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Hepatoprotective activity of methanol extract of *Asteracantha longifolia* in rats

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Indu Tripathi

Dr. Indu Tripathi received her B.V.Sc & A.H in 2005 and M.V.Sc. in 2007 in Veterinary Pharmacology & Toxicology from the Veterinary College, Hebbal, Bangalore, Karnataka Veterinary, Animal & Fisheries Sciences University, Bidar, Karnataka. Currently she is pursuing her PhD in Veterinary Pharmacology and Toxicology at Veterinary College, Hebbal, Bangalore.

Indu Tripathi[1], Shridhar, N.B[2], Jayakumar, K[3], Sathyanarayana, K[4], Sathyanarayana, M.L[5], Vinay Tikare [6] and Murthy, G.S[7].

1,2,3,6 Department of Veterinary Pharmacology and Toxicology, Veterinary College, KVAFSU, Hebbal, Bangalore-560 024

4 Department of Animal Husbandry Extension, Veterinary College, KVAFSU, Hebbal, Bangalore-560 024

5 Department of Veterinary Pathology, VeterinaryCollege, KVAFSU, Hebbal, Bangalore-560 024

7 Herbal Science Trust, Bangalore

Corresponding author:
Dr. N.B. Shridhar MVSc, PhD.
Associate Professor, Department of Veterinary Pharmacology and Toxicology
Veterinary College, KVAFSU, Hebbal, Bangalore-560 024, India
Phone: 0944805977, Email: sridhar_vet@rediffmail.com

Abstract

The study was conducted to assess the hepatoprotective activity of *Asteracantha longifoli*a in carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. The hepatoprotective effect of methanolic extract of whole plant of *Asteracantha longifolia* was studied by monitoring serum aspartate amino transferase (AST), serum alanine amino transferase (ALT) and histopathological alterations. Pre-treatment with methanol extract of *Asteracantha longifolia* in rats treated with CCl_4 showed a significant inhibition ($P<0.001$) of rise in serum ALT and AST at all the doses tested (600, 900 or 1200 mg/kg). Histopathological studies provided the added evidence for biochemical analysis. Histopathological studies showed total recovery of liver damage caused by CCl_4 on treatment with silymarin as well as *Asteracantha* and such treated groups showed very less hepatic damage with few areas of fatty changes. These studies revealed that the methanol extract of *Asteracantha longifolia* has potent hepatoprotective activity. It showed even better efficiency than the well known hepatoprotective agent silymarin in reducing the ALT and AST concentrations in CCl_4 treated rats.

Key words: *Asteracantha longifolia,* methanol, hepatoprotective, carbontetrachloride, silymarin

Introduction

Asteracantha longifolia is described in Ayurvedic literature as Ikshura, Ikshugandha, and Kokilasha. *Asteracantha longifolia* (syn. *Hygrophila auriculata*). It is a stout herb with fasciculate, erect and sub quadrangular stem. The plant is widely distributed throughout India, Srilanka, Burma, Malaysia and Nepal. The whole plant, roots, seeds and ashes of the plant are extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction, pain, urinary infections, oedema and gout. It is classified in Ayurvedic system as Seethaveeryam, Mathuravipaka and used for the treatment of premeham (diabetes), athisaram (dysentery) etc.

(Nadkarni, 1978; Chopra *et al.,* 1986). Further, it is known for possessing antitumor (Mazumdar *et al.,* 1997; Ahmed et *al*., 2001), hypoglycemic (Fernando *et al.,* 1991) and antibacterial (Boily and Vanpuyvelde, 1986) activities. The main constituents of this plant are mucilage, fixed oil and phytosterol (Misra et *al*., 2001). However, the hepatoprotective property of *Asteracantha longifolia* is not documented very well. Hence, the present study was undertaken to explore the hepatoprotective property of the plant.

