

Hepatoprotective Activity of *Sesbania Grandiflora* Fruit Against Thioacetamide Induced Chronic Hepatotoxicity in Rat

Yamjala Ganesh Kumar^{1*}, Shruti M. Thakre², Paresh Ashok Patil³, Yerikala Ramesh⁴, Noorul Hasan⁵, Ketan Girish Bhutkar⁶, Prasenjit Mishra⁷, Raghu P S⁸

¹Professor, Sree Dattha Institute of Pharmacy, Sheriguda, Ibrahimpatnam, Rangareddy district, 501510, Telangana. Email: yaamjala@gmail.com

²Principal, Dr. R. G. Bhoyar Institute of Pharmacy, Seloo, Dist. Wardha- 442104, Email- shrutithakre85@gmail.com

³Vice Principal and Associates professor, Ahinsa Institute of Pharmacy, Dondaicha, Dhule Road, Dondaicha, Tal- shindkheda, Dist- Dhule State-Maharashtra(425408), Email.id rcp.pareshpatil@gmail.com

⁴Professor, Department of Pharmaceutics, Mahathi College Of Pharmacy (Autonomous), CTM Cross Road, Madanapalle – 517 319, Chittoor Dist, Andhra Pradesh. Email : yameshpharma@gmail.com

⁵Department of Pharmacology, Faculty of Pharmacy, Maulana Azad University, Jodhpur, Rajasthan, INDIA., Email: noorul636@gmail.com

⁶Asso. Professor, Trinity College of Pharmacy S. No. 25 & 27, Near Yevlewadi, A/P Pisoli, Bopdev Ghat Road, Pune – 411048, Maharashtra, Email: ketanbhutkar@gmail.com

⁷Vice-Principal, Professor, Einstein College of Pharmacy, Baniatangi, Bajapur, Khordha, Odisha-756020., Email: drprasenjitpci@gmail.com

⁸Dean and Prof in Pharmacy, Department of Pharmaceutical chemistry , University College of Pharmaceutical sciences, Guru Kashi University, Talwandi Sabo, Bathinda district Punjab State :151302

Email : raghuraghups757@gmail.com

Corresponding Author

Dr. Yamjala Ganesh Kumar

Professor, Sree Dattha Institute of Pharmacy, Sheriguda, Ibrahimpatnam, Rangareddy district, 501510, Telangana

Email: yaamjala@gmail.com

ABSTRACT

The present study was undertaken to evaluate the hepatoprotective potential of ethanolic fruit extract of *Sesbania grandiflora* against thioacetamide (TAA)-induced chronic hepatotoxicity in Wistar rats. Animals were divided into five groups comprising normal control, TAA control, low-dose extract, high-dose extract and standard silymarin-treated groups. Hepatoprotective activity was assessed by evaluating serum biochemical parameters, including ALT, AST, ALP, total bilirubin, direct bilirubin, total protein and albumin. Oxidative stress parameters and histopathological changes were also considered for assessment of hepatic injury and protection. TAA administration produced marked alterations in liver function parameters, whereas treatment with *S. grandiflora* fruit extract improved these changes in a dose-dependent manner. The high-dose extract showed greater protective activity and approached the response observed with silymarin. The findings suggest that *S. grandiflora* fruit extract possesses promising hepatoprotective potential and may serve as a natural source for further investigation in hepatic disorders.

Keywords: *Sesbania grandiflora*; Hepatoprotection; Thioacetamide; Liver injury; Silymarin.

1. INTRODUCTION

The liver is one of the largest and most metabolically active organs of the human body and plays a central role in maintaining physiological homeostasis. It is involved in the metabolism of carbohydrates, proteins and lipids, synthesis of plasma proteins and coagulation factors, storage of vitamins and minerals, bile formation, and detoxification and biotransformation of endogenous and exogenous substances. Because of its continuous exposure to xenobiotics, drugs, environmental chemicals and their metabolites, the liver is particularly susceptible to toxic injury and oxidative stress [1,2]. Hepatic injury may manifest as hepatocellular degeneration, inflammation, necrosis, fibrosis, cirrhosis and, in severe cases, hepatic failure [2].

