

Bio Activity of *Sesbania Grandiflora* against Hepatic Damage in Albino Rats

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Abstract

A phytotherapeutic approach to modern drug development can provide many invaluable drugs from traditional medicinal plants. Medicinal plants have been considered as important therapeutic aid for alleviating ailment of humankind. Numerous plants and polyherbal formulations are used for the treatment of liver diseases. This present investigation was aimed to assessing the hepatoprotective activity of aqueous, ethanol and acetone extract of *Sesbania grandiflora* leaves against carbon tetra chloride (CCl₄) induced liver damage in albino rats. Silymarin as standard drug for comparing the activity. The activity was assessed by comparing the biochemical parameters in serum levels such as serum glutamate pyruvate transaminase, serum glutamate oxalate transaminase, total bilirubin, alkaline phosphatase of plant extracts treated group with carbon tetrachloride treated animals. Results showed, ethanolic extract treated group showed highly significant activity ($p < 0.001$), whereas aqueous extract treated group has shown the significant ($p < 0.01$) action but less compared with ethanolic extract, acetone treated group showed moderate action. Plant extracts restores biochemical enzymes and brings down to normal as compared to standard drug silymarin. This results shows and confirms the significant protective activity against CCl₄ induced hepatotoxicity.

Keywords: Phytotherapeutic, *Sesbania Grandiflora*, Antioxidant, Carbon Tetra Chloride, Hepatotoxicity.

Introduction

Liver is very important organ in the human body. It regulates metabolic functions such as detoxification and play vital role in bio-chemical conversion. During the process of elimination there is chance of accumulation different kinds of toxic materials inside the hepatocytes and there is chance of liver infection, and hepatic disorders such as hepatitis ¹. Liver diseases caused by various toxic chemicals, chemotherapeutic agents, excessive consumption of alcohol and microorganisms. Hepatotoxicity is an acute adverse effect in liver in caused by over dosages of drugs, toxic chemicals, viruses, bacteria and parasites ².

Hepatotoxicity is a slight changes in hepatic structure and function which may result hypertension, ascites, jaundice, increased bleeding and cause multiple metabolic changes affecting other organs ^{3,4}. The magnitude of derangement of liver by disease or hepatotoxin is generally measured by the level of glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST), alkaline phosphatase (ALP), bilirubin, albumin, and whole liver homogenate ^{4,5}.

CCl₄ is a widely used industrial chemical and a potent hepatotoxin. It induces hepatotoxicity by producing free radical, putting oxidative stress hence causing lipid peroxidation in liver tissues, consequently necrotic liver damage ^{6,7}. Liver diseases such as hepatitis, cirrhosis and fatty liver are worldwide. Various commercial synthetic drugs are used to treat liver disorders also cause side effect to the liver. Hence, Herbal drugs have become increasingly popular and their use is widespread. Herbal medicines have been used in the treatment of liver diseases for a long time. In India numerous medicinal plants are used for treatment of liver disorders ⁸. Hepatoprotective effect of some plants like *Spirulina maxima* ⁹ *Eclipta alba* ¹⁰, *Boehmerianivea* ¹¹, *Cichorium intybus* ¹², and *Picrorrhiza kurroa* ¹³, *Boswellia Serrata* ⁴, *Psidium quajava* ¹⁴, *Coccinia indica* ¹⁵ has been well documented.

Sesbania grandiflora **Figure. 1** fast growing tree belongs to the family, Fabaceae, is commonly known as agathi in regional language Tamil. The leaves, used as greens for cattle and poultry, have got anthelmintic property against selected helminthes ^{16, 17}. The bark, leaves, flowers and roots are also used medically herbs distributed in the tropical regions of the globe ¹⁸. Juice of leaves and flowers is popular remedy for nasal catarrh and headache when it is sniffed up the nostrils. Juice of the flowers is squeezed into the eyes to relieve the dimness of vision.

Juice of flower is ideal as expectorant ¹⁹. The leaves of the plant have been reported to have anxiolytic and anticonvulsant effect while the flowers have been reported to have anti-microbial activity ²⁰. It shows hypolipemic, anti-ulcer and anti-inflammatory properties as well. Therefore, to justify the traditional claims, we have assessed the hepatoprotective effect of *Sesbania grandiflora* leaves extract in albino rats using biochemical enzyme based analysis.



Figure 1. *Sesbania grandiflora* leaves

Materials and methods

Chemicals: Analytical grade carbon tetra chloride, Silymarin and other chemicals were purchased from Himedia laboratories private limited, Mumbai. *Sesbania grandiflora* plant was collected from, Tiruvannamalai, South India.

