EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF AGLAIA ELEAGNOIDEA (A. JUSS) BENTH STEM EXTRACT AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN ALBINO RATS

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Abstracts

Objective: The present study was intended to evaluate the hepatoprotective activity of hydro alcoholic extract of stem of Aglaia eleagnoidea (A. Juss) Benth. It is a medicinal plant found in the dense and moist forests in Western and Eastern Ghats, and also in many drier parts of Indian states like Andhra Pradesh, Karnataka, Kerala, Bihar, Andaman and Nicobar Islands.

Method: The hydro alcoholic extract and its hexane, ethyl acetate and methanol fractions of Aglaia eleagnoidea (A. Juss) Benth., at the doses of 150,300 & 600 mg/kg and two isolated phytoconstituents (50mg/kg) was administered orally by suspending in Sodium CMC to the Wister albino rats with carbon tetra chloride(1ml/kg) induced hepatotoxicity. Silymarin (50mg/kg) was given as reference standard drug. This dose dependant study was followed by histopathological studies of ccl4 and plant extract treated liver tissue.

Result: Studies of blood samples of carbon tetra chloride treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by paracetamol. It was evident from the results that after treatment with the plant extract, there was a significant reduction in the levels of serum biochemical parameters serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALKP) and total bilirubin (TBL) induced by carbon tetrachloride hepatotoxicity. The histopathological observations also showed that plant extract treated liver sections against carbon tetrachloride induced hepatotoxicity revealed the well preserved cellular architecture of the liver tissue.

Conclusion: The present study indicates the potential hepatoprotective activity of Aglaia eleagnoidea stem and its bioactive phytoconstituents predominantly stigmasterol, β-sitosterol, Daucosterol and unidentified polyphenols. These compounds probably by suppressing the activity of reactive oxygen species which cause cellular damage, there by offering protection to the targeted tissue against the hepatotoxicity induced by carbon tetra chloride in albino rats.

Keywords: Aglaia eleagnoidea, Carbon tetrachloride, Hepatotoxicity, Hepatoprotective activity, antioxidants, Histopathological study, phytoconstituents

1. Introduction
   A large number of populations suffer from hepatic diseases of unknown origin. The development of antihepatotoxic drugs being a major thrust area has drawn the attention of workers in the field of natural product research. Herbal drugs play a major role in the treatment of hepatic disorders. In the absence of reliable liver protective drugs in modern medicine, in India, a number of medical plants and their formulations are used to cure hepatic disorders in traditional systems of medicine1. Only a small portion of the hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their efficacy.

Genus Aglaia is represented by more than 100 species belonging to the Mahogany family (Meliaceae). Certain species of Aglaia
such as A. Lawii. *A. eleagnoidea* is a traditional medicinal plant having been used for the treatment of bacterial infection, liver, tumour diseases and headaches. Some species of *Aglaia* were listed in the data base on ethnomedicinal plants of Western Ghats, India by Indian Council of Medicinal Research. The fruit is acidic in taste and edible. It is cooling and astringent and employed in inflammations and febrile complaints. The seeds are said to be useful in painful micturitions. The leaf juice of *A. roxburghiana* Miq., is used to treat warts and cancerous ulcers, and contain bisamide alkaloids, odorin and 5’-epi-odorine. These alkaloids are found to inhibit the growth of the vinblastin-resistant KB cells by enhancing the anticancer activity of vinblastine.

Species of *Aglaia* were widely studied for their insecticidal, anti-cancer properties and isolation of several bisamide alkaloids, triterpenoid lingnanes, benzofuran derivative and cycloartenol derivatives. *Aglaia roxburghiana* var. *courtallensis* was identified as possessing antitumor and antiviral activities. Anti-inflammatory activity of *Aglaia roxburghiana* var. *beddomei* extract and triterpenes roxburghiadiol A and B was carried out. The bioefficacy of aglaroxin B and aglaroxin C from *Aglaia eleagnoidea* (syn. *A.roxburghiana*) was assessed using the gram pod borer, *Helicoverpa armigera* (Hübner) and Asian armyworm, *Spodoptera litura*. The compounds exhibited strong growth inhibition in diet bioassay. Both aglaroxin B and aglaroxin C were toxic to various stadia. Nutritional analysis revealed the antifeedant properties of the compounds.

