Hepatoprotective and antioxidant efficacy of Anethum graveolens linn in carbon tetrachloride induced hepatotoxicity in albino rats

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ABSTRACT

In the present study, the hepatoprotective and antioxidant efficacy of Anethum graveolens Linn was evaluated in CCl₄ induced hepatotoxicity in albino rats. Hepatotoxicity was induced in albino rats by subcutaneous injection of carbon tetrachloride. Hepatoprotective efficiency of 100 mg/Kg wt of ethanolic extract of Anethum graveolens Linn was evaluated. Hepatotoxicity in experimental rats was evidenced by enhancement in the activity of SGOT, SGPT, MDA, & ALP and significant decrease in the activity of GSH, GPX, SOD & CAT. In plant extract treated animals, the toxicity effect of CCl₄ was controlled significantly by restoration of the activity of above serum enzymes and antioxidant enzymes compared to the normal and standard drug silymarin treated groups. Therefore the results of present study support the hepatoprotective effect of Anethum graveolens Linn.

INTRODUCTION

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. The liver helps to purify the blood by changing potentially harmful chemicals into harmless ones[6]. Hepatotoxicity implies chemical-driven liver damage. Chemicals that cause liver injury are called hepatotoxins. CCl₄ is a volatile organic alkyl halogen that is present in the environment largely because of release from manmade sources. It is a clear color less & non inflammable heavy liquid with a characteristic odor. High exposure to CCl₄ can cause liver, kidney damages. The nervous system including the brain is affected and headaches, dizziness, sleeping, nausea & vomiting [1].

In this study are selected Anethum graveolens Linn was selected to evaluate its in hepatoprotective function on CCl₄ induced hepatotoxicity in albino rats. Anethum graveolens Linn is a common ingredient in gripe water, give to relive colic pain in babies. Anethum graveolens Linn stimulates milk flow in lactating mothers. An infusion of the flowering plant is recommended for urinary complaints[8].

EXPERIMENTAL SECTION

Hepatotoxicity inducer
CCl₄ was used as the hepatotoxicity inducer in animal purchased from Chemie PvtLtd.

Collection of plant materials
Anethum graveolens Linn whole plants materials were collected from the fields of Thiruvarur in Tamilnadu.
Preparation of plant extract
*Anethum graveolens Linn* whole plants were dried at 40°C for 7 days. Powdered using electric grinders and stored in a container. This fine crude powder was used as herbal drug.

Preparation of Ethanol extract
Weighed amount of dried powder of *Anethum graveolens Linn* leaves taken and added 50ml of 99.9% hot ethanol mixture evaporated at 55°C by using hot air oven, the collected the extract hepatotoxicity studies.

Animals
Healthy young male albino rats (130-150 gm) were purchased from animal house of St, Joseph arts and science college, Tirchy. The groups of rats were kept separately individual stainless steel hoppers. The test animals should be characterized by stain, source, sex, weight and age. The animals were kept individually for feeding in conventional laboratory diets with an unlimited supply of drinking water.

Experimental design
The rats were randomly divided into four groups of five animals each.

**Group 1:** Control rats.
**Groups 2:** Hepatotoxicity induced rats-CCl₄ at a dose 0.5ml/kg/b.wt.
**Groups 3:** Rats treated only with *Anethum graveolens Linn* (100mg/kg/b.wt).
**Groups 4:** Rats treated with CCl₄ along with *Anethum graveolens Linn* (100mg/kg/b.wt).
**Groups 5:** Rats treated with silymarin as standard (50 ml/kg/wt).

Sample collection
The standard and test formulations were administrated for 21 days, once in a day. At the end of experiment rats were sacrificed by cervical decapitation. Blood was collected and centrifuged for serum separation and tissue was dissected out .Weighed using ice cold saline solution. The resulting supernatant was used for biochemical assays.

Biochemical analysis
Serum enzymes such as SGOT, SGPT, MDA &ALP are estimated in CCl₄ intoxicated of plant treated rats. Antioxidants enzymes such as GSH, GPX, SOD & CAT analysed in liver homogenate of CCl₄ intoxicated of plant treated rats.

RESULTS AND DISCUSSION
Table 1: Shows the elevation of serum enzymes in CCl₄ in rats. After treatment with plant extract those enzyme returns to normal level. Table 2: Shows a significant return in the antioxidant enzymes activity in tixic rats, while treating with plant enzymes activity in toxic rats, while treating with plant extract those enzymes restores their original activity.*Anethum graveolens Linn* has been used to treat some chronic liver diseases such as hepatitis & hepatocirrhosis. We have been trying to search for hepatoprotective compounds of *Anethum graveolens Linn*[3]. The present in vitro study also indicated that *Anethum graveolens Linn* had a protective effect against CCl₄ induced injury to primarily cultured rat hepatocytes. Since the extraction & isolation of *Anethum graveolens Linn* are relatively simple and have a high content in the leaves of *Anethum graveolens Linn* [10]. We may take these advantages to further study its mechanisms of hepatoprotective effect and develop a new drug from it.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT</th>
<th>SGPT</th>
<th>MDA</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>124.80±23.71</td>
<td>53.25±1.71</td>
<td>12.72±0.65</td>
<td>179±3.70</td>
</tr>
<tr>
<td>Group II</td>
<td>302.40±59.40*</td>
<td>163.30±10.30*</td>
<td>14.15±0.75*</td>
<td>186±4.60*</td>
</tr>
<tr>
<td>Group III</td>
<td>156.80±9.30**</td>
<td>163.30±10.30**</td>
<td>13.25±0.76**</td>
<td>182±5.90**</td>
</tr>
<tr>
<td>Group IV</td>
<td>153.30±8.3**</td>
<td>105.50±3.1**</td>
<td>12.34±0.82**</td>
<td>175±7.80**</td>
</tr>
<tr>
<td>Group V</td>
<td>140.80±8.5**</td>
<td>199.8±0.11**</td>
<td>12.9±0.94**</td>
<td>176±8.60**</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD (n=6)

