Hepatoprotective Activity Of *Sesamum Indicum* Linn. Against Ccl₄-Induced Hepatic Damage In Rats

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ABSTRACT
In present study, the hepatoprotective activity of ethanolic extracts of *Sesamum indicum* Linn. seeds were evaluated against carbon tetrachloride (CCL₄) induced hepatic damage in rats. The extract at two different doses (400mg/kg and 700mg/kg) was administered orally once daily. The substantially elevated serum enzymatic level of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), alkaline phosphatase (ALP), Acid phosphatase (ACP), Total Protein, Albumin and Total Bilirubin were restored towards normalization significantly by the extract. The biochemical observations were supplemented with histopathological examination of rat liver sections. The results of this study strongly indicate the *Sesamum indicum* Linn. Seeds have potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats.

Keywords: *Sesamum indicum* Linn.; carbon tetrachloride; Marker enzymes; Hepatoprotective; Histopathology.

INTRODUCTION
Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body.[¹] It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences.[²] Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug or another non-infectious agent.[³] Hepatitis is one of the most prevalent diseases in the world[⁴] and hepatic problems for a significant number of liver transplantation and death recorded worldwide, available pharmacotherapeutic option for liver diseases are very limited and there is a great demand for the development of new effective drugs.[⁵] In ayurveda many indigenous plants have been mentioned and well established as hepatoprotective agents.[⁶]

*Sesamum indicum* Linn. (Pedaliaceae) is commonly known as Sesame, Til in Hindi, Sesame in French, Wijen in Japanese, Sesamo in Spanish and Vanglo in German. It is annual or perennial herbs, or occasionally shrubs found in the warmer region of Africa, Asia and Australia. About six species are recorded in India of which *Sesamum indicum* is widely cultivated.[⁷] Sesame oil is used in the preparation of liniment, ointment, and soaps and act as laxative, demulcent and has got emollient properties.[⁸] Scientifically, most pharmacological studies on *Sesamum indicum* seed is reported hypoglycemic effect in genetically diabetes,[⁹] antitumor effect,[¹⁰] antiestrogenic activity,[¹¹] benefits in the parkinsonism disease,[¹²] antihypertensive effect[¹³] and increases vitamin E concentration with out use of vitamin E supplements.[¹⁴] In the present study we investigated the hepatoprotective activity of *Sesamum indicum* seeds against carbon tetrachloride induced hepatotoxicity in wistar rats.

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MATERIAL AND METHODS
Preparation of plant extract: The seeds of the plant *Sesamum indicum* Linn. were collected from the local supplier of Udaipur District, Rajasthan, India and authenticated by Department of Pharmacognosy, B. N. College of Pharmacy, Udaipur, Rajasthan, India. The seeds were shaded-dried for one week reduced to coarse powder using mixer grinder. The powder obtained was successively extracted with alcohol. The extract was concentrated under reduced pressure.

Phytochemical study: The extract of *Sesamum indicum* was subjected for phytochemical study. [15]

Experimental animals: The young healthy albino rats of Wistar strains of either sex, weighing between 200 to 225 gm. were used for the experiment. They were housed in the cages under the laboratory standard condition c. (23±2 °C, 60 -70%, 12 hr light/dark cycles) and given standard pellet diet. Water was given ad libitum. The study was permitted by the institutional animal ethical committee at the B. N. College of Pharmacy, Udaipur with Reg. no. 870/ac/05/CPSEA. Initial body weight of each animal was recorded.

Acute toxicity studies: *Sesamum indicum* at different doses (50-2000 mg/kg) was administered orally to normal rats. During the first hours after the drug administration, the animals were observed for gross behavioral changes if any for 7 days. The parameters such as hyper activity, grooming, convulsions, sedation, hypothermia, mortality were observed and doses selected were 400mg and 700mg/kg/day.

