Preliminary hepatoprotective activity of Jigarine CL on Carbon Tetra Chloride induced hepatic damage in rats

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Abstract
Jigarine CL is an herbal preparation of Hamdard laboratories waqf Pakistan that has been used as hepatoprotective agent. In this study, the protective effects of Jigarine CL against liver damage were evaluated in carbon tetrachloride (CCl4)-induced chronic hepatotoxicity in rats. Sprague-Dawley (SD) rats were divided in to four groups (I, II, III, IV). Groups I received olive oil 10ml/kg p.o served as control and group II and III were orally fed with Jigarine CL (20ml/kg) for 14 consecutive days while Groups IV were orally fed with saline. After the last dose of jigarine CL and saline group III and IV received a single dose of CCl4 (0.3 ml/kg body weight in a 20% olive oil) was injected intraperitoneally 30 min after the last dose of Jigarine CL and the animals were starved for 24 h. The degree of hepatic-protection was measured using biochemical parameters (serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) and Bilirubin) and histopathology. Data obtained from results were compared using student’s t-test. The results showed that the treatment of Jigarine CL significantly lowered the CCl4-induced serum levels of hepatic enzyme markers (GOT, GPT and total bilirubin) indicated hepatoprotective effect of the Jigarine CL. Histopathological examination of liver sections confirmed that, pre-treatment with Jigarine CL decreased the degree of hepatic damage induced by CCl4. The significant reversal of the biochemical and histological changes induced by CCl4 treatment in rats, indicating promising hepatoprotective activity of Jigarine CL in rats.

Keywords: CCl4, Hepatoprotective, Hepatic enzymes marker, Jigarine CL,

1. Introduction
Liver diseases is a leading cause of health in both developed and under developed countries. In affluent countries, the incidence of liver cirrhosis is correlated with increasing consumption of alcohol1. Apart from alcohol consumption, nutritional, genetic and immunological factors are also associated with the development of liver injury. In several developing countries where alcohol consumption is not very significant, viral infections, especially viral hepatitis is one of the major causes of liver injury leading to both cirrhosis and cancer2.

These chronic diseases constitute a fast increasing burden to society. The increasing cost of treatment and non-availability of effective drugs in the modern system of medicine led to several studies on possible hepatoprotective action of traditional drugs3. In different countries wealth of medicinal plants are considered to have hepatoprotective properties. Certain levels of protection have been reported with extracts of Casuarina equisetifolia, Cajanus cajan, Glycosmis pentaphylla, Bixa orellana, Argemone mexicana, Physalis minima, Caesalpinia bonduc4, Epaltes divaricata5 and Barrisal a herbal preparation6.

Jigarine CL is one of the herbal medicines formulated by Hamdard Laboratories (Waqf) Pakistan, used as a hepatoprotective in liver disorders. Jigarine CL is composed of following eight medicinal plants.

1. Achillea millifolium
2. Artemisia absinthium
3. Tamarix dioica
4. Melia azadirachta
5. Rosa damascena
6. Sphaeranthus indicus
7. Rheum emodi
8. Allium sativum

Compositae
Compositae
Tamaricaceae
Meliaceae
Rosaceae
Compositae
Polygonaceae
Liliaceae
a. *Achillea millefolium*: the aqueous-methanol extract has reported to have hepatoprotective, antispasmodic and calcium antagonist activities 7.
b. *Artemisa Absinthium* used in traditional medicine possesses protective effect against acute liver injury 8.
c. *Tamarix dixia* is used as a carminative, diuretic and for the treatment of hepatic and splenic inflammation in traditional system of medicine 9.
d. *Melia azadridichra* is also possessing hepatoprotective activity 10.
e. *Rosa damascena* has an anticonvulsant effect 11.
f. *Rheum emodi* crude aqueous extract inhibits hepatic microsomal P450 enzyme 12.
g. *Sphaeranthus indicus* is widely used in Ayurvedic system of medicine to treat mental illness, hemicrania, jaundice and hepatopathy 13.
h. *Allium sativum* (Allium sativum) has been shown to have antiviral activity 14.

2. Material and Methods

2.1 Drug: Jigarine CL (syrup) was provided by Director Quality Control Division, Hamdard Laboratories (Waqf) Pakistan. It is pink thick liquid.

