Hepatoprotective Activity of *Calotropis gigantea* Root Bark Experimental Liver Damage Induced by D-Galactosamine in Rats

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**ABSTRACT:** The suspensions of alcoholic extract of root bark of the plant *Calotropis gigantea* in 0.6% carboxy methyl cellulose (CMC) were evaluated for hepatoprotective activity in Wistar albino rats by inducing hepatic injury with D-galactosamine (400 mg/kg). Alcoholic extract of root bark of the plant *Calotropis gigantea* at an oral dose of 200 mg/kg and 400 mg/kg exhibited a significant (P<0.001, P<0.01 and P<0.05) protective effect by normalizing the levels of aspartate amino transferase (ASAT/GOT), alanine amino transferase (ALAT/GPT), alkaline phosphatase (ALP), total bilirubin (TB), lactate dehydrogenase (LDH), which were significantly (P<0.001) increased in rats by treatment with 400 mg/kg i.p. of D-galactosamine. Silymarin (25 mg/kg), a known hepatoprotective drug used for comparison exhibited significant activity (P<0.001).

**KEYWORDS:** Calotropis gigantea; D-galactosamine; Histopathology; Hepatoprotective activity.

**Introduction**

Diabetes mellitus is a debilitating and often life-threatening disease. Liver regulates various important metabolic functions. Liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. Hepatic damage is associated with distortion of these metabolic functions. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. However, we do not have a satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver. So the search for effective hepatoprotective drug continues.

*Calotropis gigantea* R.Br. (Asclepiadaceae) commonly known as milk weed or swallow-wort is a common waste land weed in India. Traditionally *Calotropis gigantea* is used alone or with other medicinal plants to treat common diseases such as fevers, rheumatism, indigestion, elephantiasis, asthma, diarrhea (Kirtikar KR et al., 1975).

The plant is reported to possess free radical scavenging (Mueen A et al., 2003), anti-diarrhoeal activity (Chitme HR et al., 2004). The plant cures toothache and earache (Allen TF, 1994 and Aminuddin RD, 2001) in sprain (Manandhar MP, 1990), in anxiety (Boericke W, 2001) cures pain (Sharma V, 2001), in epilepsy (Jain SK et al., 2001) and in mental disorders (Upadhyaya AS et al., 1994). It is also used in some parts of India in wound healing in combination with other plants (Biswas TK, 2003). Extensive literature survey indicates that there is no substantial work was carried out on the root bark of the plant *C. gigantea*. Hence the present study was carried out to determine effect of ethanol extract of *C. gigantea* root bark on hepatoprotective activity in rats by D-galactosamine induced hepatotoxicity models in rats.

**Materials and methods**

**Plant material**

Root bark was collected from Mandsaur, Madhya Pradesh, India during April 2006. A voucher specimen was deposited in B R Nahata college of Pharmacy, Mandsaur. Root bark of the plant was dried in shade. The dried roots bark was powdered (3 Kg) defatted with petroleum ether (60–80 °C) and soaked in ethanol (95%) and kept aside for four days. After four days, the ethanolic layer was decanted off. The process was repeated for four times. The
solvent from the total extract was distilled off and the concentrate was evaporated on a water bath to a syrupy consistency and then evaporated to dryness (yield 7.2%).

**Animals**

Wistar albino rats of either sex weighing between 180 – 200 g of either sex were obtained from animal house of B R Nahata college of Pharmacy, Mandsaur. The study was approved Institutional Ethics Committee for animal experimentation B R Nahata college of Pharmacy, Mandsaur, (approval no:BRNCP/Fac/07-67).

These animals were used for the acute toxicity, wound healing activity. The animals were stabilized for 1 week; they were maintained in standard condition at room temp; 60 ± 5% relative humidity and 12 h light dark cycle. They had been given standard pellet diet supplied by Hindustan Lever Co. Mumbai and water ad-libitum throughout the course of the study.

**Hepatoprotective activity**

Animals were divided in five groups of six rats each. Group 1 served as normal control and received. In Group 2 rats Liver damage was induced by administration of D - galactosamine at dose of 400-mg/kg i.p. on 14th day (Binduja S et al., 1996). Group 3 rats were treated with Silymarin (25 mg/kg p.o.) was used as positive standard (King EJ and Armstrong AR, 1934). Group 4 rats were pretreated with *Calotropis gigantea* root bark extract at a dose of 200 mg/kg orally as a fine suspension in 0.6% sodium carboxy methyl cellulose (CMC) for 14 days prior to the administration of D - galactosamine. Group 5 rats were pretreated with *Calotropis gigantea* root bark extract at a dose of 400 mg/kg orally as a fine suspension in 0.6% sodium carboxy methyl cellulose (CMC) for 14 days prior to the administration of D - galactosamine.

