Original Research

Hepatoprotective Activity of Methanolic Extract of *Oldenlandia herbacea* Against D-Galactosamine Induced Rats

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Summary. The present study is to evaluate the hepatoprotective activity of methanolic extract of *Oldenlandia herbacea* against D-Galactosamine (D-GalN) induced rats. Hepatoprotective activity was performed by using the toxicant D-GalN (200 mg/kg) in Wistar rats. Methanolic extract of the whole plant of *O. herbacea* was administered orally at doses of 100 and 200 mg/kg/day for 8 days. Silymarin (100 mg/kg) was used as standard drug. Levels of various biochemical parameters in serum and histopathology of liver were assessed to study the hepatoprotective effect of the extract. The extract showed significant reduction in the D-GalN induced liver damage and symptoms of liver injury by restoration of the deviated levels of various biochemical parameters of liver which were observed in toxic control group. Histopathology of the liver sections confirmed that the extract prevented hepatic damage induced by D-GalN. The methanolic extract of *O. herbacea* showed significant hepatoprotective activity.

Industrial relevance. The herbal drugs continue to serve as an important source of conventional therapies for diverse disease conditions and reactions. This is a preliminary study and this will support us to isolate the biological active phyto-constituents for treating liver disorders. This study will help in discovering a new or active phytochemicals and thus create a novel hepatoprotective moiety with less adverse effect for the betterment of the human society and more affordable and accessible to the users.

Keywords. *Oldenlandia herbacea*; D-galactosamine; Hepatoprotective activity; Silymarin.

INTRODUCTION

Liver is the second largest organ in the body. Liver diseases remain one of the serious health problems (Baranisrinivasan P, et al, 2009). The importance of herbal medicine in curing various ailments has been already established. There are potent indigenous herbal medicines available for liver disorders in various parts of the world and much of them were not yet scientifically validated and if done may lead to the development of cost effective drugs (Asha VV, et al, 1998; Subramoniam A, 1995).

*Oldenlandia herbacea* Roxb. (*Hedyotis herbacea*) (family- Rubiaceae) is an erect, glabrous annual shrub found in temperate and tropical regions of Africa and Asia was shown in (Figure 1). Its extract or decoction has been reported to be useful in the treatment of malaria. Decoction of the herb has been used for bathing rheumatic patients and the powdered herb administered with honey for rheumatic fever and swellings. The herb is boiled in oil and the oil used for elephantiasis and pains in the body. The leaves have been employed as expectorant in Asthma (Kirtikar KR, et al, 1987). Ethnomedically, this plant is recognized as one of the ingredient in Jaundice treatment. Ursolic acid, kaempferol-3-o-arabinino pyranoside, kaemferol-3-o-rutinoside, 23-ethyl-cholest-23-en-3-ol and 9, 9-dimethyl hexacosane have been reported to be present in this plant (Ahmad SH, et al, 1996; Pandian S, et al, 2008). The present study was performed to evaluate the methanolic extract of *O. herbacea* for its hepatoprotective activity against D-galactosamine induced rats.

MATERIALS AND METHODS

**Plant collection and extraction.** The whole plant of *O. herbacea* was collected during January 2008 from Karisakkadu village, Pudukkottai district of Tamilnadu, India and identified and authenticated by Dr. S. Rajan, Government Arts College Ootacamund, where a voucher specimen was preserved for further reference. The whole plant was shade dried, powdered and
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extracted (500 g) with methanol in a soxhlet extractor for 20 h. The extract was concentrated under reduced pressure at controlled temperature (40-50°C) using rotavapour (R-205, Buchi Laboratory Equipments, Flawil, Switzerland). The concentrated extract was dried in oven at 40°C to constant weight. The methanol extract thus obtained (Yield: 24 g, 4.8% w/w) was dark green semisolid in nature.

**Figure 1. O. herbacea aerial parts**

**Acute toxicity study as per OECD guideline 425.** Acute oral toxicity study of the extract was carried out in Wistar rats as per the OECD 425 guidelines. The test was performed using five rats at a dose of 2000 mg/kg body weight. Methanolic extract of *O. herbacea* was suspended in distilled water using sodium carboxymethylcellulose (sodium CMC, 0.3%) and administered orally to the animals with the help of an intragastric catheter. Animals were observed individually once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days. Animal was observed for clinical signs, physical abnormalities, changes in body weight and pre-terminal deaths (OCED 425 guidelines, 2001).