2.2 Experimental Animals: Adult Sprague Dawley rats (n=40) of both sexes (200 to 225 g) were used for hepatoprotective, toxicological and histopathological studies, while NMR-1 mice (n=40: weight 20-30g) were used only for toxicological studies. They were obtained from Animal House of Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences (Dr. HMIIPHS) and were housed in groups of 6 per cage for seven days prior to experimentation with free access to standard feed and tap water ad libitum and kept on a 12 h light/dark cycle. All animals were housed in an air-conditioned room at 23±1°C during the quarantine period. The experimental procedures were performed according to Guidelines for Care and Use of Laboratory Animals in Biomedical Research (2011) 15. All experimental Procedure was approved by review board of departmental research committee.

2.4 Toxicological studies

2.4.1 Acute Toxicity Test: Mice were divided into four groups. Each group has 10 mice of both sexes (5 Male and 5 Female). Jigarine CL was administered orally Forty consecutive days with a dose of 10ml/kg, 20ml/kg, and 30ml/kg and one group served as a control administration 0.9% NaCl. Mortality and behavioral changes were noted 2hrs after administration and animals were kept under observation for two weeks 16, 17.

2.5 Protective effect of Jigarine CL on carbon tetrachloride induced acute hepatotoxicity in rats: Sprague Dawley rats weighing between 200 to 225 g of either sex were divided into four groups, each group consists of eight rats.

Group I: Animals received olive oil 10ml/kg p.o served as control.

Group II: Animals received Jigarine CL 20ml/kg p.o served as control.

Group III: The dose of 20ml/kg body weight of Jigarine CL was administered orally in rats for seven consecutive days, and at day 14th they were also administered a single dose of CCl4 0.3ml/kg in olive oil intraperitoneally 30min. after the last dose of Jigarine CL, and the starved the animals for 24 hours 18.

Group IV: animals served as reference control, which received a single dose of 0.3ml/kg of CCl4 intraperitoneally and then starved for 24 hrs 19. After 24 hours animals were anaesthetized with Pentothal sodium 10mg/kg i.p. 24 hours after the last dose of treatment 19 and the blood samples approximately (3 to 4ml) were withdrawn from cardiac puncture serum was separated by centrifugation (2000 rpm for 15min).

The serum level of SGOT, SGPT and Bilirubin were determined spectrophotometrically on the same day.

2.5.1 Histopathological studies: Liver fragments were taken immediately after the blood was collected, fixed in 10% neutral formalin, dehydrated in graded (80-100%) alcohol, cleared in xylene, and embedded in paraffin. Six µm sections were prepared, and then deparaffinated in xylene, passed through 80% to 100% alcohol, and stained with hematoxylin and eosin (H&E.) for assessment of liver morphology 20.

2.6 Statistical Analysis: Data obtained from results regarding serum biochemical estimations, body weight and weight of vital organs were compared using student’s t-test (paired and unpaired) and P<0.05 were considered to be significant.

3. Results

3.1 Acute Toxicity: A shown in (Table 1) the drug was administered for 14 consecutive days in different group of mice did not show any mortality and overt changes in behavior or symptoms of toxicity at the end of two weeks period. Jigarine CL was found to be safe up to dose 30ml/kg in mice.

Table – 1. Effects of Jigarine CL in Mice

<table>
<thead>
<tr>
<th>Dose (ml/kg)</th>
<th>Route of Administration</th>
<th>Weight of Mice X±SEM (g)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>p.o.</td>
<td>24.64±0.35</td>
<td>NIL</td>
</tr>
<tr>
<td>10ml</td>
<td>p.o.</td>
<td>25.19±0.49</td>
<td>NIL</td>
</tr>
<tr>
<td>20ml</td>
<td>p.o.</td>
<td>24.34±0.49</td>
<td>NIL</td>
</tr>
<tr>
<td>30ml</td>
<td>p.o.</td>
<td>23.64±0.47</td>
<td>NIL</td>
</tr>
</tbody>
</table>

3.2 Autopsy: Autopsy revealed that no gross changes in organs viz. liver, spleen, heart and kidney.

3.3 Protective effect of Jigarine CL on carbon tetrachloride induced acute hepatotoxicity in rats: Oral administration of jigarine CL did not cause any significant changes in the levels of marker enzymes namely SGOT, SGPT and Bilirubin as compared to control rats. In rats, hepatic damage induced by CCl4 caused significant rise in marker enzymes SGOT, SGPT, and Total serum bilirubin (Table 2). A single dose of CCl4 0.3ml/kg increased the serum level of SGOT (1810.6±10.6 IU/l), SGPT (1546±14.23) and bilirubin (4.9±0.36) statistically significantly as compared to control.