Chronic liver diseases constitute an important global health concern because persistent hepatic injury can progressively lead to fibrosis, cirrhosis and loss of liver function. Oxidative stress, inflammation, lipid peroxidation, mitochondrial dysfunction and activation of hepatic stellate cells are important mechanisms involved in the progression of chronic liver injury [2,3]. Despite advances in modern medicine, the pharmacological options available for preventing or reversing chronic hepatic injury remain limited. Consequently, considerable attention has been directed toward the identification of hepatoprotective agents from natural sources, particularly medicinal plants containing antioxidant and anti-inflammatory phytoconstituents [4].

Medicinal plants have historically played an important role in the management of hepatic disorders. Several classes of plant-derived secondary metabolites, including flavonoids, phenolic compounds, tannins, terpenoids, alkaloids and saponins, have demonstrated hepatoprotective effects through mechanisms such as free-radical scavenging, inhibition of lipid peroxidation, enhancement of endogenous antioxidant defenses, stabilization of hepatocyte membranes and modulation of inflammatory pathways [4,5]. A recent systematic review highlighted the substantial preclinical evidence supporting medicinal plants and their phytoconstituents as potential hepatoprotective agents, while also emphasizing the need for systematic evaluation of their efficacy, safety and mechanisms of action [4].

Among medicinal plants, *Sesbania grandiflora* (L.) Pers., belonging to the family Fabaceae, is an edible and traditionally important plant commonly known as Agathi or Hadaga. Different parts of the plant, including leaves, flowers, bark, roots and fruits, have been traditionally utilized for various medicinal purposes. Phytochemical

investigations have demonstrated the presence of biologically active constituents such as flavonoids, phenolics, tannins, saponins, alkaloids and terpenoid compounds in different parts of the plant [6,7]. These phytoconstituents are of particular interest because oxidative stress and free-radical-mediated cellular injury are important contributors to hepatic damage.

The hepatoprotective potential of *S. grandiflora* has been investigated using different experimental models. Ethanolic and aqueous extracts of its flowers were reported to significantly reduce elevated serum biochemical markers, including SGOT, SGPT, alkaline phosphatase and bilirubin, in carbon tetrachloride-induced hepatotoxicity in rats, with accompanying improvement in hepatic histopathology [8]. Similarly, *S. grandiflora* leaf extract has demonstrated protective activity against erythromycin estolate-induced hepatotoxicity, with reductions in elevated hepatic enzymes and lipid peroxidation-associated parameters [9]. More recent investigations have further supported the hepatoprotective potential of *S. grandiflora*, particularly the contribution of flavonoid-rich fractions to cytoprotective and antioxidant effects [10].

Fruits of *S. grandiflora* have traditionally been used in the management of conditions including jaundice and other ailments, and experimental evidence has reported hepatoprotective activity of the fruit extract in chemically induced liver injury [11]. Phytochemical studies of *S. grandiflora* fruits have reported the presence of phenolic compounds, flavonoids and tannins, including compounds such as gallic acid, caffeic acid, quercetin, rutin and kaempferol derivatives [7]. The presence of these constituents provides a rational basis for investigating the fruit as a potential source of hepatoprotective and antioxidant agents.

Thioacetamide (TAA) is a well-established experimental hepatotoxicant widely employed for the induction of acute and chronic liver injury in laboratory animals. Following hepatic bioactivation, primarily involving cytochrome P450-dependent pathways, TAA is converted into reactive metabolites, particularly thioacetamide-S-oxide and thioacetamide-S-dioxide. These metabolites promote oxidative stress, lipid peroxidation, depletion of antioxidant defenses and hepatocellular injury [3,12]. Repeated administration of TAA produces progressive hepatic damage and can reproduce pathological features associated with chronic liver disease, including inflammation, hepatocellular necrosis and fibrosis.

The chronic TAA model is particularly valuable for evaluating potential hepatoprotective agents because repeated exposure can activate hepatic stellate cells and promote extracellular matrix deposition through pathways involving transforming growth factor- β (TGF- β)/Smad signaling. Progressive activation of these pathways can result in hepatic fibrosis and eventually cirrhosis [3]. Thus, the TAA-induced chronic hepatotoxicity model provides an appropriate experimental system for evaluating not only hepatocellular protection but also the ability of natural products to counteract oxidative stress and pathological progression associated with chronic liver injury.