Preparation of plant extracts

Sesbania grandiflora leaves were collected and shade dried at room temperature. The shade dried leaves were powdered and extracted by using aqueous, ethanol and acetone. Aqueous extracts was prepared by subjecting a 100 g of dried powdered leaves in to 100 ml of distilled water and incubated in water bath shaker for 12 h at 40°C. Ethanol and acetone extract prepared by the coarsely powdered leaves was extracted using soxhlet and extracted with 80 % ethanol and 70 % acetone for 24 h at 60 °C and 55° C, respectively. The extracted were collected and concentrated by drying under vacuum and semisolid suspensions were obtained. These suspensions were used to assess hepatoprotective activity.

Experimental design for hepatoprotective activity of sesbania grandiflora

Adult male Wister albino rats maintained at the college weighing between 150g-170g were used for the hepatoprotective studies. Animals were divided into six groups in six rats each:

Group I (Normal): Orally received distilled water for 7 days.

Group II (Induced): Orally received carbon tetra chloride (2g/kg body weight) only for 7 days.

Group III (Standard): Orally received Silymarin (20 mg/kg body weight) along with CCl₄ (2g/kg body weight) for 7 days.

Group IV (Treatment): Orally received Aqueous leaf extracts (300mg/kg body weight) along with CCl₄ (2g/kg body weight) for 7 days.

Group V (Treatment): Orally received ethanol leaf extracts (300mg/kg body weight) along with CCl₄ (2g/kg/body weight) for 7 days.

Group VI (Treatment): Orally received acetone leaf extracts (300mg/kg body weight) along with CCl₄ (2g/kg body weight) for 7 days.

Silymarin was used as positive control for comparing hepatoprotective potential of different leaves extract of *Sesbania grandiflora*.

Hepatoprotective activity of *S. grandiflora*

Collection of blood and biochemical analysis

On the 8th day, all the animals were scarified and blood samples were collected in glass tube from retro-orbital puncture to obtain haemolysis for 30 min at 37° C. Serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, serum bilirubin and alkaline phosphatase and Serum protein^{21, 22} were obtained from serum following centrifugation process was used for the biochemical analysis.

Antioxidant activity of *S. grandiflora*

Liver homogenate preparation

Liver homogenates were prepared by using a 100mM KCl buffer (pH7.0) containing 0.3mM EDTA and centrifuged at 6000 rpm for 45min at 4°C. After completion of centrifugation process collect the supernatant was used for estimation of antioxidant levels were analyzed Superoxide dismutase (SOD)²³, Catalase (CAT)²⁴ and Glutathione Peroxidase (GP)²⁵.

Statistical analysis

The difference of biochemical parameters were measured using the statistical method i.e. Analysis of Variance (ANOVA). Analysis of Variance refers to the examination of differences among the samples and the results are expressed as mean± SEM and $p < 0.05$, $p < 0.01$, $p < 0.001$ was considered to be statistically significant.

Results and discussion

The hepatoprotective and antioxidant activity of *S. grandiflora* leaves extracts are shown in **Figure. 2-4**. the biochemical parameters such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, serum bilirubin and alkaline phosphatase were estimated to assess the liver function. The marked increase in SGOT, SGPT and ALP levels were observed in CCl₄ treated group II animals are 94.98 ± 0.69 , 29.98 ± 0.67 and 299.68 ± 0.12 IU/L, respectively. The increased level of SGOT, SGPT, ALP and bilirubin is conventional indicator of liver injury. However these levels were reversed to near normal levels of group I animals with treatment of aqueous, ethanol and acetone extract of *Sesbania grandiflora*, which are statistically significant. The activities of extracts were comparable to a standard drug. These extracts has restored the all the biochemical parameters levels in serum. And also the standard silymarin has restored the biochemical levels of SGOT, SGPT, and ALP significantly ($p < 0.01$) i.e. 69.42 ± 0.38 , 24.42 ± 0.33 , and 170.12 ± 0.25 IU/L respectively in serum. In case of bilirubin and total protein there was a noticeable increase i.e. in serum levels treating with CCl₄. Treatment with aqueous, ethanol and acetone extract has reversed the serum bilirubin and total protein in serum levels to (0.50 ± 0.07 and 6.85 ± 0.12 mg/dl), (0.45 ± 0.09 and 6.59 ± 0.32 mg/dl), (0.47 ± 1.06 and 6.26 ± 1.07 mg/dl), respectively which are statistically highly significant ($p < 0.001$) when compared with CCl₄ treated animals.

