Hepatoprotective Activity of the Fruits of Cucumis Sativus (L.)

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ABSTRACT

Hepatoprotective activity of the ethanolic extract of the fruits of Cucumis sativus was studied against paracetamol induced toxicity in albino rats. Hepatotoxicity was induced by paracetamol showed significant biochemical, and histological deteriorations in the liver of experimental animals. Treatment with ethanolic extract of the fruits of Cucumis sativus had significant protection against hepatic damage by maintaining the biochemical parameters such as glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), γ-glutamyl transferase (GGT), total bilirubin, conjugated bilirubin, unconjugated bilirubin and lipid peroxidase (LPO) as well as the levels of liver homogenates glutathione peroxidase (GPx), glutathione reductase (GRD), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) within normal range which were evidently showed in histopathological study. Liver histopathology showed that the ethanolic extract of the fruits of Cucumis sativus reduced the space formation, loss of cell boundaries and hepatic necrosis induced by paracetamol in albino rats. The ethanolic extract of the fruits of Cucumis sativus has highly significant hepatoprotective effect at 500 mg/kg on the liver of paracetamol induced toxicity along with silymarin as a standard hepatoprotective agent.

Keywords: Cucumis sativus, Hepatoprotective, Ethanol extract, Paracetamol, Silymarin.

INTRODUCTION

Liver is the vital organ of metabolism and excretion. About 20,000 deaths found every year due to liver disorders. This is a major public health problem, contributing to life-threatening complications of portal hypertension, liver failure and increased incidence of hepatocellular carcinoma. In spite of tremendous strides in the modern medicine, there are still only a few drugs available for the treatment of liver disorders. There are potent indigenous herbal medicines available for the treatment of liver disorders in various parts of the world and most of them have not yet scientifically been validated. If they are conducted, it could lead to the development of cost effective drugs.

The plant Cucumis sativus (Fam. Cucurbitaceae) commonly known as "Mullu vellari" in Tamil, “Sakusa” in Sanskrit, “Kheera” in Hindi and “Cucumber” in English is reported to possess a number of medicinal values. The botanical family Cucurbitaceae, commonly known as cucurbits also displays a rich diversity of sex expression, and the cucumber has served as a primary model system for sex determination studies. The cucurbits are also model plants for the study of vascular biology, as both xylem and phloem sap can be readily collected for studies of long-distance signaling events. Cucumis sativus fruit is shown to possess various activities such as antihyperglycemic activity, inhibitory effects on protein kinase C (PKC) activity, antioxidant activity, amylolytic activity, anticancer activity, antilactogenic activity, and antimutagenicity. The juice is used in many beauty products. C. sativus is amongst the constituents of cosmetics marketed as treatments for skin inflammations and other skin disorders, and as skin protectants.

MATERIALS AND METHODS

Collection of plant materials

The fruits of Cucumis sativus was collected in the month of July from Alangulam, Tirunelveli District, Tamil Nadu and identified by Prof. P. Jayaraman, Plant Anatomy Research Centre, West Thambaram, Chennai- 600 045, Tamil Nadu, India.

A voucher specimen (MSU/PHAR/HER-141) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli – 627 012, Tamil Nadu, India.

Experimental animals

Wistar albino rats (weighing 150-200 g) were used for hepatoprotective studies. The animals were fed with standard pellet diet supplied by Hindustan Lever Ltd., Kolkata, India and fresh water ad libitum. They were housed in standard stainless-steel cages at a 12 h cycle of light and dark. The room temperature was kept at (25±3°C), and humidity was maintained at 50%.

Drugs and chemicals

Paracetamol was purchased from S.D. Fine Chemicals Ltd. (India), Silymarin was obtained as gift sample from Ranbaxy (Devas, India), Standard kit of SGPT, SGOT, SALP, bilirubin and total protein were obtained from Jain Scientific Industries, Moradabad, India. All other reagents used were of analytical grade.
Preparation of extracts
The collected fruits were cut into pieces, shade-dried at room temperature and powdered. The dried fruit powder (500 g) was successively extracted using petroleum ether (40°-60°C), benzene, chloroform, ethanol and water by using Soxhlet apparatus. The last trace of solvent was removed under reduced pressure distillation and then vacuum dried. The dried crude ethanolic extract was used for the study.

Acute toxicity
Acute toxicity study was performed for the ethanolic extract of the fruits of Cucumis sativus as per OECD guidelines. Albino rats received 2000 mg/kg b.w. of ethanolic extract orally. The animals were observed for toxic symptoms continuously for the first 4 h after dosing. The rats were continuously observed for their mortality and behavioral response for 48 h and thereafter once in a day for 14 days. There was no mortality recorded. Therefore the drug should be free from toxicity.