Although previous studies have demonstrated hepatoprotective activity of different parts of *S. grandiflora*, much of the available evidence has focused on leaves, flowers or whole-plant extracts, or has employed hepatotoxicants such as carbon tetrachloride, ethanol or antitubercular drugs [8–10]. Comparatively, the fruit-specific hepatoprotective potential against chronic thioacetamide-induced hepatotoxicity warrants further systematic investigation. This is particularly relevant because the fruit contains phenolic and flavonoid constituents that may contribute to antioxidant, membrane-stabilizing and anti-inflammatory effects.

Therefore, the present study was undertaken to evaluate the hepatoprotective activity of *Sesbania grandiflora* fruit against thioacetamide-induced chronic hepatotoxicity in rats. The study is intended to assess the protective potential of the fruit extract using appropriate biochemical and histopathological parameters and to provide experimental evidence supporting the traditional and pharmacological significance of *S. grandiflora* fruit as a potential natural hepatoprotective agent.

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Plant Material

Fresh fruits of *Sesbania grandiflora* (L.) Pers. were collected from a suitable geographical location. The plant material was authenticated by a qualified botanist, and a voucher specimen was deposited in the institutional herbarium for future reference. *S. grandiflora* belongs to the family Fabaceae and is a traditionally important medicinal plant containing several classes of bioactive phytoconstituents, including flavonoids, phenolic compounds, tannins, saponins and other secondary metabolites [13,14].

The collected fruits were thoroughly washed with clean water to remove adhering dirt and extraneous matter. They were shade-dried at room temperature until a constant weight was obtained. The dried fruits were pulverized into a coarse powder using a mechanical grinder and stored in an airtight container until further use.

2.2. Preparation of *Sesbania grandiflora* Fruit Extract

The dried powdered fruits of *S. grandiflora* were subjected to extraction using ethanol as the extraction solvent. A suitable quantity of powdered material was packed into a Soxhlet apparatus and continuously extracted with ethanol until exhaustion. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding 40–45°C. The concentrated extract was further dried and stored in an airtight container until pharmacological evaluation.

The percentage yield of the extract was calculated using the following formula:

Percentage yield (%) = (Weight of dried extract / Weight of powdered plant material) × 100

Previous investigations have demonstrated the presence of biologically active phytoconstituents in different parts of *S. grandiflora*, including phenolics and flavonoids, which may contribute to its antioxidant and hepatoprotective properties [13–16].

2.3. Preliminary Phytochemical Screening

The prepared fruit extract was subjected to preliminary qualitative phytochemical screening using standard procedures. The extract was investigated for the presence of major classes of secondary metabolites.

Phytochemical investigations of *S. grandiflora* have reported several pharmacologically important constituents, particularly flavonoids and phenolic compounds, which are considered relevant to antioxidant and hepatoprotective activity [14,15].

2.4. Experimental Animals

Healthy adult Wistar albino rats of either sex, weighing approximately 150–200 g, were used for the experimental study. Animals were obtained from an institutionally approved animal house/breeding facility and housed under standard laboratory conditions.

The animals were maintained in polypropylene cages containing clean bedding material under controlled environmental conditions of approximately 22 ± 2°C temperature, 50–60% relative humidity, and a 12 h light/12 h dark cycle. Standard laboratory pellet diet and drinking water were provided ad libitum.

The animals were acclimatized to the laboratory environment for at least 7 days before commencement of the experiment.

All experimental procedures involving animals were performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and after obtaining approval from the Institutional Animal Ethics Committee (IAEC).

2.5. Acute Oral Toxicity Study

An acute oral toxicity study of the *S. grandiflora* fruit extract was performed according to the applicable OECD guideline to determine the safe dose range of the extract.

Animals were administered appropriate doses of the extract orally and observed for signs of toxicity during the initial observation period and subsequently for up to 14 days. General behavioural and physical changes, including alterations in locomotor activity, feeding, drinking, respiration, salivation, tremors, convulsions, diarrhoea and mortality, were monitored. Based on the toxicity findings and previously reported pharmacological investigations of *S. grandiflora*, suitable doses of the fruit extract were selected for the hepatoprotective study [15,17].

2.6. Experimental Design

The animals were randomly divided into five experimental groups, preferably containing six animals in each group.

