

Evaluation of Hepatoprotective activity of fruits of *Sesbania grandiflora L. Pers* against thioacetamide and ranitidine induced hepatotoxicity in rats

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Abstract

The Petroleum ether extract of *Sesbania grandiflora L. pers* fruits showed a significant dose dependent (100mg, 200mg/kg p.o.) protective effect against thioacetamide and ranitidine induced hepatotoxicity in Wistar albino rats. The degree of protection was measured by using biochemical parameters like Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Alkaline phosphatase (ALP), Total bilirubin (BRN), Total Cholesterol (TC), Total protein and Histopathological alterations. The fruit extract completely prevented the toxic effects of Thioacetamide and Ranitidine on the above serum parameters. The petroleum ether extract of *Sesbania grandiflora L. pers*fruits produced significant protection.

Key Words: Hepatoprotective, Ranitidine, *Sesbania grandiflora* and Thioacetamide,...

Introduction

The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of metabolic functions. [1] Additionally, it is the key organ of metabolism and excretion is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. The toxins absorbed from the intestinal tract gain access first to the liver resulting in a variety of liver ailments. Thus liver diseases remain one of the serious health problems. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drugs available for the treatment of liver disorders. [2] Therefore, many folk remedies from plant origin are evaluated for its possible antioxidant and hepatoprotective effects against different chemical-induced liver damage in experimental animals.

The plant genus *Sesbania* is comprised of about 60 species which are widely distributed throughout tropical and subtropical regions. Most species are annual herbs or shrubs, but a few are small trees. [3] *Sesbania grandiflora L. pers* (Fabaceae), commonly known as *sesbania* and *agathi* has been used as an important dietary nutritive source in Southeast Asian countries. (Figure 1) Ayurvedics, believing the fruits to be alexeteric, laxative and intellectually stimulating, prescribe them for anaemia, bronchitis, fever, pain, thirst, ozoena and Quartan fever. Yuani consider the tonic levels useful in biliousness, fever and nyctalopia. Indians apply the roots in rheumatism, the juice of the

leaves and flowers for headache and nasal catarrh. [4] Various parts of this plant are used in Indian traditional medicine for the treatment of a broad spectrum of illness including leprosy, gout, rheumatism and liver disorders. It also possess anti-inflammatory, analgesic, antipyretic, anxiolytic, and anticonvulsive activities.[5] Previous studies have reported that *S. grandiflora* has protective effect against oxidative damage and hypolipidemic property on cigarette smoke-exposed rats. [5] [4]

The experimental liver damage induced by long exposure and/or high doses of thioacetamide results in histological and biochemical changes that present most similarities with the human disease. [6] Fatty acids profile is also modified in rats with cirrhosis induced by thioacetamide. [7] Ranitidine is a histamine-2 (H₂) receptor antagonist available both by prescription and over-the-counter for treatment of duodenal ulcers and esophageal reflux. A small percentage of people taking ranitidine have developed idiosyncratic liver injury.[25] Hepatotoxicity associated with ranitidine is characterized by increases in serum markers of hepatocellular injury with more modest increases in indicators of cholestatic injury and, typical of idiosyncrasy, the time of onset of hepatotoxicity relative to initiation of therapy varies greatly. [26]



Figure 1. *Sesbania grandiflora L. pers.* Plant

The literature survey revealed that there are no scientific studies carried out regarding hepatoprotective activity of the fruits of *Sesbania grandiflora L. pers.* Based on the previous results on acute models of liver toxicity, in the present study has investigated for the effects of the oral dietary administration of *Sesbania grandiflora L. pers* fruits petroleum ether extract in an experimental model of cirrhosis induced by the intake of thioacetamide and ranitidine. In present investigation biochemical parameters, plasma fatty acids profile and liver histology parameters were analyzed.

Materials and methods

Plant material

Fruits of *Sesbania grandiflora L. pers* were collected from Ooty, Tamilnadu, India. The plant was authenticated by Emerald by Field Botanist Dr. Rajan S, Ooty. The fruits were dried at 40°C in hot air oven for 24 hrs to be removed any moisture present.

