The study was designed to evaluate the hepatoprotective activity of 70% ethanolic extract of leaves of 
Albizzia lebbeck (70% EELAL) in experimental liver damage induced by thioacetamide (100 mg/kg, s.c) in 
albino rats. Hydroalcoholic extract of leaves of Albizia lebbeck was prepared and subjected to acute toxicity 
study as per CPCSEA guideline no. 420. The two doses i.e. 100 mg/kg and 200mg/kg were selected for 
 further study. The degree of hepatoprotection was determined by estimating levels of tissue GSH, lipid 
peroxidation, physical parameters (wet liver weight and liver volume) and biochemical markers like SGPT, 
SGOT, Bilirubin (Total and Direct) and ALP. The extract at the dose of 100mg/kg and 200 mg/kg produced 
significant protective effect as indicated by decreased the activity of serum enzymes, bilirubin, tissue lipid 
peroxidation and physical parameters and increased levels of tissue GSH in a dose dependent manner. The 
effects of extract were comparable to that of standard drug silymarin. Histopathological observation also 
confirms these findings. These results suggest that 70% EELAL possess potential hepatoprotective activity 
against thioacetamide induced hepatic damage in rats.

KEY WORDS: Albizia lebbeck, Hepatoprotection, thioacetamide

1. INTRODUCTION

Liver diseases are worldwide problem. Management of liver disease has become a major concern in 
medical science. No reliable drugs are available in allopathic medical practice. So, there is phenomenal 
increase in the search for the herbal hepatoprotectants throughout the world. Keeping with the tone of trends, 
we in our laboratory also have undertaken research to search for herbal hepatoprotectants that are available at 
a hand enhance stretch. As the part of our search, we normally undertake field survey and contact with the 
native practitioner.

In one of our survey, we found big tree and botanically named as Albizziabebbeck. The literature 
survey of the plant revealed that traditionally this plant is used for snake bite, leucoderma, opthalmia etc. 
(Kritikar KR and Basu BD, 1998) (Asolkar LV and Kakkar KK, 1965). In modern literature, the reports are 
indicating that plants possess tannins, flavonoids and saponins etc. (Arvind K, 2007). In addition, there are 
reports that the plant possesses anti-inflammatory (Pramanik KC, 2005), nootropic (Chintawar SD, 2002) 
(Une HD, 2001) anxiolytic (Une HD, 2001), anticonvulsant (Kasture VS, 1996) (Kasture VS, 2000), 
antifertility (Gupta RS, 2004) and anti diarrheal activity (Besra SE, 2002). However, there are no reports 
either in the traditional and modern literature regarding hepatoprotective property of the plant, but there are 
reports that flavonoids possess hepatoprotective role (Tiwari KA, 2001). Since, the leaves of the plant 
Albizzia lebbeck are known to contain flavonoids (Arvind K, 2007). Hence, the leaves of the plant were 
selected to screen for hepatoprotective effect against experimentally induced liver damage in rats.

Thioacetamide was originally used as fungicide to protect the decay of organs (Childs JFL, 1946). It 
is recognized as a potent hepatotoxin and carcinogen in rats (Fitzhugh OG and Nelson AA, 1948). Therefore, 
in the present study this model of hepatitis in rats was used to assess the hepatoprotective effect of 70% 
ethanolic extract of leaves of Albizia lebbeck (70% EELAL).

2. MATERIALS AND METHODS

2.1. Plant Material: The leaves of plant Albizia lebbeck were collected from fields of Anand, Gujarat in the 
month of December 2010. It was identified and authenticated by Prof. G.C. Jadeja, Dept of Agricultural 
Botany, Anand Agricultural University. The leaves were shade dried at room temperature and pulverized. 
The 70% ethanolic extract (12.82%) was prepared by using 70% ethanol in a soxhlet apparatus after de-
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fatting with petroleum ether. Preliminary phytochemical investigation showed the presence of flavonoids, tannin, saponins glycoside in 70% EELAL. So 70% EELAL was selected for the present activity.

2.2. Animal: Wistar albino rats (150-200g) and mice (18-25 g) of either sex were used for the study, procured from National Toxicology centre, Pune, Maharashtra. After one week of acclimatization the animals were used for further experiments. Approval from the institutional animal Ethical committee for usage of animal in the experiment was obtained as per the Indian CPCSEA guidelines.

2.3. Acute Toxicity studies: The acute toxicity was determined on albino mice by fixed dose method of OECD Guide line no 420 given by CPCSEA. Groups of 6 mice were administered test drug by oral route in the range of 200-300 mg/kg and mortality was observed for 24 hr.