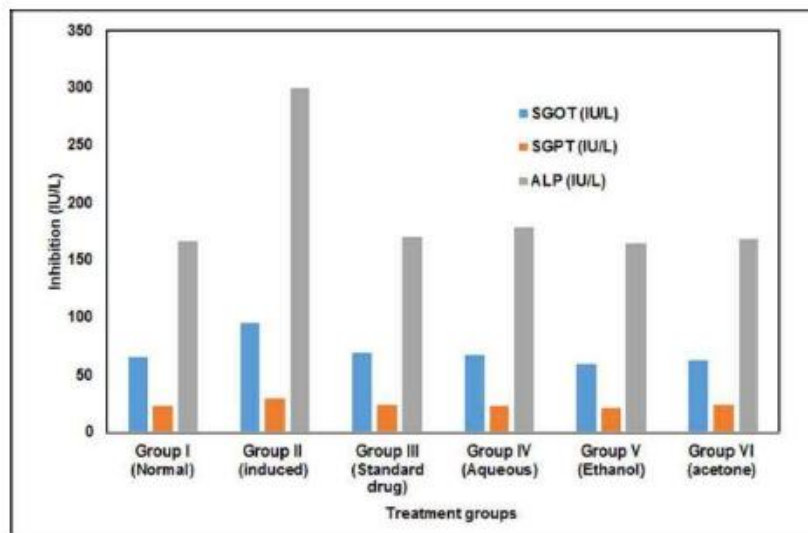


Figure. 2. Hepatoprotective activity in carbon tetra chloride induced hepatotoxic model shows changes serum enzymes sgot, sgpt and alp in serum

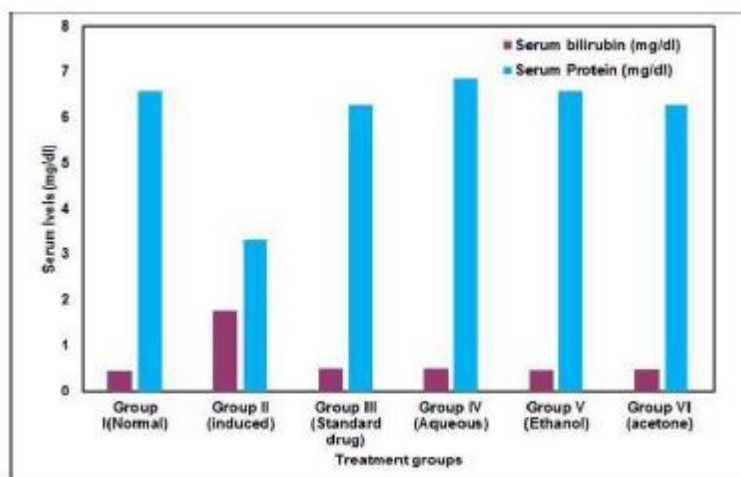


Figure 3. Hepatoprotective activity in carbon tetra chloride induced hepatotoxic model shows changes serum bilirubin and total protein

The restoration of biochemical factors in serum was also noticed in treating with the standard drug silymarin (0.49 ± 0.04 and 6.26 ± 0.16 mg/dl). It is stipulated that the extract treated group was protected from hepatic cell damage caused by CCl_4 induction. The extract at a dose of 300 mg/kg body wt. exhibited orally, significant protective effect by lowering the serum levels of transaminases (SGOT and SGPT), bilirubin and alkaline phosphatase (ALP).

The effects produced were comparable to that of a standard hepatoprotective agent silymarin. In ethanol extract treated animals, the toxicity effect of carbon tetrachloride was controlled significantly by restoration of the levels of serum bilirubin and enzymes as compared to the normal and standard drug silymarin-treated groups. Antioxidant activities of hepatic SOD, CAT and GPx were estimated and shown in the **Table 3**, **Figure. 4** SOD, CAT and GPx activities were significantly ($p < 0.001$) enhanced only in the orally received ethanol extract of *S. grandiflora* leaves. The antioxidant activities of aqueous, ethanol and acetone extract shows significant activity near to the normal group of animals.

Table 1. Hepatoprotective activity in carbon tetra chloride induced hepatotoxic model shows changes serum enzymes sgot, sgpt and alp in serum

Parameters	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)
Group I (Normal)	65.15±0.14***	23.15±0.17***	166.15±0.22***
Group II (induced)	94.98±0.69*	29.98±0.67*	299.68±0.12*
Group III (Standard drug)	69.42±0.38**	24.42±0.33***	170.12±0.25***
Group IV (Aqueous)	67.48±0.39**	23.48±0.38**	178.48±0.28***
Group V (Ethanol)	59.59±0.76***	21.59±0.74***	164.51±0.17***
Group VI (acetone)	62.58±0.34***	24.58±0.35**	168.59±0.73***

*p < 0.05, **p < 0.01, ***p < 0.001 value are considered statistically significant (BMRT)