Hepatoprotective activity against paracetamol-induced toxicity in albino rats
Rats were divided into six groups, each group consisting of six animals.

Group I: Controls received the vehicle viz. normal saline (2 ml/kg p.o.).

Group II: Received paracetamol orally (750 mg/kg) at every 72 h for 10 days.

Group III: Received ethanolic extract of the fruits of Cucumis sativus at the oral dose of 100 mg/kg for 10 days and simultaneously administered paracetamol 750 mg/kg every 72 h. (Low dose)

Group IV: Received ethanolic extract of the fruits of Cucumis sativus at the oral dose of 250 mg/kg for 10 days and simultaneously administered paracetamol 750 mg/kg every 72 h. (Moderate dose)

Group V: Received ethanolic extract of the fruits of Cucumis sativus at the oral dose of 500 mg/kg for 10 days and simultaneously administered paracetamol 750 mg/kg every 72 h. (High dose)

Group VI: Received silymarin 50 mg/kg orally for 10 days and simultaneously administered paracetamol 750 mg/kg every 72 h. (Standard drug).

At the end of the experimental period, all the animals were sacrificed by cervical decapitation. Blood samples were collected, and the serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for the various biochemical parameters.

Assessment of liver damage
Liver damage was assessed by the estimation of serum activities of serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), γ-glutamyl transpeptidase (GGTP), total bilirubin, conjugated bilirubin, unconjugated bilirubin, total protein, albumin, and globulin according to the method by using commercially available test kits. Lipid peroxidase (LPO), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) were estimated in liver homogenate.

Histopathological studies
The livers were removed from the animals and the tissues were fixed in 10 % formalin for at least 24 h. Then, the paraffin sections were prepared (Automatic tissue processor, Autotechnique) and cut into 5 µm thick sections using a rotary microtome. The sections were then stained with Haematoxylin-Eosin dye and studied for histopathological changes, such as fatty changes, necrosis, vacuole, space formation, loss of cell boundaries for microscopic observations.

Statistical analysis
The values were expressed as Mean ± SD. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison test and data on liver weight variations were analyzed using Student’s ‘t’ test. The levels of significance are mentioned as * P ≤ 0.05, ** P ≤ 0.01.

RESULTS AND DISCUSSION
Paracetamol produces hepatic necrosis when ingested in very large doses. It is metabolised in the liver primarily to glucuronide and sulphate conjugates. Paracetamol toxicity is due to formation of toxic metabolites when a part of it is metabolised by cytochrome P<sub>450</sub>. Induction of cytochrome P<sub>450</sub> or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity. Therefore, the antihepatotoxic activity of the drug may be due to: inhibition of cytochrome P<sub>450</sub>; promotion of glucuronidation; stimulation of hepatic regeneration; activation of the functions of reticuloendothelial systems; or inhibition of protein biosynthesis.

The body weight in the control and various experimental groups is presented in Table 1. There was a significant reduction in body weight in the paracetamol-treated control rats when compared to the corresponding normal rats. Treatment with ethanolic extract of the fruits of Cucumis sativus (100 mg/kg p.o. 250 mg/kg p.o. 500 mg/kg p.o.) and silymarin (50 mg/kg p.o.) to paracetamol-induced hepatic damaged rats caused a marked increase in the body weight. A considerable increase in body weight was noticed in the 500 mg/kg p.o. dose.

The serum biochemical parameters in the control and various experimental groups are presented in Table 1 and Table 2. Rats treated with paracetamol showed a significant hepatic damage as observed from elevated serum level of hepatospecific enzymes as well as severe alteration in different liver parameters. Because they are cytoplasmic in location and released into circulation after
cellular damages indicating development of hepatotoxicity. Serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), γ-glutamate transpeptidase (GGTP), total bilirubin, conjugated bilirubin, and unconjugated bilirubin in serum were increased in paracetamol intoxicated control animals as well as reduction in the levels of total protein, albumin, and globulin compared to those of the control rats.