**Evaluation of serum enzymes**

The serum levels aspartate transaminase (AST), alanine transaminase (ALT) (King J, 1965), alkaline phosphatase (ALP) (Reitman S and Frankel SA, 1957), lactate dehydrogenase (LDH) (Rossalki SB and Rau D, 1972), gamma glutamyl transferase (γ-GT) and levels of bilirubin (Mallory E and Evelyn K, 1987) were assayed.

**Histopathological studies**

The livers were removed from the animals and the tissues were fixed in 10% formalin for at least 24 h. Then the paraffin sections were prepared and cut into 5 μm thick sections. The sections were then stained with Haematoxylin– Eosin dye and were studied for Histopathological changes, i.e. necrosis, fatty changes, ballooning degeneration, and lymphocyte infiltration.

**Statistical analysis**

The data’s were expressed as mean ± SD. The data of hepatoprotective activity was analyzed by one way analysis of variance (ANOVA) followed by Dunnet’s-‘t’ test. A p value less than 0.05 was considered as statistically significant.

**Results**

Rats intoxicated with D-galactosamine alone (Group 2) developed hepatocellular damage as evident from a significant elevation (< 0.05) in the serum activities of ASAT, ALAT, ALP, LDH, γ-GT and bilirubin level when compared with control (Table-1). Pretreatment of *Calotropis gigantea* root bark extract afforded a significant protection against D-galactosamine -induced liver injury by maintaining the levels to near normal. In pretreatment animals, the levels of ASAT, ALAT, LDH, γ-GT and bilirubin group II were significantly elevated by D-galactosamine administration. Treatment with alcoholic extracts of *Calotropis gigantea* root bark extract at a dose of 200 and 400 mg/kg showed a significant decrease of ASAT, ALAT, ALP, LDH, γ-GT and bilirubin levels. Standard control drug, Silymarin (25 mg/kg) also prevented the elevation of serum enzymes (Table 1). The results obtained so are statistically significant and comparable to the silymarin treated group as shown in the (Table 1).
**Table 1.** Effect of alcoholic extracts of *Calotropis gigantea* root bark (p.o) on D-galactosamine induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Treatments</th>
<th>ASAT (U/L)</th>
<th>ALAT (U/L)</th>
<th>ALP (U/L)</th>
<th>γ-gt (U/L)</th>
<th>ldh (U/L)</th>
<th>TB (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Control (normal saline)</td>
<td>67.83±1.4</td>
<td>36.17±3.7</td>
<td>428.0±11.3</td>
<td>7.45±0.02</td>
<td>423.3±2.6</td>
<td>0.30±0.01</td>
</tr>
<tr>
<td>2</td>
<td>D-Galactosamine 400 mg/kg, ip.</td>
<td>138.3±1.7</td>
<td>74.17±3.0</td>
<td>888.5±6.2</td>
<td>16.35±1.99</td>
<td>732.2±3.6</td>
<td>2.21±0.01</td>
</tr>
<tr>
<td>3</td>
<td>D-Galactosamine 400mg/kg, ip. Silymarin 25 mg/kg (p.o)</td>
<td>84.50±3.4a</td>
<td>45.67±3.4a</td>
<td>492.3±5.9a</td>
<td>8.88±0.34a</td>
<td>5.8.7±4.2a</td>
<td>0.48±0.01a</td>
</tr>
<tr>
<td>4</td>
<td>D-Galactosamine 400mg/kg, ip. + Calotropis root extract (200mg/kg body.wt) (p.o.)</td>
<td>119.2±1.4***</td>
<td>68.33±1.2***</td>
<td>761.7±3.6***</td>
<td>11.12±.062***</td>
<td>684.1±3.3***</td>
<td>1.38±0.02***</td>
</tr>
<tr>
<td>5</td>
<td>D-Galactosamine 400 mg/kg, ip. + Calotropis root extract (400mg/kg body.wt) (p.o.)</td>
<td>106.7±2.7***</td>
<td>62.67±3.0***</td>
<td>749.6±6.8***</td>
<td>9.40±0.11***</td>
<td>674.2±2.8***</td>
<td>1.20±0.01***</td>
</tr>
</tbody>
</table>

TB—total bilirubin, ASAT— aspartate amino transferase, ALAT—alanine amino transferase, ALP—alkaline phosphatase, LDH—lactate dehydrogenase *P<0.001*

