Effect of *Asteracantha longifolia* Nees. against CC1₄ induced liver dysfunction in rat

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Significant recovery after treatment with the whole plant slurry of *A. longifolia* Nees. was observed in plasma AST, ALT and cholesterol levels in CCl₄ induced hepatotoxic rats. This was amply supported by electron micrographs, which indicated normalization of cytoarchitecture of mitochondria and endoplasmic reticulum. The results suggest that the slurry of the plant is useful as a liver tonic.

**Keywords:** *Asteracantha longifolia*, Albino Wistar rats, Hepatoprotectant, Liver disorder.

**IPC Code:** Int Cl A61

The importance of traditional systems of medicine has now been recognized world over. Traditional medicines derive their scientific heritage from rich experiences of ancient civilization. Hence, it is not surprising that traditional medicines claim comes for several "difficult to cure" diseases like hepatitis, diabetes and even cancer. *Asteracantha longifolia* Nees. (Acanthaceae) known as Kokilaksha in Sanskrit, is used in various herbal formulations used against liver disorder. The earlier reported work on *A. longifolia* is an efficacy study by using the mice model. In this study, liver dysfunction was induced by CCl₄ and ethanolic extracts of leaves and seeds of *A. longifolia* were used as hepatoprotectants. The hepatoprotective activity on the methanolic extract of the seeds against paracetamol and thioacetamide intoxication in rats has been reported. More recent reports on the antitumor activity of methanolic extract of seeds of *A. longifolia* on hepatocarcinogenesis in rats, have been documented.

The present work investigates the efficacy of *A. longifolia* whole plant slurry on CCl₄ induced liver dysfunction. The efficacy level has been compared with a known hepatoprotectant, silymarin.

**Materials and Methods**

*Asteracantha longifolia* whole plant was collected from Thane, Maharashtra, India. Herbarium of the plant was authenticated from Raw Materials Herbarium & Museum (RHMD), Delhi of National Institute for Science Communication And Information Resources (NISCAIR), CSIR, New Delhi and National Botanical Research Institute (NBRI), CSIR, Lucknow. A voucher specimen of the plant material has been deposited at RHMD, NISCAIR (No. 1857) and NBRI. After collection of the plant, it was carefully segregated, washed and dried at 45°C to constant weight. The dried plant free of moisture was powdered and sieved through a BSS Mesh No. 85 sieve and stored in an air tight Pearlpot container.

Albino wistar rats of either sex weighing 110-150 g were procured from Haffkin Biopharmaceuticals, Parel, Mumbai, India. The animals were maintained in an animal house with standard facilities having CPCSE approval (No. 25/1/99-A WD). They were kept under uniform conditions of light and water ad libitum. They were fed with rat feed (AMRUT feed). The animals were randomly divided into following 5 groups of 10 (5 male and 5 female) each; Group I: normal control, Group II: toxicant (CCl₄) control, Group III: toxicant CCl₄ natural recovery, Group IV: toxicant (CCl₄) and plant treated, and Group V: toxicant (CCl₄) and silymarin treated as positive control. The animals from Group I received an ip injection of 0.5 ml of liquid paraffin and those from...
Groups II, III, IV and V received an ip injection of 0.7 ml/kg of CCl4 in 0.5 ml liquid paraffin / animal on the first day of the study6-12. The animals from Groups I, II and III received an oral dose of 2 ml of distilled water (d/w) once daily. The animals from Group IV received an oral dose of 0.709 g/kg of sieved whole plant powder13 of A. longifolia suspended in 2 ml of distilled water per animal. A dose of 0.007 g/kg silymarin14 (in the form of Silybon tablets manufactured by Ranbaxy Laboratories, India) suspended in 2 cm² of distilled water was administered orally to each rat of Group V. The animals from Groups I, II, IV and V were sacrificed on the fourth day (72 hr after dosing) and the ones from Group III were sacrificed on seventh day of the study (Table 1).

Before sacrifice the animals were weighed. Blood was collected by cardiac puncture under light ether anesthesia. The blood was transferred to heparinised tubes. The plasma was separated by centrifugation for estimation of tissue biochemical assays like DNA, RNA, total proteins, glycogen and tissue cholesterol. DNA and RNA were estimated using standard methods15-17. All values were expressed as mean ± SE and statistically evaluated at 95% confidence using Student’s t test. The extent of liver recovery was compared with the known hepatoprotectant, silymarin.

