Hepatoprotective activity of glinus lotoides linn against paracetamol induced liver damage in albino rats

Sudha Rameshwari. K
Assistant Professor and ¹II M.Sc. Student, Department of Biochemistry, V.V.Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India
Corresponding author email: sudharameshwari@vvvcollege.org

Thilagaarasi. T
Assistant Professor and ¹II M.Sc. Student, Department of Biochemistry, V.V.Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India

Abstract---Aim: To evaluate the hepatoprotective activity of the chloroform extract of the selected Glinus lotoides linn plant by using Paracetamol induced hepatic injury in albino rats. Methods: The chloroform extract of Glinus lotoides linn was allowed for screening hepatoprotective activity on Paracetamol induced Albino rats and they were compared with standard and negative control. Results: The drug treatment Chloroform Extract of Glinus lotoides linn [CEGL] was carried out at two low dose levels 200mg and 400mg/ kg, both of which along the standard Silymarin 100mg/kg treated group showed a significant reduction in the elevated enzyme levels (P < 0.01). Liver section shows normal cells compared to control. Conclusion: These data suggests a dose dependent hepatoprotective activity of CEGL. At the end of this study, a strong conclusion can be drawn that the Chloroform Extract of Glinus lotoides linn possess hepatoprotective activities induced by Paracetamol in Albino rats.

Keywords---Glinus lotoides linn, paracetamol, hepatoprotective activity.

Introduction

Herbal medicines have recently attracted much attention as alternative medicines useful for treating (or) preventing life style related disorder but very little knowledge is available about their mode of action (Chatterjee TK, 2000; Handa SS & Sharma A, 1982). About 80 percent of the world population in developing countries mainly relies on the use of traditional medicine which is predominantly
based on plant materials (Atal CK et al., 1984). Liver diseases have become one of the major causes of morbidity and mortality all over the world. Among, Drug Induced Liver Injury (DILI) is one of the most common causative factors that pose a major clinical and regulatory challenge. Paracetamol (PCM) is also known as acetaminophen taken in overdose can cause severe hepatotoxicity and nephrotoxicity (Russmann et al., 2009; Vermeulen et al., 1992). Hepatic dysfunction caused by various hepatotoxic substances remains one of the problems (Mossa JS et al., 1991). Therapeutic effectiveness of certain plants (or) plant extract in clinical studies (Jemal Demma et al., 2007). Traditional system of drugs has been the starting point for the discovery of many important modern drugs. This fact led to chemical and pharmacological screening programs of plant not only in India but all over the world. So for studies have been carried out to elevate effect of herbal plant extracts on paracetamol induced hepatotoxicity (Subramanian A & Pushpangadan P, 1999). There are numerous plants and polyherbal formulation claimed to have hepatoprotective activity (Sachdeva et al., 2001). From the existing literature and the information from “napralet” we have selected the hepatoprotective activity which is screening for the present work which could not be attempted by any researcher earlier. Glinus Lotoideas Linn (Siruserupadai in Tamil) is a smelling aromatic herb belonging to the family mollunginanceae. It is being used for cough, tuberculosis, Hick up, thirsty cough, tuberculosis, asthma and AIDS. The juice of this plant is taken internally to strengthen weak children and also has an anti-tumor activity. Chloroform extract of Glinus Lotoideas Linn. Possesses wound healing action and antibacterial activity. Flavanoids, Glycosides may be attributed to the wound healing activity (Sudha Rameshwari K et al., 2013 ). Thus the present study was conformed to evaluate the hepatoprotective activity of the chloroform extract of the Glinus Lotoideas Linn plant by using paracetamol induced hepatic injury in albino rats.

Materials and Methods

The Glinus Lotoideas Linn powder was collected from Sri Harshini herbal at Madurai, Tamilnadu, India.

Preparation of plant extracts

The plant powder was extracted with petroleum ether, chloroform and ethyl acetate by successive cold extraction method for 72 hours. These three sample extracts were taken to carry out the phytochemical analysis.