In the third group, jigarine CL (20 ml/kg) treated (14 days) rats were used for additional treatment with CCl4 for a period of 24 h. Such treatment was found to reduce theserum transaminase activity as shown in Table 2. This activity was assessed due to significant reduction in bilirubin (P<0.05), SGPT (P<0.05), SGOT (P<0.05), compared to CCl4 treated 4th group.

Table – 2. Effect of Jigarine CL on carbon tetrachloride induced acute hepatotoxicity in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Bilirubin mg/dl X±SEM</th>
<th>SGPT IU/l X±SEM</th>
<th>SGOT IU/l X±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Olive oil</td>
<td>2.81±0.44</td>
<td>100.41±0.09</td>
<td>113.9±6.0</td>
</tr>
<tr>
<td>2</td>
<td>Jigarine CL p.o.</td>
<td>2.4±0.11</td>
<td>116.5±0.14</td>
<td>114.6±3.05</td>
</tr>
<tr>
<td>3</td>
<td>Jigarine CL p.o + CCl4 i.p.</td>
<td>3.1±0.5</td>
<td>261.5±10.14</td>
<td>186.6±14.05</td>
</tr>
<tr>
<td>4</td>
<td>CCl4 + olive oil i.p.</td>
<td>4.9±0.36</td>
<td>1546±14.23</td>
<td>1810.6±10.6</td>
</tr>
</tbody>
</table>
3.4 Hepatoprotective effect of Jigarine CL on liver histology: The histopathological examination clearly reveals that the hepatic cells are normal in jigarine treated rats group and hepatic cell are same as in control group (Fig 1a and b). While after CCl₄ hepatic cell showed complete disorganization (Fig 1c). The hepatocytes were severely damaged and ascites retention was observed. In group three jigarine treated rats has prevented CCl₄ to induced liver damage. The hepatic pictures of rats treated with Jigarine CL+CCL₄ showed a prevalence of morphologically normal hepatocytes and resulted in a significant reduction of all morphological alterations caused by CCl₄ intoxication. Only some hepatocytes with early necrotic lesions were encountered (Fig 1d).

Fig. 1a Liver section of control rats (20X)  
Fig. 1b Liver section of rat treated with Jigarine CL (20ml/kg) (20X)  
Fig. 1c Liver section of rat intoxicated with CCl₄ (20X)  
Fig. 1d Liver section of rat treated with Jigarine CL (20ml/kg) and intoxicated with CCl₄ (20X)

4. Discussion  
In the present study rats treated with a single dose of CCl₄ developed hepatic damage that was observed as a substantial increase in the serum levels of SGOT, bilirubin and decrease in serum level of total protein. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver[21]. The reduction in the levels of Marker enzyme (SGPT, SGOT, and Bilirubin) by Jigarine is an indication that it prevents the hepatic damage caused by CCl₄. In this study the results clearly indicate that administration of Jigarine would effectively protect liver against CCl₄ induce hepatotoxicity and this effect was verified by both biochemically and histopathologically. Jigarine CL is composed of eight different medicinal plant and all were reported to possess hepatoprotective activities and antiviral effects in previous studies. All these ingredients collectively protecting the hepatic damage caused by CCl₄.

5. Conclusion  
Jigarine CL did not produce any morphological changes on liver in experimental animals. It may be safely given to patient suffering from jaundice. Long terms and clinical experimental studies should be carried out to confirm these findings.

Acknowledgement  
Authors are grateful to Dr. S.I Ahmed (Late) Former Dean Faculty of Pharmacy, University of Karachi, Hamdard University Karachi and Director of HMIPHS, Hamdard University Karachi for his support and encouragement at every step of this study.

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