Hepatoprotective effect of *Cassia fistula* against carbon tetrachloride induced hepatotoxicity in wistar rats: Pathomorphological studies

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Abstract

Experimental trial was conducted to evaluate hepatoprotective effects of *Cassia fistula* in carbon tetrachloride induced hepatotoxic wistar rats and its pathomorphological study for a period of 28 days. The study was conducted on 48 Wistar rats through six groups. Group I was taken healthy control. The group II, III, IV, V and VI being the treatment groups were given carbon tetrachloride for induction of hepatotoxicity for a period of 28 days. Group III treated with standard drug Silymarin and group IV, V and VI with different doses of aqueous extract of *Cassia fistula*. Toxicated rats show Behavioural changes, decrease in body weight and increase in relative organ weight. Gross and histopathological examination revealed mild to moderate changes in liver, kidney, lung, brain and testes in CCl₄ treated rats. Daily dosing of aqueous extract of leaves of *Cassia fistula* showed ameliorative against CCl₄ toxicated rats.

Keywords: *Cassia fistula*, carbon tetrachloride, pathomorphological

Introduction

Chronic liver diseases one of the primary health issues all over the world with liver cirrhosis and drug induced liver injury. Untreated liver diseases may results in liver failure and turn fetal. Among all digestive diseases liver diseases are recognized as 2nd leading cause of mortality in world. Untreated liver diseases may results in liver failure and turn fetal. The liver is considered one of the most important organs that act as a metabolic center for nutrients such as carbohydrates, proteins, lipids and the excretion of waste metabolites. Many chemicals have a strong effect on damaging liver cells such as carbon tetrachloride, thioacetamide etc. (Mohamed Saleem et al., 2010) [13].

Liver injury in rats due to Carbon tetrachloride (CCl₄) was first reported in 1936 (Cameron et al., 1936) [3]. The course of CCl₄-induced hepatotoxicity is determined by the partial oxygen pressure in the tissues, due to the low partial pressure of oxygen in the tissue which leads to the predominant formation of CCl₃* and CHCl₂* radicals and to the covalent bond with the metabolites (De Groot et al., 1988 [7] Masuda and Nakamura, 1990) [12]. High oxygen partial pressure shifts the CCl₄ metabolism to form the CCl₃-OO * radical followed by lipid peroxidation and forces the cell from steatosis to apoptosis or fatty liver (Kiezka and Kappus, 1980; De Groot et al., 1988) [10-7].

"Green medicines" are healthier and safer than synthetic ones. The entire world population is transformed into natural drugs. Acceptance by the public and the medical profession is increasing as understanding of the mechanisms by which herbs can positively influence health and quality of life. (Dawada et al., 2012) [6]. *Cassia fistula* Linn. (Cassia) The family of the Caesalpiniaaceae commonly known as Amulthus and popularly known in English as "Indian Laburnum" has been widely used in the Ayurvedic system of medicine for various disorders. (R. K. Gupta, 2010) [8].

Considering these facts, the present study was planned to evaluate the hepatoprotective effect of *Cassia fistula* in carbon tetrachloride induced hepatotoxic Wistar rats: Pathomorphological studies.

Material and Methods

The plant material was identified by taking help of Botanist from Department of Agriculture Botany, VNMKV, Parbhani and was used in present study.

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The aqueous extract of *Cassia fistula* leaves was prepared and same was used for hepatoprotective study in Wistar rats. Carbon tetrachloride was procured from chemical supplier. Standard drug Silymarin was purchased from the market.

**Experimental animals**

The present study was conducted on 48 Wistar rats of either sex, age 4-6 weeks and having 180-200 g body weight. All the rats were procured from the Laboratory Animal House, Department of Veterinary Pharmacology and Toxicology, College of Veterinary & Animal Sciences, Parbhani. The Wistar rats were housed in standard laboratory conditions in polypropylene cages, provided with food and water ad-libitum in the experimental room of Laboratory animal house, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani. The Institutional Animals Ethics Committee (IAEC) approved the experimental protocol as per the guidelines of Committee For The Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with Resolution no. IAEC/40/19 dated 02/03/2019.