Table 1: Effect of ethanolic extract of the fruits of Cucumis sativus on the body weight, liver SGOT, SGPT, SALP, GGTP on Paracetamol-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg p.o.)</th>
<th>Body weight</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Control</td>
<td>2 mL saline</td>
<td>161.14±6.56</td>
<td>168.53±4.12</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>750</td>
<td>178.59±7.84</td>
<td>171.16±6.41</td>
</tr>
<tr>
<td><em>Cucumis sativus</em> extract</td>
<td>100</td>
<td>163.66±5.26</td>
<td>168.74±3.31</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>174.81±5.94</td>
<td>176.29±5.13</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>187.59±7.12</td>
<td>196.31±6.64</td>
</tr>
<tr>
<td>Silymarin</td>
<td>50</td>
<td>181.26±6.43</td>
<td>189.63±4.28</td>
</tr>
</tbody>
</table>

Values are Mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P < 0.05; **P < 0.01 as compared with normal control to liver damaged control; #P<0.05; ##P<0.01 as compared with liver damaged control to drug treated animal; NS: not significant

Table 2: Effect of ethanolic extract of the fruits of Cucumis sativus on the liver total bilirubin, conjugated bilirubin, unconjugated bilirubin, total protein, albumin, globulin, A/G ratio on paracetamol-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg p.o.)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Conjugated bilirubin (mg/dl)</th>
<th>Unconjugated bilirubin (mg/dl)</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A/G Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 mL saline</td>
<td>0.76±0.03</td>
<td>0.21±0.05</td>
<td>0.55±0.02</td>
<td>8.31±0.34</td>
<td>4.56±0.61</td>
<td>3.75±0.51</td>
<td>1.2:1</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>750</td>
<td>2.65±0.11**</td>
<td>1.55±0.06**</td>
<td>1.10±0.05**</td>
<td>6.12±0.24**</td>
<td>3.10±0.14**</td>
<td>3.02±0.22</td>
<td>1.0:1</td>
</tr>
<tr>
<td><em>Cucumis sativus</em> extract</td>
<td>100</td>
<td>1.26±0.06</td>
<td>0.31±0.02</td>
<td>0.95±0.07**</td>
<td>7.21±0.21</td>
<td>4.05±0.24</td>
<td>3.16±0.51</td>
<td>1.2:1</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1.12±0.04</td>
<td>0.26±0.01</td>
<td>0.81±0.05**</td>
<td>7.74±0.56</td>
<td>4.11±0.74</td>
<td>3.57±0.34</td>
<td>1.1:1</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.93±0.05</td>
<td>0.20±0.03</td>
<td>0.73±0.02**</td>
<td>7.96±0.36</td>
<td>4.17±0.72</td>
<td>3.85±0.26</td>
<td>1.0:1</td>
</tr>
<tr>
<td>Silymarin</td>
<td>50</td>
<td>0.88±0.07</td>
<td>0.24±0.04</td>
<td>0.64±0.04</td>
<td>8.16±0.12</td>
<td>4.64±0.11</td>
<td>3.52±0.16</td>
<td>1.3:1</td>
</tr>
</tbody>
</table>

Values are Mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P < 0.05; **P < 0.01 as compared with normal control to liver damaged control; #P<0.05; ##P<0.01 as compared with liver damaged control to drug treated animal; NS: not significant

Table 3: Effect of ethanolic extract of the fruits of Cucumis sativus on the liver LPO, GPX, SOD, CAT, GSH on Paracetamol-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg p.o.)</th>
<th>LPO (nm MDA/mg protein)</th>
<th>GPX (U/mg protein)</th>
<th>GRD (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GSH (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 mL saline</td>
<td>1.754±0.014</td>
<td>3.729±0.151</td>
<td>0.598±0.041</td>
<td>0.204±0.014</td>
<td>3.093±0.019</td>
<td>27.15±0.68</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>750</td>
<td>4.129±0.024**</td>
<td>1.119±0.136**</td>
<td>0.124±0.014**</td>
<td>0.092±0.004**</td>
<td>1.112±0.051**</td>
<td>14.56±0.74**</td>
</tr>
<tr>
<td><em>Cucumis sativus</em> extract</td>
<td>100</td>
<td>2.892±0.051**</td>
<td>1.816±0.108**</td>
<td>0.263±0.056**</td>
<td>0.129±0.013**</td>
<td>1.934±0.056**</td>
<td>14.66±0.73</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>2.114±0.063**</td>
<td>2.004±0.116**</td>
<td>0.321±0.014**</td>
<td>0.146±0.070**</td>
<td>2.262±0.024**</td>
<td>16.89±0.56</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>1.808±0.074**</td>
<td>2.621±0.173**</td>
<td>0.391±0.025**</td>
<td>0.169±0.033**</td>
<td>2.514±0.022**</td>
<td>19.26±0.68</td>
</tr>
<tr>
<td>Silymarin</td>
<td>50</td>
<td>1.786±0.031**</td>
<td>3.428±0.158**</td>
<td>0.565±0.019**</td>
<td>0.196±0.021**</td>
<td>3.163±0.018**</td>
<td>23.74±0.71</td>
</tr>
</tbody>
</table>

Values are Mean ± SD of 6 animals in each group. Statistical analysis ANOVA followed by Dunnett t-test. *P < 0.05; **P < 0.01 as compared with normal control to liver damaged control; #P<0.05; ##P<0.01 as compared with liver damaged control to drug treated animal; NS: not significant
Graph 1-6: Comparison of body weight, SGOT, SGPT, SALP, GGTP, total bilirubin, conjugated bilirubin, unconjugated bilirubin, total protein, albumin, globulin, LPO, GPx, GRD, SOD, and CAT levels before and after treatment on paracetamol-induced hepatotoxicity.