Preparation of plant extracts

The dried fruits of *Sesbania grandiflora L. pers* were powdered and exhaustively extracted with petroleum ether (60–80 C) in a soxhlet apparatus for 24h. Petroleum ether was evaporated and the residue was dissolved in a known volume of 10% Dimethyl sulfoxide (DMSO). The extract was stored in the refrigerator until used.

Experimental animals

The study was carried out on mixed sex of Wistar albino rats (150–200g). The rats were procured from Sri Raghavendra enterprises, Bangalore, Karnataka. Rats were fed with a standard pellet and water ad libitum. The rats were kept in standard environmental conditions (temperature 25–28 C and 12h light/12h dark cycle). The study has got the clearance from the Institutional Animal Ethical Committee (IAEC) (Approval no.DSCP/M Pharm Col/IAEC/24/09-10) of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity study

Wistar albino rats were divided into five groups of six animals each. First group served as normal control. Petroleum ether extract of *Sesbania grandiflora L. pers* fruits was administered orally to remaining groups at the dose level of 250, 500, 1000 and 2000 mg/kg p.o. body weights. All animals were observed for toxic symptoms and mortality for 72 h.

Thioacetamide-induced hepatotoxicity

Thioacetamide was obtained from Loba Chemie, Mumbai. Six rats were used per group in the study. Group I, which served as control and received the vehicle. Group II served as toxicant control and received a single dose subcutaneous injection of thioacetamide (100mg/kg) as a 2%w/v solution in distilled water. Group III received thioacetamide and Silymarin 100mg/kg. Group IV received thioacetamide and petroleum ether extracts of *Sesbania grandiflora L. pers* fruits 100mg/kg p.o., Group V received thioacetamide and petroleum ether extracts of *Sesbania grandiflora L. pers* fruits 200mg/kg p.o., This study was performed in 10 days.[8]

Group I	Vehicle (Distilled water 10ml/kg p.o.)
Group II	Toxicant (Thioacetamide (100 mg/kg s.c.)
Group III	Toxicant + Silymarin 100mg/kg p.o.
Group IV	Toxicant + Petroleum ether extracts of <i>Sesbania grandiflora L. pers</i> fruits 100mg/kg p.o.,
Group V	Toxicant + Petroleum ether extracts of <i>Sesbania grandiflora L. pers</i> fruits 200mg/kg p.o.,

Ranitidine-induced hepatotoxicity

Ranitidine was obtained from Loba Chemie, Mumbai. Six rats were used per group in the study. Group I, which served as control and received the vehicle. Group II served as toxicant control and received a oral administration of ranitidine (50 mg/kg). Group III received thioacetamide and Silymarin 100mg/kg Group IV received ranitidine and petroleum ether extract of *Sesbania grandiflora L. pers* fruits 100mg/kg p.o., Group V received ranitidine and petroleum ether extract of *Sesbania grandiflora L. pers* fruits 200mg/kg p.o., Group I–V received the following treatments were carried out 3 weeks period in rats. [9]

Group I	Vehicle (Distilled water 10ml/kg)
Group II	Toxicant (Ranitidine 50mg/kg p.o.)
Group III	Toxicant + Silymarin 100mg/kg p.o.
Group IV	Toxicant + Petroleum ether extracts of <i>Sesbania grandiflora L. pers</i> fruits 100mg/kg p.o.
Group V	Toxicant + Petroleum ether extracts of <i>Sesbania grandiflora L. pers</i> fruits 200 mg/kg p.o.

Assessment of liver functions

Twenty-four hours after the toxicant administration, the rats of each group were anaesthetized and blood was collected directly from the heart. The blood samples were allowed to clot for 20–30min. Serum was separated by centrifugation at 37 C and used for estimation of various biochemical parameters.