**Cassia fistula leaves extract**

Aqueous extract of *Cassia fistula* leaves was prepared by cold extraction method. Leaves of the plant were allowed to dry completely under shade. Shed dried leaves were ground to powder with the help of an electrically operated grinder. Then 20% of aqueous solution was made by dissolving 200 grams of powder in 1 litre of distilled water. It was mixed thoroughly and allowed to soak for 48 hours at 4°C in refrigerator. It was shaken intermittently with an electrical operated flask shaker. Thus, resulting solution was first filter and then by whatman filter paper onto glass plates. They were allowed to dry and aqueous extract of *Cassia fistula* was obtained. Resulting extract were scraped off the glass plate and stored in a plastic container at 4°C for dosing of rats during experiment.

**Induction of hepatotoxicity**

Total 48 Wistar rats were used for the present investigation. Amongst 40 rats from II to VI induced hepatotoxicity by daily intraperitoneal administration of carbon tetrachloride 0.1 ml and liquid paraffin 0.1 ml, i. e. @ 0.2 ml/rats. (Slater, 1978) [19].

**Experimental design**

The 48 Wistar rats were divided into 6 different groups, each group comprised of 4 male and 4 female rats as detailed below.

**Group I:** Healthy control

**Group II:** Treated with CCl₄ in liquid paraffin 1:1 @ 0.2 ml/rat intraperitoneal route

**Group III:** Treated with CCl₄ in liquid paraffin 1:1 @ 0.2 ml/rat intraperitoneal route and standard drug Silymarin @ 25 mg/kg body weight orally.

**Group IV:** Treated with CCl₄ in liquid paraffin 1:1 @ 0.2 ml/rat intraperitoneal route and aqueous extract of *Cassia fistula* @ 200 mg/kg body weight orally.

**Group V:** Treated with CCl₄ in liquid paraffin 1:1 @ 0.2 ml/rat intraperitoneal route and aqueous extract of *Cassia fistula* @ 400 mg/kg body weight orally.

**Group VI:** Treated with CCl₄ in liquid paraffin 1:1 @ 0.2 ml/rat intraperitoneal route and aqueous extract of *Cassia fistula* @ 600 mg/kg body weight orally.

Daily dosing was carried out for the experimental period of 28 days

**Collection of Sample**

Rats were sacrificed on day 28 of experiment by using excess dose of inhalation anesthesia (di-ethyl ether) at the end of experiment. Relative organ weights, Gross necropsy and histopathological examination were performed. Liver, kidney, lungs, brain, ovaries and testes were collected in 10% formalin for histopathological examination.

**Parameters studied**

Various types of parameters were studied, these include general observations (behavioral changes, body weight and organ weight) pathological observations (gross pathology and histopathology) were performed.

**Behavioral changes**

All the experimental animals in the treatment groups were daily examined for any abnormal behavioral changes. if any and compared with control group animals.

**Body weight**

The body weights were taken on day 0th, 7th, 14th, 21st and day 28th i.e. on the termination day of the experiment.

**Relative Organ weight**

At the end of the study, the relative organ weights of liver, kidney, lungs, brain, ovaries and testes of experimental rats were noted.

**Pathomorphological study**

Gross necropsies of the organs were observed for the presence of any abnormal gross pathological change. Liver, kidney, lungs, brain, ovaries, and testes tissue were collected in 10% formalin. After fixation, the tissue pieces were processed as per the standard procedure (Culling, 1974) [5].

**Statistical analysis**

The data obtained from various parameters from all groups were analyzed as per the method suggested by (Panse and Sukhatme, 1967) [15] using factorial randomized block design (FRBD) or completely randomized block design (CRD).

**Results and Discussion**

The present experiment was designed to assess the hepatoprotective effect of *Cassia fistula* in carbon tetrachloride induced hepatotoxic Wistar rats. The results are interpreted in the present topic.