The results of administration of the ethanolic extract of the fruits of *Cucumis sativus* orally (100 mg/kg, 250 mg/kg, and 500 mg/kg) for 10 days to hepatotoxic bearing rats had produced significant protective effect on paracetamol-induced hepatic damage in albino rats. Treatment with ethanolic extract of the fruits of *Cucumis sativus* decreased the levels of elevated serum enzymes like, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), γ-glutamyl transpeptidase (GGTP), total bilirubin, conjugated bilirubin, and unconjugated bilirubin as well as increased the levels of total protein, albumin, and globulin. In 500 mg/kg p.o. dose of the ethanolic extract of the fruits of *Cucumis sativus* treated groups normalizing the elevated levels of biochemical parameters. From these results the degree of protection observed was maximum with higher dose of the ethanolic extract of the fruits of *Cucumis sativus* (500 mg/kg p.o.).
The levels of lipid peroxides and activity of enzymic antioxidants, glutathione peroxidase (GPx), glutathione reductase (GRD), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in liver homogenates are presented in Table 3. Marked increase in the lipid peroxide levels and concomitant decrease in enzymic antioxidants levels were observed in hepatotoxic rats, while the ethanolic extract of the fruits of *Cucumis sativus* treatment has brought down the elevated level of LPO and also significantly enhanced the reduced levels of GPx, GRD, SOD, CAT, and GSH. Comparison of in vivo effects of the three doses of the ethanolic extract of the fruits of *Cucumis sativus* and silymarin on paracetamol-induced changes in biochemical parameters in rats are presented in (Graph 1-Graph 6).

**Figure 1**: Histopathology of the ethanolic extract of the fruits of *Cucumis sativus* on paracetamol - induced hepatotoxicity in albino rats.

A-Control group; B-PCM treated group; C-PCM + EE of CS (100 mg/kg); D-PCM + EE of CS (250 mg/kg); E-PCM + EE of CS (500 mg/kg); F-PCM + Silymarin (50 mg/kg).

CV-Central vein, H-Hepatocyte, N-Nucleus, FC-Fatty changes, NC-Necrosis, V-Vacuole, SF-Space formation, LCB-Loss of cell boundaries, EE-Ethanolic extract, CS-*Cucumis sativus*, PCM-Paracetamol.

Liver histopathology of the ethanolic extract of the fruits of *Cucumis sativus* are presented in Figure 1. The ethanolic extract of the fruits of *Cucumis sativus* at three different doses, 100 mg/kg p.o. 250 mg/kg p.o. 500 mg/kg p.o. and silymarin followed by paracetamol-intoxication showed a sign of protection as it was evident from the absence of necrosis, space formation and vacuoles. Group I control animals treated with normal saline showed normal hepatic cells each with well-defined cytoplasm, prominent nucleus, and well brought out central vein, normal architecture of liver; while paracetamol treated liver showed disarrangement of normal hepatic cells total, centrilobular hepatic necrosis, vacuolization, loss of cell boundaries, space formation, and crowding of central vein marked level of fatty changes or degeneration and necrosis of the liver cells. GC-MS analysis of the ethanolic extract of the fruits of *Cucumis sativus* showed the presence of palmitic acid, such as n-Hexadecanoic acid. It is a hemolytic 5-α reductase inhibitor. It may be responsible for the liver disorder curing effect. The obtained results indicated ethanolic extract of the fruits of *Cucumis sativus* shows a high degree of protection against the hepatotoxic effect of paracetamol.

**CONCLUSION**

The ethanolic extract of the fruits of *Cucumis sativus* at 100 mg/kg p.o. 250 mg/kg p.o. and 500 mg/kg p.o. dose levels offered significant dose dependent protection against experimentally paracetamol induced hepatotoxicity. Among the three different doses of *Cucumis sativus* ethanolic extract, the high dose (500 mg/kg p.o.) treated group returned the injured liver to quite normal than the standard drug, silymarin. Also the present study is the biochemical evidence for the traditional use of *Cucumis sativus* fruits as a hepatoprotective drug.

**REFERENCES**