In the present study, we evaluated the hepatoprotective activity of methanol extract of *Asteracantha longifolia* in Wistar albino rats. Animals showed a significant inhibition ($P<0.001$) of rise in serum ALT and AST at all the tested doses. Histopathological studies in control group showed the normal parenchyma architecture of liver. However, centrilobular necrosis, accompanied by fatty changes and ballooning degeneration were observed in the remaining hepatocytes in the liver of rats treated with carbon tetrachloride while silymarin and *Asteracantha* treated groups showed less hepatic damage.

Materials and Methods

Collection of plant material:

Fresh plants of *Asteracantha longifolia* were collected from the marshy areas of the village Pattanayakanahalli near Tumkur district, Karnataka state during the month of November. The plant material was dried under shade for ten days. Dried plant was finely powdered and stored in air tight container until the preparation of extracts.

Preparation of plant extract:

One hundred gram of powder of whole dried plant of *Asteracantha longifolia* was taken, to which one litre of methanol was added, mixed well and kept for two days with intermittent mixing. The contents were periodically shaken using an electric shaker. After two days, contents were filtered through Buchner funnel in a conical flask and it was further concentrated by evaporating the filtrate in round bottom flask in a rotary flash evaporator maintained at 39-40^{0}C till the solvent was completely evaporated and extract settled down to bottom. The residues were weighed after concentration and their respective percentage yield was determined.

Experimental design:

Wistar albino rats, aged between seven to nine weeks and the body weight 200$\pm$10 g were procured from the Central Animal Facility, Indian Institute of Science, Bangalore. The rats were acclimatized to the experimental conditions for one week. After acclimatization, animals were grouped and housed in standard polypropylene rat cages during the entire experiment. They were maintained under standard laboratory hygienic conditions and provided standard laboratory animal feed and water *ad libitum*. The approval of the Institutional Animal Ethics Committee was obtained prior to start of the experiment.

Hepatoprotective activity:

In this experiment, methanol extract of *Asteracantha longifolia* was evaluated for its hepatoprotective activity in carbon-tetrachloride induced hepatotoxicity in Wistar albino rats. The animals were divided into six groups with six rats in each group. Animals of Group I were treated with normal saline (2 ml per animal) where as Group II animals were given a known hepatoprotective compound, silymarin orally. Group III, IV and V received the following dosages of methanol extract of Asteracantha longifolia orally 600, 900 and 1200 mg/kg b.w. respectively. Group VI received 1 ml/kg of CCl_4. The above treatment was continued for seven days. On day 8 and 9, CCl_4 was administered sub-cutaneously (1 ml/kg) to all rats except rats in Group I. The biochemical parameters (liver specific enzymes like ALT and AST) were determined 18 h after the last dose.

Clinical biochemistry:

Blood was collected from all the animals through retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30ºC for 15 min and used for the estimation of liver specific enzymes namely AST and ALT (Jain, 1990).

Histopathological studies:

After collection of blood samples, the rats were euthanized humanely and their livers were collected. The organs were fixed in neutral buffer formalin for histological examination.

Statistical analysis :

The data was analyzed by one-way ANOVA with Tukey's post test using GraphPad Prism Software (Trial version 4.03 for Windows) GraphPad Software, San Diego, California, USA.

Results

The methanol extract of *Asteracantha longifolia* was evaluated for its hepatoprotective activity in carbon-tetrachloride induced hepatotoxicity in Wistar albino rats. The extract was tested at three doses (600, 900 and 1200 mg/kg b.w.) for a period of nine days. Alanine aminotransferase (ALT) was determined in serum of different groups (Table 1). In group VI, ALT was significantly ($P<0.001$) higher than the *Asteracantha longifolia* extract treated group and silymarin treated group on day 9. The ALT concentration in group III, IV and V were significantly ($P<0.001$) lower than the group VI value on day 9.

The ALT concentration in group II was 103.14 $\pm$ 1.69 IU/L. This was significantly ($P<0.001$) lower than the group VI value of 303.00 $\pm$ 1.34 IU/L. The serum ALT concentrations in group II, III, IV and V were significantly low ($P<0.001$) compared to the group VI (CCL_4 treated group).