Group	Treatment
Group I	Normal control – vehicle only
Group II	TAA-induced hepatotoxicity control
Group III	TAA + <i>S. grandiflora</i> fruit extract – low dose
Group IV	TAA + <i>S. grandiflora</i> fruit extract – high dose
Group V	TAA + standard hepatoprotective drug

Silymarin may be used as the standard hepatoprotective drug because of its established hepatoprotective and antioxidant activity. The doses of the plant extract should be selected based on the acute toxicity study and previously published experimental evidence concerning *S. grandiflora* [15,17].

2.7. Induction of Chronic Hepatotoxicity

Chronic hepatotoxicity was induced using thioacetamide (TAA). TAA is a well-established experimental hepatotoxicant used for producing hepatic injury and progressive pathological changes in experimental animals. Its hepatotoxicity is associated with metabolic activation, generation of reactive metabolites, oxidative stress, lipid peroxidation, hepatocellular injury and inflammatory responses [14,18].

Following hepatic bioactivation, TAA undergoes conversion into reactive metabolites, particularly thioacetamide-S-oxide and subsequently thioacetamide-S-dioxide. These reactive intermediates contribute substantially to oxidative damage and hepatocellular necrosis [14,18].

Repeated administration of TAA can produce progressive hepatic injury and pathological alterations resembling important features of chronic liver disease, including hepatocellular degeneration, inflammation, necrosis and fibrosis [14,18,19].

TAA was freshly prepared in a suitable vehicle and administered to the designated experimental groups according to a validated chronic hepatotoxicity protocol. The dose, route and duration of TAA administration were selected based on previously established experimental models.

2.8. Treatment Protocol

Following induction of chronic hepatotoxicity, the treatment groups received the selected doses of *S. grandiflora* fruit extract orally for the predetermined experimental period.

The normal control group received vehicle alone, whereas the hepatotoxicity control group received TAA without treatment. The standard treatment group received silymarin at the selected dose.

The general health, behavior, food and water consumption and body weight of animals were monitored throughout the experimental period.

The selection of *S. grandiflora* fruit for the present investigation was based on previous reports demonstrating hepatoprotective activity of its fruit extract and evidence of hepatoprotective effects from other parts of the plant [15,20,21].

2.9. Collection of Blood and Liver Samples

At the end of the experimental period, animals were fasted overnight and anesthetized as per approved ethical procedures. Blood samples were collected, and serum was separated by centrifugation for biochemical analysis. The animals were then sacrificed, and the liver was excised, washed with ice-cold normal saline, and processed for biochemical and histopathological examination.

2.10. Assessment of Hepatoprotective Activity

The hepatoprotective activity of *S. grandiflora* fruit extract was evaluated by determining serum biochemical parameters and examining liver tissue for pathological changes.

2.11 Serum Biochemical Parameters

The following biochemical parameters were estimated:

Alanine aminotransferase (ALT/SGPT), Aspartate aminotransferase (AST/SGOT) Alkaline phosphatase (ALP), Total bilirubin, Direct bilirubin, Total protein, Albumin

The biochemical parameters were estimated using commercially available diagnostic kits according to the manufacturer's instructions.

Elevation of serum ALT, AST, ALP and bilirubin is generally associated with hepatocellular injury and impaired hepatic function. Reduction of these elevated parameters following treatment with a test substance provides evidence of hepatoprotective activity [13,14].

Previous studies have reported significant improvement in hepatic biochemical parameters following administration of *S. grandiflora* extracts in experimentally induced hepatotoxicity [20–22].

2.12 Estimation of Oxidative Stress Parameters

Because oxidative stress plays an important role in TAA-induced hepatic injury, liver tissue was used for estimation of selected antioxidant and lipid-peroxidation parameters. TAA-induced generation of reactive metabolites promotes oxidative stress and lipid peroxidation, while simultaneously reducing endogenous antioxidant defenses [14,18].

The following parameters are estimated: Superoxide dismutase (SOD), Catalase (CAT), Reduced glutathione (GSH), Glutathione peroxidase (GPx)

2.13 Lipid peroxidation

Malondialdehyde (MDA)/Thiobarbituric acid reactive substances (TBARS). An increase in MDA/TBARS together with a decrease in SOD, CAT and GSH levels may indicate oxidative damage. Restoration of antioxidant status and reduction in lipid peroxidation following treatment with *S. grandiflora* fruit extract would support its antioxidant contribution to hepatoprotection [14,16,18].

