

Hepatoprotective Effects of *Artemisia Scoparia* against Carbon Tetrachloride: An Environmental Contaminant

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Anwar-ul Hassan Gilani, Khalid Hussain Janbaz (Departments of Pharmacology and Faculty of Health Sciences, The Aga Khan University Medical College, Karachi.)

Abstract

The hepatoprotective activity of crude extract of *Artemisia scoparia* (aerial parts) was investigated against experimentally produced hepatic damage using carbon tetrachloride (CCl₄) as a model hepatotoxin. CCl₄ at the dose of 1.5 ml/kg, produced liver damage in rats as manifested by the rise in serum levels of AST and ALT to 395±110 and 258±61 IU/l (mean ±SEM; n=10) respectively, compared to control values of 106± 15 and 26±04. Pretreatment of rats with plant extract (150 mg/kg) significantly lowered (P<0.01), the respective serum GOT and GPT levels to 93±05 and 27±03 IU/l, indicating hepatoprotective action. Pentobarbital sodium (75 mg/kg)-induced sleeping time in mice was found to be 140.8± 1.5 min (n=10) which was similar (P>0.05) to that obtained in the group of animals preheated with the plant extract (139.9±1.8 min). CCl₄ treatment extended the pentobarbital sleeping time to 212.2± 19.1 min and pretreatment of animals with plant extract reversed the CCl₄-induced prolongation in pentobarbital sleeping time to 143.9±5.5 min (p<0.001) which further confirms the protective action of the plant extract against CCl₄-induced liver damage. These data indicate that the plant *Artemisia scoparia* is hepatoprotective and validate the folkloric use of this plant in liver damage (JPMA 44:65, 1994).

Introduction

Carbon tetrachloride (CCl₄), a potent hepatotoxin, has been used extensively as a cleansing agent, fire extinguisher, grain fumigant, vermifuge, solvent and as an intermediate in chlorofluorocarbon synthesis. Its toxicity usually follows inhalation of the vapours in a poorly ventilated environment, ingestion of contaminated food (especially grains) and ground water¹. Single exposure can rapidly lead to severe centrilobular necrosis and steatosis², whereas chronic or intermittent exposure may cause cirrhosis³ and even hepatic malignancy⁴. In modern therapeutics, hepatic damage is regarded as a sort of medical emergency because in the absence of specific therapy one has to be contented upon conservative treatment. On the other hand, traditional healers claim certain valuable recipes based on plants constituents showing preventive as well as curative potential. One such example, is *Artemisia scoparia* Thunb. (Family: Compositae; local name: Dona Than), having a folkloric reputation for its usefulness in the treatment of jaundice and other liver disorders⁵. More recently, we have reported that the crude extract of *Artemisia scoparia* afforded protection against acetaminophen-induced hepatotoxicity in rats⁶. In the present investigation, we have tested this plant extract against carbon tetrachloride (CCl₄)-induced liver injury to assess whether it also affords protection against a commonly encountered environmental hepatotoxin with a different mode of hepatocellular damage⁷. This can also be a step forward to help elucidate the possible mechanism of its hepatoprotective action.

Plant material:

Whole *Artemisia scoparia* plants were collected during the month of April, 1991 from the rural area around Thatta District in the province of Sindh, Pakistan and authenticated with the help of a botanist at the University of Karachi. The plant material was shade dried, powdered and macerated in 80% aqueous-methanol (BDH Ltd., Poole, England) for 1 week with occasional shaking. The extract was

filtered and concentrated to dark greenish brown residue under reduced pressure on a rotary evaporator.

Pharmacological materials and animals:

CCl₄, ketamine hydrochloride and pentobarbital sodium were obtained from Sigma Chemicals Company, St. Louis, Mo, USA and olive oil (P. Sasso e Figili, Oneglia, Italy) was purchased from local market. Swiss male mice (20-25g) and male albino Wistar rats (200-250g) housed at the animal house of The Aga Khan University, were used for this study. The animals were housed in air conditioned quarters and had free access to tap water and food.

Induction of hepatic Injury:

Hepatic injury was produced by oral administration of 1.5 ml/kg CCl₄ diluted upto 20% by olive oil and the animals in the control group received an equal volume of olive oil.

