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Preventive and Curative Effects of *Artemisia absinthium* on Acetaminophen and CCl_4-induced Hepatotoxicity

ANWAR-UL HASSAN GILANI* and KHALID HUSSAIN JANBAZ

Department of Pharmacology, The Aga Khan University Medical College, Faculty of Health Sciences, Karachi-74800, Pakistan [Fax: (92) 21 493 4294]

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Abstract—1. Effect of aqueous-methanolic extract of *Artemisia absinthium* (Compositae) was investigated against acetaminophen- and CCl_4-induced hepatic damage.

2. Acetaminophen produced 100% mortality at the dose of 1 g/kg in mice while pretreatment of animals with plant extract (500 mg/kg) reduced the death rate to 20%.

3. Pretreatment of rats with plant extract (500 mg/kg, orally twice daily for two days) prevented ($P < 0.01$) the acetaminophen (640 mg/kg) as well as CCl_4 (1.5 ml/kg)-induced rise in serum transaminases (GOT and GPT).

4. Post-treatment with three successive doses of extract (500 mg/kg, 6 hr) restricted the hepatic damage induced by acetaminophen ($P < 0.01$) but CCl_4-induced hepatotoxicity was not altered ($P > 0.05$).

5. Plant extract (500 mg/kg) caused significant prolongation ($P < 0.05$) in pentobarbital (75 mg/kg)-induced sleep as well as increased strychnine-induced lethality in mice suggestive of inhibitory effect on microsomal drug metabolizing enzymes (MDME).

6. These results indicate that the crude extract of *Artemisia absinthium* exhibits hepatoprotective action partly through MDME inhibitory action and validates the traditional use of plant in hepatic damage.

Key Words: *Artemisia absinthium*, preventive, curative, acetaminophen, CCl_4, hepatotoxicity

INTRODUCTION

Artemisia absinthium Linn. (Family: Compositae), commonly known as "Wormwood" or "Vilayati afsanteen" is a perennial herb growing wild in northern hilly areas of Pakistan (Haq, 1983). The plant is also commonly grown in the west and recognized for its medicinal value particularly in hepatobiliary complaints and in helminth infections (Keville, 1991). The herbal material (leaves and flowering tops) is regarded as anthelmintic, antiseptic, febrifuge and stomachic in the indigenous system of medicine and has been employed successfully to alleviate chronic fever, dyspepsia and hepatobiliary ailments (Nadkarni, 1976; Said, 1982).

The plant has undergone extensive phytochemical investigations and the presence of a variety of chemical constituents such as ascorbic acid (Klyshev and Alyukina, 1971; Slepetys, 1975), flavonoids (Hoffmann and Herrmann, 1982), carotenoids (Sergeeva and Zakharova, 1977), tannins (Slepetys, 1975) and lignans (Greger and Hofer, 1980) have been identified. Similarly, the phytopharmacological evaluation showed the presence of anti-inflammatory (Sommer *et al.*, 1965), antipyretic (Ikram *et al.*, 1987), antifertility (Rao *et al.*, 1988), antibacterial (Kaul *et al.*, 1976), antifungal (Maruzzella *et al.*, 1960), anti-helmintic (Caius and Mahasker, 1920), antimollusk (Gurevich, 1948), anti-amoebic (Tahir *et al.*, 1991) and antimalarial (Hernandez *et al.*, 1990; Zafar *et al.*, 1990) activities.

However, scientific studies on its usefulness in liver damage are few and the aim of the present study was to confirm and validate the traditional hepatic efficiency of *A. absinthium* by using different animal models of hepatotoxicity.

*To whom all correspondence should be addressed.

MATERIALS AND METHODS

Plant extract

Artemisia absinthium (Aerial parts) were purchased from local herbal stores and authenticated with the help of a botanist at The University of Karachi. The plant material was powdered and macerated in 80% aqueous-methanol (BDH Ltd. Poole, U.K.) for one week with occasional shaking. The extract was filtered and concentrated to dark greenish brown residue under reduced pressure on a rotary evaporator, with an approximate yield of 8%.