Assay of serum transaminases

The activities of serum glutamate oxaloacetate transaminase (SGOT) and of serum glutamate pyruvate transaminase (SGPT) were estimated by the method of Reitman and Frankel (1957). The enzyme activity was expressed as U/l. [10]

Assay of alkaline phosphatase

The activity of serum alkaline phosphatase (ALP) was estimated by the method of Kind and King. The enzyme activity was expressed as U/l. [11]

Estimation of bilirubin and total protein

Serum bilirubin (BRN) and total protein (TP) were estimated by the methods of Malloy and Evelyn and Wooten, respectively. The units were expressed as mg/dl. [12]

Estimation of total Cholesterol

The activity of total Cholesterol (TC) was estimated by the method of Zalatkis. The enzyme activity was expressed as mg/dl. [13]

Histological investigation

The liver samples fixed for 48h in 10% formal saline were dehydrated by passing successively in different mixtures of ethyl alcohol–water (50, 80, and 95%, and finally in absolute alcohol), cleared in xylene and embedded in paraffin. Sections (4–5 mm thick) were prepared and then stained with hematoxylin and eosin dye for microscopic observation of cell necrosis, fatty change, hyaline degeneration, ballooning degeneration, and infiltration of kupffer cells and lymphocytes.

Statistical analysis

Results of the biochemical estimations are reported as Mean±S.E.M. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA), Dunnet's *t* test was used for determining significance. [14]

Results

Thioacetamide-induced hepatotoxicity

Biochemical parameters SGOT, SGPT, ALP, BN and TC were significantly elevated ($P < 0.05$) in the group of rats given thioacetamide (200 mg/kg s.c.) (Table 1). The pretreatment of petroleum ether extract of *Sesbania grandiflora L. pers fruits* exhibited inhibition of thioacetamide induced increase in the levels of all the five biochemical parameters, resulting in significant restoration towards their control values. Table.1 depicts that treatment with thioacetamide reduced serum total protein which was significantly brought towards normal values by pretreatment with the petroleum ether extract of *Sesbania grandiflora L. pers fruits*. The maximum protection against thioacetamide induced hepatic aberrations was achieved with the higher dose of the extract. (Figure 2&3)

Histopathological examination

The hepatoprotective effect of the test drug was further confirmed by histopathological examination of the livers of control, thioacetamide treated and thioacetamide with Standard drug and extract treated groups. The liver samples of Thioacetamide treated rats showed fatty change and degeneration with loss of nuclear architecture like centrilobular necrosis and hydropic degeneration. The histopathological pattern of the livers of the rats treated with thioacetamide with higher dose of the extract showed minimal necrosis in centrilobular and regeneration of hepatocytes. (Figure 4.)

Ranitidine-induced hepatotoxicity

Biochemical parameters SGOT, SGPT, ALP, BRN and TC were significantly elevated ($P < 0.05$) in the group of rats given ranitidine (50mg/kg. p.o) (Table 2). The pretreatment of petroleum ether extract of *Sesbania grandiflora L. pers fruits* exhibited inhibition of ranitidine induced increase in the levels of all the five biochemical parameters, resulting in significant restoration towards their control values. Table 2. depicts that



Table 1. Effect of extract of fruits of *Sesbania grandiflora L. pers* on serum parameters in thioacetamide induced hepatic damage in rats

Group	Treatment	SGOT	SGPT	SALP	Total Bilirubin	Total Protein	Total Cholesterol
I	Normal control 10 ml/kg p.o	72.5 ± 7.18	40.28 ± 5.65	105.58 ± 4.35	0.32 ± 0.06	13.25 ± 0.65	56.12 ± 3.01
II	Toxicant control (Thioacetamide 100mg/kg, s/c)	135.58 ± 9.53	120.65 ± 9.51	198.52 ± 8.62	1.26 ± 0.02	7.41 ± 0.85	81.5 ± 1.25
III	Silymarin (100mg/kg, p.o) + Thioacetamide	75.35 ± 12.5**	45.58 ± 7.65**	125.54 ± 9.45***	0.54 ± 0.009***	12.32 ± 0.42***	61.5 ± 2.43***
IV	Petroleum ether extract (100mg/kg, p.o) + Thioacetamide	101.85 ± 8.25*	85.52 ± 4.23*	181.5 ± 6.25 ^{ns}	1.09 ± 0.028**	10.58 ± 0.67**	75.26 ± 3.26 ^{ns}
V	Petroleum ether extract (200mg/kg, p.o) + Thioacetamide	87.52 ± 7.35**	72.56 ± 9.25**	152.5 ± 8.45**	0.65 ± 0.03***	11.68 ± 0.21***	62.41 ± 2.87***

Values are mean ± SEM (n=6) one way ANOVA followed by Dunnett's 't' test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, *** represents very significant at p<0.001 and ns represents non significant.

