Hepatoprotective activity of Hydroalcoholic extract of *Momordica charantia* Linn. leaves against Carbon tetrachloride induced Hepatopathy in Rats.

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Abstract: The plant *Momordica charantia* Linn. belonging to family *Cucurbitaceae* traditionally used in Ayurvedic system as antidiabetic, anthelminitic, antibacterial, antitumor and also used in liver disorders. The aim of present study was to evaluate the hepatoprotective activity of hydroalcoholic extract of *Momordica charantia* Linn. leaves against carbon tetrachloride induced hepatotoxicity in albino wistar rats. Hydroalcoholic extract of *Momordica charantia* leaves (100mg/kg and 200mg/kg p.o.) were administered to the experimental rats for seven days. The hepatoprotective activity of hydroalcoholic extract of *Momordica charantia* leaves were evaluated by estimation of SGOT, SGPT, ALP and total bilirubin. Histopathology of the liver was also studied. In the hydroalcoholic extract of *Momordica charantia* leaves treated animals, the toxic effect of carbon tetrachloride was controlled significantly by restoration of the increased levels of SGOT, SGPT, ALP and total bilirubin as compare to the toxicant control. The hydroalcoholic extract of leaves of *Momordica Charantia* showed significant hepatoprotective activity.

Keywords: *Momordica charantia* leaves; hepatoprotective activity; CCl₄; SGOT; SGPT; ALP; total bilirubin; histopathology.

Introduction
The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions¹ . Additionally, it is the key organ of metabolism and excretion, thus it is continuously and variably exposed to xenobiotics because of its strategic placement in the body. The toxins absorbed from the intestinal tract go first to the liver resulting in a variety of liver ailments. Thus liver ailments remain one of the serious health problems. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drugs available for the treatment of liver disorders ²³. Therefore, many folk remedies from plant origin are evaluated for its possible antioxidant and hepatoprotective effects against different chemical-induced liver damage in experimental animals. CCl₄-induced hepatotoxicity model is frequently used for the investigation of hepatoprotective effects of drugs and plant extracts⁴⁵.

Leaves of *Momordica charantia* Linn. Family *Cucurbitaceae* are effective in bilious affections as emetic and purgative. Leaves are administered internally in leprosy, piles, jaundice. It is active as galactoguge, it is also applied round the eye orbit for night blindness. Leaf juice is rubbed to soles in burning of the feet, and used in liver complaint of childrens. In cambodia and in Gold coast, leaves are also considered to be antipyretic.⁶.

Experimental

Plant Material
The *Momordica charantia* Linn. leaves for the proposed study were collected from area of Chikhli Dist. Buldhana and were authenticated by department of botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. The freshly collected leaves of *Momordica charantia* Linn. were shade dried and then powdered to coarse size. About 1 kg of leaves powder of *Momordica charantia* Linn. was subjected
to cold maceration with (mixture of 50% ethanol and 50% distilled water) hydroalcoholic mixture. It was kept for 8 days. After maceration, the solvent was distilled off and the extract was concentrated on water bath. The extract was evaluated for hepatoprotective activity.

**Animals**

Wistar albino rats (150-200 g) of either sex were procured from Anuradha College of Pharmacy, Chikhli, and used for the study. The animals were kept in polypropylene cages and were fed with standard pelleted feed and water ad libitum. The study has got the approval from the Institutional Animal Ethical Committee (IAEC) of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

**Source of Chemicals:**

Analytical Laboratory grade chemicals, solvents were used for the studies, which were procured from s. d. fine and span diagnostic Ltd.

**Experimental design for hepatoprotective activity**

Animals were divided into five groups, each group containing six animals

- **Group I** (normal control) received distilled water for 7 days.
- **Group II** (induction control) received CCl<sub>4</sub> 1ml/kg, i. p. 1:1 dilution with coconut oil (Lin et al., 1998) on 5<sup>th</sup> day.
- **Group III** received liver tonic (5ml/kg, p.o.) for 7 days and CCl<sub>4</sub> induction on 5<sup>th</sup> day.
- **Groups IV-V** received hydroalcoholic extract of leaves (100mg/kg and 200mg/kg p.o) for 7<sup>th</sup> days and CCl<sub>4</sub> induction on 5<sup>th</sup> day. On the 8<sup>th</sup> day, the animals were sacrificed under ether anesthesia, blood and liver samples were collected. The blood was allowed to clot for 30 min; serum was separated by centrifuging and was used for biochemical estimations.

For histopathological study, liver tissue was quickly removed after autopsy and fixed in 10% formo saline.

**Assessment of hepatoprotective activity**

The activities of serum glutamate pyruvate transaminase (SGPT), and serum glutamate oxaloacetate transaminase (SGOT) were assessed by the method of Reitman and Frankel. Estimation of serum ALP and serum bilirubin (Jendrassik Groff method) were also carried out to assess the acute hepatic damage caused by CCl<sub>4</sub>.