Pharmacological materials and animals

Acetaminophen (acetaminophen, 4-hydroxy acetanilide), CCl_4, ketamine hydrochloride, pentobarbital sodium and methylcellulose were obtained from Sigma Chemical Company, St Louis, MO, U.S.A. and olive oil (P. Sasso e Figili, Oneglia, Italy) was purchased from a local market. Acetaminophen and CCl_4 were suspended in 1% methylcellulose (50 mg/mL) and olive oil (20% v/v) respectively.

Swiss male mice (20–25 g) and male albino Wistar rats (200–250 g) housed at the Animal House of The Aga Khan University, maintained at 23–25°C were used for this study.

Lethality study in mice

Preliminary experiments were performed on mice to estimate the protective effect of plant extract against lethal dose of acetaminophen (1 g/kg). Animals were divided into 2 groups of 10 animals each. One group was treated orally with plant extract (500 mg/kg) followed after 1 hr by oral administration of acetaminophen (1 g/kg). The second group served as a control and received the same treatment except that normal saline (0.9% NaCl) was administered instead of plant extract. The mortality was observed for 48 hr post-administration of acetaminophen.

Induction of hepatic injury

Hepatic injury in rats was induced separately by oral administration of acetaminophen (640 mg/kg) as well as CCl_4 (1.5 mL/kg). The control animals received an equal volume of vehicle.

Multiple dose pre-treatment in rats

Rats were divided into 3 groups of 10 animals each. Group 1 served as vehicle control and received normal saline (10 mL/kg) and vehicle (i.e. 1% methylcellulose; 13 mL/kg) orally. Group 2 was given 4 doses of normal saline at 12 hr intervals and acetaminophen was administered orally 1 hr post-treatment of the last dose. Group 3 was treated similarly to that of group 2, except that plant extract (500 mg/kg, dissolved in 10 mL saline) was administered instead of saline.

In a parallel study on 3 similar groups of rats ($n = 10$), the treatment remained the same as mentioned in the above study except that acetaminophen was replaced by CCl_4 and consequently the vehicle was also changed to olive oil (7.5 mL/kg).

Animals were anaesthetized with ketamine (100 mg/kg, i.m.) 24 hr after the last treatment and blood (3 mL) was collected by cardiac puncture using sterile disposable syringes. Serum was separated by centrifugation (3000 r.p.m., for 15 min) and serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were estimated on the same day spectrophotometrically using Merck diagnostic kits.

Multiple dose post-treatment in rats

Rats were divided into 3 groups of 10 animals each. Group 1 served as vehicle control and received vehicle (1% methylcellulose; 13 mL/kg), followed by normal saline orally. Group 2 was given acetaminophen orally at 0 hr and then after every 6 hr, normal saline (10 mL/kg) was administered orally until 24 hr of toxin administration. Group 3 was treated similarly to group 2, except that plant extract (500 mg/kg, dissolved in 10 mL saline) was administered instead of saline.

In another study on 3 similar groups of rats ($n = 10$), the same protocol was followed except the toxin (acetaminophen) was replaced by CCl_4 and olive oil served as vehicle. All other procedures i.e. blood collection, serum separation and enzyme estimation were performed at 24 hr as mentioned above.

Pentobarbital-induced sleeping time in mice

The effect of plant extract on pentobarbital-induced sleeping time was studied in mice as described by Montilla and colleagues (1990). Animals were divided into 2 groups of 10 animals each (Table 1). Group 1 received normal saline (10 mL/kg), while group 2 was given plant extract (500 mg/kg) as a single oral dose and pentobarbital

Table 1. Effect of pretreatment with methanolic extract of *Artemisia absinthium* on pentobarbital sleeping time in mice

Treatment	Sleeping time (min)
Control (Vehicle + pentobarbital) (10 ml/kg + 75 mg/kg)	81 ± 02
Treated (Extract + pentobarbital) (500 mg/kg + 75 mg/kg)	117 ± 16*

Each value represents mean ± SEM of 10 determinations.
*$P < 0.05$.