Table 2. Effect of extract of fruits of *Sesbania grandiflora L. pers* on serum parameters in ranitidine induced hepatic damage in rats

Group	Treatment	SGOT	SGPT	SALP	Total Bilirubin	Total Protein	Total Cholesterol
I	Normal control 10 ml/kg p.o	72.5 ± 7.18	40.28 ± 6.52	105.58 ± 4.350	0.32 ± 0.06	13.25 ± 4.25	52.51 ± 4.25
II	Toxicant control (Ranitidine 50mg/kg, s/c)	126.85 ± 7.24	110.56 ± 4.58	195.62 ± 7.52	1.13 ± 0.45	6.51 ± 0.711	86.5 ± 3.23
III	Silymarin (100mg/kg, p.o) + Ranitidine	76.52 ± 6.52***	48.23 ± 8.21***	154.32 ± 9.65**	0.57 ± 0.052***	12.95 ± 0.51***	54.2 ± 4.65**
IV	Petroleum ether extract (100mg/kg, p.o) + Ranitidine	89.65 ± 8.25*	76.58 ± 9.25*	174.52 ± 6.85 ^{ns}	0.98 ± 0.025*	11.21 ± 0.22**	71.5 ± 9.54 ^{ns}
V	Petroleum ether extract (200mg/kg, p.o) + Ranitidine	82.5 ± 7.25**	62.52 ± 7.87**	151.52 ± 4.68**	0.71 ± 0.054***	11.95 ± 0.45***	54.85 ± 4.85**

Values are mean ± SEM (n=6) one way ANOVA followed by Dunnett's 't' test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, *** represents very significant at p<0.001 and ns represents non significant



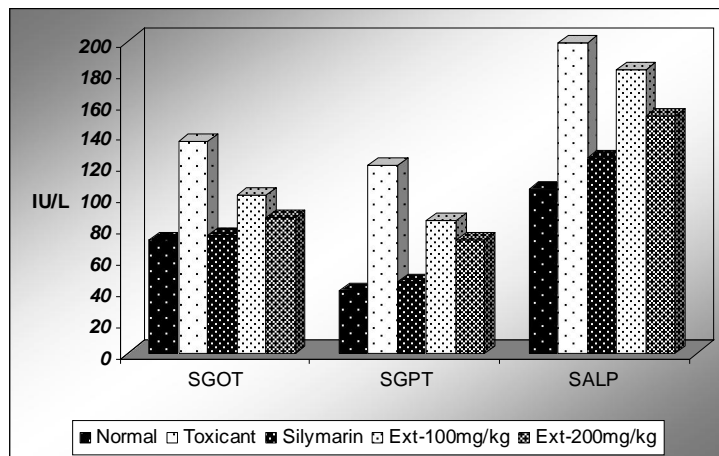


Figure 2. Effect of extract of fruits of *Sesbania grandiflora L. pers* on SGOT, SGPT & SALP in thioacetamide induced hepatic damage in rats

