HEPATOPROTECTIVE ACTIVITY OF THE ETHANOLIC EXTRACT OF THE FRUITS OF CUCUMIS TRIGONUS ROXB.

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ABSTRACT

Objective: Hepatoprotective activity of the ethanolic extract of the fruits of Cucumis trigonus Roxb. extract has been evaluated by paracetamol-induced liver damage model in rats.

Methods: In hepatoprotective activity study, paracetamol significantly increased the levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), γ-glutamyl transpeptidase (GGTP), total bilirubin, unconjugated bilirubin and lipid peroxidase (LPO) as well as decreased the levels of liver homogenates, glutathione peroxidase (GPOx), glutathione reductase (GRD), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH).

Results: Three different doses of the ethanolic extract of the fruits of Cucumis trigonus (100 mg/kg body weight, 250 mg/kg body weight and 500 mg/kg body weight) inhibited the serum marker enzymes. The decrease in the liver homogenates was comparable with the reference standard, silymarin (50 mg/kg body weight).

Conclusion: In 500 mg/kg body weight dose, of the ethanolic extract of the fruits of Cucumis trigonus fatty changes, necrosis, vacuole, space formation, loss of cell boundaries were completely absent. Histopathological study also confirms the hepatoprotective activity of the ethanolic extract of the fruits of Cucumis trigonus.

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

INTRODUCTION

Liver is the key organ in metabolism, detoxification and secretory function in the body. It also regulates important metabolic functions. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction [1]. Therefore, maintenance of a healthy liver is essential for the overall well-being of an individual. In India, numerous medicinal plants and their formulations are used in ethno medical practices and traditional system of medicine for liver disorders [2]. Therefore, searching for effective and safe drugs for liver disorders are continues to be an area of interest.

Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost [3]. In spite of the tremendous advances made in allopathic medicine, no effective antihepatotoxic medicine is available till date [4]. Plant drugs are known to play a vital role in the management of liver diseases. The plant Cucumis trigonus belongs to the Cucurbitaceae family. It is commonly known as “Thummittikai” in Tamil, “Vishala” in Sanskrit and “Bitter gourd” in English and is indigenous to India, Ceylon, Malaya, North Australia, Afghanistan and Persia [5]. The fruits of Cucumis trigonus are reported to be useful in treating leprosy, fever, jaundice, diabetes, cough, bronchitis, anemia, constipation and other abdominal disorders [6, 7]. Alcoholic extract of Cucumis trigonus fruit is shown to possess various activities such as anabolic activity [8] analgesic, anti-inflammatory and diuretic activity [9]. Recently it’s proteolytic and serine protease activity has been reported [10, 11]. No other biochemical investigation has been carried out on hepatoprotective activity of the fruits of Cucumis trigonus in experimental rats. Hence the present investigation has been carried out to study the hepatoprotective activity of the ethanolic extract of the fruits of Cucumis trigonus on paracetamol-induced liver toxicity in albino rats.

MATERIALS AND METHODS

Collection of plant materials

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

A voucher specimen (MSU/PHAR/HER-140) 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. Room temperature was kept at (25±3°C), humidity 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, Morabad, 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, ethanoll and water by a Soxhlet apparatus. The last trace of the 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 trigonus as per OECD guidelines [12]. Albino rats received 2000 mg/kg body weight of ethanol extract. 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.

**Paracetamol-induced experimental liver damage**

Rats were divided into six groups, each group consisting of six
animals [13].

Group I: Controls received the vehicle viz normal saline
(2 mL/kg body weight).

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

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

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

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

Group VI: Received silymarin orally 50 mg/kg body weight for 10 days
and simultaneously administered paracetamol 750 mg/kg body weight
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
SGOT, SGPT, serum alkaline phosphatase (SALP), γ-glutamate
transpeptidase (GGTP), total bilirubin, conjugated bilirubin,
unconjugated bilirubin, total protein, albumin, and globulin
according to the method by using commercially available test kits
[14,15,16]. Lipid peroxidase (LPO) [17] glutathione peroxidase
(GPx) [18] glutathione reductase (GRD) [19] superoxide dismutase
(SOD) [20] catalase (CAT) [21] and reduced glutathione (GSH) [22]
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
to 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 [23].