With the back ground of its ability to cure hepatic tumors, the present study was aimed at evaluating hepatoprotective activity of *Aglaia eleagnoidea*.

### 2. Materials and Methods

#### 2.1 Plant material

The stem of *Aglaia eleagnoidea* were collected from the forests of Eastern Ghats of Paderu, Visakhapatnam District, Andhra Pradesh, India in the month of August 2010 and its identity was confirmed by the department of Botany, Andhra University, Visakhapatnam. The herbarium specimen of the plant was deposited in the department of Botany, Andhra University with the Voucher no: VPJ-KMK/DOB/AE1410.

#### 2.2 Preparation of extracts

The shade dried stems of about 500g were ground to coarse powder, and was then extracted with 80% methanol using Soxhlet apparatus till exhaustion for about 48 hours and further concentrated under vacuum to get the residue. The percentage yield was found to be 8% (wt/wt). The qualitative phytochemical screening of hydro-alcoholic extract of *Aglaia eleagnoidea* stem revealed the presence of steroids, triterpenoids, flavonoids, glycoside, saponins and tannins.

#### 2.3 Experimental animals

Healthy Wistar-Albino rats of either sex, weighing 150-250g, obtained from Mahavir Enterprises, Hyderabad were used in the study. The animals were maintained at standard housing conditions (room temperature 23-25°C, relative humidity 55%). A controlled 12 h light/12 h dark cycle was maintained. The animals were fed with standard pellet diet and water *ad libitum*. All procedures were performed according to the Institutional Animal Ethics Committee’s approval.

#### 2.4 Toxicity studies

Acute toxicity study was performed for hydro-alcoholic extract according to the acute toxic classic methods as per OECD, 1986. albino rats The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose of 400mg/kg and observed for 14 days. Mortality of two out of three animals, in the given dose administered was assigned as toxic dose and in case of no mortality; the procedure was repeated with further higher doses. From the studies it was found that the hydro-alcoholic extract of *Aglaia eleagnoidea* stem was non-toxic below 1500mg/kg. Therefore the doses of the extract selected for testing and evaluation of hepatoprotective activity were 150, 300 and 600 mg/kg, body weight.

#### 2.5 CCL4-induced hepatotoxicity

The Wistar albino rats of either sex were divided into six groups of six animals (n=6) each. Group-I served as normal control and received vehicle (Sodium CMC) + olive oil suspension in the ratio of 1:1 (1 ml/kg. p. o) once daily for 3 days. Group – II served as hepatotoxic treated group (negative control), received vehicle on 1st and 2nd day and CCL4 (1ml/kg s.c. suspended in olive oil in the ratio of 1:1) on the third day. Group-III, (positive control) received Silymarin (50mg/kg, i. p. suspended in sodium CMC) once daily for 3 days and CCL4 (1ml/kg s.c.) on the third day. The three test groups (IV – VI) received oral administration of 80% hydro-alcoholic extract of *Aglaia eleagnoidea* stem at
doses of 150, 300 and 600mg/kg p.o in sodium CMC suspension once daily for 3 days followed by CCl₄ (1ml/kg s.c) on the third day as per Suresh Kumar and Mishra with slight modification. 24 h after CCl₄ treatment, blood was collected from all the groups, and allowed to clot for the separation of serum. The blood was centrifuged at 3000rpm for 15 min to separate the serum. The serum was used for estimation of biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALKP) and total bilirubin (TBL). All the determinations were carried out using standard SPAM diagnostic kits by an Autoanalyser.