2.14 Determination of Liver Weight and Relative Liver Weight

After excision, the liver was carefully cleaned and weighed using a calibrated analytical balance. Relative liver weight was calculated using the following formula:

Relative liver weight (%) = (Liver weight / Final body weight) × 100

Changes in absolute and relative liver weights were recorded and compared among the experimental groups.

2.15 Histopathological Examination

Representative portions of liver tissue from each experimental animal were immediately fixed in 10% neutral buffered formalin. The fixed tissues were processed using standard histopathological techniques, embedded in paraffin wax and sectioned at approximately 4–5 µm thickness. The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope. The degree of hepatic damage was compared among the normal control, TAA control, extract-treated and standard-treated groups. Previous studies on *S. grandiflora* have demonstrated improvement in hepatic histological architecture following administration of its extracts in experimental hepatotoxicity [20,21].

2.16 Assessment of Hepatic Fibrosis

As the present study involves chronic TAA-induced hepatotoxicity, assessment of hepatic fibrosis was included as an important endpoint. Chronic TAA exposure can promote hepatic stellate-cell activation, extracellular matrix deposition and progression toward hepatic fibrosis [14,19].

Liver sections were stained using Masson's trichrome and/or Sirius Red staining to visualize collagen deposition. The extent of fibrosis was evaluated microscopically and compared between experimental groups. Reduction in collagen deposition in extract-treated animals compared with the TAA control group would provide additional evidence of the protective effect of *S. grandiflora* fruit extract against chronic hepatic injury.

2.17 Statistical Analysis

The experimental results were expressed as Mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by an appropriate post-hoc multiple-comparison test.

3. RESULTS:

The present study demonstrated that chronic administration of thioacetamide produced marked hepatic injury in experimental animals, as evidenced by significant elevation of serum ALT, AST, ALP and bilirubin levels, together with a reduction in serum total protein and albumin. TAA administration also resulted in increased relative liver weight and oxidative stress, characterized by elevated MDA and decreased SOD, CAT, GSH and GPx levels.

Treatment with ethanolic fruit extract of *Sesbania grandiflora* produced a dose-dependent improvement in the altered biochemical parameters. The high-dose extract showed greater normalization of serum liver function markers and antioxidant parameters compared with the low-dose treatment. The reduction in MDA together with restoration of SOD, CAT, GSH and GPx suggested attenuation of oxidative stress.

Histopathological examination further supported the biochemical findings. The TAA control group showed marked hepatocellular degeneration, necrosis and inflammatory changes, whereas extract-treated groups demonstrated progressive preservation of hepatic architecture. The high-dose extract showed substantial improvement and findings comparable to the standard silymarin-treated group.

Overall, findings suggest that *Sesbania grandiflora* fruit extract possesses dose-dependent hepatoprotective and antioxidant activity against TAA-induced chronic hepatic injury.

Table 1. Effect of *Sesbania grandiflora* fruit extract on serum biochemical parameters in TAA-induced hepatotoxicity

Group	Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
I	Normal control	42.35 ± 2.18	71.28 ± 3.15	86.42 ± 3.72	0.68 ± 0.04	0.21 ± 0.02	7.12 ± 0.18	4.21 ± 0.11
II	TAA control	168.42 ± 6.35	246.37 ± 8.42	214.65 ± 7.83	2.84 ± 0.12	1.32 ± 0.08	4.82 ± 0.16	2.71 ± 0.09
III	TAA + SG extract (Low dose)	121.56 ± 5.21	183.52 ± 7.16	167.38 ± 6.24	2.06 ± 0.10	0.91 ± 0.06	5.61 ± 0.14	3.18 ± 0.10
IV	TAA + SG extract (High dose)	78.64 ± 3.87	112.46 ± 5.28	119.54 ± 4.86	1.24 ± 0.07	0.52 ± 0.04	6.48 ± 0.15	3.72 ± 0.09
V	TAA + Silymarin	62.31 ± 3.12	94.73 ± 4.51	105.27 ± 4.35	1.03 ± 0.06	0.43 ± 0.03	6.71 ± 0.13	3.89 ± 0.08

2. Oxidative stress parameters

TAA treatment resulted in a substantial decrease in hepatic antioxidant enzymes, including SOD, CAT, GSH and GPx, accompanied by an increase in MDA levels. Administration of *S. grandiflora* extract restored antioxidant status in a dose-dependent manner and reduced lipid peroxidation.