Qualitative Phytochemical Analysis

Aqueous, ethanol and methanol extracts were subjected to qualitative phytochemical analysis to identify the presence of phytoconstituents such as tannin, terpenoids, alkaloids, glycosides, steroids, flavonoids, phenol, protein, carbohydrate, quinine, Anthroquinone and saponins (Khandelwal KR, 2006; Anees Ahmad Siddiqui and Mohammed Ali ,1997; Harborne JB, 2009).
Pharmacological study

Wistar strain albino rats of either sex weighing between 150 and 180 g were procured from animal house of Sankaralingam Bhuvaneswari College of Pharmacy (Regd. No. 622/02/C/CPCSEA) used for this study. They were maintained under standard conditions (28±2°C; 55-60% relative humidity) and fed a standard diet for rats (Hindustan lever, Bangalore) and given water ad libitum. The animals were divided into V groups of VI animals in each group. Group I animals served as control and received 1ml/Kg of normal saline. Group II animals were similarly treated as Group I. Group III animals were treated with Silymarin 100mg/kg .p.o. Group IV and V animals were treated with chloroform extract of Glinus lotoides linn (CEGL) 200mg and 400mg/kg, p.o respectively for seven days. On the seventh day, paracetamol suspension was given by oral route in a dose of 750mg/kg to all rats except rats in group I. After 36 hours, all the rats were sacrificed under light anaesthesia; blood was collected in sterile centrifuge tubes and allowed to clot. Serum was separated by centrifuging at 2500rpm for 10 minutes and used for the estimation of SGOT, SGPT (Reitman S, Frankel S, 1957), SALP (Kind PR, King EJ, 1954). Malloy and Evelyn method (1937) was used to determine the serum bilirubin.

Histopathological examination

After the animals were sacrificed, the abdomen was cut open and the liver was removed. A portion of the liver in each group was fixed in 10% formalin and protected for histopathology. In a serial sections of 5µm thickness were made from the fixed liver tissues and then studied with haemoxylin and eosin to evaluate the details of hepatic architecture in each group microscopically (Henry R et al., 1960).

Statistical analysis

Results were presented as mean and standard error (Mean ± S.E). The statistical significance between the control and each of the treated groups were determined by Dunnnett’s test after one-way ANOVA. The level of significance was set at $P < 0.01$. (C. W. Dunnet, 1964)

Results

The different solvent extracts of Glinus lotoides linn such as Petroleum ether, Chloroform and ethyl acetate were subjected to preliminary analysis of phytochemical constituents and the results were tabulated in Table 1. All the extracts have protein, carbohydrates, sterols, Flavanoids, flavones, phytosterols, phenols and tri terpenoids. Alkaloids present in the petroleum ether and chloroform extract. Ethyl acetate extract lacks alkaloids and glycosides. Quinones is absent in all extracts. Chloroform extract only shows the presence of glycosides compare with other two extracts. During the present study, chloroform extract was selected for the hepatoprotective activity because the maximum numbers of phyto constituents such as protein, carbohydrate, sterol, phytosterol, glycosides, phenols, flavonoids and alkaloids these compounds are present in the extracts alone. The flavonoids were identified by TLC method using chloroform: methanol
(9:1 v/v) solvent system. A bright fluorescent spot was observed and this indicated the presence of flavonoids. Based on the qualitative phytochemical analysis, we choose chloroform extract for the further studies of pharmacological activity.

The results of the hepatoprotective activity are present paracetamol causes significant increase in the activity of SGOT, SGPT, Alkaline phosphatase and serum bilirubin levels as compared to normal control rats, indicating liver damage was tabulated in Table 2. Results indicate that chloroform extracts of *Glinus lotoides linn* provides significant protection against the paracetamol induced toxic effect on liver. In paracetamol induced toxic hepatitis toxicity begins with the changes in the endoplasmic reticulum which loss of metabolic results in the enzymes located in intra cellular structures. The blood samples of the paracetamol alone treated animals showed increase in the levels of liver weights (5.50gm) SGOT (223) SGPT (1009) alkaline phosphatase (236) and total bilirubin (2.24) as compared to control phosphatase (88.5) and total bilirubin (0.67) elevation of serum enzyme and bilirubin levels is considered as an index of liver damage at liver necrosis. Histopathological sections of the paracetamol treated animals showed fatty changes, necrotic changes as compared to the intact arrangement of cells of the control animal were shown in figure 1. Administration of silymarin and chloroform extracts of *Glinus lotoides linn* showed protective effect against the toxic effects of paracetamol. Among the extract significant hepatoprotection was noticed in the group – V animals treated with chloroform extract at high doses of 400mg/kg, was comparable to that of standard drug silymarin while the low doses of 200mg/kg treated animals exhibited the least hepatoprotective activity. Histopathological study of the test extract treated animals showed recovery against the paracetamol induced necrosis in their normal compact arrangement of hepatic cells as compared to control.