2.6 Histopathological studies

One animal from each of the treated group showing maximum activity as indicated by improved biochemical parameters was used for this purpose. The rats were sacrificed by cervical dislocation and the abdomen was cut open to remove the livers. The liver samples of gross lesion were excised, washed thoroughly with saline water and the weight and volume of the wet liver was estimated. The livers were then fixed in 10% neutral buffered formalin solution for 24 hours and embedded in paraffin using conventional methods. Later they were cut into 5μm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene (DPX). The sections were examined under light microscope for histopathological changes in liver architecture and their photomicrographs were taken using Nickon binocular microscope with image analyser.

2.7 Statistical analysis

The mean values ±S.E.M. were calculated for each parameter and for determining the significant inter-group differences for each parameter and the data was analysed by ANOVA. Individual comparisons of the group mean values were done as per Dunnet’s, 1964.

2.8 Phytochemical evaluation

Column chromatography was done by standard procedure using silica gel (Qualigens), 60-120 mesh. The column was eluted with n-hexane, n-hexane: ethyl acetate, ethyl acetate and ethyl acetate: methanol by step gradient. The bioactivity guided fractionation yielded five compounds, lenoleic acid stigmasterol and sitosterol from hexane - ethyl acetate fraction, Daucosterol from hexane - ethyl acetate fraction. The structures of the three compounds were elucidated with the help of 1D NMR i.e., ¹H NMR, ¹³C NMR, IR and Mass spectra.

3. Results

Acute toxicity studies were performed for the extract according to the toxic classic methods as per guidelines - 423 prescribed by OECD. The hydro-alcoholic extract did not cause any mortality up to 1500mg/kg and hence considered as safe.

The results of serum biochemical parameter levels have been presented as mean ±SEM. The percentage decrease or increase was calculated by considering the enzyme level difference between hepatotoxic treated and control rats as 100% level of reduction (Tables 1). The comparative efficacy of the extract tested for its hepatoprotective activity, the relationship between dose and percentage reduction in each case was depicted in the form of a bar diagram (Figure: 1).

Carbon tetrachloride (1ml/kg s.c.) intoxication in normal rats produced significantly elevated levels of serum biochemical parameters SGOT (86.07±1.83 to 550.48±12.33 IU/L), SGPT (46.00±0.35 to 456.18 ± 8.38 IU/L), ALP (160.08 ± 1.60 to 375.66 ± 5.46 IU/L) and TB (0.31 ± 0.06 to 1.86 ± 0.14 mg/dl). The liver showed significant increase in its weight (Liv.Wt 9 gms), and volume (Liv. Vol -10 cc) indicating acute hepatocellular damage, severe fatty changes and biliary obstruction. The percentage reduction of various serum biochemical parameters in case of standard drug silymarin (50 mg/kg, i.p.) in CCl₄ intoxicated rats revealed a significant reduction (p<0.01) in the levels of SGOT (91.27%), SGPT (88.92%), ALP(83.80%) and TB(96.77%), Liver Weight(93.75%) and Liver Volume.(91.27%), SGPT (88.92%), ALP(83.80%) and TB(96.77%), Liver Weight.(93.75%) and Liver Volume.(100%) respectively (Table 1). Treatment with hydro-alcoholic extract of Aglaia elaegnoidea stem (150, 300 and 600 mg/kg p.o doses) on CCl₄ intoxicated rats revealed a significant dose dependant reduction (p<0.01) in the levels of SGOT, SGPT, ALP, TB, Liver Weight as well as Liver Volume respectively (Table 1 ; Fig: 1, 2 & 3), compared to that of CCl₄ intoxicated group.

Histopathological studies of liver section of the control group showed normal cellular architecture with distinct hepatocytes showing well preserved cytoplasm, prominent nucleus, nucleolus, sinusoidal spaces and central vein. There was no sign of inflammation, fatty change or necrosis in these animals (Fig: 3A). The liver section of CCl₄ intoxicated group showed complete disarrangement of normal hepatic cells...
with intense centrilobular necrosis, vacuolization, neutrophile infiltration, fatty changes and sinusoidal hemorrhages and dilatation (Fig: 3B). The liver sections of silymarin treated rats at 50mg/kg p.o dose showed apparently normal liver lobule with no sign of necrosis in centrilobular area and portal vein but only a few inflammatory cells in the centrilobular area. They showed a normal hepatic architecture with normal hepatocytes, sinusoidal spaces, less vacuole formation, absence of necrosis and less visible changes as compared to control (Fig: 3C).

