Protective effect of *Hemidesmus indicus*
against rifampicin and isoniazid-induced hepatotoxicity in rats

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Abstract

Oral treatment with the ethanol extract of *Hemidesmus indicus* roots (100 mg/kg, for 15 days) significantly prevented rifampicin and isoniazid-induced hepatotoxicity in rats. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Hemidesmus indicus*; Antihapatotoxic activity

1. Introduction

Rifampicin (RMP) and isoniazid (INH), alone or in association, are still widely used in most antitubercular chemotherapeutic regimens [1]. However, these drugs are also well known as hepatotoxic agents [2]. The rate of hepatotoxic reaction was reported to be much higher in Indian patients [3,4] receiving these antitubercular drugs as compared to that reported from developed countries [5] even when a similar dose schedule was used. RMP, a powerful inducer of mixed function
oxidase (MFO) in man [6] and rats [7], contributes to the hepatotoxicity of INH by enhancing the production of toxic metabolites.

*Hemidesmus indicus* (L.) R.Br. (Asclepiadaceae), Indian name ‘Magrabu’, is a medicinal plant found in India from Kashmir to Kanyakumari. In Indian ayurvedic medicine, the oral administration of the extract of its roots has been used to cure various liver disorders. In traditional medicine, the plant has also been used as a blood purifier and to cure fever, leprosy, rheumatism and snake bite [8,9]. The present study was aimed to assess the antihepatotoxic potential of *H. indicus* root ethanol extract (HI) on mitochondrial function in antitubercular drugs (RMP and INH)-induced hepatotoxicity in rats.

2. Experimental

2.1. Drugs and chemicals

RMP and INH were obtained from Lupin Laboratories, Bombay, India. All other chemicals used were of analytical grade. HI, prepared (yield: 16.3%) from roots of *H. indicus*, was supplied by Captain Srinivasamurti Drug Research Institute for Ayurveda, Arumbakkam, Chennai, India. HI contains oligoglycosides, steroids, tannins, saponins and two coumarino lignoids, hemidesmin-1 and hemidesmin-2 [10].

2.2. Animals

Male Wistar rats (weighing 150–180 g) were used. They were housed under standard environmental conditions and allowed standard pelleted diet (M/s Hindustan Lever Foods, Bangalore, India) and water ad libitum.

2.3. Antihepatotoxic activity

Animals were divided into four groups of six rats each. Group I were normal control rats provided only with standard diet; group II were normal animals, orally treated with HI (100 mg/kg per day) for 15 days; group III animals were intraperitoneally (i.p.) injected with RMP and INH (each at doses of 50 mg/kg, dissolved in sterile distilled water (for 15 days)) [11]; and group IV animals were orally administered with HI (100 mg/kg per day) and i.p. injected with RMP and INH (each at doses of 50 mg/kg per day) for 15 days.

At the end of the experiment, animals were killed by decapitation. The liver was excised immediately, mitochondria were isolated by the method of Johnson and Lardy [12] and used for the estimation of protein [13], isocitrate dehydrogenase [14], α-ketoglutarate dehydrogenase [15], succinate dehydrogenase [16], malate dehydrogenase [17], NADH dehydrogenase [18], cytochrome c oxidase [19], lipid peroxides [20], catalase [21], and superoxide dismutase [22].
Table 1
Effect of the ethanol extract of *Hemidesmus indicus* roots (HI; 100 mg/kg per day, p.o., for 15 days) on the levels of mitochondrial protein and mitochondrial enzymes in isoniazid + rifampicin (INH + RMP, each at 50 mg/kg per day, i.p. for 15 days)-intoxicated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II (A)</th>
<th>Group III (B)</th>
<th>Group IV (A + B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.42 ± 3.17</td>
<td>32.74 ± 3.98</td>
<td>22.17 ± 1.72***</td>
<td>31.14 ± 3.06**</td>
</tr>
<tr>
<td>HI-treated</td>
<td>32.14 ± 3.86</td>
<td>81.61 ± 68.9</td>
<td>710.14 ± 65.2***</td>
<td>798.21 ± 71.3**</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>812.73 ± 78.4</td>
<td>811.61 ± 68.9</td>
<td>48.04 ± 5.12***</td>
<td>61.84 ± 6.13**</td>
</tr>
<tr>
<td>α-Ketoglutarate dehydrogenase</td>
<td>68.87 ± 6.38</td>
<td>69.87 ± 6.89</td>
<td>48.04 ± 5.12***</td>
<td>61.84 ± 6.13**</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>26.57 ± 2.13</td>
<td>27.74 ± 2.69</td>
<td>9.64 ± 1.15***</td>
<td>20.19 ± 1.68***</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>349.71 ± 25.64</td>
<td>354.31 ± 29.08</td>
<td>247.15 ± 21.74***</td>
<td>307.53 ± 25.14**</td>
</tr>
<tr>
<td>NADH dehydrogenase</td>
<td>31.07 ± 1.94</td>
<td>29.51 ± 2.86</td>
<td>46.17 ± 3.82***</td>
<td>32.94 ± 3.05</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>46 × 10^{-2} ± 0.31</td>
<td>44 × 10^{-2} ± 0.46</td>
<td>98 × 10^{-2} ± 0.71***</td>
<td>5.4 × 10^{-2} ± 0.51**</td>
</tr>
</tbody>
</table>