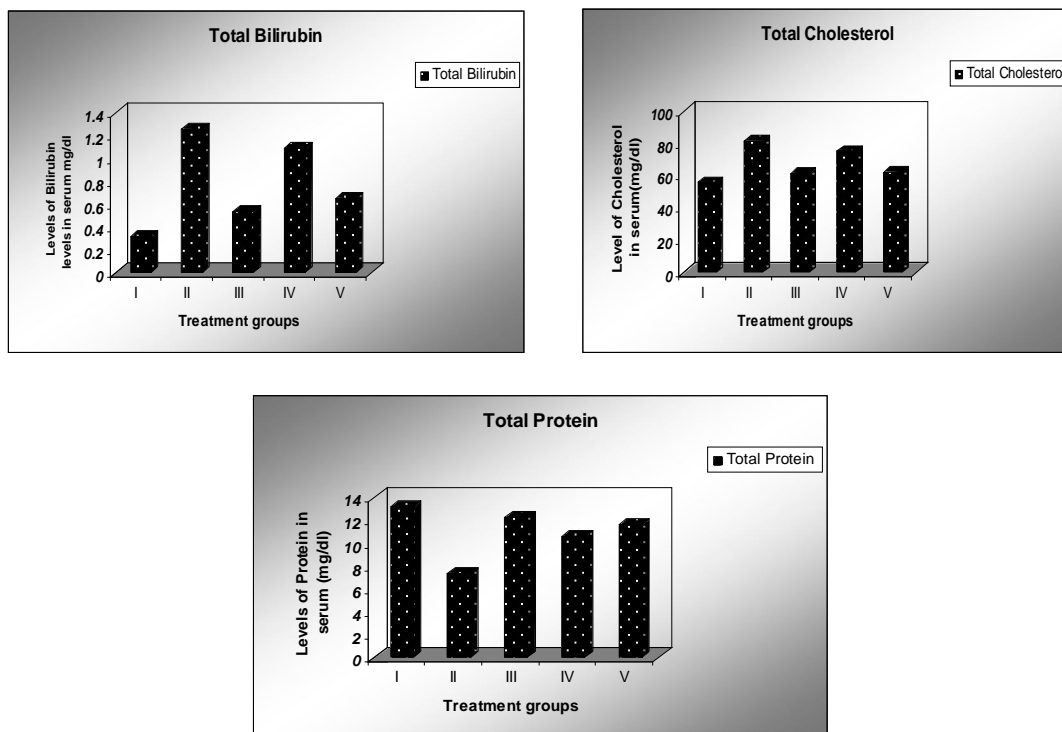
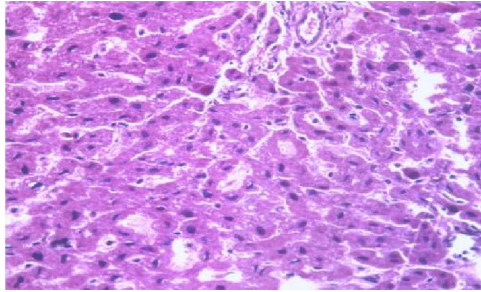
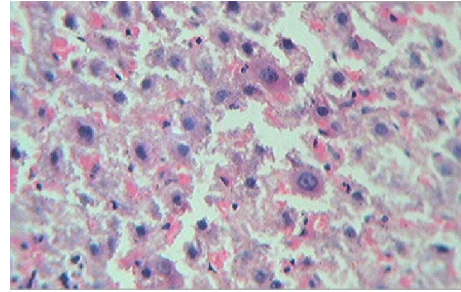


Figure 3. Effect of extract of fruits of *Sesbania grandiflora L. pers* on Total bilirubin, Cholesterol & Protein in thioacetamide induced hepatic damage in rats

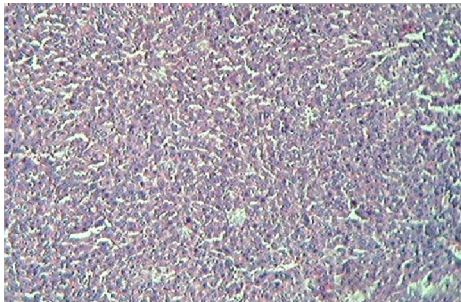




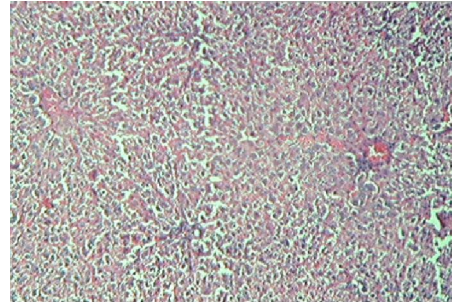
Normal control



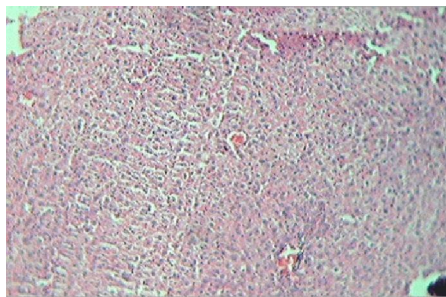
Toxicant



Standard



low dose (100mg/kg)