2.4. Thioacetamide induced hepatotoxicity: Healthy albino rats were divided into 5 groups of 6 animals each. Group-I and Group II, which served as negative and positive control groups, received normal saline (1ml/kg) for 9 days. Group III received 100 mg / kg silymarin (standard drug) orally for 9 days. Group IV and Group V received daily dose of 70% EELAL (orally) for 9 days (100 mg/kg and 200 mg/kg). But on 7th day 30 minutes after administration of saline, silymarin and test extract; animals of group II-V received 100mg/kg thioacetamide (s.c). The animals were fasted for 12 hr before administration of thioacetamide (Chattopadhyay RR, 2003) Blood samples were collected under mild ether anaesthesia and were sacrificed by cervical dislocation and liver tissue was collected after 48 hr of thioacetamide injection.

2.5. Biochemical studies: Blood was obtained from all the animals by puncturing retro-orbital plexus. Collected blood was centrifuged (2000 rpm for 10 mins) to get clear serum and was used to estimate various biochemical markers like SGPT, SGOT, ALP, total bilirubin (0.63±0.03) and direct bilirubin (2.17±0.06). Since there was death of one animal in the group treated with 300mg/kg dose and all the animals died in the group of animals treated with 2000mg/kg dose. Therefore 1000mg/kg was treated as LD_50 and 1/10th and 1/5th (i.e. 100mg/kg and 200 mg/kg) of 1000mg/kg were selected for further study.

Increased levels of liver volume (5.84±0.097), wet liver weight (5.49±0.11), SGOT (324.05±6.41), SGPT (201.06±3.46), ALP (332.03±7.358) total bilirubin (2.17±0.06) and direct bilirubin (0.63±0.03) observed in thioacetamide treated group. The treatment with 70% EELAL brought back the elevated levels of physical parameters and biochemical markers of hepatitis in a dose dependant manner [table 1].

Thioacetamide was found to reduce tissue GSH and increase the lipid peroxidation levels. Administration of 70 % EELAL significantly increased the tissue GSH levels and reversed lipid peroxidation change to near normal level [table 2].

In histopathological assessment, hepatic cells showed extensive fatty degeneration and ballooning of hepatocytes, central vein also was congested. However, treatment with 70% EELAL showed dose dependant
improvement in the liver architecture as indicated by the histopathological observations that there was mild inflammation, slight congestion and improvement of portal vein was obtained.

The reversal of wet liver weight, liver volume, levels of biochemical markers and histopathological observations reveal that the 70% EELAL possess hepatoprotective activity against thioacetamide induced hepatotoxicity in albino rats.

Table 1 - Effects of 70% ethanolic extract of *Albizzia lebbeck* leaves on physical parameters and biochemical markers in thioacetamide induced hepatotoxicity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Physical parameters</th>
<th>Biochemical parameters</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver Volume (ml/100g)</td>
<td>Wet liver Weight (gm/100g)</td>
<td>SGOT IU/L</td>
</tr>
<tr>
<td>Negative control (1ml distilled water P.O.)</td>
<td>3.91 ± 0.0860</td>
<td>3.48 ± 0.1078</td>
<td>140.05 ± 2.62</td>
</tr>
<tr>
<td>Thioacetamide 100mg/Kg s.c. (Positive control)</td>
<td>5.84 ± 0.09799</td>
<td>5.49 ± 0.1101</td>
<td>324.05 ± 6.41</td>
</tr>
<tr>
<td>Thioacetamide 100mg/kg s.c. + Silymarin 100mg/kg p.o.</td>
<td>4.07 ± 0.02***</td>
<td>3.65 ± 0.04***</td>
<td>162.15 ± 5.40***</td>
</tr>
<tr>
<td>Thioacetamide 100 mg/kg s.c. + 70% EELAL 100mg/kg p.o.</td>
<td>4.73 ± 0.07***</td>
<td>4.27 ± 0.04***</td>
<td>215.53 ± 4.33***</td>
</tr>
<tr>
<td>Thioacetamide 100 mg/kg s.c. +70% EELAL 200mg/kg p.o.</td>
<td>4.28 ± 0.11***</td>
<td>3.86 ± 0.15***</td>
<td>200.37 ± 5.48***</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. of six rats / treatment. Significance *P<0.05, **P <0.01, and *** P<0.001, compared to thioacetamide treatment.