**Result and Discussion**

**Hepatoprotective Activity**

This study shows that hepatic injury induced by CCl<sub>4</sub> caused significant rise in marker enzymes SGOT, SGPT, ALP and total bilirubin. The serum enzymes like SGOT, SGPT, ALP and total bilirubin of treated animals were significantly reduced (p<0.01) by seven days pretreatment of hydroalcoholic extract of leaves of *Momordica charantia* Linn. at two dose levels 100mg/kg and 200mg/kg, when compared with CCl<sub>4</sub> treated control (group II ). From the result it is clear that the drugs show dose dependent activity. The effects on serum marker enzymes and total bilirubin are shown in Table.

**Light Microscopic Examination**

In general, CCl<sub>4</sub> caused marked damage of rat hepatocytes in the form of fatty degeneration, cytoplasmic vacuolation, focal and confluent hepatocellular necrosis, and portal tract fibrosis with endothelial swelling and disruption (Fig 2). Pretreatment with hydroalcoholic extract of *Momordica Charantia* leaves at two dose level markedly attenuated the CCl<sub>4</sub> induced histopathological changes in rat liver (fig. 4 and 5).

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT IU/L</th>
<th>SGPT IU/L</th>
<th>Alkaline phosphatase (U/L)</th>
<th>Total Billirubin mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal control)</td>
<td>55.33±2.56</td>
<td>38.66±6.3</td>
<td>164.33±5.10</td>
<td>0.75±0.11</td>
</tr>
<tr>
<td>Group II (CCl&lt;sub&gt;4&lt;/sub&gt; treated control)</td>
<td>166.33±4.18</td>
<td>107.33±7.2</td>
<td>278.50±1.32</td>
<td>1.64±0.23</td>
</tr>
<tr>
<td>Group III (CCl&lt;sub&gt;4&lt;/sub&gt; + Liver Tonic)</td>
<td>61.50±3.83</td>
<td>56.50±5.52</td>
<td>202.33±4.41</td>
<td>0.85±0.02</td>
</tr>
<tr>
<td>Group IV (CCl&lt;sub&gt;4&lt;/sub&gt; + hydroalcoholic extract of <em>M.charantia</em> leaves 100mg/kg)</td>
<td>69.66±3.87</td>
<td>51.50±4.24</td>
<td>193.52±6.40</td>
<td>0.91±0.14</td>
</tr>
<tr>
<td>Group V (CCl&lt;sub&gt;4&lt;/sub&gt; + hydroalcoholic extract of <em>M.charantia</em> leaves 200mg/kg)</td>
<td>61.33±3.98</td>
<td>57.50±6.57</td>
<td>190.50±4.41</td>
<td>0.87±0.19</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD from six observations. One way ANNOVA is followed by Dunnet’s t-test. Group III, IV, V is compared with Group II (p<0.01).
Discussion

Hepatoprotective activity:

Since the changes associated with CCl₄-induced liver damage are similar to that of acute viral hepatitis⁷. 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 lipids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage⁸. Amino transferases are present in high concentration in liver, an important class of enzymes linking carbohydrate and amino acid metabolism. Alanine amino transferase and aspartate amino transferase are well known diagnostic indicators of liver disease. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream⁹. Alkaline phosphatase is a membrane bound enzyme and its elevations in plasma indicate membrane disruption in the organ. Alkaline phosphatases, although not a liver specific enzymes, the liver is the major source of this enzyme. The level of this enzyme increases in cholestasis.¹⁰. Hepatotoxicity is characterized by cirrhotic liver condition which in turn increased the bilirubin release¹¹. In the present study, the activities of these enzymes, total bilirubin were found to increase in the hepatotoxic animals¹², and were significantly reduced in groups of hydroalcoholic extract of leaves of Momordica charantia administered rats as compared to that of toxicant rats. The effect was more pronounced with these extracts, this might be due to the higher contents of flavonoids. It probably did so by reducing the accumulation of toxic CCl₃ derived metabolites, which may contribute to the changes in the rough endoplasmic reticulum and the disturbance of protein metabolism in liver.

Comparative histopathological study of the liver from different groups of rats corroborated the hepatoprotective efficacy of hydroalcoholic extract of leaves of Momordica charantia (fig. 1 to 5). Various pathological changes like steatosis, centrilobular necrosis and vacuolization seen in group II (toxicant rats) is due to oxidative damage by free radical generation. These pathological changes were prevented to moderate extent in both test groups. This might be due to presence of flavonoids and ascorbic acid. Antioxidant property is claimed to be one of the mechanism of hepatoprotective drugs. Further Flavonoids and ascorbic acid have been suggested to act as antioxidants by free radical scavenging. Thus the hepatoprotective activity of Momordica charantia leaves may be attributed to the presence of flavonoids and ascorbic acid.
Fig.No.5  H. E. of M. charantia leaves (200mg/kg)

References