### Results

#### Biochemical parameters

The observations obtained after the evaluation of biochemical parameters3-8,14,16,21-28 are enumerated in Tables 2 and 3.

#### Histopathological observations

Normal rat liver showed characteristic features of the hepatic lobule10,20,29 (Fig. 1). The liver of rat treated with CCl4 showed dilated sinusoids with vacuolation in hepatocytes and also congestion10,23,27,29,31,33 (Fig. 2). CCI4 treated liver after natural recovery almost was cut and fixed in Bouin's fixative. Fixed tissues were processed for routine haematoxylin and eosin staining and evaluated. For electron microscopy, 1 mm of fixed liver pieces were transferred to fresh ice-cold fixative for 2 hr and then for 4 hr in 0.1 M cacodylate buffer (pH 7.4). The tissue was then washed in cacodylate buffer and postfixed for 1 to 2 hr in 1% osmium tetroxide (OsO4). Tissues were dehydrated in alcohol and cleared in propylene oxide. The cleared tissues were embeded in resin, polymerized at 60°C and blocks were prepared in araldite. Ultrathin sections showing gold interference (0.7 μ) were cut on LEICA ULTRACUT R ultramicrotome, picked up on copper grids, stained with urayl acetate and lead citrate for final viewing. Sections were observed and photographed on a JEOL-JEM 1010 Electron microscope. The remaining liver was used for the estimation of tissue biochemical assays like DNA, RNA, total proteins, glycogen and tissue cholesterol.

### Table 1—Efficacy study of A. longifolia on CCl4 treated rats—dosage regimen

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5 ml liquid paraffin ip and 2 ml d/w oral</td>
<td>0.7 ml/kg CCl4 in 0.5 ml liquid. paraffin. ip and 2 ml d/w oral</td>
<td>0.7 ml/kg CCl4 in 0.5 ml liquid. paraffin. ip and 2 ml d/w oral</td>
<td>0.7 ml/kg CCl4 in 0.5 ml liquid. paraffin. ip and 2 ml d/w oral</td>
<td>0.7 ml/kg CCl4 in 0.5 ml liquid. paraffin. ip and 0.007 g/kg Silymarin in 2 ml d/w oral</td>
</tr>
<tr>
<td>2</td>
<td>2 ml d/w oral</td>
<td>2 ml d/w oral</td>
<td>2 ml d/w oral</td>
<td>2 ml d/w oral</td>
<td>0.709 g/kg plant powder in 2 ml d/w oral</td>
</tr>
<tr>
<td>3</td>
<td>2 ml d/w oral</td>
<td>2 ml d/w oral</td>
<td>2 ml d/w oral</td>
<td>2 ml d/w oral</td>
<td>0.709 g/kg plant powder in 2 ml d/w oral</td>
</tr>
<tr>
<td>4</td>
<td>Sacrifice</td>
<td>Sacrifice</td>
<td>2 ml d/w oral</td>
<td>Sacrifice</td>
<td>0.007 g/kg Silymarin in 2 ml d/w oral</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>—</td>
<td>2 ml d/w oral</td>
<td>—</td>
<td>Oral 0.007 g/kg Silymarin in 2 ml d/w oral</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>—</td>
<td>2 ml d/w oral</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td>—</td>
<td>2 ml d/w oral</td>
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<td>—</td>
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</tbody>
</table>

Note: The above dosage is for an individual animal of the group. Each group had ten animals (5 of either sex).
The liver after CCl4 treatment showed classical fatty liver as indicated by significant increase in tissue cholesterol. The tissue cholesterol levels reduced after natural recovery and silymarin treatment. A. longifolia treatment caused classical fatty liver as indicated by significant increase in tissue cholesterol. The tissue cholesterol levels reduced after natural recovery and silymarin treatment. A. longifolia treatment indicated protective action of the plant treatment to CCl4 induced damage. Peak changes in plasma enzymes were noticed at 24 hr after CCl4 administration but complete recovery was slow and took about 14 days. The removal of necrotic debris starts by 48 hr after CCl4 administration and is usually complete by one week after exposure. Alteration of serum enzyme levels can be monitored to evaluate the hepatocellular damage caused by various foreign compounds. CCl4 treatment causes significant increase in blood and tissue parameters studied. CCl4 treatment caused a marked elevation in the transaminases.