When compared to those of CCl4 intoxicated group, groups treated with methanolic extract of Aglaia elaegnoidea stem (150mg/kg p.o, 300mg/kg p.o and 600mg/kg p.o) in CCl4 intoxicated rats revealed a significant reduction (p<0.01) in the levels of SGOT (64.79%, 79.19%, 87.93%), SGPT (54.12%, 68.08%, 73.60%), ALP (55.67%, 66.43%, 69.68%), TB (71.51%, 79.11%, 88.60%) respectively, and the effect was dose dependent at the three dose levels.

Out of the groups treated with hexane, ethyl acetate and methanol soluble fractions at the dose of 300mg/kg p.o, ethyl acetate and methanol soluble fractions have shown a significant reduction (p<0.01) in serum biochemical parameters SGOT (65.78%, 76.42%), SGPT (69.23%, 82.35%), ALP (71.51%, 79.11%, 88.60%) respectively. The methanol soluble fraction was found to be more potent than ethyl acetate fraction because of its higher percentage reduction with elevated levels of biochemical parameters, even though both have showed a significant hepatoprotective effect at same p value (p<0.01). Whereas hexane soluble fraction showed no or less activity and exhibited poor reduction (p<0.01) in serum biochemical parameters SGOT (20.32%), SGPT (32.84%), ALP (19.32%), TB (65.80%).

The histopathological examination of rats administered with hydro-alcoholic extract of Aglaia elaegnoidea stem (150mg/kg p.o) showed necrosis, fatty changes and inflammatory neutrophils and with higher dose of 300mg/kg p.o and 600mg/kg p.o showed reduced necrosis, fatty changes and vacuolization in a dose dependant manner.

When compared to CCl4 treated group, the histopathological examination of rats treated with hydro-alcoholic extract (150 mg/kg p.o) showed absence of necrosis, fatty changes, vacuolization and sinusoidal dilatation and with the higher dose 300mg/kg p.o and 600mg/kg p.o showed less disarrangement and degeneration of hepatocytes and mild fatty changes indicating protective activity of extract. But the histopathological examination of rats treated with hexane fraction (300mg/kg p.o) showed severe necrosis, high fatty changes, intense vacuolization and neutrophils infiltration indicating no or poor hepatoprotective activity. Whereas ethyl acetate and methanol soluble fractions (300mg/kg p.o each) showed mild necrosis, fatty changes, vacuolization and neutrophils infiltration. The group treated with methanol fraction showed less disarrangement and degeneration of hepatocytes with mild fatty changes than ethyl acetate fraction.

Among the isolated compounds tested, though both of them showed significant protective effect, the unidentified polyphenolic compound (88.76%, 71.96%, 75.81% & 88.38%) showed better activity almost similar to the standard silymarin compared to Daucosterol (Table: 1; fig. 2). The histopathological examination of rats treated with isolated compounds showed less disarrangement and degeneration of hepatocytes with mild fatty changes and looked almost normal indicating that the compounds offered significant amount of hepatoprotection against CCl4 induced hepatotoxicity.

The carbon tetrachloride mechanism begins with the trichloromethyl radical (CCl3) by the action of the mixed function of cytochrome P450 oxygenase system. This free radical, which is initially formed as non-reactive, reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical. Both these radicals are capable of binding with proteins / lipids or abstracting a hydrogen atom from an unsaturated lipid, thus initiating lipid peroxidation. This process of lipid peroxidation can significantly damage hepatic plasma membranes. The increased levels of SGOT, SGPT, ALP and TB are conventional indicators of liver injury. The ability of hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin is the index of its protective effect. Hepatocellular necrosis leads to elevation of the serum marker enzymes, which are released from the liver in to the blood serum and increase in the levels of SGOT, SGPT, ALP and TB upon exposure to CCl4, indicating a considerable hepatocellular injury.