Experimental design: Liver toxicity was induced by administrating carbon tetrachloride subcutaneously in lower abdomen of rats, in suspension of liquid paraffin (LP) in the ratio 1:2 v/v at the dose of 1ml/kg body weight on alternate days for seven days. [16]

Rats were divided in to five groups I-V, consisting of six animals each. Rats of group I served as a control and were received liquid paraffin (1ml/kg) on alternate days for 21 days. Group II animals served as treated control and were received carbon tetrachloride (1ml/kg) on alternate days for seven days. Group III animals were given silymarin (25mg/kg) for 21 days plus carbon tetrachloride (1ml/kg) was given on alternate days for seven days. Group IV and V animals were given ethanolic extract of *Sesamum indicum* (400mg/kg) and (700mg/kg/day) respectively for 21 days plus carbon tetrachloride (1ml/kg) was given on alternate days for seven days. Replenishing a known quantity of fresh food daily at 9.00am and thereby measuring the food intake of the previous day carried out measurement of daily food consumption. Body weights of rats were recorded weekly to assess percentage of weight gain of each animal. After 21st day of treatment, animals were kept starved overnight fasting and sacrificed by cervical dislocation. At the end of treatment, blood sample were collected by retro-orbital puncture under light ether anesthesia and the serum was used for assay of marker enzymes viz. Serum Glutamate Oxaloacetate Transaminase(SGOT), [17] Serum Glutamate Pyruvate Transaminase (SGPT), [17] Alkaline phosphatase (ALP), [18] Acid phosphatase (ACP), [19] Total Protein, [20] Albumin [20] and Total Bilirubin. [21] The results were expressed as units/liter (U/L).

Histopathology: A portion of liver tissue in each group was fixed in 10% formalin (formalin diluted to 10% with normal saline) and proceeded for histopathology. Sections were stained with ehrlich’s hematoxylin and eosin.

Statistical analysis: Values were expressed as Mean ± SD. Data were evaluated using one-way analysis of variance (ANOVA) followed by Scheff’s/Dunnet’s test for determining statistical significance of difference in serum marker enzymes, proteins and bilirubin level between different groups. Results were considered statistically significant at P <0.05. [22]

RESULTS
Phytochemical study: The extract of *Sesamum indicum* for phytochemical study showed the presence of lignans, proteins, amino acids, carbohydrates and lipids.

Acute toxicity studies: No mortality observed with oral administration of *Sesamum indicum* even at the highest dose (2000mg/kg). Both the doses of *Sesamum indicum* had no toxic effect on the normal behavior of the rats.

Effect on food consumption and weight gain: The effect of food consumption and weight gain significantly increased in groups III and IV animals as compared to II group.

Effect on biochemical parameters: There was a significant elevation of marker enzymes viz., SGOT, SGPT, ALP, ACP levels in the carbon tetrachloride treated group II, as compared to
control untreated group I. The groups III-V treated with Silymarin and different doses of *Sesamum indicum* (400mg/kg and 700mg/kg), above activities of enzymes were found to significantly decreased, as compared to carbon tetrachloride treated group II (Table 1 and Fig. 1). The group II animals treated with carbon tetrachloride significantly increased the level of total protein and albumin whereas the level of total bilirubin was decreased significantly. In groups III-V, treatment with silymarin and different doses of *Sesamum indicum* (400mg/kg and 700mg/kg) reversed these parameters significantly, as compared to carbon tetrachloride treated group II (Table 2 and Figs. 2, 3).

