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HEPATOPROTECTIVE AND ANTIOXIDANT EFFECTS OF *ALBIZZIA LEBBECK* AGAINST THIOACETAMIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The study was designed to evaluate the hepatoprotective activity of 70% ethanolic extract of leaves of *Albizzia lebeck* (70% EELAL) in experimental liver damage induced by thioacetamide (100 mg/kg, s.c) in albino rats. Hydroalcoholic extract of leaves of *Albizzia lebeck* 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: *Albizzia lebeck*, 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 *Albizzialebeck*. The literature survey of the plant revealed that traditionally this plant is used for snake bite, leucoderma, ophthalmia 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 lebeck* 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 damages 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 *Albizzia lebeck* (70% EELAL).

2. MATERIALS AND METHODS

2.1. Plant Material: The leaves of plant *Albizzia lebeck* 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-

Journal of Chemical and Pharmaceutical sciences

fattening 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 (Bradley DW, 2003), SGOT (Rej R, 1973), ALP (McComb RB and Bowers GN, 1972), Bilirubin (total and direct) (Pearlman PC and Lee RT, 1974).

2.6. Histopathology: The liver was excised from the animals and washed with the normal saline. The materials were stored in 10% buffered neutral formalin and then subjected to histopathological examination.

2.7. In vivo tissue GSH estimation: Tissue Glutathione measurements were performed using a modification of Ellamn procedure (Aykae G, 1985). Liver tissue samples were homogenized in ice cold trichloroacetic acid (1gm tissue in 10 ml 10% TCA) in an ultra trux tissue homogenizer. The mixture was centrifuged at 3000 rpm for 10 mins. Then 0.5 ml of supernatant was added to 2ml of (0.3M) disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4mg/ml in 1% sodium acetate) was added and absorbance was taken at 412 nm.

2.8. In vivo lipid peroxidation estimation: The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation (John BA and Steven AD, 1978). Combine 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-2.0 μ mol of lipid phosphate) with 2.0 ml of TCA-TBA-HCL and mixed thoroughly. Solution was heated for 15 mins and cooled. Then precipitate was removed by centrifugation at 1000 rpm for 10 mins and absorbance of sample was determined at 535 nm against a blank that contain all the reagents minus lipid.

2.9. Statistical analysis: Results were expressed as mean \pm SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Turkey-Kramer multiple comparisons test. P value less than 0.05 was considered to be statistically significant (p<0.05).

3. RESULTS

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 LD₅₀ 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 \pm 0.097), wet liver weight (5.49 \pm 0.11), SGOT (324.05 \pm 6.41), SGPT (201.06 \pm 3.46), ALP (332.03 \pm 7.358) total bilirubin (2.17 \pm 0.06) and direct bilirubin (0.63 \pm 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

Journal of Chemical and Pharmaceutical sciences

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 lebeck* leaves on physical parameters and biochemical markers in thioacetamide induced hepatotoxicity

Treatment	Physical parameters		Biochemical parameters Mean \pm SEM				
	Liver Volume (ml/100g)	Wet liver Weight (gm/100g)	SGOT IU/L	SGPT IU/L	ALP IU/L	Total Bili-rubin mg/dl	Direct Bili-rubin mg/dl
Negative control (1ml distilled water P.O.)	3.91 \pm 0.0860	3.48 \pm 0.1078	140.05 \pm 2.62	62.32 \pm 4.47	158.37 \pm 3.70	0.90 \pm 0.01	0.24 \pm 0.02
Thioacetamide 100mg/Kg s.c. (Positive control)	5.84 \pm 0.09799	5.49 \pm 0.1101	324.05 \pm 6.41	201.06 \pm 3.46	332.03 \pm 7.358	2.17 \pm 0.06	0.63 \pm 0.03
Thioacetamide 100mg/kg s.c. + Silymarin 100mg/kg p.o.	4.07 \pm 0.02***	3.65 \pm 0.04***	162.15 \pm 5.40***	89.98 \pm 2.78***	193.37 \pm 6.90***	0.922 \pm 0.06***	0.32 \pm 0.03***
Thioacetamide 100 mg/kg s.c. + 70% EELAL 100mg/kg p.o.	4.73 \pm 0.07***	4.27 \pm 0.07***	215.53 \pm 4.33***	133.05 \pm 4.52***	231.40 \pm 3.27***	1.53 \pm 0.041**	0.45 \pm 0.01**
Thioacetamide 100 mg/kg s.c. +70% EELAL 200mg/kg p.o.	4.28 \pm 0.11***	3.86 \pm 0.15***	200.37 \pm 5.48***	101.18 \pm 3.70***	210.34 \pm 4.20***	1.05 \pm 0.02***	0.39 \pm 0.02***

Values are the mean \pm 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 peroxidation levels in thioacetamide induced hepatotoxicity

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

Values are the mean \pm 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

Journal of Chemical and Pharmaceutical sciences

(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, TNF α , 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 TNF α . 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.