*P*< 0.05 compared with normal control

**P**<0.05 compared with hepatitis control
The present study, it was obtained that, the rats treated with CCl₄ resulted in significant hepatic damage as shown by the elevated levels of serum markers. These changes in the marker levels will reflect in hepatic structural integrity. The rise in the SGOT is usually accompanied by an elevation in the levels of SGPT, which play a vital role in the conversion of amino acids to ketoacids[9].

Table II: Level of antioxidant enzymes in CCl₄ intoxicated of plant treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH</th>
<th>GPX</th>
<th>SOD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>319±2.33</td>
<td>8.0±3.51</td>
<td>6.26±2.93</td>
<td>67±3.95</td>
</tr>
<tr>
<td>Group II</td>
<td>206±2.57*</td>
<td>5.51±3.68*</td>
<td>5.54±2.75*</td>
<td>38±3.91*</td>
</tr>
<tr>
<td>Group III</td>
<td>308±2.64**</td>
<td>8.21±3.74**</td>
<td>7.00±3.76**</td>
<td>59±3.76**</td>
</tr>
<tr>
<td>Group IV</td>
<td>290±2.55**</td>
<td>6.31±3.71**</td>
<td>5.92±2.51**</td>
<td>57±3.32**</td>
</tr>
<tr>
<td>Group V</td>
<td>286.50±2.60**</td>
<td>6.31±3.65**</td>
<td>6.61±2.19**</td>
<td>51±3.96**</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD (n=6)
P*< 0.05 compared with normal control
P**<0.05 compared with hepatitis control

Lipid peroxidation has been postulated to the destructive process of liver injury due to CCl₄ administration. In the present study, the elevations in the levels of end products of lipid peroxidation in the liver of rat treated with CCl₄ were observed. The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with ethanol extract of *Anethum graveolens* Linn significantly reversed these changes. Hence, it may be possible that the mechanism of hepatoprotection by ethanol extraction of *Anethum graveolens* Linn is due to its antioxidant effect[5].

The alkaline phosphatase is the prototype of these enzymes that reflects the pathological alteration in biliary flow 20. The CCl₄ induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubins content. The ethanol extract of *Anethum graveolens* Linn CCl₄ induced suppression of the increased ALP activity with the concurrent depletion of raised bilirubins suggests the possibility of the extract to have ability to stabilize biliary dysfunction in rat liver during hepatic injury by CCl₄. Thus administration of ethanol extract of aerial part of *Anethum graveolens* Linn is against the toxic effect of CCl₄[10].

GSH is an important endogenous anti-oxidant substance. The decrease of GSH content may be due to increased GSH consumption as it participates in the detoxification system for the metabolism ofCCl₄, and results in an enhanced susceptibility of hepatocytes to CCl₄ toxicity[11]. Our results showed that CCl₄ obviously decreased GSH content in the hepatocytes, but *Anethum graveolens* Linn could partly reverse it. This suggested that the nature of *Anethum graveolens* Linn protecting-SH compounds (such as GSH) from CCl₄ injury may be the third mechanism of its hepatoprotection.

In the present investigations, CCl₄ intoxicated rats decreased the content of GPX in liver, whereas, treatment with ethanol extract of *Anethum graveolens* Linn (100 and 200mg/kg) able to reverse such effects. Superoxide dismutase (SOD) is a key defense enzyme and catalyses the dismutation of superoxide anions. Catalase (CAT) is a haemeprotein that catalyses the reduction of H₂O₂ and able to prevent the tissue from reactive free oxygen and hydroxyl radicals. Decrease in SOD activity can result in the removal of superoxide anions that may inactivate SOD thereby causing an inactivation of H₂O₂ scavenging enzymes. Administration of ethanol extract of *Anethum graveolens* Linn treated rats able to prevent effectively the decrease in SOD and CAT activities, which may be directly correlated to scavenging or neutralizing of radicals by ethanol extract of *Anethum graveolens* Linn resulting in protection of these important defense enzymes[2].

**CONCLUSION**

In this work, the hepatoprotective efficacy *Anethum graveolens* Linn in drug induced hepatotoxic rats was studied. In CCl₄ intoxicated rats, the serum enzymes showed elevation in their activity and antioxidant enzymes showed a marked decline in its action. In plant treated rats, the serum marker enzymes and antioxidant enzymes reversed to its original level. In conclusion, oral administration of *Anethum graveolens* Linn is effective in suppressing CCl₄ induced hepatotoxicity.
REFERENCES

[8] Prajgapati, Rodriguez FFl, Contreras CM, Molina MH (2000); Estudio preliminary del as possible acciones antidepresive as de Anethum graveolens linn., resume de ponencias del primer congreso nacional de plants medicinales de Mexico, pp 69-74.