The Aspartate aminotransferase (AST) concentration in group VI was significantly ($P<0.001$) higher than *Asteracantha* treated group (Table 2) on day 9. The AST concentration in groups II, III, IV and V were significantly ($P<0.001$) lower than the group VI (CCL_4 treated group).

Histopathological studies also provided supportive evidence. The control group (Fig. 1) showed the normal parenchyma architecture with cords of hepatocytes, portal tracts and central veins without noticeable alterations compared to the control group. Centrilobular necrosis accompanied by fatty changes and ballooning degeneration were observed in the remaining hepatocytes in the liver of rats treated with carbon tetrachloride (Fig. 2). Silymarin (Fig. 3) and *Asteracantha* (Fig. 4, 5 and 6) treated groups showed less hepatic damage with few areas of fatty changes.

Discussion

Present study was conducted to evaluate the hepatoprotective effect of methanol extract of *Asteracantha longifolia* against CCl_4- induced hepatic damages in Wistar albino rats. In the present study, it has been observed that CCl_4 induced a significant elevation ($P<0.001$) in the concentrations of serum marker enzymes like ALT and AST which is indicative of liver damage. High levels of AST indicate liver damage which may be due to necrosis or membrane damage that releases the enzyme into circulation. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, ALT is more specific to the liver and is thus a better parameter for detecting liver injury.

Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Clarke and Clarke, 1975). On day 9, there was a significant decrease ($P<0.01$) in the concentration of serum AST or ALT when compared to the CCL_4 control group. This indicated the hepatoprotective effect of the plant extract. The hepatoprotective effect of the plant extract was comparable to the standard hepatoprotective drug silymarin. This correlates with the earlier findings of Hewawasam *et al.* (2003) who reported the aqueous extract of whole plant of *Asteracantha longifolia* had the hepatoprotective activity in CCl_4-induced hepatotoxicity in mice. In the present study, the hepatoprotective effect of the plant extract was both dose and time-dependent. Maximum protective activity of the extract was observed when administered once daily at a dose of 1200 mg/kg b.w. for 7 days before CCl_4 administration.

Histopathological studies also provided supportive evidence for the biochemical analysis. The control group (Group I) showed the normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins without noticeable alterations. Centrilobular necrosis accompanied by fatty changes and ballooning degeneration were observed in the remaining hepatocytes of rats treated with carbon tetrachloride. *Asteracantha longifolia* and silymarin treated groups showed less hepatic damage with few areas of fatty changes which is suggestive of hepatoprotective activity of *Asteracantha longifolia*. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells.

Flavonoids are known to be antioxidants, free radical scavengers and antilipoperoxidants leading to hepatoprotection. The observed protective effect of the plant extract against carbon tetrachloride may be attributed to the presence of flavonoids, ascorbic acid, carotenoids, tannins and lignins among the plant constituents which are hepatoprotective in nature (Hewawasam *et al.*, 2003).

The present study suggested that the methanol extract of whole plant of *Asteracantha longifolia* possesses potent hepatoprotective activity which had protected the liver against CCl4-induced hepatotoxicity.

Conclusions

Methanol extract of *Asteracantha longifolia* was found to be a very good hepatoprotective agent on CCl_4-induced hepatotoxicity in rats with significant inhibition ($P<0.001$) of rise in serum ALT and AST at all the tested doses (600, 900 or 1200 mg/kg b.w.). Histopathological studies showed that the control group showed the normal parenchyma architecture whereas centrilobular necrosis, accompanied by fatty changes and ballooning degeneration were observed in the remaining hepatocytes of rats treated with carbon tetrachloride. Silymarin and *Asteracantha* treated groups showed significantly less hepatic damage with few areas of fatty changes. These studies revealed that the methanol extract of *Asteracantha longifolia* has potent hepatoprotective activity at the normal dose of 900 mg/kg and even better than the well known hepatoprotective agent silymarin in reducing the ALT and AST concentration in CCl_4 treated rats.

