HEPATOPROTECTIVE ACTIVITY STUDIES OF CUCUMIS TRIGONUS ROXB. AGAINST RIFAMPICIN-ISoniazid-INDUCED TOXICITY IN RATS.

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ABSTRACT
The fruits of Cucumis trigonus Roxb. was analysed for the hepatoprotective activity against albino rats with liver damage induced by rifampicin-isoniazid. Rifampicin (RIF) plus isoniazid (INH) treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury. The ethanolic extract of the fruits of Cucumis trigonus showed normalization of body weight, biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), γ-glutamyl transpeptidase (GGTP), total bilirubin (TB), total protein (TP) as well as the levels of liver homogenates, Lipid peroxidase (LPO), glutathione peroxidase (GPx), glutathione reductase (GRD), superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH). The effects of ethanolic extract of the fruits of Cucumis trigonus (100 mg/kgbw ip 250 mg/kgbw ip & 500 mg/kgbw ip) was compared with that of the standard drug, silymarin. The ethanolic extract showed significant hepatoprotective activity in 500 mg/kg ip dose. The hepatoprotective activity has also been supported by histopathological studies of liver tissue.

KEYWORDS: Hepatoprotective, Cucumis trigonus, Histopathology.

INTRODUCTION
Rifampicin (RIF) and isoniazid (INH), the two front-line drugs have been used in the treatment of tuberculosis, that is known to be potentially hepatotoxic. Rifampicin, which is generally co-administered with isoniazid in the treatment of tuberculosis, is toxic to hepatocytes. A meta analysis of studies involving the use of a multiplicity of antituberculosis drug regimens predominantly in adults have shown an incidence of liver injury of 1.1 % in patients with RIF alone, 1.6 % in patients with INH alone, and 2.55 % in patients with RIF plus INH [1]. Reactive oxygen species play a key role in RIF-INH-induced hepatotoxicity [2]. Since oxidative stress has been regarded as the major mechanism of antituberculosis drug-induced hepatotoxicity [3], antioxidants might be used as potential antihepatotoxic drugs against RIF-INH caused liver injury [4].

A major defense mechanism involves the antioxidant enzymes, including SOD, CAT and GSH-Px, which convert active oxygen molecules into non-toxic compounds [5]. The pathogenesis of the hepatotoxicity is involved in all the hepatic cell types via death and regeneration processes, and liver diseases often progress from subclinical icteric hepatitis to hepatic fibrosis, cirrhosis and hepatocellular carcinoma [6].

Cucumis trigonus (Fam. Cucurbitaceae) is commonly known as “Thummittikai” in Tamil, “Bitter gourd” in English, and “Vishala” in Sanskrit. The fruits of Cucumis trigonus are reported to be useful in treating leprosy, jaundice, diabetes, and other abdominal disorders. Cucumis trigonus fruit is shown to possess various activities such as antidiabetic activity [7], anabolic activity [8], cardioprotective activity [9], analgesic and anti-inflammatory [10], and diuretic activity [11].

Hepatoprotective activity of the ethanolic extracts of the fruits of Cucumis trigonus and Cucumis sativus against paracetamol-induced toxicity in albino rats have been already performed by our group [12, 13]. In the present study hepatoprotective activity studies of the ethanolic extract of the fruits of Cucumis trigonus on RIF-INH-induced liver toxicity in albino rats have been carried out.

MATERIAL AND METHODS
Collection of plant materials
The fruits of Cucumis trigonus was collected in the month of March from Alangulum, Tirunelveli District, Tamil Nadu and identified by Prof. P. Jayaraman, Plant Anatomy Research Centre, West Thambaram, Chennai-600 045, Tamil Nadu, India (Reg.No of the authentification certificate: PARC/2013/2048).
Experimental animals
Male 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
Rifampicin and isoniazid were purchased from Micro Labs, India. Silymarin was obtained as gift sample from Ranbaxy (Devas, India), Standard kits of SGPT, SGOT, SALP, bilirubin and total protein were obtained from Jain Scientific Industries, Moradabad, India. All other reagents used for the study were of analytical grade.

Preparation of extract
The collected fruits were cut into pieces, shade-dried at room temperature and powdered. The dried fruit Powder(500g) 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 trigonus as per OECD guidelines. Albino rats received 2000 mg/kgbw ip of the ethanolic 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 behavioural 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.

Induction of experimental hepatotoxicity
Each 50 mg/kg bw ip of RIF + INH solutions were prepared separately in sterile distilled water. Rats were divided into nine groups, each group consisting of six animals.