**Discussion**

Food and water consumption decreased after CCl4 treatment. This is an indication of toxic response by the treated animals. Decrease in body weight after CCl4 treatment in treated animals further confirmed the toxic response. Increase in body weight after A. longifolia treatment indicated protective action of the plant treatment to CCl4 induced damage.
Figs 1-5—Light micrograph of liver from (1) normal rat showing central vein (long arrow); sinusoids (short arrow); hepatocyte (arrow head); (2) CCl₄ treated rat showing vacuolated hepatocytes (long arrow); sinusoids (short arrow) dilated sinusoids (arrow head); central vein (CV); (3) rats recovering from CCl₄ treatment showing vacuolated hepatocytes (arrow heads); dilated sinusoids (long arrow); central vein (CV); (4) rats treated with CCl₄ and plant and slurry showing vacuolated hepatocytes (long arrow); infiltration by mononuclear cells (arrow heads); central vein (CV); congestion (thick arrow) and (5) rats treated with CCl₄ and silymarin, showing vacuolated hepatocytes (long arrow); dilated sinusoids (short arrow); congestion (arrow head) [Figs 1-5, magnification × 100].
treatment caused significant reduction in tissue cholesterol of female animals. The liver after CCl₄ and silymarin treatment shows regions of recovery, which are more focused as against overall recovery seen in case of A.longifolia slurry treatment.

The results of animals treated with A.longifolia suggest that treatment with plant slurry can control the increase in plasma AST, ALT and cholesterol levels. Transaminase has been reported to attain normal levels with the healing of liver parenchyma and regeneration of liver cells. Comparison of enzyme levels in animals after natural recovery phase indicates that animals treated with A.longifolia recovered in the liver functions enzyme levels within
two days after CCl₄ administration, whereas animals after natural recovery phase showed reduction in these enzyme levels after six days.

Treatment with *A. longifolia* after CCl₄ induced hepatotoxicity caused reduction in tissue cholesterol levels, triglycerides and blood AST and ALT levels as compared to those in animals of normal control and silymarin treated group. Female rats showed more significant response with greater reduction in levels of blood AST and ALT. Cholesterol and triglyceride levels were, however, more than normal control and silymarin treated group. The results show that CCl₄ increased the levels of AST, ALT in plasma significantly. The results of natural recovery animals indicate that the levels do not attain the values of animals in the control group even after a recovery period of 6 days.

Changes in RNA indicate toxicant induced changes in protein synthesis. Variations in DNA level of liver observed in the present study suggests increase in cell division after CCl₄ induced cellular damage. The plant slurry treated group showed elevated levels of DNA and RNA as compared to silymarin treated group, which indicated tissue rejuvenation in these animals.

Significant variation in tissue glycogen after treatment with CCl₄ indicated impairment of liver metabolism. CCl₄ treatment caused reduction in tissue glycogen levels. The plant slurry treated group showed a distinct decrease in tissue glycogen as compared to CCl₄ control group, which suggests mobilization of glycogen in the liver tissue of plant treated group. The recovery of membrane bound enzymes after the plant slurry treatment was well supported by observations made in the electron microscopic studies. The normal cytoarchitecture of mitochondria and endoplasmic reticulum were adequate indications of these recoveries.

**Conclusion**

The early recovery of enzyme levels seen in animals administered with plant slurry as compared to animals undergoing natural recovery, indicate hepatoprotective efficacy of plant slurry. The significant accumulation of lipids seen in electron micrographs is a clear indication of CCl₄ induced hepatic dysfunction. Many compounds cited in literature exhibit liver protection against CCl₄ induced lipid peroxidation. The improved histology of liver as observed in histopathological evaluation on animals treated with plant slurry as compared to those seen in animals administered CCl₄ alone indicates the ability of plant slurry to induce accelerated regeneration of liver cells, reducing the leakage of AST and ALT into
the blood. This is supported by the recovery of membrane bound enzymes after the plant slurry treatment as observed in the electron micrographs. The normal cytoarchitecture of mitochondria and endoplasmic reticulum are adequate indications of these recoveries.

The present study brings out the hepatoprotective action of A longifolia slurry against CCl₄ induced liver dysfunction in rats. The use of whole plant powder as liver tonic seems to be effective. To rationalise the dose and also to establish the best formulation for therapeutic use of the plant, however, more work needs to be carried out at different dosage regimen.

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