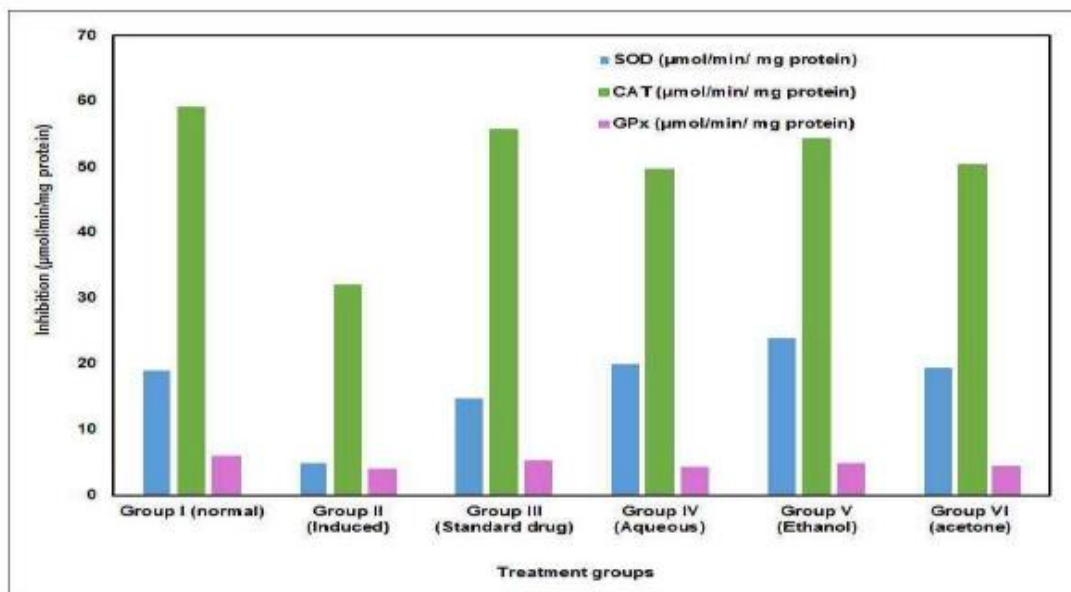


Figure 4. Antioxidant levels in carbon tetra chloride induced hepatotoxic model shows changes in the levels of sod, cat and GPx

Table 2. Hepatoprotective activity in carbon tetra chloride induced hepatotoxic model shows changes serum bilirubin and total protein

Parameters	Serum bilirubin (mg/dl)	Serum Protein (mg/dl)
Group I(Normal)	0.44±0.03***	6.58±0.06***
Group II(induced)	1.76±0.10*	3.32±0.04*
Group III(Standard drug)	0.49±0.04***	6.26±0.16**
Group IV (Aqueous)	0.50±0.07**	6.85±0.12**
Group V (Ethanol)	0.45±0.09***	6.59±0.32***
Group VI (acetone)	0.47±1.06**	6.26±1.07***

*p < 0.05, **p < 0.01, ***p < 0.001 value are considered statistically significant (BMRT)

Table 3. Antioxidant levels in carbon tetra chloride induced hepatotoxic model
Shows changes in the levels of sod, cat and GPX

Parameters	SOD ($\mu\text{mol}/\text{min}/\text{mg protein}$)	CAT ($\mu\text{mol}/\text{min}/\text{mg protein}$)	GPx ($\mu\text{mol}/\text{min}/\text{mg protein}$)
Group I (Normal)	18.91 \pm 0.31***	58.99 \pm 4.80***	5.98 \pm 2.46***
Group II(induced)	04.94 \pm 0.21*	31.89 \pm 3.43*	3.98 \pm 0.35*
Group III(Standard drug)	14.73 \pm 0.39**	55.67 \pm 3.13**	5.34 \pm 2.11**
Group IV (Aqueous)	20.01 \pm 0.17**	49.68 \pm 0.55***	4.32 \pm 1.92**
Group V (Ethanol)	23.92 \pm 0.27***	54.23 \pm 3.17***	4.84 \pm 1.70***
Group VI (acetone)	19.46 \pm 0.11***	50.38 \pm 0.18***	4.42 \pm 1.02***

*p < 0.05, **p < 0.01, ***p < 0.001 value are considered statistically significant (BMRT).

Conclusion

In the present report stated that the aqueous, ethanol and acetone extract of commonly available plant *Sesbania grandiflora* leaves was extensively investigated for its hepatoprotective potential against CCl₄ induced hepatotoxicity. There was a significant increase in serum levels of bilirubin, alanine transaminase, aspartate transaminase and alkaline phosphatase with a decrease in total protein level, in the CCl₄ treated animals, reflecting liver injury. In the extracts treated animals there was a decrease in serum levels of the markers and significant increase in total protein, indicating the recovery of hepatic cells. A strong conclusion can be drawn that, extract of *Sesbania grandiflora* possess most significant (p<0.001) hepatoprotective activity compared with the standard drug silymarin. So that the development of medicines using the extracts of plant materials or bioactive compounds with standards of safety and efficacy can revitalize treatment of liver disorders and hepatoprotective activity.

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