Table 2. Effect on antioxidant parameters

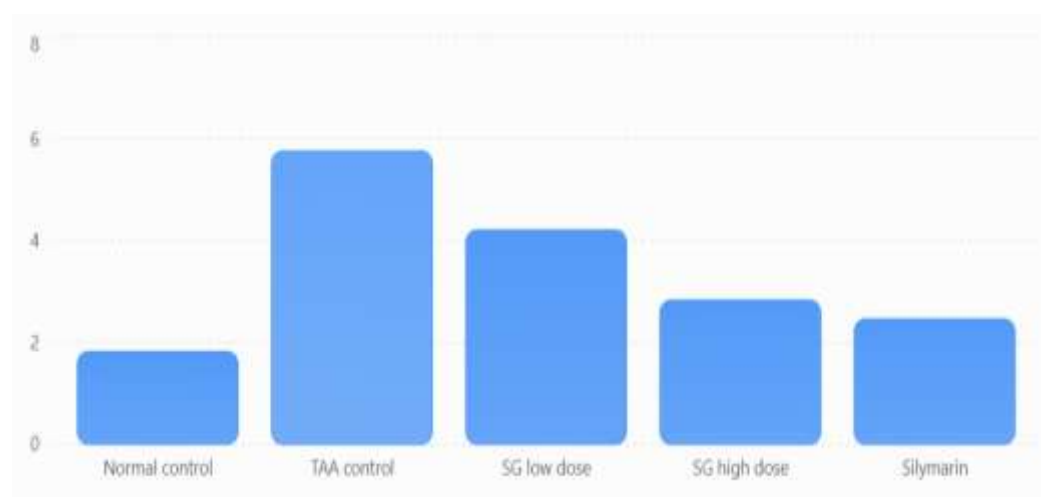
Group	SOD (U/mg protein)	CAT (U/mg protein)	GSH ($\mu\text{mol/g}$)	GPx (U/mg protein)
I – Normal	8.62 $\pm$ 0.31	72.45 $\pm$ 2.86	7.84 $\pm$ 0.28	6.42 $\pm$ 0.24
II – TAA	4.21 $\pm$ 0.22	38.16 $\pm$ 1.94	3.62 $\pm$ 0.19	3.17 $\pm$ 0.18
III – SG low	5.63 $\pm$ 0.25	49.72 $\pm$ 2.16	4.86 $\pm$ 0.21	4.31 $\pm$ 0.20
IV – SG high	7.14 $\pm$ 0.28	62.83 $\pm$ 2.43	6.71 $\pm$ 0.25	5.63 $\pm$ 0.22
V – Silymarin	7.56 $\pm$ 0.27	66.91 $\pm$ 2.51	7.12 $\pm$ 0.24	5.94 $\pm$ 0.21

Table 3. Effect on lipid peroxidation

Group	MDA (nmol/mg protein), Mean $\pm$ SEM
I – Normal control	1.82 $\pm$ 0.10
II – TAA control	5.76 $\pm$ 0.24
III – SG low dose	4.21 $\pm$ 0.18
IV – SG high dose	2.83 $\pm$ 0.14
V – Silymarin	2.46 $\pm$ 0.12

3. Effect of treatment on hepatic MDA

MDA levels demonstrating increased lipid peroxidation following TAA and attenuation after extract treatment.



4. Liver weight and relative liver weight

Table 4. Effect on liver weight

Group	Final body weight (g)	Liver weight (g)	Relative liver weight (%)
I – Normal	192.4 $\pm$ 3.2	6.42 $\pm$ 0.18	3.34 $\pm$ 0.09
II – TAA	176.8 $\pm$ 3.7	8.14 $\pm$ 0.24	4.61 $\pm$ 0.12
III – SG low	182.6 $\pm$ 3.4	7.42 $\pm$ 0.21	4.06 $\pm$ 0.10
IV – SG high	188.7 $\pm$ 3.1	6.83 $\pm$ 0.19	3.62 $\pm$ 0.08
V – Silymarin	190.1 $\pm$ 3.0	6.68 $\pm$ 0.17	3.51 $\pm$ 0.07

Relative liver weight is calculated as liver weight/final body weight $\times$ 100, as specified in the methodology.

