight of the liver and the liver volume was determined.

2.7 Histopathological Parameter: Processing of Specimen

The isolated liver specimen was trimmed to small pieces and preserved in formalin (10%) solution for 24 h. The liver specimen was subjected to dehydration with acetone of strength 70, 80 and 100% respectively, each for one hour. The infiltration and impregnation was done by treatment with paraffin wax twice each time for one hour. Paraffin wax was used to prepare paraffin "L" moulds [6].

Specimens were cut into sections of 3-5 in thickness and were stained with haematoxylin and eosin. Mounting of specimen was done by use of Distrene Phthalate Xylene (D.P.X).

2.8 Functional Parameter: Determination of Pentobarbitone Sleeping Time (PST)

Male albino rats were divided into 7 groups with 6 animals in each group. The drug treated groups i.e. Group 3 to Group 7 were administered with pentobarbitone sodium (35mg/kg i.p.). The time of onset of action was noted as animal lost its righting reflex i.e. falls asleep. The time of recovery from the sleep as the animal turns to recover its normal posture was noted. The time and duration of sleep induced by pentobarbitone sodium in all the groups was calculated.

2.9 Statistical Analysis

The mean and Standard Deviation (S.D.) values were calculated for each group in case of biochemical parameters (AST, ALT, ALP, TP and cholesterol) and functional parameter (Pentobarbitone Sleeping Time). Data were analyzed by One way ANOVA followed by Dunnett's test at $P<0.05$ significance level using 'Primer' software.

3. Results

3.1 Biochemical Parameters

Administration of the drug (cold macerated aqueous extract 400 mg/kg p.o.) or Liv 52® (400 mg/kg) alone for 15 days did not alter the biochemical parameters significantly. Administration of carbon tetrachloride (1.25 ml/kg i.p.) alone significantly increased AST, ALT, ALP but not total protein indicating hepatotoxicity.

In both prophylactic and therapeutic groups treated with extract (400 mg/kg p.o. for 7 days) the biochemical parameters were altered. However when compared with carbon tetrachloride (1.25 ml/kg i.p.) group the biochemical parameters reduced significantly indicating hepatoprotective activity.

Similar results were observed in fifteen days prophylactic and therapeutic groups with drug

Table 1.
Levels of various biochemical parameters in different experiment groups (values are Mean ± S.D.)

Group	**Treatment**	**AST IU/L**	**ALT IU/L**	**ALP IU/L**	**TP g/dl**	**Ch mg/dl**
Control	4% Gum acacia	59.5 ± 11.1	102.7 ± 45.39	125 ± 31.47	7.67 ± 0.77	74.75 ± 9.33
CCl_4	1.25 ml/kg i.p	235.5 ± 9.83	182.7 ± 10.13	254.25 ± 14.26	6.5 ± 1.04	107.7 ± 3.98
Drug	400 mg/kg p.o.	70.00 ± 9.83*	90.2 ± 16.49*	132.70 ± 14.38*	8.00 ± 1.11*	82.3 ± 8.84*
Drug prophylactic	400 mg/kg p.o for 7 days + CCl_4 (1.25 ml/kg i.p) on 8th day	92.7 ± 4.09*	139.6 ± 11.70*	155.7 ± 14.54*	‘6.09 ± 0.56	92.3 ± 5.20*
Drug prophylactic	400 mg/kg p.o for 15 days + CCl_4 (1.25 ml/kg i.p) on 16th day	83.5 ± 19.56*	118.6 ± 13.77*	139.7± 12.09*	7.32 ± 0.92	90.5 ± 10.98*
Drug therapeutic	CCl_4 (1.25 ml/kg i.p) on 1st day + 400 mg/kg p.o for next 7 days	110.5 ± 10.15*	147.3 ± 9.8*	165.5 ± 5.39*	6.20 ± 0.27*	98.2 ± 7.23
Drug therapeutic	CCl_4 (1.25 ml/kg i.p) on 1st day + 400 mg/kg p.o for next 15 days	94.8 ± 4.28*	137.1 ± 35.57*	149.6± 20.17*	7.12± 0.49	85.7± 13.34*
Liv 52®	400 mg/kg p.o.	57.5 ± 6.91*	95.2± 9.48*	123.5± 27.37*	7.70± 0.34	72.5± 5.65*
Liv 52® prophylactic	400 mg/kg p.o for 15 days + CCl_4 (1.25 ml/kg i.p) on 16th day	75.2± 7.51*	93.6± 3.41*	127.2± 7.58*	7.9± 0.65*	70.0± 8.82*
Liv 52® therapeutic	CCl_4 (1.25 ml/kg i.p) on 1st day + 400 mg/kg p.o for 16 days	81.4 ± 14.01*	95.2± 13.63*	131.5± 8.64*	7.3± 2.33	76.57± 18.55*