4. Discussion

It was evident from the results that after treatment with the plant extract, there was a...
significant reduction in the levels of serum biochemical parameters induced by CCl₄ hepatotoxicity. The histopathological observations also showed that plant extract treated liver sections against CCl₄ induced hepatotoxicity revealed the absence of necrosis and well preserved cellular architecture. Silymarin is used as a standard hepatoprotective drug. In the present investigation 50mg/kg of silymarin showed significant difference compared to the extracts. Boigk et al and Bhadauria et al 20,21 observed similar cases of effectiveness of silymarin at the same dose.

The effects of CCl₄ are generally observed after 24hrs of its administration. Hence withdrawal of the blood for biochemical parameters should be carried out only after 24hrs of CCl₄ intoxication. From table- 2 it is evident that the hydro-alcoholic extract was able to reduce all the elevated biochemical parameters and thereby reducing the hepatotoxic intoxication as well as the levels of total proteins and albumin. The reduction is attributed to the damage which is generally localized in the endoplasmic reticulum. This results in the loss of P₄₅₀ its functional failure with a decrease in protein synthesis and accumulation of triglycerides. Intoxication with CCl₄ also resulted in inhibition of synthesis of the bile acids from cholesterol which is synthesized in live or derived from plasma lipids, leading to increase in cholesterol levels. Suppression of cholesterol levels suggests inhibition of the synthesis of bile acids from cholesterol is reversed by the extract. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by CCl₄. Reduction of ALKP levels with concurrent depletion of raise in bilirubin level suggests the stability of the biliary function during injury with CCl₄. The raise in protein and albumin levels suggests the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by the hydro-alcoholic extract is similar to silymarin treatment.

Histological examination of the liver sections revealed that the normal liver architecture was disturbed by hepatotoxic intoxication. In the sections obtained from the rats treated with methanolic extract and intoxicated with hepatotoxic, the normal cellular architecture was retained compared to that of silymarin, thereby confirming the protective effect of the extract. The decrease in the necrosed area demonstrated by the extract as well as decrease in the infiltration of the inflammatory cells in the liver lobules is indicative of therapeutic efficacy of the plant extracts. This is an indication that the cellular damage caused by hepatotoxin (CCl₄) was either prevented or repaired by the bioactive phytoconstituents of the plant, indicating their protective effect.

The present study indicates the potential hepatoprotective activity of Aglaia eleagnoida stem and its bioactive phytoconstituents predominantly stigmasterol, β-sitosterol, Daucosterol and unidentified polyphenols. These compounds probably by suppressing the activity of reactive oxygen species which cause cellular damage, there by offering protection to the targeted tissue.

The reduction in levels of SGOT and SGPT by the extracts is an indication of stabilization of plasma membrane as well as a repair of hepatic tissue damage caused by CCl₄. This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes 22. The alkaline phosphate is the prototype of these enzymes that reflects the pathological alteration in biliary flow 23. The CCl₄ induced elevation of this enzymatic activity in the serum is in line with high level of serum bilirubin content. The hydro-alcoholic extract induced suppression of the increased SALP activity with the concurrent depletion of raised bilirubin suggests the possibility of the extracts to have ability to stabilize biliary dysfunction in rat liver during hepatic injury by CCl₄. Thus, administration of hydro-alcoholic extract of Aglaia eleagnoida stem revealed hepatoprotective activity against the toxic effect of CCl₄, which was also supported by histopathological studies. The preliminary phytochemical analysis of the extract has shown the presence of steroids, triterpenoids, flavonoids, glycoside, saponins and tannins, which have been known for their antioxidant and hepatoprotective activities 24.

