“HEPATOPROTECTIVE ACTIVITY OF DIFFERENT EXTRACTS OF AERIAL PARTS OF 
LI MNOPHILLA INDICA” BY USING PARACETAMOL AND ETHANOL INDUCED MODELS

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ABSTRACT

The present study was carried out to evaluate the hepatoprotective activity of leaves of Limnophilla indica of family Scrophulariaceae (Dog flower family). The plant extract was prepared and examined against CCI4-induced liver damage in wistar rats using silymarin as standard. The extract was screened for alkaloids, steroids, proteins, flavonoids, saponins, carbohydrates, tannins and glycosides. Enzyme activities of Serum glutamate oxaloacetate (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase, Total bilirubin and Total protein levels are analysed. Results indicate that the methanolic leaf extract had protective activity over paracetamol and ethanol induced hepatotoxicity when compared with toxic control and silymarin groups. Results of present investigation confirm the hepatoprotective activity of Limnophilla indica. The statistical analysis was carried out using one way analysis of varience (ANOVA) followed by Dunnet’s t-test. P- values <0.05 were considered as significant.

Keywords: Limnophilla indica, SGOT, SGPT, TB, TP, ALP, CCI4

INTRODUCTION

Nature always stands as golden mark to exemplify the outstanding phenomena of symbiosis. Nature serves humans with medicines which were used to maintain health, to treat and heal many ailments. For the treatment of human diseases a basic product from Natural products like plant, animal and minerals were used[1]. Some plants are identified as rasayanas in Indian Ayurvedic system of medicine having various pharmacological properties such as immunostimulant, tonic, neurostimulants, antiaging, antibacterial, antiviral, anti-inflammatory, anticancer, adaptogen etc[2]. An entire section of Materia Medica of Ayurveda is devoted to drugs entitled as ‘Rasayana’ for enhancement of body resistance[3]. Rasayana, listed as a class in the texts of traditional Indian Medicine literature, consists of a number of plants reputed to promote physical and mental health, improve defense mechanisms of the body and enhance longevity. These attributes are similar to the modern concept of adaptogenic agents, which are known to afford protection of the human physiological system against diverse stressors[4].

Medicinal herbs used in liver disorders are Silybum marianum, Andrographis paniculata, Glycyrrhiza glabra, Picrorrhiza kurroa, Phyllanthus niruri are potential candidates, Solanum nigrum, Taraxacum officinale, Cichorium intybus, Tephroia purpurea, Curcuma longa.

The liver is the largest organ in the body weighing 1200-1500 g. It is a key organ in regulating homeostasis within the body. It regulates several important functions including protein synthesis, storage and metabolism of fats and carbohydrates, detoxification of drugs and other toxins, metabolism of hormones and excretion of bilirubin. Liver diseases are associated with distortion of these metabolic functions[5]. Although viruses are the main cause of liver diseases, the liver lesions arising from xenobiotics, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon[6].

Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase, are elevated[7,8].

MATERIALS AND METHODS

Limnophilla indica (Linn.) Druce , (family: Scrophulariaceae) is originated from a Latin word that means pond-loving indicating its existence in aquatic environments. It is commonly known as ‘Ambula’ (Asian marshweed). It is a perennial from Southeast Asia, tropical to subtropical Africa, Australia, and Pacific Islands; also finds adventive distribution in North America. Limnophilla plants are widely distributed throughout India, and occupy a significant position in traditional systems of medicine. It is a small aquatic or aquatic submersed with 7.5-20 cm high aromatic herb, erect diffusely branched herb. It has a strong smell of turpentine resembling to camphor or oil of lemon; stems are subquadrangular and rooting at the lower nodes. Aerial parts are usually all whorled and pinnatilobid, 6-20 cm long, linear-oblong and serrate-dentate. Flowers are solitary, axillary or racemed; corolla is pink in colour with about 8 mm long.

Collection and autentification of plant material: The plant material was collected in the month of June 2013 from Srichalam hills and a specimen was dropped in the herbarium and the leaves was authenticated by Professor Dr. Madhavachetty S. V. University, Trupathi. The collected powdered material was shade dried and pulverized.

Solvents used for extraction: Petroleum ether and methanol

Preparation of the extract: The dried powders of leaf of Limnophilla indica were defatted with petroleum ether (60-80°C) in a Soxhlet Apparatus by continuous hot- percolation. The defatted powder material (marc) thus obtained was further extracted with methanol with same method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

PHYTOCHEMICAL SCREENING: The screening was carried out in accordance with the standard protocol as described by Trease and Evans (1983).
Test for reducing sugars (Fehling’s test): The aqueous ethanol extract (0.5 g in 5 ml of water) of individual plants was added to boiling Fehling’s solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for antraquinones: The individual plant extract (0.5 g) was boiled with 10 ml of sulphuric acid (H 2SO 4 4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia solution was added. The resulting solution was observed for orange changes.