High dose (200mg/kg)

Figure 4. Effect of extract of fruits of *Sesbania grandiflora L. pers* on histopathology studies of liver cells induced by thiocetamide



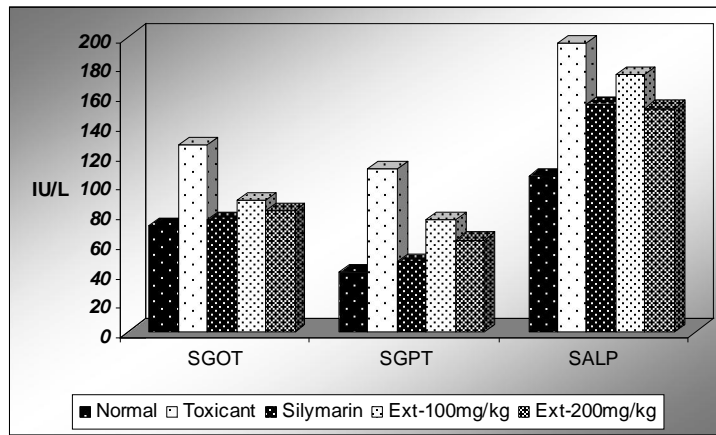


Figure 5. Effect of extract of fruits of *Sesbania grandiflora L. pers* on SGOT, SGPT & SALP in ranitidine induced hepatic damage in rats

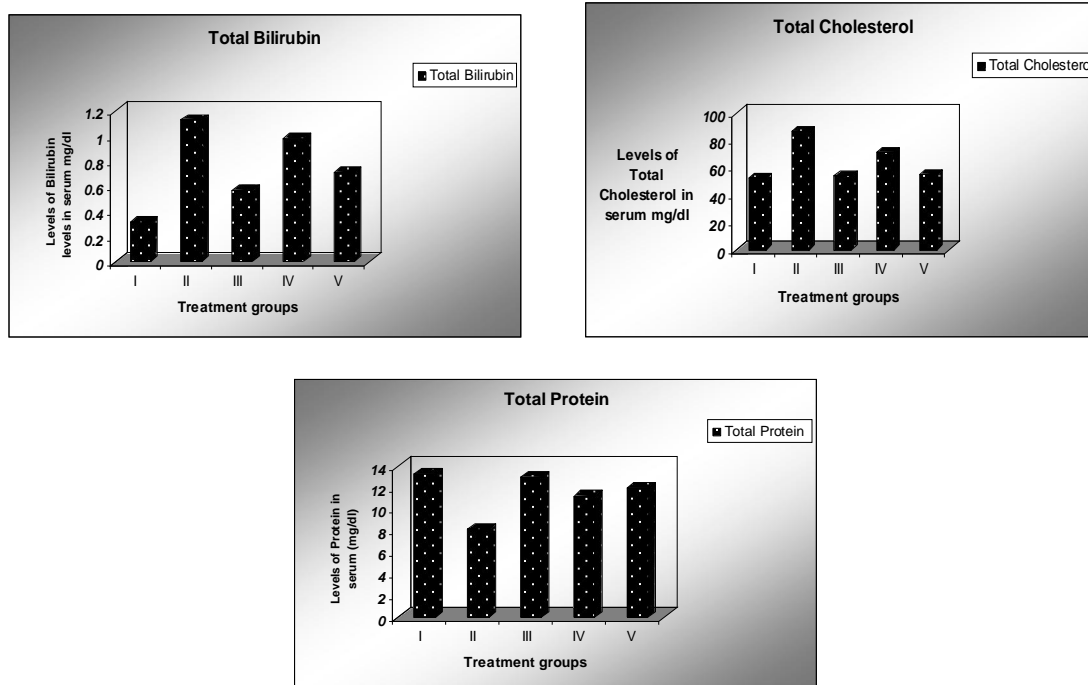


Figure 6. Effect of extract of fruits of *Sesbania grandiflora L. pers* on Total bilirubin, Cholesterol & Protein in ranitidine induced hepatic damage in rats