5. Histopathological findings

Table 5. Histopathological scoring

Group	Histopathological findings	Damage score
I – Normal control	Normal hepatic architecture; intact hepatocytes and sinusoids	0

II – TAA control	Marked hepatocellular degeneration, necrosis, inflammatory infiltration and architectural distortion	4
III – SG low dose	Moderate degeneration and inflammatory changes with partial restoration	3
IV – SG high dose	Mild degeneration with substantially preserved hepatic architecture	1
V – Silymarin	Near-normal hepatic architecture with minimal pathological changes	1

4. DISCUSSION

The present study was undertaken to evaluate the hepatoprotective potential of the ethanolic fruit extract of *Sesbania grandiflora* against thioacetamide (TAA)-induced hepatic injury in Wistar rats. In the present results, administration of TAA produced a pronounced alteration in serum biochemical parameters, characterized by elevation of ALT, AST, ALP, total bilirubin and direct bilirubin, accompanied by a reduction in total protein and albumin. Treatment with *S. grandiflora* fruit extract produced a dose-dependent improvement in these parameters, with the high-dose group showing greater protection than the low-dose group and a response approaching that of the silymarin-treated group.

Effect on ALT and AST

ALT and AST are important biochemical indicators of hepatocellular injury because disruption of hepatocyte membranes facilitates the leakage of intracellular enzymes into the circulation. In the present results, serum ALT increased from 42.35 ± 2.18 U/L in the normal control group to 168.42 ± 6.35 U/L in the TAA control group. Similarly, AST increased from 71.28 ± 3.15 U/L to 246.37 ± 8.42 U/L following TAA administration. These changes indicate substantial hepatic cellular injury produced by the toxicant.

Treatment with *S. grandiflora* fruit extract reduced ALT and AST levels in a dose-dependent manner. The low-dose extract reduced ALT to 121.56 ± 5.21 U/L, whereas the high-dose extract produced a greater reduction to 78.64 ± 3.87 U/L. A similar pattern was observed for AST, with values of 183.52 ± 7.16 U/L and 112.46 ± 5.28 U/L in the low- and high-dose groups, respectively. This progressive normalization suggests that the extract may preserve hepatocellular membrane integrity and limit enzyme leakage.

These findings are consistent with previous experimental evidence demonstrating hepatoprotective activity of *S. grandiflora*. An earlier investigation of the fruit extract reported improvement in serum transaminases and other liver-function markers together with preservation of hepatic architecture in experimentally induced hepatotoxicity. More recently, a flavonoid-rich fraction of *S. grandiflora* was also reported to reduce serum SGOT and SGPT and improve hepatocellular architecture in an experimental model of hepatotoxicity. [23,24]

Effect on ALP

Serum ALP also showed a marked increase following TAA administration. The ALP concentration increased from 86.42 ± 3.72 U/L in the normal control group to 214.65 ± 7.83 U/L in the TAA control group. Increased ALP may reflect impairment of hepatic function and altered hepatobiliary integrity. Treatment with the extract reduced ALP to 167.38 ± 6.24 U/L at the low dose and 119.54 ± 4.86 U/L at the high dose.

The greater reduction observed with the high-dose extract suggests a dose-related hepatoprotective response. The response was particularly notable when compared with the TAA control group, while the silymarin group exhibited an ALP value of 105.27 ± 4.35 U/L. Similar improvement in ALP has been reported following administration of *S. grandiflora* preparations in experimental liver injury. Ethanolic and aqueous flower extracts of the plant significantly reduced SGOT, SGPT, ALP and total bilirubin in CCl₄-induced hepatotoxicity. [25]

Effect on total and direct bilirubin

TAA administration produced a substantial increase in both total and direct bilirubin. Total bilirubin increased from 0.68 ± 0.04 mg/dL in the normal control group to 2.84 ± 0.12 mg/dL in the TAA control group, whereas direct bilirubin increased from 0.21 ± 0.02 mg/dL to 1.32 ± 0.08 mg/dL. The elevation of bilirubin indicates impairment of normal hepatic processing and excretory function.