***, **, * P<0.001, P<0.01, P<0.05, respectively. 5 μm thick sections

**Histopathological observations**

The histopathological examination of the liver sections included the examinations of five different groups for D-galactosamine induced toxicity. The microscopic examination of liver of group-I showed a normal portal triad, sinusoids, and cord arrangement of hepatocytes (Fig-A). The microscopic examination of liver of group-II showed marked to moderately severe fatty change of liver with presence of large fat vacuoles in the cytoplasm pushing the nuclei at the periphery. At places many fat vacuoles are seen united and are forming small fat cysts as well. Areas in this group are also showing degeneration and necrosis of hepatocytes (Fig-B). The microscopic examination of liver of group III showed almost normal appearing hepatocytes and no fatty change, or absence of fatty change in hepatocytes, this indicated that silymarin provided significant Hepatoprotection from fatty change (Fig-C). The microscopic examination of liver of group-IV revealed that the test drug when used in 200mg/kg body wt. was not able to provide proper protection from fatty change in liver as the sections of liver at this dose showed moderately severe fatty change (Fig-D). The microscopic examination of liver of group-V revealed almost normal hepatocytes with only occasional fine fat vacuoles and mild inflammation. No significant fatty change or necrosis or marked inflammation was seen (Fig-E).
Fig. 1  Histopathological observation of liver slides in normal, D-galactosamine intoxicated, silymarin treated and Calotropis gigantea root bark extract treated rats (hematoxylin and eosin, 100 ×).  

(A) Normal liver showing normal histology normal portal triad, sinusoids, and cord arrangement of hepatocytes, 5 μm (H&E) (100 ×).  

(B) D-Galactosamine treated group - moderately severe fatty change of liver with presence of large fat vacuoles in the cytoplasm pushing the nuclei at the periphery, 5 μm (H&E) (100 ×).  

(C) Silymarin treated group - normal appearing hepatocytes and no fatty change, or absence of fatty change in hepatocytes, 5 μm (H&E) (100 ×).  

(D) Calotropis gigantea root bark extract 200mg/kg. (p.o) Moderately severe fatty changes, normally appearing hepatocytes, 5 μm (H&E) (100 ×).  

(E) Calotropis gigantea root bark extract 400mg/kg. (p.o), almost normal hepatocytes with only occasional fine fat vacuoles and mild inflammation. No significant fatty change or necrosis or marked inflammation, 5 μm (H&E) (100 ×).

Discussion
Exogenous administration of D-galactosamine has been found to induce liver damage, which closely resembles human viral hepatitis (Taniguchi H et al., 2004 and Decker K and Keppler D, 1972). The toxicity of D-galactosamine results from inhibition of RNA and protein synthesis in the liver (Endo Y et al., 1992 and Manabe A et al., 1996). The metabolism of D-Galactosamine may deplete several uracil nucleotides including UDP-glucose, UDP-galactose and UTP (Tsai CC et al., 1997) which are trapped in the formation of uridine-diphospho-galactosamine. Accumulation of UDP-sugar nucleotides (Mitra SK et al., 1998) may contribute to the changes in the rough endoplasmic reticulum and to the disturbance of protein metabolism.
Further, intense galactosamination of membrane structures is thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium pump, with consequent increase in the intracellular calcium is considered to be responsible for cell death. An evidence of hepatic injury is leakage of cellular enzymes into the plasma. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage.

In present study D- galactosamine in larger dose produced liver necrosis. This may be due to depletion of several uracil nucleotides including UDP-glucose, UDP-galactose and UTP. This resulted in elevation in levels of quantitative markers in the serum i.e. ASAT, ALAT, ALP, γ-GT, LDH, Total bilirubin. Pretreatment with Calotropis gigantea alcoholic root bark extracts brought down the elevated levels of ASAT, ALAT, ALP, γ-GT, LDH, and Total bilirubin. The histopathological observations supported the evidence.

The hepatoprotective action may be mediated through the inhibition of UDP-sugar derivatives, enhancement of glycoprotein biosynthesis and stabilization of cell membrane and inhibition of lipid accumulation by its hypolipidemic property. The hepatoprotective property of the extract may be attributed to the presence of flavonoids, which are present in the plant. Previous phytochemical investigations of Calotropis gigantea described the isolation and structural determination of a flavonoid (Sen S et al., 1992). The inhibitory activity of this flavonoid in free radical production could be related to the hepatoprotective effect.

Conclusions

The alcoholic extract of root bark of plant Calotropis gigantea found to have significant hepatoprotective activity by D- galactosamine induced hepatic injury method. The effect is almost comparable to silymarin or slightly less. Alcoholic extract of root bark of plant Calotropis gigantea in the doses of 200mg/kg, 400mg/kg body wt, reduced the levels of serum ASAT, ALAT, ALP, γ-GT, LDH and Total bilirubin significantly. Though the extract showed significant hepatoprotective activity, it is also necessary to determine the exact compound responsible for this activity. Further research work is needed to determine exact compound responsible for hepatoprotective activity and exact mechanism of hepato-protective activity of the plant.

References


Sharma V. Dravyaguna Vigyan, Chaukhambhala Bharti Academy, Varanasi, 2001, p 435.