Table 2 - Effect of 70% EELAL on tissue GSH and lipid per oxidation levels in thioacetamide induced hepatotoxicity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH levels Absorbance Mean ± SEM (% Increase)</th>
<th>Lipid per oxidation Absorbance Mean ± SEM (% Inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (1ml dist. Water p.o.)</td>
<td>0.950 ± 0.012</td>
<td>0.203 ± 0.013</td>
</tr>
<tr>
<td>Thioacetamide (positive control) (100ml/kg s.c + 100 mg/kg s.c.)</td>
<td>0.566± 0.015</td>
<td>0.639 ± 0.029</td>
</tr>
<tr>
<td>Thioacetamide+ Silymarin (100ml/kg s.c +100 mg/kg, p.o.)</td>
<td>0.924 ± 0.048*** (63.25%)</td>
<td>0.224 ± 0.019*** (64.94%)</td>
</tr>
<tr>
<td>Thioacetamide+70% EELAL (100mg/kg s.c +100mg/kg p.o.)</td>
<td>0.759 ± 0.018*** (34.09%)</td>
<td>0.441 ± 0.017*** (30.98%)</td>
</tr>
<tr>
<td>Thioacetamide + 70% EELAL (100mg/kg s.c +200mg/kg p.o.)</td>
<td>0.875 ± 0.017*** (54.59%)</td>
<td>0.336 ± 0.020*** (47.41%)</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. of six rats / treatment. Significance *** P<0.001, compared to thioacetamide treatment.

DISCUSSION

In the assessment of liver damage by thioacetamide (TAA) the determination of enzyme level such as SGPT, SGOT, ALP, direct and total bilirubin are largely used. Our result revealed that all the biomarker enzymes of liver toxicity were elevated by thioacetamide treatment and treatment with test extract brought back the elevated levels to the near normal levels. The effect of test extract was comparable to that of standard silymarin 100 mg/kg.

Chronic thioacetamide exposure produces cirrhosis in rat (Chieli E and Malvadi G, 1985). Thioacetamide is metabolized by liver CYP 450 2E1 to thioacetamide-5-oxide, which is a direct hepatotoxin
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(Kumar G, 2004). The thioacetamide-5-oxide is responsible for the change in cell permeability, increased intracellular concentration of calcium, increases in nuclear volume and enlargement of nucleoli and also inhibits mitochondrial activity that leads to cell death (Neal RA and Halpert J, 1982) (Ambrose AM, 1950).

In vivo administration of thioacetamide to rodents results in cell death in centrilobular zones both by apoptosis and necrosis. The cellular changes induced by apoptosis occur after a cascade of cell signaling and caspase mediated events and are triggered by two major pathways: extrinsic and intrinsic pathway. The extrinsic pathway implicates death ligands such as Fas ligand, TNFα, TRAIL and their receptors. The intrinsic pathway includes apoptotic stimuli induced by cytotoxic drugs or oxidative stress, which target mitochondria (Yang J, 2004) (Ahmad A, 2002). This pathway involves the release of cytochrome c from mitochondria to the cytosol, which induces apoptosome complex formation and results in protease procaspase-9 activation and subsequent activation of procaspase-3 through proteolytic cleavage visualized by the decrease of perform level and appearance of cleavage products. Both the pathway leads to caspase-3 activation and cleavage of limited set of essential cellular protein, leading to cell dismantlement. In the liver the apoptosis induced by thioacetamide could result from a combination of both pathways: intrinsic apoptosis pathway by generation of oxidative stress and the extrinsic apoptosis pathway by activation of kupffer cells that can secrete TNFα. In addition to this there are reports that the long term administration of TAA can cause the development of cirrhosis associated with an increased extent of lipid peroxidation (Ahmad A, 2002) (Rang HP, 2003). And hence lipid peroxidation leads to tissue damage and failure of antioxidant defence mechanism. GSH widely distributed in cells and present in high concentration in liver for long period (Ahmad A, 2002). It protects the cell against free radical, peroxides and other toxins. So the depletion of GSH level in tissue may lead to tissue damage and disorder. In this study TAA treatment depleted tissue GSH. But, treatment with test extract prevented the depletion of tissue GSH and decreased the extent of lipid peroxidation. These results of lipid peroxidation and GSH suggest that 70% EELAL is having the free radical scavenging and anti oxidant property. Exposure to hepatotoxins like TAA may over power the inbuilt antioxidant systems and cause the hepatotoxicity. Therefore anti-oxidants may protect the organs. Hence, the hepatoprotection due to 70% EELAL may be assigned to the presence of anti-oxidant principles.

In conclusion, the present study demonstrates that 70% EELAL possesses hepatoprotective activity. In addition the hepatoprotective property may be attributed to the antioxidant principles of plant, namely tannins and flavonoids. Further investigation is on to isolate, characterize and screen the active principles that possess hepatoprotective property.

Fig.1. Normal control rats (Gr. I)

Fig.2. Thioacetamide treated rats (Gr. II) showing extensive fatty degeneration and ballooning of hepatocytes, central vein was congested;
Fig. 3. Rats (Gr. III) treated with thioacetamide + 100mg/kg std-silymarin showing central vein, portal vein and kupffer cells were in normal condition.

Fig. 4. Rats (Gr. IV) treated with thioacetamide + 100mg/kg 70% EELAL showing improvement in portal vein with mild inflammation and congestion

Fig. 5. Rats (Gr. V) treated with thioacetamide + 200mg/kg 70% EELAL showing repairment of portal vein

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