Pharmacognostical and Phytochemical Studies on Some Medicinal Plants of Tirunel Veli Hills in Tamilnadu in India

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Abstract
Aqueous extracts of Securinega virosa, Phyllanthus reticulates and Breynia retusa were screened for their hepatoprotective properties against carbontetrachloride induced liver damage in Wistar albino rats. All the extracts gave promising results, thus the plants were taken up for photochemical investigation.

Fractionation of the extracts and crystallization of the compounds yielded five compounds that were identified as amyrin, thea alcohol, octacosanol, ß-sitosterol and ß-sitosterol 3-ß-D glucopyranoside.

Among the three plants screened, Securinega virosa had the best repair mechanism on blood biochemistry, and also correlated with the pathological changes seen in the liver. Histopathological examinations of the liver from the group of rats that received the extract of Securinega virosa revealed almost normal architecture of the liver.

INTRODUCTION
Several indigenous Indian herbs are reported to be useful in viral hepatitis and liver damage due to alcohol (Dhumal et al., 1989). Liver diseases are mainly caused by toxic chemicals and they damage the liver cells by inducing lipid peroxidation and other oxidative damages in liver. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. In India more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations. Only a small portion of the hepatoprotective plants and formulations used in traditional medicine have been pharmacologically evaluated for their efficacy. In cognizance with the above situation certain indigenous plants were evaluated for their hepatoprotectant potential in rats. Aqueous extracts of Securinega virosa, Phyllanthus reticulates and Breynia retusa were screened for their hepatoprotective properties against carbontetrachloride induced liver damage in Wistar albino rats. Since all the extracts gave promising results, the plants were taken up for photochemical investigation. The photochemical studies of these plants were carried out to characterize the therapeutically active constituents.

MATERIALS AND METHODS
The freshly collected plant materials were cut into pieces, shade dried and coarsely powdered. The powdered material was charged in an aspirator bottle and successively extracted with n-hexane, chloroform and methyl alcohol by cold percolation method for 72 hours. After decantation and filtering, nearly 80 percent of the solvent was removed by distillation over a boiling water bath and remaining under reduced pressure. The extracts were further dried in vacuum desiccator and the yield of the extracts was subjected to
column chromatography in order to isolate and characterize the structures of the constituents using IR and MASS spectral analysis and confirmed with authentic samples (Sethi, 1996; Wagner and Bladt, 1996).

The plant extracts were concentrated using a rotary evaporator. The concentrated extracts were dried completely, weighed and re-dissolved in 10 percent gum acacia solution and administered at 1 percent concentration. Ninety adult albino Wistar rats with body weights ranging from 150-200 g were obtained and maintained under standard laboratory conditions. The rats were randomly sorted first into three groups (T1, T2 and T3) of 30 each for the trial to be run in triplicate. Each of the group of 30 rats was again randomly divided into five sub groups of six rats each. The first sub group was served as negative control (-C) and was given vehicle gum acacia alone. The rest of the 24 rats in each major group were given a single oral administration of carbon tetrachloride at the rate of 0.1 ml per 100 g body weight in vehicle gum acacia and thus the second sub group was served as positive control (+C). The third, fourth and fifth sub groups, in addition, received oral administration of plant extracts Securungega virosa, Phyllanthus reticulatus and Breynia retusa respectively for five consecutive days.

On the sixth day, the rats were sacrificed and blood was collected from jugular vein of all the rats and frozen for assay of biochemical profile. Small pieces of liver of all the experimental animals were collected in buffer formalin for histopathological examinations. Blood samples were subjected to estimation of alanine transaminase, aspartate transaminase, urea, creatinine, cholesterol and glucose contents (Varely, 1980).

RESULTS AND DISCUSSION

Fractionation of the extracts of S. virosa, P. reticulatus and B. retusa and crystallization of the compounds yielded five compounds and identified as amyrin, thea alcohol, octacosanol, ß-sitosterol and ß-sitosterol 3-ß-D-glucopyranoside.