Multiple dose treatment in rats:

Rats were divided into three groups of 10 animals each. Group 1 served as vehicle control and received normal saline (3 ml/kg) and olive oil (7.5 ml/kg) orally. Group 2 served as toxic control and was given 4 doses of normal saline at 12 hours intervals and CCl₄ was then administered orally 1 hour post-treatment of the last dose. Group 3 was treated similar to group 2 except that plant extract (150 mg/kg) was administered instead of saline. Animals were anaesthetized with ketamine (100 mg/kg, i.m.) 24 hours after the last treatment and blood (3 ml) was collected by cardiac puncture using sterile disposable syringes. Serum was separated by centrifugation (3000 rpm, for 15 min) and serum glutamate oxaloacetate transaminase (AST) and glutamate pyruvate transaminase (ALT) were estimated on the same day spectrophotometrically using Merck diagnostic kits.

Modification of pentobarbital-induced sleeping time:

The direct effect of plant extract on pentobarbital induced sleeping time and effect of plant extract on CCl₄-induced prolongation of pentobarbital sleeping time was studied in mice by using method described by Montila and colleagues⁸, as shown in Table. To test the direct effect of plant extract on pentobarbital- induced sleeping time, animals were divided into two groups of 10 animals each. Group 1 receive. Direct effect of artemisia scoparia on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time in mice normal saline (3 ml/kg) while group 2 was given plant extract (150 mg/kg) as a single oral dose and pentobarbital (75 mg/kg, i.p.) was then administered after 1 hour to both the groups. To assess the effect of plant extract on CCl₄-induced prolongation of pentobarbital sleeping time, 2 more groups were added to the study and were treated as follows: Group 3 received 4 doses of normal saline orally at 12 hrs intervals and CCl₄ was administered as a bolus dose (1.5 ml/kg) 1 hr post-treatment of the last dose o. saline followed after 24 hrs by pentobarbital (75 mg/kg, i.p.). Animals in group 4 were treated similarly to group 3 except that plant extract (150 mg/kg) was substituted for normal saline.

Statistical analysis:

The results are expressed as Mean S.E.M. and all statistical comparisons were made by means of Student's t-test and P <0.05 was regarded as a significant.

Results

Effect of plant extract on CCl₄-induced toxicity:

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 106±15 and 26.04 IU/L respectively (Figure 1),

