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Hepatoprotective Effect of *Vitex negundo* against Carbon Tetrachloride-Induced Liver Damage

Y. Avadhoot and A. C. Rana
College of Pharmacy, Shri Govindram Seksaria Institute of Technology & Science, Park Road, Indore, M.P. 452-003, India
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Abstract □ Alcoholic extract of the seeds of *Vitex negundo* Linn. was obtained by cold maceration. A dose of 250 mg/kg (1/6 of LD_{50}) of the extract was selected to study the hepatoprotective action against carbon tetrachloride-induced liver damage. The extract was found to be effective in preventing liver damage which was evident by morphological, biochemical and functional parameters.

Keywords □ *Vitex negundo*, hepatoprotective, carbon tetrachloride.

The leaves of *Vitex negundo* Linn. are claimed to be aromatic, vermifuge and useful in dispersing the swelling of joints from acute rheumatism[1]. Banerji *et al.*[2] have isolated 4,4′-dimethoxy-*trans*-stilbene and flavonoids from the leaves and twigs of this plant. Bhargava[3,4] has reported antifertility activity of the flavonoids of the seeds of *V. negundo* and estrogenic effect of the flavone (5,7,3′-trihydroxy-6,8,4′-trimethoxy flavone). Vohra *et al.*[5] have reported that the alcoholic extract of the seeds inhibited ovulation.

V. negundo has been claimed to be useful in liver ailments[6]. The present study was, therefore, undertaken to evaluate its efficacy in preventing liver damage induced by carbon tetrachloride.

EXPERIMENTAL METHODS

Hepatoprotective action of the alcoholic extract of the seeds of *V. negundo* (=AVN) was studied in albino rats of either sex weighing 60~80g. These animals were weighed and divided into three groups of 6 animals each.

Group A: Normal animals untreated with CCl_4 or AVN.

Group B: Control animals treated with CCl_4 only

Group C: AVN treated animals

Damage was produced by using CCl_4 (1 ml/kg, with equal volume of liquid paraffin, *s.c.*) twice weekly, for a period of eight weeks. A 5% suspension of AVN was prepared with 1% carboxymethyl cellulose (CMC) in distilled water. The animals of group C were treated with 250 mg/kg of AVN *p.o.* daily for eight weeks. The animals also received simultaneously, subcutaneous injection of CCl_4 twice weekly for a period of eight weeks. The animals of group B were injected *s.c.* with CCl_4, twice weekly for eight weeks.

Assessment of damage and efficacy of AVN was evaluated with the help of three parameters[7].

1. Morphological parameters which include changes occurring in the weight of the animals, weight and volume of the liver.
2. Biochemical parameters *viz.* serum enzyme level of alanine transferase *i.e.* SGPT and alkaline phosphatase test.
3. Functional parameters: Pentobarbitone sleeping time test[8,9].

Pentobarbitone sleeping time test

The test was performed to assess the efficacy of functioning liver cells. Pentobarbitone sodium (50 mg/kg, *i.p.*) was given to the animals of each group. Sleeping time (time interval between loss and gain of righting reflex) was determined.

Collection of blood for biochemical estimation

All the animals were injected with 100 units of heparin sodium *s.c.* after 30 minutes the animals

Table I. Evaluation of hepatoprotective activity of alcoholic extract of seeds of *Vitex negundo*

Parameters	Group A normal animals mean± *s.e.*	Group B CCl_4 control mean± *s.e.*	Group C AVN treated mean± *s.e.*
Change in animal weight (g/100 g)	116.66± 1.28	120.18± 3.40*	115.10± 2.38*
Liver weight (g/100 g)	6.47± 1.16	8.16± 2.38*	6.53± 1.20*
Liver volume (ml/100 g)	5.71± 2.48	7.56± 3.12*	5.25± 1.42*
SGPT (units/l)	231.50± 6.94	542.66± 4.27ⁿ	245.0 ± 11.38#
Alkaline phosphatase (units/l)	407.20± 4.72	507.50± 3.50ⁿ	428.12± 6.48#
Sleeping time (min)	109.33± 2.51	138.0 ± 2.64ⁿ	89.14± 4.33#
Necrosis	absent	present	absent

There were 6 animals in each group.
*: The difference is statistically insignificant ($p>0.05$) when compared with group A.
#: The difference is statistically significant ($p<0.001$) when compared with group B.
ⁿ: The difference is statistically significant ($p<0.001$) when compared with group C.

were anesthetized with ether. Four ml of the blood was collected with a syringe in heparinized sample tubes by direct heart puncture[10]. The samples were immediately used for biochemical estimation. Liver was removed. The organ was observed carefully for any change in appearance and weighed. Its volume was measured by water displacement method.

RESULTS

The results of various observations are recorded in Table I.

Morphological parameters

Percent change in the weight of the animals among different groups after eight weeks study was found to be insignificant. Also, insignificant changes ($p>0.05$) were observed in morphological parameters (liver weight and volume) between the CCl_4 control group and the normal group. Insignificant changes ($p>0.05$) in liver weight and volume were observed between the group C (AVN treated) and the normal group (Group A).

Biochemical parameters

At the end of eight weeks there was a pronounced rise ($p<0.001$) in SGPT and alkaline phosphatase activity in the CCl_4 treated control group, when compared with those of the normal group.

In the AVN treated group SGPT and alkaline phosphatase level though higher than normal, were significantly less as compared to those of the CCl_4 treated control group ($p<0.001$).

Functional parameters

Sleeping time in the CCl_4 treated group was significantly more ($p<0.001$) than the normal group. In the AVN treated group sleeping time was significantly less than those of the normal and control group.

DISCUSSION

The claims put forth for efficacy of plant products as hepatoprotective agents prompted us to undertake the present study. The increase in pentobarbitone sleeping time, rise in SGPT and alkaline phosphatase level in the group B indicates the extent of liver damage.

In the group C, however, these values were closer to normal range indicating normalization of the liver function. The animals of this group also showed significant decrease in sleeping time as compared to that of the control group, indicating indirectly improvement in the functioning of the liver cells.

The hepatoprotective activity of the plant *V. negundo* can be well correlated with the phytoconstituents like flavonoids, which as a class have been shown to possess this property.

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