**Discussion**

Paracetemol toxicity is due to the formation of toxic metabolites (N-acetyl-p-benzoquinoneimine) through the action of cytochrome p450. Induction of cytochrome p450 – depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity (Concorn GB et al., 1980; Namkara W et al., 1974). Liver rich in both transaminases (GOT and GPT), contains more GPT that GOT while both transaminases are elevated in sera of patients with acute hepatic diseases, GPT, which is only slightly elevated by cardiac necrosis, is a more specific indicator of liver damage. SGOT and SGPT have been used as a marker enzyme for liver protective studies by a number of researches. Paracetamol is a well – known antipyretic and analgesic which produces hepatic necrosis at higher doses. It’s mode of action in liver is by covalent binding of its toxic metabolite, n-acetyl-p-benzoquinone amine to tissue macro molecules, resulting in cell necrosis (Albano E et al., 1985). Liver damage is always associated with cellular necrosis increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition liquid body substance levels of the many organic chemistry markers like SGOT, SGPT. Alkaline phosphatase, Serum bilirubin is elevated. (Brijesh, KT and Khosa RL, 2010 ; Gayathri R et al., 2010 ; Thangathirtupathi A et al., 2013 ). Damage to the Structural integrity of liver is reflected by an increase in the levels of serum transaminases, because they are cytoplasm in location and are released into the circulation after cellular damage. Protection against
paracetamol iatrogenic toxicity has been used as a take a look at for a possible hepatoprotective agent by many investigators (Vidhya Malar HL, Mettilda Bais M, 2009; Rajibul Islam Md., 2018).

**Conclusion**

The present study thus supports the view that chloroform extract requires further detailed studies for its bioactive principles to evaluate its potential in the treatment of liver disease. In future studies are being made to isolate and characterize the active principle to which the hepatoprotective activity can be attributed. This is surprising, as it is an ingredient of several tribal medicinal formulations used for liver diseases and viral infections.

**Acknowledgement**

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**Reference**


C. W. Dunnet, New Tables for Multiple Comparisons with a Control, Biometrics., 1964, 20(3): 482-491


Table 1: Preliminary phytoconstituents of different solvent extract of *Glinus lotoides* linn.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phyto constituents</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Sterols</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavanones</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Phyto sterols</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Tri terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Quinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2: Hepatoprotective Activity of Chloroform Extract of *Glinus Lotodies Linn* Against Paracetamol Induced Liver Damage In Albino Rats

<table>
<thead>
<tr>
<th>Design of Treatment</th>
<th>Liver weight Gm/100gm Body weight</th>
<th>SGOT IU/L</th>
<th>SCPT IU/L</th>
<th>Alkaline Phosphatase (KA Units)</th>
<th>Total (μ) Bilirubin (mg/dl)</th>
<th>Direct Bilirubin (mg/dl)</th>
<th>Indirect Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.80 ± 0.098</td>
<td>49.55±0.7</td>
<td>38.03±0.7</td>
<td>88.47±0.54</td>
<td>0.67±0.01</td>
<td>0.21±0.01</td>
<td>0.46±0.02</td>
</tr>
<tr>
<td>negative control Paracetamol</td>
<td>4.59±0.127</td>
<td>223.30±12.79</td>
<td>1009.60±8</td>
<td>236.23±5.1</td>
<td>2.24±0.04</td>
<td>0.44±0.01</td>
<td>1.80±0.05</td>
</tr>
<tr>
<td>Silymarin(100 mg/kg)</td>
<td>2.79±0.08</td>
<td>49.56±1.2</td>
<td>65.56±1.2</td>
<td>56±1.2</td>
<td>12.34±0.30</td>
<td>0.75±0.01</td>
<td>0.23±0.01</td>
</tr>
<tr>
<td>200mg/kg(Tes t)</td>
<td>3.78±0.7</td>
<td>77.05±0.6</td>
<td>95.3±1.2</td>
<td>84±1.2</td>
<td>0.92±0.01</td>
<td>0.27±0.01</td>
<td>0.68±0.02</td>
</tr>
<tr>
<td>400mg/kg(Tes t)</td>
<td>3.12±0.0</td>
<td>51.53±1.1</td>
<td>73.55±1.1</td>
<td>91.77±0.21</td>
<td>0.840±0.02</td>
<td>0.26±0.02</td>
<td>0.57±0.01</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, n=6, p<0.01***, P<0.001**** vs negative control group; one way ANOVA followed by dunnett’s -t-test.
Figure 2: Histopathological sections of control, standard and test treated group

- **Figure a.**: Control Group Normal Liver
- **Figure b.**: Paracetamol treated group Fatty Changes
- **Figure c.**: Silicymarin Treated Group (100 mg / kg) Normal Liver
- **Figure d.**: CEGL Treated Group (200 mg / kg) Normal Liver
- **Figure e.**: CEGL Treated Group (400 mg / kg) Normal Liver