**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 is widely used as analgesic and antipyretic drug.
However at high dose it leads to undesirable side effects, such as
hepatotoxicity and nephrotoxicity. Paracetamol is activated and
converted by cytochrome P⁴⁵⁰ enzymes to toxic metabolite
NAPQI (N-acetyl-p-benzoquinoneimine) that causes oxidative
stress and glutathione (GSH) depletion which leads to the
cellular necrosis.

The serum biochemical parameters and liver homogenates in the
control and various experimental groups are presented in Tables 1-3.

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**Table 1: Effect of ethanolic extract of the fruits of Cucumis trigonus 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 body weight)</th>
<th>Body weight</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment (g)</td>
<td>After treatment (g)</td>
<td>SGOT (U/L)</td>
</tr>
<tr>
<td>Control</td>
<td>2 mL saline</td>
<td>16.1 ± 4.56</td>
<td>168.5 ± 4.12</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>750</td>
<td>178.5 ± 6.4</td>
<td>171.1 ± 4.61*</td>
</tr>
<tr>
<td><em>Cucumis trigonus</em></td>
<td>100</td>
<td>169.3 ± 5.88</td>
<td>173.2 ± 6.94</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>250</td>
<td>173.6 ± 2.54</td>
<td>186.3 ± 4.86*</td>
</tr>
<tr>
<td>500</td>
<td>184.3 ± 4.98</td>
<td>191.5 ± 4.53</td>
<td>31.6 ± 1.92*</td>
</tr>
<tr>
<td>Silymarin</td>
<td>50</td>
<td>181.2 ± 6.43</td>
<td>189.6 ± 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 damage d 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 trigonus on the liver total protein, albumin, globulin, A/G ratio, total bilirubin, conjugated bilirubin, unconjugated bilirubin on paracetamol-induced hepatotoxicity in rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg body weight)</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>0.7 ± 0.03</td>
<td>0.2 ± 0.05</td>
<td>0.5 ± 0.02</td>
<td>8.3 ± 0.34</td>
<td>4.5 ± 0.61</td>
<td>3.7 ± 0.51</td>
<td>1.2:1</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>750</td>
<td>2.6 ± 0.11**</td>
<td>1.5 ± 0.06**</td>
<td>1.1 ± 0.05**</td>
<td>6.1 ± 0.24**</td>
<td>3.1 ± 0.14**</td>
<td>3.0 ± 0.22</td>
<td>1:1</td>
</tr>
<tr>
<td><em>Cucumis trigonus</em></td>
<td>100</td>
<td>1.1 ± 0.24**</td>
<td>0.29 ± 0.03</td>
<td>0.83 ± 0.01**</td>
<td>8.0 ± 0.31</td>
<td>4.2 ± 0.81</td>
<td>3.6 ± 0.24</td>
<td>1:3</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>250</td>
<td>0.9 ± 0.03**</td>
<td>0.24 ± 0.01**</td>
<td>0.74 ± 0.02**</td>
<td>8.1 ± 0.28**</td>
<td>4.3 ± 0.13</td>
<td>3.7 ± 0.56</td>
<td>1:1</td>
</tr>
<tr>
<td>500</td>
<td>0.6 ± 0.02**</td>
<td>0.17 ± 0.07**</td>
<td>0.50 ± 0.02**</td>
<td>8.4 ± 0.17**</td>
<td>4.7 ± 0.61**</td>
<td>3.8 ± 0.11</td>
<td>1:1</td>
<td>1:1</td>
</tr>
<tr>
<td>Silymarin</td>
<td>50</td>
<td>0.8 ± 0.07**</td>
<td>0.24 ± 0.04**</td>
<td>0.6 ± 0.04**</td>
<td>8.1 ± 0.12**</td>
<td>4.6 ± 0.11**</td>
<td>3.5 ± 0.16</td>
<td>1:3</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 trigonus* on the liver LPO, GPx, GRD, CAT, GSH on paracetamol-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg body weight)</th>
<th>LPO (nm MDA/mg of protein)</th>
<th>GPx (U/mg of protein)</th>
<th>GRD (U/mg of protein)</th>
<th>SOD (U/mg of protein)</th>
<th>CAT (U/mg of protein)</th>
<th>GSH (µg/mg of 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 trigonus</em></td>
<td>100</td>
<td>2.691±0.017**</td>
<td>1.989±0.174**</td>
<td>0.313±0.051*</td>
<td>0.169±0.017*</td>
<td>2.443±0.054*</td>
<td>15.84±0.91**</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>250</td>
<td>1.914±0.018**</td>
<td>2.549±0.182**</td>
<td>0.384±0.038*</td>
<td>0.185±0.013*</td>
<td>2.759±0.084*</td>
<td>18.14±0.63**</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>1.628±0.021**</td>
<td>3.624±0.161**</td>
<td>0.544±0.029**</td>
<td>0.218±0.021**</td>
<td>2.993±0.063**</td>
<td>25.08±0.91**</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±0.016**</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.