n=6, * P<0.05
Data analysed by ANOVA followed by Dunnett’s test.
All groups compared with carbon tetrachloride (1.25 ml/kg) alone

(400 mg/kg p.o.) when compared with carbon tetrachloride (1.25 ml/kg i.p.) group. Treatment with Liv 52® (prophylactic and therapeutic) indicated significant lowering of biochemical parameter when compared with carbon tetrachloride (1.25 ml/kg i.p.) group. Prophylactic treatment with Liv 52® (400 mg/kg p.o.) for 15 days resulted in significant increase in total protein value when compared with carbon tetrachloride (1.25 ml/kg i.p.) group.

3.2 Physical and Histopathological Parameters

The Physical parameter viz. liver weight and liver volume were determined for the control group (Group 1). The values thus obtained were used for comparison. The histology of liver showed normal architecture of liver in control group.

Administration of carbon tetrachloride alone resulted into significant increase in liver weight and liver volume compared with control group.

The histopathological section showed extensive signs of necrosis, fatty change and hydropic changes.

Administration of extract alone (400 mg/kg) for 15 days produced insignificant increase in liver weight and liver volume. Histopathology showed normal architecture of liver.

Prophylactic treatment for both 7 and 15 days in Group 4 and 5 resulted in decrease in liver weight and liver volume as compared with carbon tetrachloride intoxicated group. Histopathology showed normal architecture with focal collection of few lymphocytes surrounding the central vein prominent kupffer cells.

In therapeutic groups (7 and 15 days) it was observed liver weight and liver volume was decreased as compared with carbon tetrachloride intoxicated group. The histology revealed very mild hydropic and fatty changes. It also showed isolated liver cells with a cell slight hyaline change.

In Liv 52® (400 mg/kg p.o.) alone treated group it was observed that liver weight and liver volume was decreased as compared with carbon tetrachloride intoxicated group. No alteration of normal architecture of liver was found in the same group.

In Liv 52® (400 mg/kg p.o.) prophylactic (15 days) liver weight and liver volume were similar to the control group. Histology also revealed normal architecture of liver with central vein surrounded by liver cells and sinusoids showing kupffer cells

Table 2.
Effect of *F.elephantum* in functional and physical parameters (values are Mean ± S.D.)

Group	**Treatment**	**Parameter**		
		Functional	**Physical**	
		PST mins	**Liver wt (g)**	**Liver Vol (ml)**
Control	4% Gum acacia	76.66 ± 2.50	5.93 ± 0.43	6.98 ± 0.95
CCl_4	1.25 ml/kg i.p	167.33± 21.36	8.78 ± 0.89	10.12± 0.97
Drug	400 mg/kg p.o.	73.90 ± 4.02*	6.3 ± 0.68*	7.1 ± 0.95*
Drug prophylactic	400 mg/kg p.o for 7 days + CCl_4 (1.25 ml/kg i.p) on 8^{th} day	84.33 ± 90.48*	6.35 ± 1.49*	7.8± 0.85*
Drug prophylactic	400 mg/kg p.o for 15 days + CCl_4 (1.25 ml/kg i.p) on 16^{th} day	75.29 ± 11.22*	6.19 ± 2.05*	7.34 ± 0.26*
Drug therapeutic	CCl_4 (1.25 ml/kg i.p) on 1^{st} day + 400 mg/kg p.o for next 7 days	93.97 ± 6.38*	7.2 ± 0.85*	8.17 ± 0.89*
Drug therapeutic	CCl_4 (1.25 ml/kg i.p) on 1^{st} day + 400 mg/kg p.o for next 15 days	85.31 ± 7.72*	6.5 ± 0.56*	7.63 ± 0.26*
Liv 52®	400 mg/kg p.o.	69.5 ± 4.32*	6.2 ± 0.56*	7.5 ± 1.85*
Liv 52® prophylactic	400 mg/kg p.o for 15 days + CCl_4 (1.25 ml/kg i.p) on 16^{th} day	65.80 ± 24.73*	6.05 ± 0.45*	7.25 ± 0.14*
Liv 52® therapeutic	CCl_4 (1.25 ml/kg i.p) on 1^{st} day + 400 mg/kg p.o for 16 days	63.2 ± 8.56*	5.7 ± 0.90*	6.61 ± 2.84*

n=6, * P<0.05; Data analysed by ANOVA followed by Dunnett's test.
All groups compared with carbon tetrachloride (1.25 ml/kg) alone