With regard to the pharmacological studies, in the negative control group, all the values were within the normal range indicating that the administration of the vehicle alone did not cause any harmful effect on the liver in living rats. The highly elevated levels of AST and also the rest of the compounds in the biochemical profile in the blood of positive control rats was indicative of disruption in the normal functioning of the liver as a result of tissue damage caused by carbon tetrachloride. This was due to necrosis and break down of hepatic cells. In fact, it is well established that the hepatotoxicity of carbon tetrachloride is due to its enzyme activation leading to the formation of CCl₃⁺ free radical, which in turn disrupts the structure and function of lipid and protein macromolecules in the cell membranes or the cell organelles and induced lipid leading to fatty liver. The ALT levels in the blood of rats administered with plant extracts were all higher than in normal rats. Among T1, T2 and T3 groups, a tendency towards return of the liver regulated enzyme ALT to normalcy was seen more in T3 group, which received extract of plant S. virosa than the other two groups. For other biochemical assay, the trend was seen to be more in favour of the plant extract of S. virosa. This had brought about better repair to the damaged liver parenchyma than the other two extracts.

Microscopic examination of livers from the positive control group of rats revealed moderate to marked congestion of central veins and sinusoids. Further the hepatocytes around the central veins showed necrotic changes with disruptions of scaffolding reticulum structure. Infiltrations of a few neutrophils and macrophages in the sinusoidal space were seen. Multi focal areas of necrotic hepatocytes were also observed in a few cases. Among the three plants screened S. virosa had the best repair mechanism on blood biochemistry, which also correlated with pathological changes seen in the liver. Histopathological examinations of the livers of the group of rats that received extract of S. virosa revealed almost normal architecture of the liver.

The isolated compounds, though not new to science, are the first report or confirmation of previous reports, for the three species namely, S. virosa, P. reticulatus and B. retusa. The results of phytochemical analyses are helpful to take up pharmacological studies and to characterize the active principles. Among the three plants
screened for pharmacology S. virosa had the best maneuver on the blood biochemistry and also in repairing the damaged liver indicating the possible pharmacological usage of the plant as a hepatoprotectant.

**Literature Cited**

**Tables**

Table 1. Effect of plant extract on blood biochemistry in carbon tetrachloride induced toxicity in rats.

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Control</th>
<th>Carbon tetrachloride</th>
<th>B. retusa T1</th>
<th>P. reticulatus T2</th>
<th>S. virosa T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alanine transaminase (IU/L)</td>
<td>23.75 ± 0.53</td>
<td>40.50 ± 0.65</td>
<td>33.50 ± 0.50</td>
<td>39.50 ± 0.10</td>
<td>30.16 ± 0.50</td>
</tr>
<tr>
<td>2</td>
<td>Aspartate transaminase (IU/L)</td>
<td>60.50 ± 0.10</td>
<td>135.00 ± 0.75</td>
<td>59.50 ± 0.10</td>
<td>97.50 ± 0.10</td>
<td>87.50 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>Urea (mg/dl)</td>
<td>20.50 ± 0.18</td>
<td>36.95 ± 0.10</td>
<td>32.50 ± 0.50</td>
<td>24.50 ± 0.00</td>
<td>30.10 ± 0.15</td>
</tr>
<tr>
<td>4</td>
<td>Creatinine (IU/L)</td>
<td>1.21 ± 0.10</td>
<td>1.40 ± 0.10</td>
<td>1.30 ± 0.10</td>
<td>1.32 ± 0.10</td>
<td>1.28 ± 0.10</td>
</tr>
<tr>
<td>5</td>
<td>Cholesterol (mg/dl)</td>
<td>80.50 ± 0.10</td>
<td>110.00 ± 0.50</td>
<td>98.55 ± 0.50</td>
<td>100 ± 0.90</td>
<td>95.00 ± 0.10</td>
</tr>
<tr>
<td>6</td>
<td>Glucose (mg/dl)</td>
<td>90.50 ± 0.75</td>
<td>60.00 ± 0.50</td>
<td>79.50 ± 0.50</td>
<td>85.50 ± 0.50</td>
<td>87.5 ± 0.10</td>
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</tbody>
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