(75 mg/kg, i.p.) was then administered after 1 hr to both the groups.

Strychnine-induced lethality in mice

Animals were divided into 2 groups of 10 mice each. One group was given vehicle (1% methylcellulose; 10 mL/kg; orally) followed after 1 hr by a sublethal dose of strychnine (0.4 mg/kg). The animals in group 2 were given similar treatment except vehicle was replaced by plant extract (500 mg/kg). The animals were monitored for the next 2 hr to count mortalities.

Acute toxicity

Different groups of 5 mice each were given graded doses of plant extract (0.5–4.0 g/kg, orally) and were kept under constant observation for 6 hr to note any behavioural changes and mortality was recorded after 24 hr of drug administration.

Statistical analysis

The results are expressed as mean ± SEM and all statistical comparisons were made by means of Student's t-test; $P < 0.05$ was regarded as significant.

RESULTS

Effect on acetaminophen-induced lethality

Acetaminophen at a dose of 1 g/kg induced 100% lethality in mice. In a group of animals pretreated with plant extract (500 mg/kg), the same dose of acetaminophen killed only 2 out of 10 animals resulting in 80% protection against lethal effect of acetaminophen.

Preventive effect on hepatotoxicity

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 98 ± 11 and 39 ± 08 IU/L, respectively (Fig. 1, top panel), while a toxic dose of acetaminophen (640 mg/kg) raised significantly ($P < 0.01$), the respective serum enzyme values to 1424 ± 454 and 741 ± 217. Group 3 was pretreated with plant extract (500 mg/kg, orally, twice daily for 2 days) to determine its effect on acetaminophen-induced rise in serum enzymes. The serum values of transaminases in pretreated group were found to be 85 ± 18 (GOT) and 34 ± 08 (GPT), which are significantly lower ($P < 0.01$) than the values of toxic control and were similar to the control values ($P < 0.05$).

In another set of experiments, the normal values of serum GOT and GPT in rats were found to be 106 ± 15 and 45 ± 11 IU/L, respectively (Fig. 1, bottom panel), which were raised significantly ($P < 0.05$) to respective values of 494 ± 155 and 305 ± 83 after administration of a toxic dose of CCl_4 (1.5 mL/kg). However, pretreatment of animals with plant extract (500 mg/kg, orally, twice daily for 2 days) returned the serum GOT and GPT values to 139 ± 18 and 76 ± 27 IU/L respectively, which are significantly lower ($P < 0.05$) than values of toxic control and were close to the control values ($P > 0.05$).

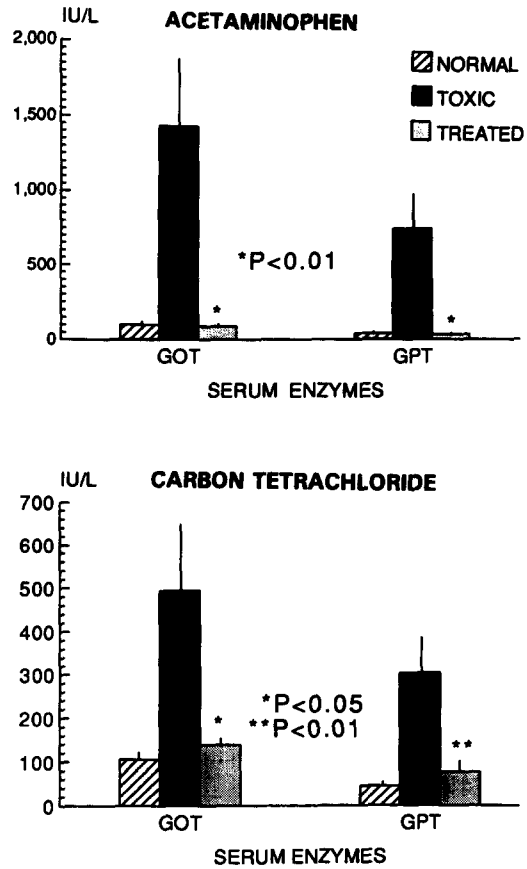

Fig. 1. Influence of pretreatment with methanolic extract of *A. absinthium* on acetaminophen- and CCl_4-induced rise in serum transaminases (GOT and GPT) levels in rats. Each bar represents mean ± SEM of 10 determinations. */**Compared with toxic control group.