There was a significant reduction in body weight (Table 1) in the paracetamol-treated control rats when compared to the corresponding normal rats. Treatment with ethanolic extract of the fruits of *Cucumis trigonus* (100 mg/kg body weight 250 mg/kg body weight 500 mg/kg body weight) and silymarin (50 mg/kg body weight) 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 body weight dose.

Administration of paracetamol to rats by oral route caused liver damage as indicated by a significant increase in serum enzymes like, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), γ-glutamte transeptidase (GGTP), total bilirubin, conjugated bilirubin, and unconjugated bilirubin (Table 1) and increased the levels of total protein, albumin, and globulin. The results indicate that the *Cucumis trigonus* possesses more hepatoprotective activity than the standard drug, silymarin.

The levels of lipid peroxides and activity of enzymic antioxidants, glutathione peroxidase (GPx), glutathione reductase (GRD), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) in liver homogenates (Table 3). Marked increase in the lipid peroxide levels and concomitant decrease in enzymic antioxidants levels were observed in carcinoma induced rats, while the ethanol extract of the fruits of *Cucumis trigonus* treatment reversed the conditions to near normal levels. Comparison of *in vivo* effects of the three doses of the ethanolic extract of the fruits of *Cucumis trigonus* and silymarin on paracetamol-induced changes in biochemical parameters in rats (Fig. 1).
Liver histopathology of the ethanolic extract of the fruits of *Cucumis trigonus* on paracetamol-induced hepatotoxicity are presented in Fig. 2. Results of histopathological studies provided supportive evidence for biochemical analysis. Histology of liver section of normal control animal exhibited normal hepatic cells each with well-defined cytoplasm, prominent nucleus, and well brought out central vein whereas that of paracetamol-intoxicated group animal showed total loss of hepatic architecture with centrilobular hepatic necrosis, fatty changes, vacuolization, loss of cell boundaries, space formation, and crowding of central vein. Treatment with ethanolic extract of *Cucumis trigonus* extract at a dose of 100 mg/kg body weight 250 mg/kg body weight and 500 mg/kg body weight showed a weak, moderate and good activity in protecting the liver cells from paracetamol-injury. Among the three different doses of *Cucumis trigonus* extract, the high dose (500 mg/kg body weight) treated group returned the injured liver to quite normal than the standard drug, silymarin. Now, it could be decided that the hepatoprotective activity was dose and time dependent. The crude ethanolic extract of the fruits of *Cucumis trigonus* had shown very potential hepatoprotective activity at a dose of 500 mg/kg body weight. GC-MS analysis [24] of the ethanolic extract showed the presence of bile acids such as glycodeoxycholic acid, and 3α,7α,12α–trihydroxy coprostanic acid. These compounds may be responsible for the liver disorder curing effect. These findings suggest that the ethanolic extract of the fruits of *Cucumis trigonus* could protect liver against the paracetamol-induced liver damage in rats.
CONCLUSION

Based on the enzymatic levels and histopathological observations the ethanolic extract of the fruits of Cucumis trigonus treated group, it can be concluded that the fruits possess significant hepatoprotective activity. Hence it is advised that if one happens to take paracetamol in overdose they can consume Cucumis trigonus fruit extract as a hepatoprotective agent. Also the present study is the biochemical evidence for the traditional use of Cucumis trigonus fruits as a hepatoprotective drug.

REFERENCES


Fig. 2: Liver histopathology of the ethanolic extract of the fruits of Cucumis trigonus on paracetamol-induced hepatotoxicity.

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