Group I: Control received the vehicle viz. normal saline (2 mL/kgbw ip).
Group II: Received 50 mg/kgbw ip per day of RIF + INH each by ip route for 21 days.
Group III: Received 100 mg/kgbw ip of the ethanolic extract of the fruits of Cucumis trigonus and simultaneously received 50 mg/kgbw ip per day of RIF + INH each by ip route for 21 days. (Low dose).
Group IV: Received 250 mg/kgbw ip of the ethanolic extract of the fruits of Cucumis trigonus and simultaneously received 50 mg/kgbw ip per day of RIF + INH each by ip route for 21 days. (Moderate dose).
Group V: Received 500 mg/kgbw ip of the ethanolic extract of the fruits of Cucumis trigonus and simultaneously received 50 mg/kg ip per day of RIF + INH each by ip route for 21 days. (High dose).

Group VI: Received 2.5 mg/kgbw ip of silymarin (Standard drug) and simultaneously received 50 mg/kgbw ip per day of RIF + INH each by ip route for 21 days.

At the end of 21 days, 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. Body weights of the rats were measured daily for 21 days. Daily changes in body weights were recorded.

Assessment of liver damage
Liver damage was assessed by the estimation of serum activity of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), γ- glutamyl transpeptidase (GGTP), total bilirubin (TB), conjugated bilirubin, unconjugated bilirubin, total protein (TP), albumin, and globulin according to the method by using commercially available test kit. Lipid peroxidase (LPO) [23] glutathione peroxidase (GPx) [24] glutathione reductase (GRD) [25] superoxide dismutase (SOD) [26] catalase (CAT) [27] and reduced glutathione (GSH) [28] 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 were mentioned as * P ≤ 0.05, ** P ≤ 0.01.

RESULTS AND DISCUSSION
Rifampicin is a first line drug used in the treatment of tuberculosis and leprosy. It possesses the ability to eliminate semi dormant or persisting organism. Short course chemotherapy containing rifampicin and isoniazid in combination has proved to be highly effective in the treatment of tuberculosis, but one of its adverse effects is hepatotoxicity. RIF induces cytochrome P450 enzyme causing an increased production of toxic metabolites from acetyl hydrazine (AcHz). RIF can also increase the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half life of AcHz (metabolite of INH) is shortened by RIF and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which...
is related to the higher incidence of liver necrosis caused by INH and RIF in combination. Damaged hepatocytes or biliary epithelium may release cell constituents (e.g., enzymes) into blood resulting in increased levels of these analytes. The more commonly measured ‘liver’ enzymes are alanine aminotransferase (ALT, formerly SGPT), aspartate aminotransferase (AST, formerly known SGOT), serum alkaline phosphatase (SALP), and γ-glutamyl transeptidase (GGTP). Although there will be an increase of ALT and AST in heart and liver diseases, total bilirubin (TB), a byproduct of the breakdown of red blood cells in the liver is a good indicator of liver function. High levels will cause icterus and are indicative of damage to the liver and bile duct. Administration of RIF + INH combination only, showed a significant derangement of liver function as assessed by change in serum enzymes SGOT, SGPT, SALP, GGTP, TB, TP, albumin, globulin as well as the levels of liver homogenates, LPO, GPx, GRD, SOD, CAT, and GSH and also liver histopathology.

Table-1 shows the levels of SGOT, SGPT, SALP, GGTP in the serum and bodyweight. There was a significant increase in the levels of SGOT, SGPT, SALP, GGTP in serum of rats treated with RIF + INH when compared with that of the control rats. Whereas the levels of body weight in RIF + INH treated rats were decreased. There is a gain in body weight in all the drug treated groups. Pretreatment of rats with the ethanolic extract of the fruits of Cucumis trigonus caused a significant reduction in the levels of enzymes leading to a significant reversal of hepatotoxicity.