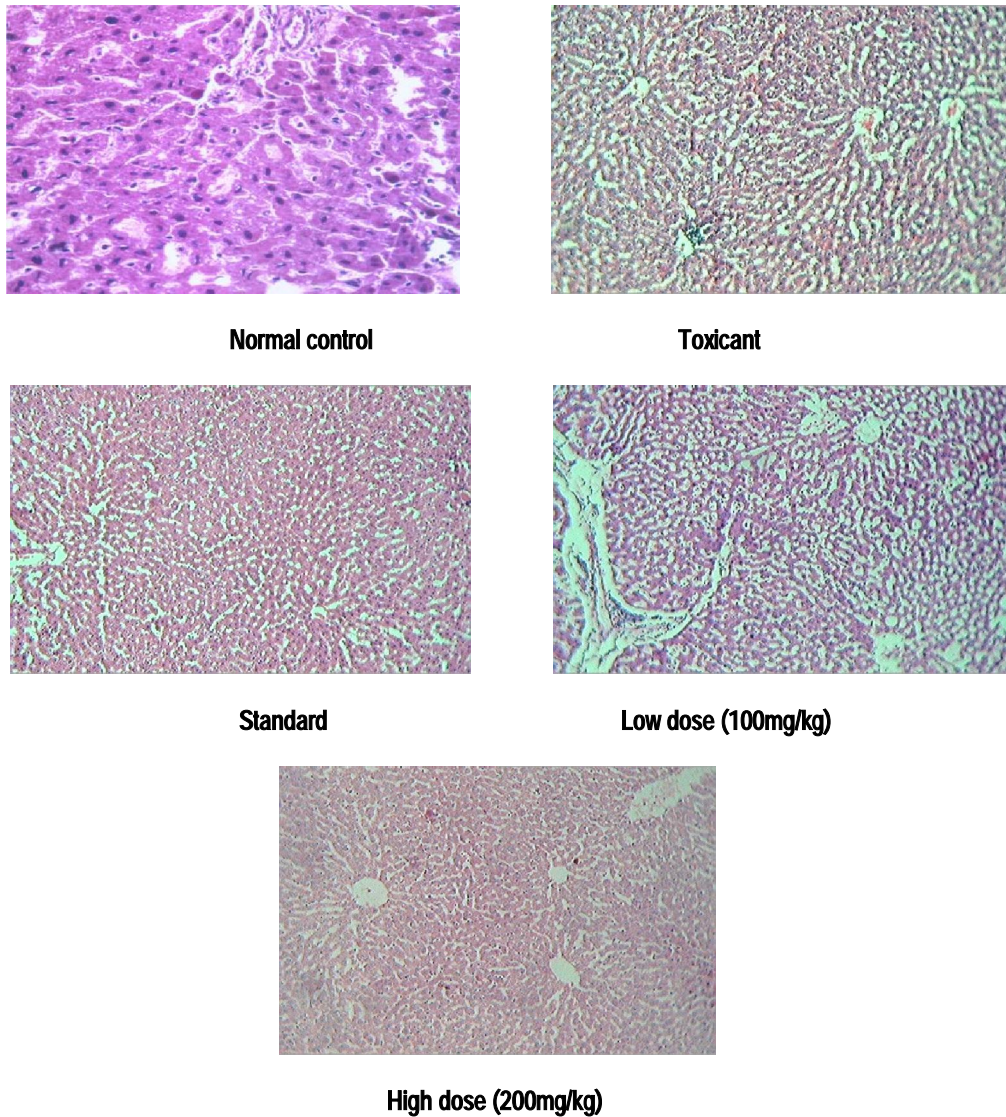


Figure 7. Effect of extract of fruits of *Sesbania grandiflora L. pers* on histopathology studies of liver cells induced by ranitidine

treatment with ranitidine reduced serum total protein which was significantly brought towards normal values by pretreatment with the petroleum ether extract of *Sesbania grandiflora L. pers* fruits. The maximum protection against ranitidine induced hepatic aberrations was achieved with the higher dose of the extract. (Figure 5&6)