**Effect on liver histopathology:** Histological profile of the control untreated group I animals showed normal hepatocytes (Fig. 4). Group II animals treated with carbon tetrachloride

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**Table 1. Effect of Sesamum indicum (SI)Linn. on different parameters in the Serum of CCl₄ treated rats.**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Biochemical Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGOT U/L</td>
</tr>
<tr>
<td>Control (LP only)</td>
<td>73±2.19</td>
</tr>
<tr>
<td>CCl₄ only</td>
<td>211±2.66***</td>
</tr>
<tr>
<td>CCl₄ + Silymarin</td>
<td>84.5±2.73***</td>
</tr>
<tr>
<td>CCl₄ +SI(400mg/kg)</td>
<td>107.5±4.92***</td>
</tr>
<tr>
<td>CCl₄+SI(700mg/kg)</td>
<td>99±3.28***</td>
</tr>
</tbody>
</table>

All values are represented as Mean ± SD (n=6)
P value: +++ < 0.001 When compared with control untreated animals.
***<0.001 when compared with carbon tetrachloride induced hepatotoxic rats models.

**Table 2. Effect of Sesamum indicum (SI) Linn. on different parameters in the serum of CCl₄ treated rats.**

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Biochemical Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TOTAL PROTEIN (g/dl)</td>
</tr>
<tr>
<td>Control (LP only)</td>
<td>7.69±0.26</td>
</tr>
<tr>
<td>CCl₄ only</td>
<td>5.29±0.09***</td>
</tr>
<tr>
<td>CCl₄ + Silymarin</td>
<td>6.63±0.35***</td>
</tr>
<tr>
<td>CCl₄ +SI(400mg/kg)</td>
<td>5.46±0.32</td>
</tr>
<tr>
<td>CCl₄+SI(700mg/kg)</td>
<td>6.29±0.20***</td>
</tr>
</tbody>
</table>

All values are represented as Mean ± SD (n=6)
P value: +++ < 0.001 When compared with control untreated animals.
* < 0.05,**<0.01;***<0.001 When compared with carbon tetrachloride induced hepatotoxic rats models.
exhibited intense centrilobular necrosis, macrovascular fatty changes (Fig. 5). The group III-V animals treated with drug silymarin and ethanolic extract of *Sesamum indicum* exhibited significant liver protection against the toxicant as evident by the presence of lesser fatty infiltration and absence of cell necrosis (Figs. 6, 7 and 8).

Fig. 4. Control treated group I rats showing normal hepatocytes

Fig. 5. CCl4 treated group II rats showing hepatic damage.

Fig. 6. CCl4 + Silymarin treated group III rats showing almost normal hepatocytes.

Fig. 7. CCl4 + Sesamum indicum (400mg/kg) treated group IV rats showing less damage.

Fig. 8. CCl4 + Sesamum indicum (700mg/kg) treated group V rats showing near normal hepatocytes.

DISCUSSIONS

The CCl4 is one of the most commonly used hepatotoxins in the experimental study of liver diseases.[23] The hepatotoxicity induced by CCl4 is due to its metabolite CCl3-, a free radical that alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage.[24] The magnitude of damage can be assessed conventionally by measuring serum marker enzymes released into the blood.[25] The present study revealed a significant increase in the activities of SGOT, SGPT, ALP, ACP and serum bilirubin on exposure to CCl4, indicating considerable injury. Administration of extract of *Sesamum indicum* at two different doses (400mg/kg and 700mg/kg) attenuated the increased levels of serum enzymes, produced by CCl4 and caused a subsequent recovery toward normalization almost like that of Silymarin treatment. Further plant extract has increased the levels of total proteins and albumin, suggesting hepatoprotective effect of *Sesamum indicum* extract which was confirmed by the liver histopathological examination. Histological changes were found balloon generation, fatty changes, cell necrosis in carbon tetrachloride induced hepatic damage (Fig. 5). Liver section observed less damage, no centrilobular necrosis and lesser fatty degeneration in the groups treated with extract of *Sesamum indicum* Linn. and Silymarin comparable with carbon tetrachloride
The present study has lead to conclusion that extract of *Sesamum indicum* has the potential to protect liver from toxic substances. Further studies on any other models and extensive clinical trials are needed to confirm these results. In most of the developed and developing countries, the incidence of viral hepatitis is more so, the investigation for an efficient hepatoprotective drug from the natural resource is an urgent necessity.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