Curative effect on hepatotoxicity

Control (saline + vehicle) serum values of GOT and GPT in rats were found to be 108 ± 22 and 42 ± 11 IU/L ($n = 10$), respectively, (Fig. 2, top panel), while a toxic dose of acetaminophen (640 mg/kg) raised significantly ($P < 0.001$), the respective serum enzyme values to 1125 ± 281 and 833 ± 195. The treatment with the plant extract (500 mg/kg, orally) was started to group 3 animals (6 hr after acetaminophen administration) to evaluate its curtailing effect upon the acetaminophen-induced progression of hepatic damage duly monitored by

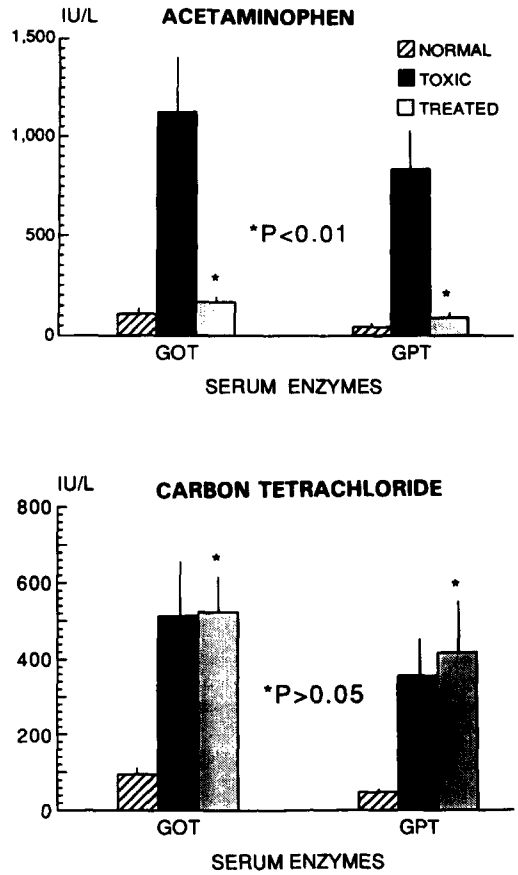

Fig. 2. Influence of post-treatment with methanolic extract of *A. absinthium* on acetaminophen- and CCl_4-induced rise in serum transaminases (GOT and GPT) levels in rats. Each bar represents mean ± SEM of 10 determinations. *Compared with toxic control group.

serum transaminases. The serum values of enzymes in the post-treated group were found to be 167 ± 28 (GOT) and 88 ± 26 (GPT), which were significantly lower than the serum values of toxic control group ($P < 0.01$) and were comparable with the normal values ($P > 0.05$).

The administration of toxic dose of CCl_4 (1.5 mg/kg, orally) raised significantly ($P < 0.05$) the serum values of GOT and GPT to 511 ± 165 and 353 ± 101 IU/L, respectively, compared to respective control values of 95 ± 13 and 48 ± 10 (Fig. 2, bottom panel). The group 3 animals were treated with multiple doses of plant extract, following CCl_4 intoxication. The serum values in the treated group were found to be 523 ± 94 (GOT) and 415 ± 130 (GPT), which were similar to the values of the toxic control group ($P < 0.05$) and higher than those of normal values ($P > 20.05$).

Effect on pentobarbital-induced sleep

Effect of plant extract on pentobarbital sleeping time was studied in mice and the results are shown in Table 1. Pentobarbital at a dose of 75 mg/kg, i.p., caused sleep in mice for a period of 81 ± 02 min (mean ± SEM, $n = 10$), whereas the sleeping time in the group of animals pretreated with plant extract was found to be 117 ± 16 min, which was significantly higher than that in the control group ($P < 0.05$).