**Behavioral Changes**

All experimental rats appeared alert, active and healthy during period of acclimatization without displaying any unfavorable manifestations suggested adequate acclimatization. All the rats were observed daily during the experimental period, experimental rats of treatment groups did not revealed any observable behavioral changes, except in group 2, carbon tetrachloride control group animals. In which rats showed dullness, restlessness, reduced feed intake.
Body weight
The details of Average body weight (gm/week) of all the groups on 0th, 7th, 14th, 21st and 28th day of experiment are summarized in Table 1 and fig.1

<table>
<thead>
<tr>
<th>Groups of rat</th>
<th>Average body weight of rats (gm/week)</th>
<th>Stat</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intervals of study</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 day</td>
<td>7th day</td>
<td>14th day</td>
</tr>
<tr>
<td>I</td>
<td>187.67±2.80</td>
<td>189.08±2.64</td>
<td>191.12±2.64</td>
</tr>
<tr>
<td>II</td>
<td>187.85±1.76</td>
<td>186.85±1.65</td>
<td>182.30±1.38</td>
</tr>
<tr>
<td>III</td>
<td>187.85±2.28</td>
<td>188.13±2.30</td>
<td>190.66±2.37</td>
</tr>
<tr>
<td>IV</td>
<td>186.67±2.61</td>
<td>189.95±2.62</td>
<td>190.20±2.60</td>
</tr>
<tr>
<td>V</td>
<td>187.70±2.14</td>
<td>187.96±2.18</td>
<td>190.42±2.29</td>
</tr>
<tr>
<td>VI</td>
<td>187.77±1.89</td>
<td>189.03±1.89</td>
<td>191.47±1.82</td>
</tr>
<tr>
<td>Stat</td>
<td>NS</td>
<td>NS</td>
<td>S</td>
</tr>
<tr>
<td>CD</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Different superscripts a, b, c shows significance difference between different groups on specific day (p<0.05)

Relative Organ weight
The Relative organ weights (gm) of all the groups of experiment are summarized in Table 2 and fig.2

<table>
<thead>
<tr>
<th>Groups of rat</th>
<th>Relative organ weight of experimental rats (gm)</th>
<th>Stat</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Lung</td>
</tr>
<tr>
<td>I</td>
<td>3.66±0.25</td>
<td>0.84±0.02</td>
<td>1.07±0.08</td>
</tr>
<tr>
<td>II</td>
<td>5.87±0.15</td>
<td>1.27±0.07</td>
<td>1.17±0.10</td>
</tr>
<tr>
<td>III</td>
<td>3.73±0.19</td>
<td>0.83±0.05</td>
<td>0.94±0.04</td>
</tr>
<tr>
<td>IV</td>
<td>4.66±0.18</td>
<td>1.03±0.06</td>
<td>1.02±0.08</td>
</tr>
<tr>
<td>V</td>
<td>4.08±0.23</td>
<td>0.89±0.06</td>
<td>0.97±0.04</td>
</tr>
<tr>
<td>VI</td>
<td>3.76±0.21</td>
<td>0.85±0.05</td>
<td>0.98±0.06</td>
</tr>
<tr>
<td>CD values</td>
<td>0.601</td>
<td>0.162</td>
<td>-</td>
</tr>
<tr>
<td>Statistics</td>
<td>S</td>
<td>S</td>
<td>NS</td>
</tr>
</tbody>
</table>

The mean Average body weight in group II animals on day 7th, 14th, 21st and 28th were observed to be significantly decreased when compared to group I (healthy control) animals.

Table 1: Value of Average body weight (gm/week) in experimental rats in different groups at different intervals of study

The mean Average body weight in group III, V and VI animals was observed to be significantly lower when compared to group II (control). In group III, V and VI animals were observed to be significantly lower when compared to group II values due to administration of aqueous extract of Cassia fistula. In group IV was observed to be significantly decreased when compared to group II however, significantly increased when compared to group I, III and VI values. Similar results reported by Adewole et al. (2007) [1] in CCl-induced kidney damage in rats.

The relative weight of lung, brain, testes and ovaries does not vary significantly.

Gross Pathology
The gross pathological examination showed congestion, enlargement of liver (hepatomegaly), increased fragility and focal necrosis of liver from the rats of group II (CCl4 treated rats). The earlier reports in respective gross pathological examination in CCl4 intoxicated rats published by Uehara et al. (2013) [10] goes parallel with present observation. However, kidney, lung, brain, testes and ovaries did not show any appreciable gross pathological changes in any of rats from control as well as treatment groups.