Test for terpenoids (Salkowski test): To 0.5 g each of the individual extract was added 2 ml of chloroform. Concentrated H 2SO 4 (3 ml) was carefully added to form a layer. A reddish brown coloration was confirmed for the presence of terpenoids.

Test for flavonoids: A portion of the individual plant extract (0.5 g) was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for saponins: To 0.5 g of each plant extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

S.No Constituents Test Methanolic extract
1. Alkaloids A) Mayer’s reagent Present
   B) Wagner’s reagent
   C) Dragendorff’s reagent
   D) Hagner’s reagent
2. Carbohydrates a) Molisch’s reagent Present
   b) Benedict’s reagent
   c) Fehling’s solution
3. Glycosides a) Modified borntrager’s test
   b) Legal test Present
4. Phytosterol a) Salkowski’s test Absent
   b) Libermann Burchard’s test
5. Saponins a) Froth test Absent
   b) Foam test
6. Tannins Gelatin test
7. Proteins a) Xanthoprotein test Absent
   b) Ninyhydrin test
8. Flavonoids a) Alkaline reagent test Present
   b) Lead acetate test
   c) Shinoda test
9. Diterpenes Copper acetate test Present

ACUTE TOXICITY STUDY

Acute oral toxicity study
Acute oral toxicity test was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for 3-4 hrs providing only water, after which the extracts were administered orally at the dose level of 5mg/kg by intra gastric tube and observed for 3 days. If mortality was observed in 2-3 animals then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg.

Hepatoprotective activity

Screening methods for hepatoprotective activity
Paracetamol induced hepatotoxicity model.
Arsenic induced hepatotoxicity model.
Isoniazide induced hepatotoxicity model.
Carbon tetra chloride induced hepatotoxicity model.
Glucocorticoids induced hepatotoxicity model.
NSAIDS associated toxicity of liver.

Models chosen for the study: paracetamol and ethanol induced hepatotoxicity model.

Paracetamol induced hepatotoxicity in rats

Animals were divided into 5 groups of six animals each
Group I: Treated as Normal control and receives 5% Gum Acacia.
Group II: Treated as toxic control and is administered with paracetamol to induce hepatotoxicity.
Group III : Administered with hepatotoxin and standard drug silymarin at a dose of 25 mg/kg.
Group IV: Administered with hepatotoxin and MELI extract at a dose of 250 mg/kg.
Group V: Administered with hepatotoxin and MELI extract at a dose of 500 mg/kg.

After thirty minutes of paracetamol and ethanol administration Group III, IV and Group V rats were treated with Silymarin (25 mg/kg/day), MELI 250 mg/kg/day and MELI 500 mg/kg/day respectively. All the animals were sacrificed by cervical decapitation under light ether anesthesia on the Ninth day. Blood was collected by heart puncture and centrifuged (300 rpm for 10 mins) to obtain...
serum. The serum was used for the assay of total bilirubin, alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and total protein.

Statistical analysis

The results obtained in biochemical assays were given in terms of mean ± SEM. The statistical significance of the data was assessed by one way analysis of variance (ANOVA) followed by Dunnet’s ‘t’ test between different groups. Toxic, Standard and Test groups were compared with normal group. Standard and all Test groups were compared with the toxic group. p<0.05 was considered as statistically significant.

RESULT AND DISCUSSION

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>ALT (M/L)</th>
<th>AST (M/L)</th>
<th>ALP (M/L)</th>
<th>TB (M/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>86.66±2.801</td>
<td>33.32±1.89</td>
<td>46.83±1.64</td>
<td>0.268±0.02</td>
</tr>
<tr>
<td>Group II</td>
<td>Paracetamol</td>
<td>245.3±2.985</td>
<td>165.5±3.38</td>
<td>90.16±2.509</td>
<td>5.378±0.03</td>
</tr>
<tr>
<td>Group III</td>
<td>Paracetamol+Silymarin</td>
<td>93.66±2.108***</td>
<td>41.5±1.995***</td>
<td>47.3±1.641***</td>
<td>0.276±0.019***</td>
</tr>
<tr>
<td>Group IV</td>
<td>Paracetamol+MELI 250mg/kg</td>
<td>116.33±3.49***</td>
<td>80.83±2.45***</td>
<td>54.6±2.27***</td>
<td>0.75±0.03***</td>
</tr>
<tr>
<td>Group V</td>
<td>Paracetamol+MELI 500mg/kg</td>
<td>162.1±6±4.2***</td>
<td>130.8±2.006***</td>
<td>60.6±2.81***</td>
<td>1.93±2.27***</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM for 6 rats, *P<0.5, **P<0.01, ***P<0.001. Compared with control, data were analysed by using one-way ANOVA followed by Dunnet’s test, standard Silymarin (5 ml/kg; p.o) MELI- Methanolic extract of Limnophila indica, dose (250mg/kg of B.W) MELI-Methanolic extract of Limnophila indica (500mg/kg of B.W)

RESULT

Hepatoprotective activity of methanolic extracts Limnophila indica on Serum marker enzymes (ALT,AST,ALP) and Total bilirubin on Paracetamol induced hepatotoxicity, in rats.