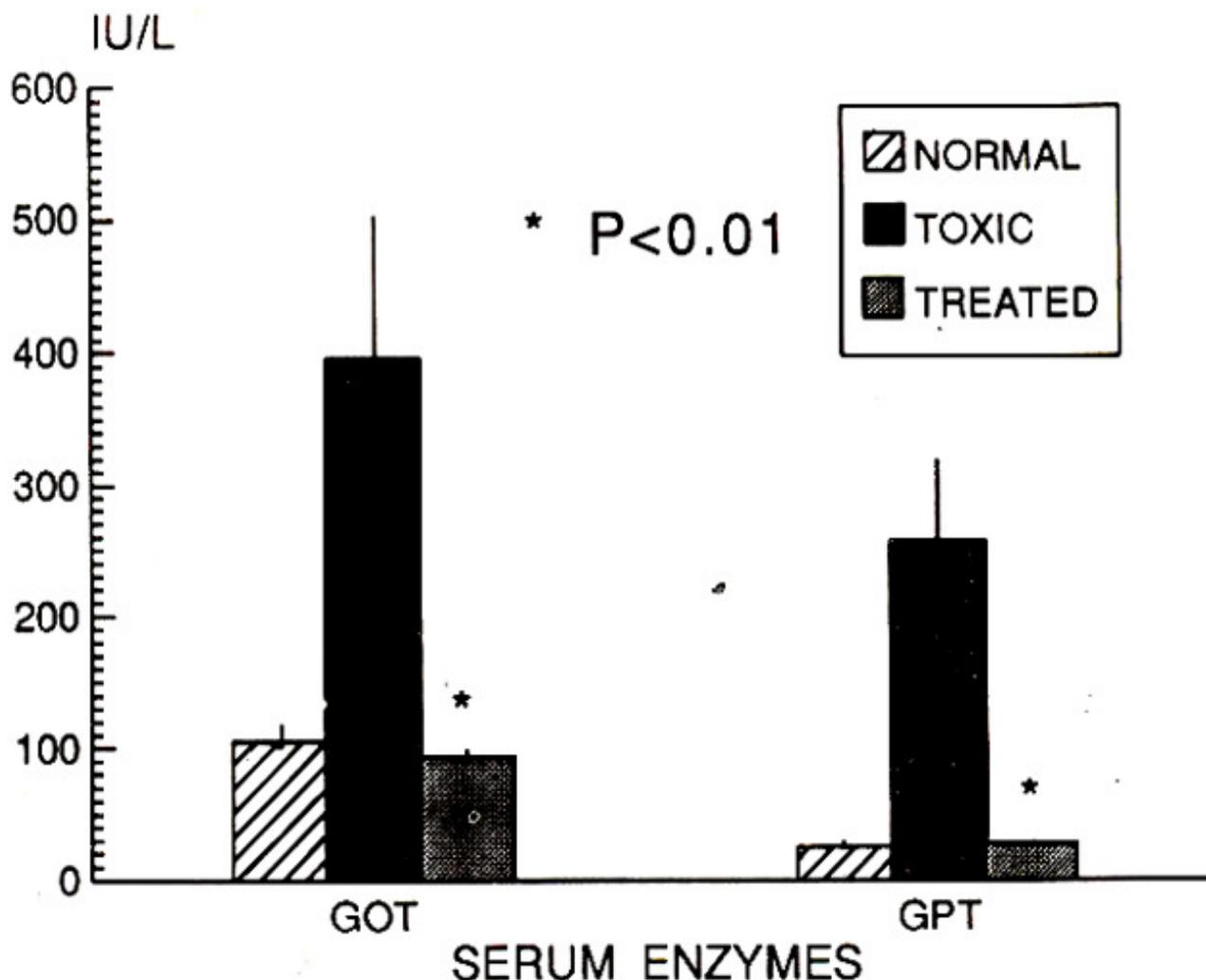


Figure. Effect of crude extract of *artemisija scoparia* on CCl₄- induced rise in serum transaminases (AST and ALT) in rats.

while a toxic dose of CCl₄ (1.5 ml/kg) raised significantly ($P < 0.01$), the respective serum enzyme values to 395 ± 110 and 258 ± 61 IU/L. Group 3 was pretreated with plant extract (150 mg/kg, orally, twice daily for 2 days) to determine its effect on CCl₄-induced rise in serum enzymes. The serum values in pretreated group were found to be 93 ± 05 (GOT) and 27 ± 03 IU/L (GPT), which are significantly lower ($P < 0.01$) than the values of toxic control and close to normal values ($P > 0.05$).

Effect of plant extract on pentobarbital-induced sleep:

Effect of plant extract on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time was studied in mice and the results are shown in Table.

Table. Direct effect of artemisia scoparia on pentobarbital sleeping time as well as on CCl₄-induced prolongation of pentobarbital sleeping time in mice.

Group	Treatment	Sleeping time (min)
1	Saline + pentobarbital (3 ml/kg + 75 mg/kg)	140.8 ± 1.5
2	Extract + pentobarbital (150 mg/kg + 75 mg/kg)	139.9 ± 1.8
3	Saline + CCl ₄ + Pentobarbital (3 ml/kg + 1.5 ml/kg + 75 mg/kg)	212.2 ± 19.1*
4	Extract + CCl ₄ + Pentobarbital (150 mg/kg + 1.5 ml/kg + 75 mg/kg)	143.9 ± 5.5**

Each value represents the Mean ± S.E.M. of 10 determinations. Salines/extract/CCl₄ was given orally, while pentobarbital was given intraperitoneally.

*P < 0.001 (level of significance for difference between values of groups 1 and 3).

Pentobarbital at a dose of 75mg/kg, i.p., caused sleep in mice for a period of 140.8±1.5 min (Mean±S.E.M., n= 10). Pentobarbital sleeping time in the group of animals pretreated with plant extract was found to be 139.9± 1.8 min which is similar to that in the control group (P>0.05). Whereas pretreatment of animals with CCl₄, prolonged the pentobarbital sleeping time to 212.2±19.1 min, the value that is significantly higher (P < 0.001) than that of control (Table). However, prior treatment of animals with the plant extract returned this CCl₄-induced prolongation of pentobarbital sleeping time to 143.9±5.5 min, which is close to the control sleeping time (P > 0.05).