Histopathological Investigation
The histarchitecture of liver from rat of control were within normal histological limits. The histarchitecture study of liver revealed congestion, minimal to severe, focal to diffuse, fatty changes and necrosis. There was dilation of central vein, mononuclear cell infiltration as well as vascular degenerative changes in hepatic parenchyma of rats of group II which were intoxicated with CCl4 (plate1). However, treatment related restoration of histarchitecture of liver was noted and same was evidence with less intense histopathological changes in rats of groups III, IV, V and VI also the restoration appeared to be treatment and dose dependant (plate 2).The earlier reports in respective histopathological examination of liver in CCl4 intoxicated rats and restoration of histarchitecture of liver by Cassia fistula published by Bhakta et al. (1999) [2] Dawada et al. (2012) [6] goes parallel with present observation. In CCl4 toxicity, trichloromethyl free radical was produced which is toxic reactive metabolite. This toxic metabolite binds covalently to the lipid membrane of the adipose tissue and causes peroxidative degradation which results in fats from the adipose tissue are translocated and accumulated in the hepatocyte. (Singh et al., 2014) [18].

The histomorphological studied of the kidney from rats of group II showed mild to moderate, focal to multifocal cistic degenerative changes, vascular degenerative changes, congestion, necrotic changes and occasional hyaline caste in lumen of convuluted tubules. (plate 3). The earlier reports in respective histopathological examination of kidney in CCl4 intoxicated rats published by Adewole et al. (2007) [1] Saber et al. (2012) [16] goes parallel with present observation. Similar but less intense histopathological changes were noted in rats of group III, IV, V and VI and the restoration appeared to be treatment and dose dependant. However, the section of the kidney from the rats of the control group found to be within histological limits.

The histopathological study of section of lung from rats of group II showed congestion and inflammatory cell infiltration with variation in extends of intensity, more intense pneumopathy (plate 4). The earlier reports in respective histopathological examination of lung in

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CCl₄ intoxicated rats published by Khan et al. (2012)⁹ goes parallel with present observation. The brain on histopathology revealed mononuclear cell infiltration and vacuolation in the rats of group II (plate 5). The earlier reports in respective histopathological examination of brain in CCl₄ intoxicated rats published by Simeonova et al. (2019)¹⁷ goes parallel with present observation. However, the section of brain from all other treatment of control group did not showed any appreciable histopathological changes. The histopathological examination of testes from rats of intoxicated non treated group showed minimal to mild loss of spermatogonial cells, increase in interstitial cells and distortion of basement membrane at places. (plate 6) The earlier reports in respective histopathological examination of testes in CCl₄ intoxicated rats published by Ojo et al. (2016)¹⁴ goes parallel with present observation. The sections of testes from treated rats were with less intensity and the same were found to be dose dependent. The sections of ovaries from the control as well other experimental groups did not showed any appreciable histopathological change.

Plate 1: Section of liver with fatty changes and necrosis in rat of group II (400X) H and E stain

Plate 2: Section of Liver with mononuclear cell infiltration in rat of group VI (100X) H and E stain

Plate 3: Note cystic degenerative changes, congestion and MNC infiltration in kidney from rat of group II (100X) H and E stain

Plate 4: Section of lungs from rat of group II showing congestion and severe inflammatory cell infiltration. (100X) H and E stain

Plate 5: Section of brain showing MNC infiltration and vacuolation from rat of group II (400X) H and E stain
Plate 6: Note occasional loss of spermatogonial cell in testes from seminiferous tubule in rat of group II (100X) H and E stain

Conclusion
From present investigation it can be concluded that, Use of carbon tetrachloride at the dose rate of 0.1 ml/rat by intraperitoneal injection for 21 days can induce hepatotoxicity in wistar rats. The carbon tetrachloride showed damaging results in gross and histopathological observations where-as the treatment with Cassia fistula leaves extract at the dose rate of 600 mg/kg body weight showed promising results.

References