Interaction with strychnine

In preliminary experiments, the median lethal dose (LD_{50}) of strychnine in mice was found to be 0.9 mg/kg, whereas 0.4 mg/kg was proved as a sub-lethal dose. However, pretreatment with a single dose (500 mg/kg) of plant extract 1 hr prior to strychnine administration potentiated the effect of strychnine causing almost 60% mortality rate.

The plant extract up to an oral dose of 4 g/kg was found to be devoid of any lethal effect and no apparent behavioural change was observed.

DISCUSSION

Acetaminophen- and CCl_4-induced hepatic injuries are commonly used models for the screening of hepatoprotective drugs (Slater, 1965; Plaa and Hewitt, 1982) and the extent of hepatic damage is assessed by the level of released cytoplasmic transaminases (GOT and GPT) in circulation (Chenoweth and Hake, 1962; Sallie *et al.*, 1991). The aqueous-methanolic extract of *A. absinthium* when administered prophylactically, exhibited protection against both acetaminophen- and CCl_4-induced liver injuries as manifested by the reduction in toxin-mediated rise in serum transaminases in rats as well as protection against lethal doses of acetaminophen in mice.

Both acetaminophen and CCl_4 share the common property of being converted into their respective reactive metabolites *N*-acetyl-*p*-benzoquinoneimine (NAPQI) and halogenated free radicals (HFR) by hepatic cytochrome *P*-450 (Packer *et al.*, 1978; van de Straat *et al.*, 1987). The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and α-tocopherol, etc.), ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes (Pesh-Imam and Recknagel, 1977; Aldridge, 1981).

The inhibitors of microsomal drug metabolizing enzymes (MDME) can impair the bioactivation of acetaminophen and CCl_4 into their respective reactive species and thus provide protection against the prevailing hepatocellular damage (Castro *et al.*, 1974; Nelson *et al.*, 1980). Since the MDME inhibitory activity is reported to be common in medicinal plants (Shin, 1989), the plant extract was subjected to

pentobarbital sleep study to see if it also exhibits inhibitory effects on MDME. The duration of pentobarbital-induced sleep in intact animals is considered as a reliable index for the activity of hepatic MDME (Conney, 1967). Pentobarbital is metabolized by the hepatic MDME to inactive metabolites and any drug with inhibitory effect on MDME is likely to prolong pentobarbital-induced sleeping time (Fujimoto *et al.*, 1960).

The pretreatment of animals with plant extract resulted in the prolongation of pentobarbital sleeping time ($P < 0.05$), therefore, it is not unreasonable to speculate that the plant extract might contain MDME inhibitory constituents that cause hepatoprotection. However, sleep potentiation of pentobarbital can also be achieved by CNS depressing drugs without alteration in MDME activity (Shin, 1989). Strychnine toxicity test was performed to see whether the plant extract mediated potentiation of pentobarbital sleep is due to enzyme inhibitory action or sedative effect. The strychnine is a substrate for MDME (Adamson and Fourts, 1959) and most known inhibitors of MDME increase the toxicity of strychnine through potentiation of its CNS stimulant activity (Kato, 1968). The observed mortality at the sub-lethal dose of strychnine is suggestive of the potentiating effect of the plant extract and confirms its inhibitory effect upon MDME.

It is reported earlier that the compounds with a methylene-dioxybenzene group are likely to exhibit an inhibitory effect on MDME (Anders, 1968). The literature survey revealed the presence of sesartemin in this plant (Ahmad *et al.*, 1986) and interestingly, this compound carries methylene-dioxybenzene group in its structure (Greger and Hofer, 1980), thus suggesting that the possible inhibitory effect of plant extract on MDME may be due to the presence of sesartemin as a plant constituent. The exact mode of hepatoprotective action of the plant extract may be speculative at this stage but these results indicate that the possible presence of enzyme inhibitory effect may be partially responsible for the hepatoprotective effect of plant extract.