*aValues are mean ± S.D., n = 6; **P < 0.01 ***P < 0.001; Group III vs. Group I, Group IV vs. Group III; Student's t-test.*

*bMicrogram/g.

*cNanomoles of α-ketoglutarate formed/h per mg protein.

*dNanomoles of ferrocyanide formed/h per mg protein.

*eMicromoles of succinate oxidised/min per mg protein.

*fMicromoles of NADH oxidised/min per mg protein.

*gChange in optical density/min per mg protein.
Table 2
Effect of the ethanol extract of *Hemidesmus indicus* roots (HI; 100 mg/kg per day, p.o., for 15 days) on the levels of lipid peroxides, superoxide dismutase and catalase in isoniazid + rifampicin (INH + RMP, each at 50 mg/kg per day, i.p. for 15 days)-intoxicated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I Control</th>
<th>Group II HI-treated (A)</th>
<th>Group III RMP + INH (B)</th>
<th>Group IV A + B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxides</td>
<td>168.71 ± 15.43</td>
<td>164.33 ± 14.89</td>
<td>285.78 ± 25.16***</td>
<td>188.94 ± 17.18***</td>
</tr>
<tr>
<td>Catalase</td>
<td>128.71 ± 11.32</td>
<td>125.93 ± 10.97</td>
<td>68.83 ± 5.99***</td>
<td>96.45 ± 7.94**</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>42.14 ± 3.28</td>
<td>43.60 ± 3.74</td>
<td>28.18 ± 1.78***</td>
<td>33.74 ± 3.02**</td>
</tr>
</tbody>
</table>

*Values are mean ± S.D., n = 6; **P < 0.01, ***P < 0.001; Group III vs. Group I, Group IV vs. Group II; Student’s t-test.

a Nanomoles of malondialdehyde formed/mg protein.

b One unit of SOD activity is the amount enzyme required to give 50% inhibition of epinephrine auto-oxidation.

c Nanomoles of H$_2$O$_2$ decomposed/min per mg protein.

2.4. Statistical analysis

Results are expressed as mean ± S.D. Student’s t-test was used to assess statistical significance.

3. Results and discussion

The level of liver mitochondrial protein and the activities of isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, NADH dehydrogenase and cytochrome c oxidase were significantly decreased in RMP and INH-intoxicated rats as compared with control rats. Intraperitoneal administration of RMP and INH also induced a significant increase in mitochondrial lipid peroxidation with a significant decrease in the activities of antiperoxidative enzymes (CAT and SOD). Oral co-treatment with the ethanolic extract of *Hemidesmus indicus* roots (100 mg/kg per day, for 15 days) significantly prevented these alterations and maintained enzyme levels at near normal values (Tables 1 and 2).

The observed effects are probably due to a free radical scavenging activity of the coumarino lignoids hemidesmin-1 and hemidesmin-2 present in the extract [10].

References