### TABLE 1: Effect of the ethanolic extract of the fruits of Cucumis trigonus on the body weight and other biochemical parameters on rifampicin-isoniazid-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg ip)</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Weight gain↑ / loss ↓ (g)</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>SALP (U/L)</th>
<th>GGTP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 mL saline</td>
<td>192.54±4.31</td>
<td>194.6±5.34</td>
<td>02.12 ↑</td>
<td>27.27±0.94</td>
<td>36.59±0.93</td>
<td>191.26±10.11</td>
<td>8.59±1.21</td>
</tr>
<tr>
<td>RIF + INH</td>
<td>100</td>
<td>198.33±5.39</td>
<td>174.14±5.84**</td>
<td>24.19 ↓</td>
<td>144.51±4.56**</td>
<td>163.59±2.80**</td>
<td>263.16±11.36**</td>
<td>19.63±1.44**</td>
</tr>
<tr>
<td>Cucumis trigonus</td>
<td>100</td>
<td>185.56±4.34</td>
<td>192.39±4.69#</td>
<td>06.85 ↑</td>
<td>83.14±1.36#</td>
<td>69.36±4.54#</td>
<td>193.56±8.24#</td>
<td>17.66±0.94#</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>195.15±6.34</td>
<td>204.65±5.89#</td>
<td>09.50 ↑</td>
<td>41.33±2.64#</td>
<td>28.24±5.64#</td>
<td>163.22±3.84#</td>
<td>16.08±0.48#</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>212.63±6.84</td>
<td>228.33±5.93#</td>
<td>15.70 ↑</td>
<td>29.11±2.66#</td>
<td>20.26±1.87#</td>
<td>152.54±8.14#</td>
<td>11.27±0.73#</td>
</tr>
<tr>
<td>Silymarin</td>
<td>2.5</td>
<td>214.68±5.84</td>
<td>227.55±6.84**</td>
<td>12.87 ↑</td>
<td>32.63±1.69#</td>
<td>29.59±1.94#</td>
<td>182.69±3.91#</td>
<td>11.84±0.17#</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; ns: not significant.

Table-2 shows the changes in the levels of total bilirubin, conjugated bilirubin, unconjugated bilirubin, total protein, albumin, and globulin in the serum of different experimental rats. In comparison with the control group, in the RIF + INH treated rats, significant increase in the levels of total bilirubin, conjugated bilirubin, unconjugated bilirubin (p < 0.01) were noticed. There was a significant reduction in the levels of total protein, albumin, and globulin (p < 0.05). Interestingly, in the RIF + INH-induced rats, the levels of total bilirubin, conjugated bilirubin, unconjugated bilirubin, total protein, albumin, and globulin in the liver could be normalized by the pretreatment with the ethanolic extract of the fruits of Cucumis trigonus. 500 mg/kg bw ip of Cucumis trigonus showed better activity.

### TABLE 2: Effect of the ethanolic extract of the fruits of Cucumis trigonus on the biochemical parameters on rifampicin-isoniazid-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg ip)</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>0.63±0.04</td>
<td>0.22±0.012</td>
<td>0.41±0.021</td>
<td>7.94±0.51</td>
<td>4.11±0.26</td>
<td>3.83±0.12</td>
<td>1.0:1</td>
</tr>
<tr>
<td>RIF + INH</td>
<td>100</td>
<td>3.84±0.84**</td>
<td>1.80±0.014**</td>
<td>2.04±0.037**</td>
<td>6.24±0.36**</td>
<td>3.94±0.53</td>
<td>2.30±0.16*</td>
<td>1.4:1</td>
</tr>
<tr>
<td>Cucumis trigonus</td>
<td>100</td>
<td>2.04±0.92*</td>
<td>1.19±0.033</td>
<td>0.83±0.021</td>
<td>7.68±0.14</td>
<td>4.28±0.26</td>
<td>3.40±0.32</td>
<td>1.2:1</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1.02±0.76</td>
<td>0.32±0.010**</td>
<td>0.70±0.011</td>
<td>8.14±0.73**</td>
<td>4.21±0.33</td>
<td>3.46±0.11</td>
<td>1.2:1</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.59±0.021#</td>
<td>0.21±0.040**</td>
<td>0.38±0.042**</td>
<td>8.33±0.51**</td>
<td>4.87±0.34</td>
<td>3.93±0.23*</td>
<td>1.2:1</td>
</tr>
<tr>
<td>Silymarin</td>
<td>2.5</td>
<td>0.96±0.02</td>
<td>0.26±0.03#</td>
<td>0.70±0.16**</td>
<td>7.89±0.53**</td>
<td>4.02±0.62</td>
<td>3.87±0.16*</td>
<td>1.0:1</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.