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References

Ahmed S, Rahman A, Mathur M, Athur M, Sultana S (2001). Anti-tumor promoting activity of Asteracantha longifolia against experimental hepato carcinogenesis in rats. *Food. Chem. Toxicol*. 39: 19-28.

Boily Y, Vanpuyvelde L (1986). Screening of medicinal plants of Rwanda. *J. Ethnopharmacol.* 16: 1-13.

Chopra RN, Nayar SL, Chopra IC (1986). Glossary of Indian Medicinal Plants. Chandigarh. pp 29-35.

Clarke EGC, Clarke ML (1975). Veterinary Toxicology. 1st Edn., Bailliere Tindall, London. pp 10-21.

Fernando MR, Nalinie SD, Thabrew MI, Ariyananda PL, Karunanayake EH (1991). Effect of *Artocarpus heterophyllus* and *Asteracanthus longifolia* on glucose tolerance in normal human subjects and in maturity onset diabetic patients. *J. Ethnopharmacol*. 31: 277-282.

Hewawasam RP, Jayatilaka KAPW, Pathirana C, Mudduwa LKB (2003). Protective effect of *Asteracantha longifolia* extract in mouse liver injury induced by carbon tetrachloride and paracetamol. *J. Pharmacy Pharmacol.* 55:1413-1418.

Jain NC (1990). Schalm's Veterinary Haematology. 4th Edn., KM Verghese Co., New Delhi. pp 45-69.

Mazumdar UK, Gupta M, Maiti S, Mukhejee D (1997). Antitumor activity of *Hygrophila spinosa* in Ehrlich ascites carcinoma and sarcoma-180 induced mice. *Indian. J. Exp. Biol*. 35: 473-477.

Misra TN, Singh RS, Pandey H S, Singh, BK, Pandey RP (2001). Constituents of *Asteracantha longifolia. Fitoterapia.* 72:194-196.

Nadkarni KM (1978). *Indian Materia Medica.* 1: 667-669.

Table 1: Effect of methanol extract of *Asteracantha longifolia* on serum alanine aminotransferase concentration (ALT) in carbon tetrachloride induced hepatotoxicity in rats

ALT (U/L)		
Groups	**Day 0**	**Day 9**
Group I (Control)	65.50±1.69	77.83 ± 5.15
Group II (600 mg/kg)	62.72±2.02	144.16±0.16**
Group III (900 mg/kg)	63.37±2.20	112.87±2.13***
Group IV (1200 mg/kg)	60.97 ±1.28	149.83±2.06***
Group V (Silymarin, 50mg/kg)	59.89±1.67	133.83±1.69***
Group VI (CCL_4, 1 mg/kg)	62.37±2.13	303±1.34

Compared with the control group values of respective days. Values are mean ±SE, **P<0.05, ***P<0.001, n = 6

Table 2: Effect of methanol extract of *Asteracantha longifolia* on serum aspartate aminotransferase (AST) concentration in CCL_4 induced hepatotoxicity

AST (U/L)		
Groups	**Day 0**	**Day 9**
Group I (Control)	127.16±5.64	137.00±3.56
Group II (600 mg/kg)	142.33±10.87	214.33±2.06***
Group III (900 mg/kg)	147.33±10.77	208.167±2.57***
Group IV (1200 mg/kg)	138.33±3.18	201.83±1.85***
Group V (Silymarin, 50mg/kg)	128.37±2.06	202.16±3.09***
Group VI (CCL_4, 1 mg/kg)	119.33±4.19	323.5±7.64

Compared with the control group values of respective days. Values are mean ±SEM, ***P<0.001, n = 6

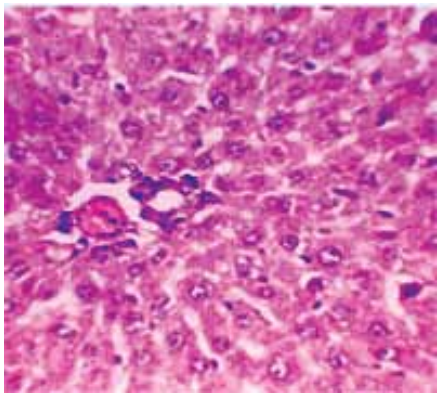

Fig. 1 : Section of liver from rat given normal saline 2ml, showing normal hepatocytes. (H & E X 500)