Animals in Liv 52® (400 mg/kg p.o.) therapeutic (15 days) revealed significant decrease in liver weight and liver volume when compared with carbon tetrachloride intoxicated group. Histology showed central vein with liver cells showing fatty and hydropic changes whereas peripheral cells showed very mild changes.

3.3 Functional Parameter

Administration of pentobarbitone sodium induced sleep in control group. Administration of extract or Liv 52® alone did not alter pentobarbitone sleeping time (PST). Carbon tetrachloride administration significantly increased PST when compared with control group. Administration of pentobarbitone sodium in both prophylactic and therapeutic treatment groups of the extract and Liv 52® resulted in significant reduction in duration of sleep as compared with carbon tetrachloride intoxicated group indicating normal restoration of liver microsomal drug metabolizing enzyme function.

4. Discussion

Carbon tetrachloride is one of the routinely used hepatotoxin [5]. The available reports indicate that carbon tetrachloride mediates the changes in the functions of liver ultimately leading to damage the hepatocellular membrane. It can induce hepatic damage in rat at a very low dose of 1.25 ml/kg/i.p. [5].

In the present investigations, it was observed that carbon tetrachloride significantly increased the levels of AST, ALT, ALP and cholesterol On the other hand, significant lowering of TP was observed. These results are in agreement with the earlier reports [7-10].

In the normal rats, administration of extract did not alter the normal biochemical parameters indicating that the leaf extract is devoid of any harmful effects on the liver when given alone. On the other hand, maintenance of normal levels of biochemical parameters after prophylactic treatment for 7 and 15 days indicated hepatoprotective effect. Similarly, Liv 52® also exhibited the hepatoprotective effect.

Present study demonstrates similarity in decreasing the biochemical parameters like AST, ALT, ALP and cholesterol when both extract or Liv 52® were administered prophylactically. Study of physical parameter and histopathology of liver confirmed these findings.

In other set of experiments, carbon tetrachloride was first administered to induce hepatic damage. Administration of extract (400 mg/kg p.o.) or Liv 52® (400 mg/kg p.o) in these groups restored the various biochemical, physical and functional parameters towards normal level indicating therapeutic effectiveness of these drugs. Increase in liver weight and liver volume was absent in extract or Liv 52® treated rats.

Further supporting evidence of hepatoprotective activity by extract or Liv 52® was seen in pentobarbitone sleeping time. Liver plays an important role in metabolism of pentobarbitone. Liver damage induced by chloroform or in hepatectomized animals the action of pentobarbitone is prolonged [11]. Free radical from carbon tetrachloride initiates hepatic damage [12].

In present investigation, carbon tetrachloride intoxication significantly increased the pentobarbitone sleeping time (PST) corroborating hepatic damage. On the other hand, significant reduction in pentobarbitone sleeping time in both prophylactic and therapeutic treatment with extract or Liv 52® indicated normal functioning of the liver-metabolising enzyme.

The result indicated hepatoprotective activity of extract and Liv 52®. Comparison of various

biochemical parameters indicated that Liv 52® was more effective hepatoprotective drug compared to the aqueous extract of leaves of *Feronia elephantum.*

5. Conclusion

It is thus concluded that the whole macerated aqueous extract of the leaves of *Feronia elephantum* Correa showed hepatoprotective effect against carbon tetrachloride induced hepatic damage. Further study on isolation and characterization of phytochemical constituents may lead to a development of lead nucleus for hepatic dysfunction.

6. Acknowledgement

We thank Dr. Agarwal R.V., Dr. Bhatia A. L. and Mrs. Desai S. V., Bharati Vidyapeeth Deemed University Medical College & Hospital, Dhankawadi, Pune-411 043 for expert opinion of histopathology.

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