DISCUSSION

Acetaminophen is an antipyretic and analgesic drug, which is activated and converted by cytochrome P450 enzyme to toxic metabolite N-acetyl-para-benzoquinoneimine (NAPQI) at high doses. The reactive metabolite can be conjugated with cellular glutathione (GSH) to cause extensive GSH depletion which leads to the cellular necrosis[13]. GSH removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thioles. The GSH depletion in hepatic mitochondria is considered the most important mechanism in the paracetamol induced hepatotoxicity. Reduced GSH level was depleted in paracetamol treated group may be due to conjugation of GSH with NAPQI to form mercapturic acid. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome[11] or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity[12,13].

Normally, AST and ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatonic diseases is unusual. ALT is more selectively a liver paranchymal enzyme than AST[14]. Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and Bilirubin which are enzymes originally present higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the bloodstream in conformity with the extent of liver damage[15]. The elevated level of these entire marker enzymes observed in the group- II, paracetamol treated rats in this present study corresponded to the extensive liver damage induced by toxin. The reduced concentration of ALT, AST and ALP as a result of plant extract administration observed during the present study might probably be due in part to the presence of flavonoids. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes[16]. Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation.
and excretory capacity of hepatocyte. Decrease in serum bilirubin after treatment with extract in liver damage induced by paracetamol, indicated the effectiveness of the extract in normal functional status of the liver.

In the present study, both the methanolic extracts Limnophila indica, have the ability to reduce oxidative stress by increasing glutathione level and preventing lipid peroxidation.

Hepatoprotective activity of methanolic extracts Limnophila indica on Serum marker enzymes (ALT,AST,ALP) and Total bilirubin on Ethanol induced hepatotoxicity, in rats. Each value is the mean ± SEM for 6 rats, *P<0.5, **P<0.01, ***P<0.001. Compared with control, data were analysed by using one-way ANOVA followed by Dunnet’s test, standard Silymarin (5 ml/kg; p.o) MELI- Methanolic extract of Limnophila indica, dose (250mg/kg of B.W) MELI- Methanolic extract of Limnophila indica (500mg/kg of B.W)

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<th>TB (M/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>56.00 ± 1.527</td>
<td>8.33 ± 0.33</td>
<td>182.16±5.04</td>
<td>0.75±0</td>
</tr>
<tr>
<td>Group II</td>
<td>Ethanol</td>
<td>73 ± 1.390</td>
<td>7.33± 0.66</td>
<td>241±3.18</td>
<td>6.17±0.166</td>
</tr>
<tr>
<td>Group III</td>
<td>Silymarin + ethanol</td>
<td>38.66±2.49***</td>
<td>5.66±0.763**</td>
<td>191.51±1.89***</td>
<td>0.38±0*</td>
</tr>
<tr>
<td>Group IV</td>
<td>MELI 250mg/kg + ethanol</td>
<td>59.33±1.429***</td>
<td>7.5 ± 0.428**</td>
<td>215.33±1.68***</td>
<td>0.61±0*</td>
</tr>
<tr>
<td>Group V</td>
<td>MELI 500mg/kg + ethanol</td>
<td>56.66±1.563***</td>
<td>5.51±0.726**</td>
<td>191.93±0.90***</td>
<td>0.49±0*</td>
</tr>
</tbody>
</table>

Effect of MELI on Serum Enzymes (ALT, AST, ALP, TB) on Ethanol induced rats

RESULT
On the other hand suppression of elevated ALP activities with concur-rent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the stability of bilary dysfunction in rat liver during hepatic injuries with toxicants

DISCUSSION
There are many factors which are responsible for the liver damage or injuries such as chemicals and drugs. In the present study ethanol was used to induce hepatotoxicity, since it is clinically relevant. Ethanol produces a constellation of dose related deleterious effects in the liver. The majority of ethanol is metabolized in the liver and individuals who abuse alcohol by routinely drink-ing 50-60 g (about 4 to 5 drinks) of ethanol per day are at risk for developing alcoholic liver disease.In addition, both acute and chronic ethanol administration cause enhanced formation of cytokines, especially TNF-alpha by hepatic Kupffer cells, which have a significant role in liver injury. Besides the develop-ment of fatty liver (steatosis), another early sign of excessive ethanol consumption is liver enlargement and protein accumulation, both of which are common findings in alcoholics and heavy drinkers.

CONCLUSION
The preliminary phytochemical studies reveal the presence of flavonoids. The flavonoids known as hepatoprotective, so the observed hepatoprotective activity of Limnophila indica may be due to the presence of flavonoids. The mechanism of hepatoprotective activity may be due to activation of regeneration of hepatocytes that restores the structural and functional integrity of liver. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

REFERENCES