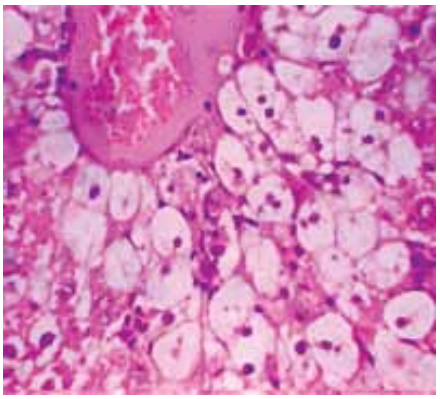

Fig. 2. Section of liver from rat given CCL4 1ml/kg showing severe fatty changes with degeneration and centrilobular necrosis with infiltration of few inflammatory cells. (H & E X 500)

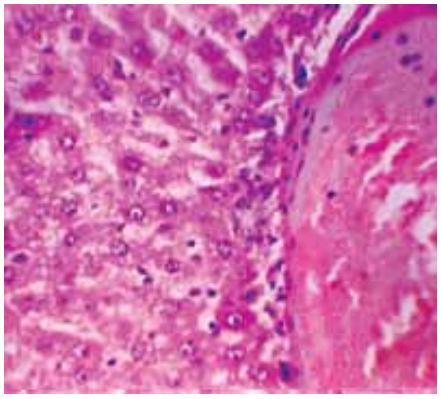

Fig. 3. Section of liver from rat given silymarin 50mg/kg showing mild fatty changes. (H & E X 500)

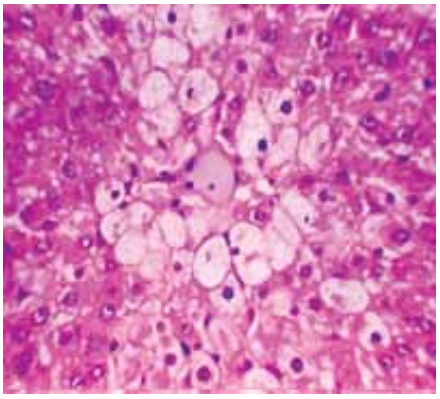

Fig. 4. Section of liver from rat given extract 600 mg/kg showing comparatively less fatty changes with intact hepatocytes (H & E X 500)

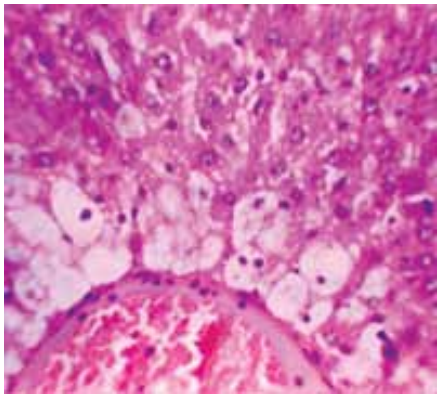

Fig. 5. Section of liver from rat given extract 900 mg/kg showing comparatively less fatty changes with intact hepatocytes (H & E X 500)

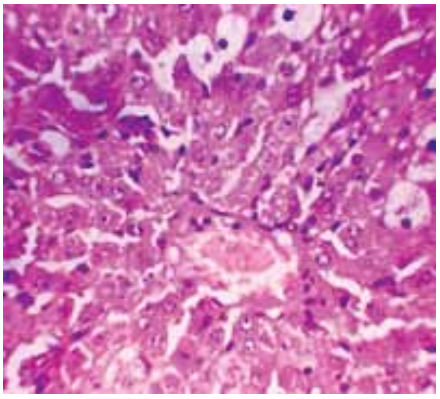

Fig. 6. Section of liver from rat given extract 1200 mg/kg showing comparatively less fatty changes with intact hepatocytes (H & E X 500)