Discussion

The crude extract of artemisia scoparia showed hepatoprotection against CCl₄-induced liver injury. The plant extract inhibited a CCl₄-induced rise in serum AST and ALT in rats. Similarly, prevention of CCl₄-induced prolongation of pentobarbital sleeping time was observed in a group of mice pre-treated with the plant extract. Liver injury induced by CCl₄ is a commonly used model for the screening of hepatoprotective drugs⁹. The rise in serum levels of AST and ALT has been attributed to the damaged structural integrity of the liver¹⁰ because these are cytoplasmic in location and are released into circulation after cellular damage¹¹. CCl₄ is metabolized by a specific isozyme of cytochrome P-450 (IIE1) to variety of chemically reactive free radical species¹². The free radical mediated toxic manifestations can effectively be minimized by cytochrome P-450 inhibitors¹⁴, GSH precursors¹⁵, free radical scavengers¹⁶, antioxidants¹⁷, sulfhydryl agents¹⁸ and Ca⁺ + channel blockers^{19,20}. The crude extract of artemisia scoparia plant used in this study seems to preserve the structural integrity of the hepatocellular membrane. This was evident from the significant reduction in the CCl₄-induced rise in serum AST and ALT levels in rats. To see whether the plant extract has inhibitory effect on hepatic microsomal drug metabolizing enzymes (MDME), it was administered with pentobarbital to mice and possible change in the duration of sleep was recorded. The duration of pentobarbital-induced sleep in intact animal is considered as a reliable index for the activity of hepatic MDME²¹. Pentobarbital is metabolized by the hepatic MDME to inactive metabolites and a drug with inhibitory effect on MDME

is expected to prolong pentobarbital sleep time. The fact that the plant extract did not modify pentobarbital sleeping time ($P > 0.05$) suggests that it is devoid of inhibitory effect on hepatic MDME such as cytochrome p-450. The damage conferred by CCl₄ to hepatocytes as well as hepatic MDME causes loss of drug metabolizing capacity of the liver, resulting in prolongation of pentobarbital-induced sleeping time²². Pretreatment of animals with plant extract prevented the CCl₄-induced prolongation of pentobarbital-sleeping time suggestive of its protective effect against CCl₄-induced damage to hepatocytes including MDME. The possible mechanism of the protective effect of artemisia scoparia would be rather speculative at this stage, however, it is clear from the results of sleeping time study that the protective effect is not mediated through inhibition of hepatic MDME. We have recently shown that the crude extract of artemisia scoparia exhibits Ca⁺⁺ channel blocking activity in isolated tissue experiments²³. Calcium content in the liver cells are increased during the process of experimental hepatic damage^{19,24} and calcium channel blocking drugs, i.e., nifedipine, diltiazem and verapamil were found to inhibit the development of hepatic damage, induced by different hepatotoxins including acetaminophen²⁵ and CCl₄. Similarly, hepatoprotective activity of the plant extract, against acetaminophen reported previously⁶ and ccl₄-induced liver damage reported in this study may be attributed to its Ca⁺⁺ channel blocking activity. Moreover, hepatoprotective action of the plant extract may also be due to the occurrence of different types of plant constituents. It is interesting that pure compounds such as rutin and other flavonoids from artemisia scoparia²⁶ have already been reported to have antioxidant, free radical scavenger and Ca⁺⁺ channel blocking-like (non-specific smooth muscle relaxant) activity in isolated tissue preparations^{27,28}, though their hepatoprotective activity was not studied. The plant extract afforded hepatoprotectivity both against acetaminophen⁶ and CCl₄ (this study) at the dose of 150 mg/kg, whereas higher dose (500 mg/kg) was required for this activity in case of other plants tested in our laboratory such as, cyperus scariosus²⁰, artemisia absinthium²⁹ and cichorium intybus³⁰. The effectiveness of the crude extract of artemisia scoparia at a dose, distinctly lower than that used for other plants may be due to the fact that this plant contains multiple constituents which may have cumulative beneficial effect in liver injury. In conclusion, this study provides the scientific basis for its traditional use in liver damage and further studies on the constituents of this plant is in progress to explore its possible mode of hepatoprotective action.

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