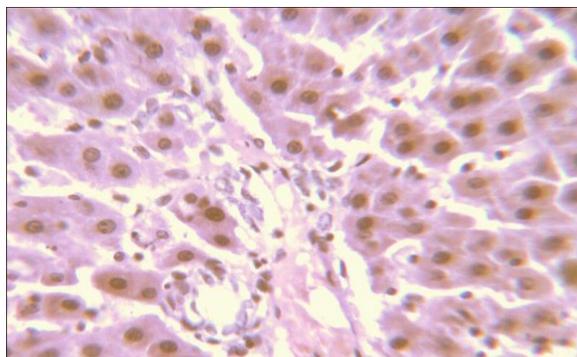


Fig.1. Normal control rats (Gr. I)

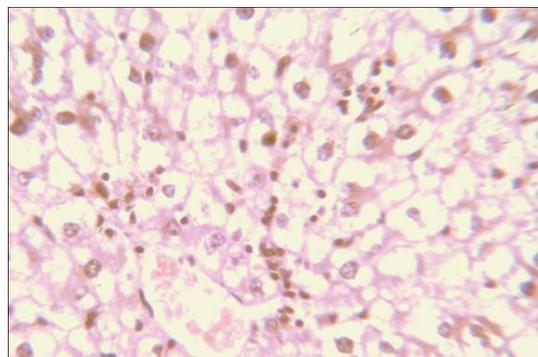


Fig.2. Thioacetamide treated rats (Gr. II) showing extensive fatty degeneration and ballooning of hepatocytes, central vein was congested;

Journal of Chemical and Pharmaceutical sciences

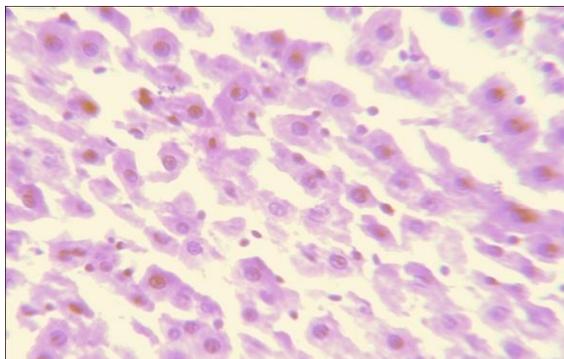


Fig.3. Rats (Gr. III) treated with thioacetamide + 100mg/kg std-silymarin showing central vein, portal vein and kupffer cells were in normal condition.

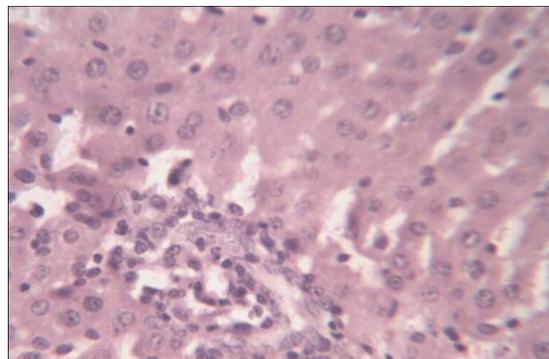


Fig.4. Rats (Gr. IV) treated with thioacetamide + 100mg/kg 70% EELAL showing improvement in portal vein with mild inflammation and congestion

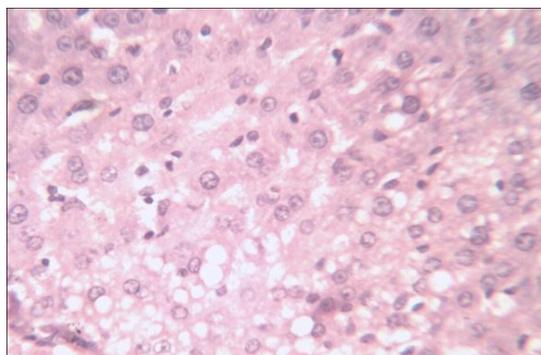


Fig. 5. Rats (Gr. V) treated with thioacetamide + 200mg/kg 70% EELAL showing repairment of portal vein

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Journal of Chemical and Pharmaceutical sciences

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