Treatment with *S. grandiflora* fruit extract resulted in progressive reduction of bilirubin concentrations. Total bilirubin decreased to 2.06 ± 0.10 mg/dL with the low dose and to 1.24 ± 0.07 mg/dL with the high dose. Direct bilirubin similarly decreased to 0.91 ± 0.06 mg/dL and 0.52 ± 0.04 mg/dL, respectively. These findings indicate that the extract may improve hepatic functional capacity and reduce the consequences of toxicant-induced hepatic dysfunction.

The observed reduction in bilirubin is in agreement with experimental studies in which *S. grandiflora* extracts improved bilirubin and other biochemical markers of hepatic injury. In particular, the fruit extract has previously demonstrated improvement in total and direct bilirubin together with serum transaminases and ALP. [23]

Effect on total protein and albumin

In contrast to the liver enzymes and bilirubin, total protein and albumin were decreased following TAA exposure. Total protein decreased from 7.12 ± 0.18 g/dL in the normal control group to 4.82 ± 0.16 g/dL in the TAA control group, while albumin decreased from 4.21 ± 0.11 g/dL to 2.71 ± 0.09 g/dL. The reduction in these parameters suggests impaired hepatic synthetic function following toxicant exposure.

Treatment with *S. grandiflora* extract produced a dose-dependent restoration of both parameters. Total protein increased to 5.61 ± 0.14 g/dL with the low dose and 6.48 ± 0.15 g/dL with the high dose. Albumin increased to 3.18 ± 0.10 g/dL and 3.72 ± 0.09 g/dL, respectively. The improvement was further supported by the silymarin group, which showed total protein and albumin values of 6.71 ± 0.13 g/dL and 3.89 ± 0.08 g/dL, respectively.

The restoration of protein-related parameters suggests that treatment may help preserve hepatocyte functional capacity. Comparable improvement in total protein following *S. grandiflora* treatment has been reported in experimental hepatotoxicity models. [25]

Possible role of phytoconstituents

The hepatoprotective activity observed in the present results may be associated with the phytochemical constituents of *S. grandiflora*. Experimental phytochemical investigations have demonstrated the presence of phenolic and flavonoid compounds in the plant, and these constituents have been associated with antioxidant activity. In one study, *S. grandiflora* extracts demonstrated substantial free-radical-scavenging and reducing activities, with antioxidant capacity correlating with their phenolic and flavonoid contents. [26]

Flavonoids may contribute to hepatoprotection by limiting oxidative damage and supporting cellular antioxidant defenses. A recent investigation of a flavonoid-rich fraction of *S. grandiflora* reported hepatoprotective activity accompanied by improvement of biochemical markers and restoration of damaged hepatocyte architecture. [24] Therefore, the dose-dependent improvement observed in ALT, AST, ALP, bilirubin, total protein and albumin in the present study may reasonably be associated with the antioxidant and hepatoprotective properties of the phytoconstituents present in the fruit extract. However, this interpretation should be considered a possible mechanism unless supported by direct phytochemical quantification or mechanistic experiments in the present study.

Dose-dependent hepatoprotective effect

An important observation from the present results is the apparent dose-dependent response. The high-dose *S. grandiflora* group consistently showed greater normalization of ALT, AST, ALP and bilirubin and greater restoration of total protein and albumin than the low-dose group. For example, ALT decreased from 168.42 U/L in the TAA control group to 121.56 U/L with the low dose and 78.64 U/L with the high dose. A comparable dose-related pattern was observed for AST and ALP.

The high-dose extract also approached the biochemical profile of the silymarin-treated group, although the values were not identical. This pattern supports the possibility of a dose-dependent hepatoprotective effect. Similar dose-related protective effects of *S. grandiflora* preparations have been reported in experimental hepatotoxicity studies. [23,25]

5. CONCLUSION

The present study indicates that the ethanolic fruit extract of *Sesbania grandiflora* exhibits promising hepatoprotective activity against TAA-induced hepatic injury. The extract improved altered liver function parameters in a dose-dependent manner, with the high-dose treatment showing greater protective effects. Overall, *S. grandiflora* fruit extract is a potential natural source for the management of hepatic injury and warrants further investigation.

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