We have recently found that the crude extract of *A. absinthium* exhibits calcium channel blocking activity in isolated tissue experiments (Gilani, 1994). Calcium contents in the liver cells are increased during the process of experimental hepatic damage (Moore *et al.*, 1985) and the plants such as *Artemisia scoparia* (Gilani and Janbaz, 1993; Gilani *et al.*, 1994a), *Cyperus scariosus* (Gilani and Janbaz, 1995b; Gilani *et al.*, 1994c) and *Rubia cordifolia* (Gilani *et al.*, 1994b; Gilani and Janbaz, 1995a) have recently been reported to share calcium channel blocking (CCB) and hepatoprotective activities. Similarly, presence of CCB activity in the crude extract of *A. absinthium* might have also contributed to its hepatoprotective activity reported in this study.

The inhibitors of MDME can provide protection against the hepatotoxicity only when they are given before the metabolic activation of the hepatotoxin and fails to provide any protection after generation of reactive metabolites. Following ingestion, acetaminophen and CCl_4 are metabolized to their respective reactive species within 6 hr (Bramanti *et al.*, 1978; Akintonwa and Essien, 1990) and hepatotoxicity can be monitored by measuring serum transaminases at 24 hr. The plant extract treatment started 6 hr after the acetaminophen administration inhibited the spread of hepatic damage as manifested by insufficient release of cytoplasmic transaminases.

The observed curative effect against acetaminophen may be attributed to the reported presence of flavonoids (Hoffmann and Hermann, 1982), ascorbic acid (Klyshev and Alyukina, 1971; Slepetys, 1975), carotenoids (Sergeeva and Zakharova, 1977), tannins (Slepetys, 1975) and lignans (Gregor and Hofer, 1980) among the plant constituents. The flavonoids are known to be anti-oxidants (Torel *et al.*, 1986; Faurè *et al.*, 1990), free radical scavengers (Bors and Saran, 1987; Hussain *et al.*, 1987) and antilipoperoxidant (Younes and Siegers, 1981; Robak *et al.*, 1986; Ratty and Das, 1988) leading to hepatoprotection (Kiso *et al.*, 1984; Handa *et al.*, 1986). Similarly, ascorbic acid serves as anti-oxidant (Demopoulos, 1973; Bus and Gibson, 1984), inhibits covalent binding of NAPQI to vital macromolecules (Lake *et al.*, 1981) and consequently can minimize toxic damage (Harman, 1985). Moreover, carotenoids are also reputed to be antioxidants (Kläui, 1982) and thus showing anti-hepatotoxic activity (Oshima *et al.*, 1984). Furthermore, the hepatoprotective potential of tannins (Hikino *et al.*, 1985) as well as lignans (Faurë *et al.*, 1990) is also well documented.

However, the anomalous observation due to inability of extract treatment to curtail progression of hepatic damage after CCl_4 activation can partly be justified on the basis of reported facts. The acetaminophen toxicity following NAPQI generation is chiefly due to oxidative stress and can effectively be ameliorated by antioxidants (Harman, 1985), whereas, the hepatic damage due to HFR may be due to lipid peroxidation (Bus and Gibson, 1979) as well as alkylation (Dogterom *et al.*, 1988). The possible presence of antioxidant and antilipoperoxidant activities to protect against NAPQI, can only inhibit the lipid peroxidation process but are unable to prevent the alkylation process due to HFR (Poli *et al.*, 1989).

In summary the crude extract of *A. absinthium* affords protection against acetaminophen and CCl_4-induced liver damage in rats as well as in mice when given before the metabolic activation of toxins. However, the protection against the metabolically generated reactive species is effective only against the acetaminophen. The hepatoprotective action may be mediated through inhibition of hepatic MDME, presence of certain antioxidants and/or calcium channel blocker(s). The plant material is safe as is obvious by the lack of any symptom of acute toxicity at an oral dose of as high as 4 g/kg and has also been reported safe after chronic treatment (Schmahl, 1956). Thus, this study provides scientific basis for the traditional use of *A. absinthium* plant in hepatobiliary diseases.

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