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Table 3 shows the levels of LPO, GPx, GRD, SOD, CAT, and GSH in the liver homogenate. The level of lipid peroxide sharply increased (6.93±0.013 nm MDA/mg protein (p < 0.01) after RIF + INH intoxication. However, the levels of GPx, GRD, SOD, CAT, GSH decreased after RIF + INH intoxication. The administration of all the three doses, viz, the low dose, moderate dose, and high dose of Cucumis trigonus decreased the level of LPO and increased the levels of GPx, GRD, SOD, CAT, GSH (p < 0.01). Among the three different doses, 500 mg/kg bw ip dose showed better activity than the standard drug, silimarin, in the case of LPO and GRD. The protective effect was dose-dependent. The hepatoprotective role of the ethanolic extract of the fruits of Cucumis trigonus might be due to the antioxidant potential of the drugs.\textsuperscript{30}

The ethanolic extract of the fruits of Cucumis trigonus improved liver function by decreasing the serum enzymes SGOT, SGPT, SALP, GGTP, TB, conjugated bilirubin, unconjugated bilirubin, LPO. However, the levels of total protein, albumin, globulin, GPx, GRD, SOD, CAT, and GSH are increased. This indicates the protective effect over liver and improvement in its functional efficiency.

### TABLE 3: Effect of the ethanolic extract of the fruits of Cucumis trigonus on the liver homogenate biochemical parameters on rifampicin-isoniazid-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg ip)</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPO (nm MDA/mg protein)</td>
<td>GPX (U/mg protein)</td>
</tr>
<tr>
<td>Control</td>
<td>2 mL saline</td>
<td>1.89±0.024</td>
</tr>
<tr>
<td>RIF + INH</td>
<td>100</td>
<td>6.93±0.013**</td>
</tr>
<tr>
<td>Cucumis trigonus</td>
<td>100</td>
<td>1.26±0.080</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>2.1±0±001</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>1.13±0.054</td>
</tr>
<tr>
<td>Silymarin</td>
<td>2.5</td>
<td>1.13±0.054</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.

### Histopathological examination

In histopathological studies of liver [Figure 1], the control showed normal gross appearance; dark maroon color of liver having smooth surfaces, microscopically normal lobular appearance having normal central vein, normal hepatic cells each with well-defined cytoplasm, prominent nucleus, well brought out central vein, normal architecture of liver, radiating cords of hepatocytes, and normal portal tract. RIF + INH treated rats showed moderate to severe liver damage characterized by disarrangement of normal hepatic cells, vacuolization, loss of cell boundaries, space formation, and crowding of centrilobular hepatic necrosis of the liver cells. RIF + INH and low dose (100 mg/kg bw ip) of the ethanolic extract of the fruits of Cucumis trigonus showed minimal necrosis, mild inflammation and less steatosis. RIF + INH and moderate dose (250 mg/kg bw ip) of the ethanolic extract of the fruits of Cucumis trigonus showed slight recovery and evidence of regeneration in some hepatocytes. RIF + INH and high dose (500 mg/kg bw ip) of the ethanolic extract of the fruits of Cucumis trigonus showed significant recovery showing absence of necrosis, space formation and vacuoles. RIF + INH and silymarin (2.5 mg/kg ip) showed normal liver architecture and occasional inflammatory cells with no traditis or necrosis.
RIF + INH + EE of CT (100 mg/kg ip)

RIF + INH + EE of CT (250 mg/kg ip)

RIF + INH + EE of CT (500 mg/kg ip)

RIF + INH + silymarin (2.5 mg/kg ip).

Figure 1. Histopathology of the ethanolic extract of the fruits of Cucumis trigonus on rifampicin-isonizid-induced hepatotoxicity in albino rats.

EE - Ethanolic extract, CT - Cucumis trigonus, RIF - Rifampicin, INH - Isonizid, CV - Central vein, H - Hepatocyte, N - Nucleus, FC - Fatty changes, NC - Necrosis, V - Vacuole, SF - Space formation, LCB - Loss of cell boundaries.

CONCLUSION
Hepatotoxicity occurs significantly with anti-TB drugs. The present study proves that the ethanolic extract of the fruits of Cucumis trigonus shows significant protective action against the hepatotoxicity induced by the drugs used in the treatment of tuberculosis. However, treatment of these extract completely protected the liver cells. GC-MS analysis indicated the presence of bile acids, carotenoids, antibiotics, steroids and phorbol ester in Cucumis trigonus. Hence the hepatoprotective effect of the extract may be due to the presence of one or more phytochemical constituents present in the Cucumis trigonus which scavenged the free radical offering hepatoprotection. Thus the ethanolic extract of the fruits of Cucumis trigonus which are useful in controlling hepatic injury in drug induced hepatotoxicity. Isolation and characterization of the active principles may yield good hepatoprotective drugs.

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