**Hepatoprotective activity of *Oldenlandia herbacea.*** Wistar rats of both sexes (180–200 g) were selected for the study and randomly divided into five groups of six animals each. All animals were maintained at a controlled temperature of 19–25 °C with 12 h light/dark cycle and fed with a standard diet and water *ad libitum*. The experiments were conducted according to the Institutional Animal Ethics Committee regulations approved by the committee for the purpose of control and supervision of experiments on animals (JSSCP/IAEC/PH. D/PH. CHEM/01/2009-10).

- **Group I** was served as normal and received the vehicle (Sodium CMC 0.3%, 5ml/kg).
- **Group II** was served as toxic control and received the vehicle.
- **Groups III and IV** was treated with the methanolic extract of *O. herbacea* at oral doses of 100 and 200 mg/kg/day for 8 days.
- **Group V** was treated with standard drug silymarin (100 mg/kg) for 8 days. On the last day of the treatment, the animals of groups II–V received a single dose of D-GalN in distilled water at 200 mg/kg intraperitoneal dose after 1 h of their respective treatments. On day 9, all animals were anesthetized using anesthetic ether and blood was collected from the retro-orbital plexus and kept for coagulation for 30 minutes at 4°C. Serum was separated by centrifugation at 2500 rpm for 15 min at 4°C and used for the estimation of various biochemical parameters.

**Analysis of biochemical parameters.** Aspartate aminotransferase (ASAT), Alanine aminotransferase (ALAT), Alkaline phosphatase (ALP), Triglycerides (TGL), Total Cholesterol (TC), Total Bilirubin (TB), Creatinine (CR), Total Protein(TP) and Albumin were measured in an autoanalyzer.

**Histopathological studies.** Liver samples were obtained after completely draining the blood, washed with normal saline, and processed separately for histological observations. A portion of the liver tissues were fixed in 10% formalin, cut into 5 µm thick sections and stained using hematoxylin-eosin and examined microscopically for histopathological changes.

**Statistical analysis.** Results obtained were expressed as mean ± SEM. The significance of the data was analyzed by one-way ANOVA followed by Tukey–Kramer multiple comparison tests and p < 0.05 was considered as statistically significant (Bolton S, 1997).Chemicals. N-nitrosodiethylamine and Phenobarbital were procured from Sigma-Aldrich,India. The assay kits for various lipid profile test were purchased from nicholas laboratories.Chemicals used in this study,1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich,India, ascorbic acid, phosphoric acid,naphthyl ethylene diamine dihydrochloride, are obtained from Sd Fine chemicals Ltd,India, and potassium ferricyanide, trichloro acetic acid, sulfanilamide were obtained from Himedia,Laboratories Pvt-Ltd,India,Folin-Ciocalteu reagent, sodium nitroprusside obtained from Qualigens fine chemicals and all the reagents used in the study were analytical grade.

**RESULTS**

The extract was found to be non-toxic at the tested dose of 2000 mg/kg in the single dose acute toxicity study. A single dose of D-GalN (100 mg/kg) administration to the animals showed marked increase in ASAT, ALAT, ALP, TGL, TC, TB, and CR with marked decrease in TP and Albumin when compared to the normal group (Table 1). Pre-treatment of the extract at both the doses
significantly reversed tested biological parameters towards the normal values thereby preventing the damage caused by D-GalN. The standard silymarin at 100 mg/kg also showed similar significant results. Dose dependent results were not obtained for the O. herbacea extract. The dose of 200 mg/kg body weight was found to be more active than the standard drug.