The present study indicates the potential hepatoprotective activity of Aglaia eleagnoida stem and its bioactive phytoconstituents predominantly stigmasterol, β-sitosterol, Daucosterol and unidentified polyphenols. These compounds probably by suppressing the activity of reactive oxygen species which cause cellular damage, there by offering protection to the targeted tissue.
Table 1: Effect of Hydro-alcoholic extract, hexane, ethyl acetate, methanol fractions and isolated compounds of Aglaia eleagnoidea stem against CCl₄ induced hepatotoxicity in albino rats

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment group</th>
<th>Serum biochemical parameters</th>
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<tr>
<td></td>
<td></td>
<td>SGOT (IU/L)</td>
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<tr>
<td>1</td>
<td>Control (olive oil 1ml/kg p.o)</td>
<td>86.07± 1.83</td>
</tr>
<tr>
<td>2</td>
<td>Toxic CCl₄ (1ml/kg i.p)</td>
<td>550.48± 12.33</td>
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<tr>
<td>3</td>
<td>Standard Silymarin (50 mg/kg p.o)</td>
<td>126.57±1.18</td>
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<tr>
<td>4</td>
<td>AEHAE (150 mg/kg p.o)</td>
<td>249.57±1.36</td>
</tr>
<tr>
<td>5</td>
<td>AEHAE (300 mg/kg p.o)</td>
<td>182.70±2.15</td>
</tr>
<tr>
<td>6</td>
<td>AEHAE (600 mg/kg p.o)</td>
<td>142.08±1.64</td>
</tr>
<tr>
<td>7</td>
<td>AEHF (300 mg/kg p.o)</td>
<td>456.08±1.22</td>
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<tr>
<td>8</td>
<td>AEEAF (300 mg/kg p.o)</td>
<td>215.64±0.82</td>
</tr>
<tr>
<td>9</td>
<td>AEMF (300 mg/kg p.o)</td>
<td>184.93±1.66</td>
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<tr>
<td>10</td>
<td>Compound–AEHE-16 (30 mg/kg)</td>
<td>212.40±0.77</td>
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<tr>
<td>11</td>
<td>Compound–AEM 10 (50 mg/kg p.o)</td>
<td>138.24±0.63</td>
</tr>
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All values are given as mean ± SEM, M=6 P<0.01 when compared to toxic (CCl₄ treated) group

*Percentage reduction of various serum biochemical parameters due to treatment with Methnolic extract of Aglaia eleagnoidea stem against CCl₄ induced hepatotoxicity in albino rats

AEHAE: Aglaia eleagnoidea hydro-alcoholic extract
AEHF: Aglaia eleagnoidea Hexane fraction
AEEAF: Aglaia eleagnoidea Ethyl acetate fraction
AEMF: Aglaia eleagnoidea Methanolic fraction
AEHE-16: Daucosterol
AEM-10: Unidentified polyphenol

Figure 1: Hepatoprotective activity of methanolic extract (80%) of Aglaia eleagnoidea stem against CCl₄ induced hepatotoxicity in albino rats
Figure 2: Hepatoprotective activity of Hexane, ethyl acetate, methanol soluble fractions and isolated compounds of *Aglaia elaegnoidea* stem against CCl₄ induced hepatotoxicity in albino rats

Fig. 3: Hepatoprotective activity of hexane, ethyl acetate and methanol soluble fractions of

*Aglaia elaegnoidea* stem

A) Normal control- 1ml olive oil/kg b.w  D) AEHAE – 300mg/kg b.w

B) CCl₄ treated-1ml/kg b.w  E) AEEAF – 300mg/kg b.w

C) Silymarin treated-50mg/kg b.w  F) AEMF – 300mg/kg b.w
5. Conclusion

In view of the reported use of Aglaia elaegnoidea for treating liver diseases in folklore medicine, an attempt has been made to test the hepatoprotective activity of the plant. The study is first of its kind. These active constituents singly or in synergy might be responsible for the observed hepatoprotective activity. Histopathological studies of the liver sections also supported the hepatoprotective activity. Histopathological studies of the liver should be due to its antioxidant activity, induced by the above mentioned compounds in the extract.

Acknowledgements

The author acknowledges University Grants Commission (UGC), New Delhi, India for allowing carrying out this work under Faculty Development Programme (FDP).

References