Histopathological examination

The hepatoprotective effect of the extract was further confirmed by histopathological examination of the livers of control, ranitidine treated, ranitidine with Standard drug and extract treated groups. The liver samples of ranitidine treated rats showed dilatation of blood sinusoids, more number of inflammatory cells in the form of lymphocytes and some incidences of bile stasis is seen. The

histopathological pattern of the livers of the rats treated with ranitidine with higher dose of the extract showed reduction in dilatation of sinusoids, decreased number of kupffer cells and lymphocytic infiltration that suggests hepatoprotective activity of the extract. (Figure 7).

Discussion

The present study reveals that the hepatoprotective activity of the petroleum ether extract of *Sesbania grandiflora L. pers* fruits against two well known hepatotoxins, viz. Thioacetamide and Ranitidine.

Thioacetamide has been used as a tool to induce hepatotoxicity in experimental animals to produce various grade of liver damage including nodular cirrhosis, liver cell proliferation, production of

pseudolobules, and parenchymal cell necrosis. [15] Several investigators have reported that a single dose of this hepatotoxin can produce centrilobular hepatic necrosis and chronic administration leads to cirrhosis in rats. Thioacetamide has been well known to cause inhibition of the respiratory metabolism of the liver due to uncontrolled entry of Ca^{2+} ions into the hepatocytes resulting in the inhibition of oxidative phosphorylation. [16] Several researchers have suggested that part of hepatocellular injury induced by Thioacetamide is mediated through oxidative stress caused by the action of cytokines through lipid peroxidation. Reduced hepatic antioxidant function has also been suggested as one of the mechanism of thioacetamide induced hepatotoxicity. [17]

Toxicity experienced by the liver during thioacetamide poisoning results from the production of a metabolite, thioacetamide *s*-oxide, which is a direct hepatotoxin.[18] It is responsible for change in cell permeability and it inhibits mitochondrial activity followed by cell death.[19] It has also been reported that chronic thioacetamide exposure produced cirrhosis in rats. [20] It is quite likely that the extract under study prevents the thioacetamide-induced hepatotoxicity due to multiple mechanisms.

Liver injury induced by ranitidine is due to its metabolite, which may leads to hepatic oxidative damage and one of its metabolite generates immunoallergic reaction. It also produces a reaction as reflected by infiltration of hepatocytes with ranitidine 50 or 30 mg/kg. Severe inflammatory changes with collagenous septa beginning to form after pronounced centrilobular and bridging necrosis. In the parenchyma there was focal liver cell necrosis with some accumulation of histocytic elements and slight steatosis and cholestasis. Portal tract shows fibrosis, bile duct proliferation, and infiltrate consisting of lymphocytes, plasma cells, polymorphs, and eosinophils. Liver injury is manifested in

terms of increased levels of serum aminotransferases, modest hepatic infiltration by both lymphocytes and eosinophils and slight focal hepatocellular necrosis also causes liver cholestasis associated with increased plasma bilirubin and alkaline phosphatase levels. [21] [22]

Silymarin is a standardized seed extract of *Silybum marianum*, which contains flavonolignans. Silymarin at doses up to 100mg/kg has been used as a standard hepatoprotective agent by numerous investigators. Dose of 100 mg/kg of silymarin was selected in the present investigation based on some published studies demonstrating the hepatoprotective activity of this dose. [23] [24] In the present study, silymarin was used as the standard to compare the activity of both of the extracts and it was found that silymarin was significantly more effective than the highest dose of each of the extracts tested in the study.

Conclusion

In conclusions, the petroleum ether extract of *Sesbania grandiflora* L. pers fruits exhibited protective effect against Thiocetamide and Ranitidine-induced hepatotoxicity. Further studies are required to isolate the active constituents involved in the hepatoprotective activity of the plant.

Acknowledgement

Authors are thankful to all the management members for providing the necessary facilities to conduct this study and are thankful to Dr.Vamseedhar. A, Asst. Professor, Sree Siddhartha Medical College, Tumkur, for his help pertaining to histopathological study.

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