**Table 1.** Effect of O. herbacea methanol extract on biochemical parameters in D-Galactosamine induced hepatotoxicity

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>ASAT (IU/l)</th>
<th>ALAT (IU/l)</th>
<th>ALP (mg/dl)</th>
<th>TGL (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>TB (mg/dl)</th>
<th>TP (g/dl)</th>
<th>CR (mg/dl)</th>
<th>Albumin (g/dl)</th>
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<tr>
<td>Normal control</td>
<td>146.83 ± 2.19</td>
<td>76.83 ± 2.24</td>
<td>179.35 ± 4.07</td>
<td>41.33 ± 1.25</td>
<td>27.23 ± 0.74</td>
<td>0.70 ± 0.02</td>
<td>8.51 ± 0.20</td>
<td>0.31 ± 0.17</td>
<td>3.01 ± 0.05</td>
</tr>
<tr>
<td>D-Galactosamine, (200 mg/kg)</td>
<td>66.54 ± 3.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.83 ± 1.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>610.66 ± 3.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.33 ± 1.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.09 ± 1.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.02 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.34 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.50 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.08 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ext (200 mg/kg) + D-GalN (200 mg/kg)</td>
<td>200.83 ± 3.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.65 ± 1.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>449.08 ± 3.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.66 ± 1.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.49 ± 0.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.71 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ext (200 mg/kg) + D-GalN (200 mg/kg)</td>
<td>197.17 ± 2.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.39 ± 2.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>329.03 ± 3.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.66 ± 1.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.35 ± 1.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.69 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.47 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.07 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Silymarin (100 mg/kg) + D-GalN (200 mg/kg)</td>
<td>173.53 ± 2.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.67 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>366.34 ± 2.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.16 ± 1.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.87 ± 0.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.80 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.57 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.69 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
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Each value represents the mean ± S.E.M., n=6;<sup>a</sup>P<0.05, <sup>b</sup>P<0.01, <sup>c</sup>Compared with normal control, <sup>d</sup>Compared with toxicant control group

Histopathological studies of liver sections of normal animals showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein. The liver sections of the rats of D-GalN control group showed disarrangement of normal hepatic cells with high degree of damage, characterized by the centrilobular necrosis, focal necrosis and bile duct proliferation. The sections of the rats treated with the methanol extract at 100 and 200 mg/kg body weight and intoxicated with D-GalN exhibited less centrilobular necrosis and bile duct proliferation compared to the D-GalN control. The standard silymarin at 100 mg/kg body weight and D-GalN treated animals showed almost normal architecture of the liver with few centrilobular fatty changes and bile duct proliferation was shown in (Figure 2).

**Figure 2.** Histology changes in liver tissues (H&E Staining) with 10X magnification in rats. A - Histological changes in liver tissue in normal control treated rat, B - Histological changes in liver tissue in D - Galactosamine treated rat, C - Histological changes in liver tissue in Silymarin treated rat, D - Histological changes in liver tissue in O. herbacea methanol extract (100 mg/kg), E - Histological changes in liver tissue in O. herbacea methanol extract (200 mg/kg).

**DISCUSSION**

The methanolic extract of O. herbacea contains flavonoids, glycosides, phenolic compounds, saponins, tannins and iridoid glycosides. Ursolic acid, kaempferol-3-α-arabino pyranoside and kaempferol-3-α-rutinoside were few of the constituents isolated from this plant. Many flavonoids and glycosides are reported for hepatoprotective activity, D-GalN is one of the commonly used substances for inducing hepatic injury in rats. GalN have been known to cause hepatotoxicity by the accumulation of UDP-GalN derivatives in the liver, followed by a depletion of hepatic UTP resulting in inhibition of mRNA and protein synthesis (Aniya Y,
et al, 2005) Free radicals are also known to play a role in the GaIN induced hepatic necrosis. It is clearly evident from table 1, that the levels of ALAT, ASAT, ALP and TB increased significantly (p<0.01) in group treated with D-GaIN comparing to normal control and it is an obvious indication of the hepatic insult (Ahmad A, et al, 2002; Friedman LS, et al, 1996). Thus the methanolic extract of *O. herbacea* supports hepatoprotective activity which was comparable to silymarin.

**CONCLUSION**

The methanolic extract at the tested doses showed significant hepatoprotective activity. The hepatoprotective activity of the extract was even found to be better than the standard silymarin. Iridoid glycosides from many plants are known for their antihepatotoxic. The hepatoprotective activity of the extract may be attributed to iridoid glycosides. Form the results of the present study it can be said that the plant can be used as a promising hepatoprotective agent.

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**REFERENCES**


