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HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *SESBANIA GRANDIFLORA* AGAINST CCl₄-INDUCED HEPATIC INJURY IN 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 an 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 at assessing the hepatoprotective activity of aqueous, ethanol and acetone extract of *Sesbania grandiflora* leaves against carbon tetrachloride (CCl₄) induced liver damage in Albino rats. Silymarin as a 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 restore biochemical enzymes and bring down to normal as compared to standard drug silymarin. This results shows and confirms the significant protective activity against CCl₄ induced hepatotoxicity.

INTRODUCTION: Liver is a very important organ in the human body. It regulates metabolic functions such as detoxification and plays a vital role in biochemical conversion. During the process of elimination, there is a chance of different accumulation kinds of toxic materials inside the hepatocytes, and there is a 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 the liver is caused by over dosages of drugs, toxic chemicals, viruses, bacteria and parasites ². Hepatotoxicity is a slight change in hepatic structure and function which may result in hypertension, ascites, jaundice, increased bleeding and cause multiple metabolic changes affecting other organs ^{3,4}. The magnitude of derangement of the 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 on 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 the treatment of liver disorders⁸. Hepatoprotective effect of some plants like *Spirulina maxima*⁹, *Eclipta alba*¹⁰, *Boehmeria nivea*¹¹, *Cichorium intybus*¹², and *Picrorhiza kurroa*¹³, *Boswellia serrata*⁴, *Psidium quajava*¹⁴, *Coccinia indica*¹⁵ has been well documented.

Sesbania grandiflora **Fig. 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 helminths^{16, 17}. The bark, leaves, flowers, and roots are also used medically herbs distributed in the tropical regions of the globe¹⁸. Juice of leaves and flowers is a 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.



FIG. 1: SESBANIA GRANDIFLORA LEAVES

Juice of flower is ideal as expectorant¹⁹. The leaves of the plant have been reported to have anxiolytic and anticonvulsant effect while the flowers have been reported to have anti-microbial activity²⁰. 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.

MATERIALS AND METHODS:

Chemicals: Analytical grade carbon tetrachloride, 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 were prepared by subjecting a 100 g of dried powdered leaves into 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 150-170g 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 tetrachloride (2 g/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 (300 mg/kg body weight) along with CCl₄ (2 g/kg body weight) for 7 days.

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

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

Silymarin was used as a positive control for comparing the 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 sacrificed, and blood samples were collected in glass tube from retro-orbital puncture to obtain hemolysis 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 45 min at 4 °C. After completion of the 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 was 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 **Fig. 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 a conventional indicator of liver injury.

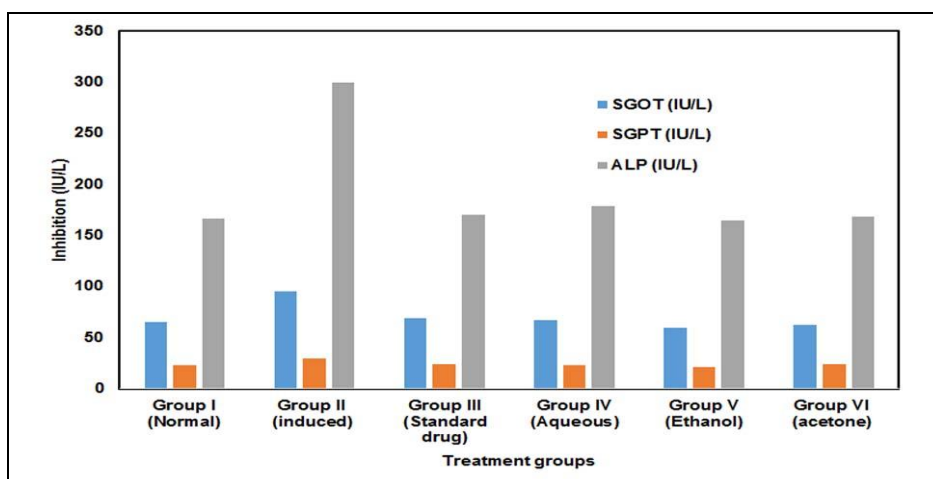


FIG. 2: HEPATOPROTECTIVE ACTIVITY IN CARBON TETRACHLORIDE INDUCED HEPATOTOXIC MODEL SHOWS CHANGES SERUM ENZYMES SGOT, SGPT, AND ALP IN SERUM

TABLE 1: HEPATOPROTECTIVE ACTIVITY IN CARBON TETRACHLORIDE INDUCED HEPATOTOXIC MODEL SHOWS CHANGES IN 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)

However, these levels were reversed to near normal levels of group I animals with the 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 have restored 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 the case of bilirubin and total protein, there was a noticeable increase, *i.e.* in serum levels treating with CCl_4 . Treatment with aqueous, ethanol and acetone extract has reversed the serum bilirubin

and total protein in serum levels to $(0.50 \pm 0.07$ and 6.85 ± 0.12 mg/dl), $(0.45 \pm 0.09$ and 6.59 ± 0.32 mg/dl), $(0.47 \pm 1.06$ and 6.26 ± 1.07 mg/dl), respectively which are statistically highly significant ($P < 0.001$) when compared with CCl_4 treated animals. 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.

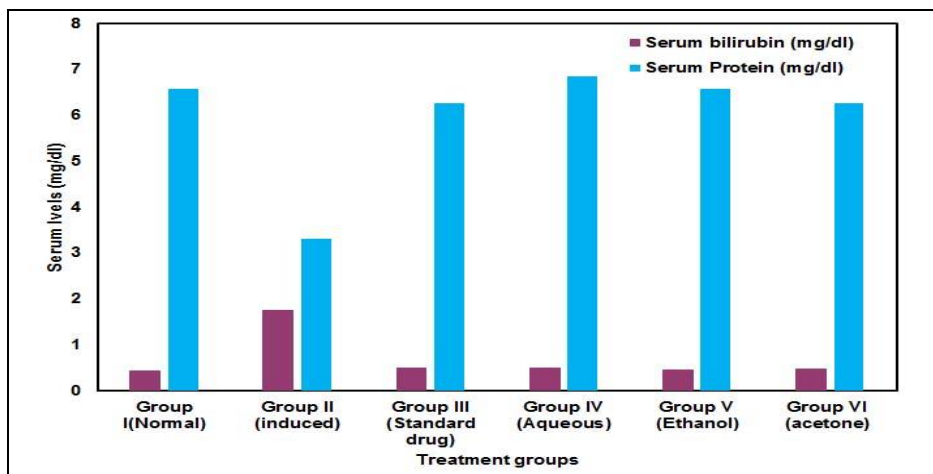


FIG. 3: HEPATOPROTECTIVE ACTIVITY IN CARBON TETRACHLORIDE INDUCED HEPATOTOXIC MODEL SHOWS CHANGES SERUM BILIRUBIN AND TOTAL PROTEIN

TABLE 2: HEPATOPROTECTIVE ACTIVITY IN CARBON TETRACHLORIDE 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).

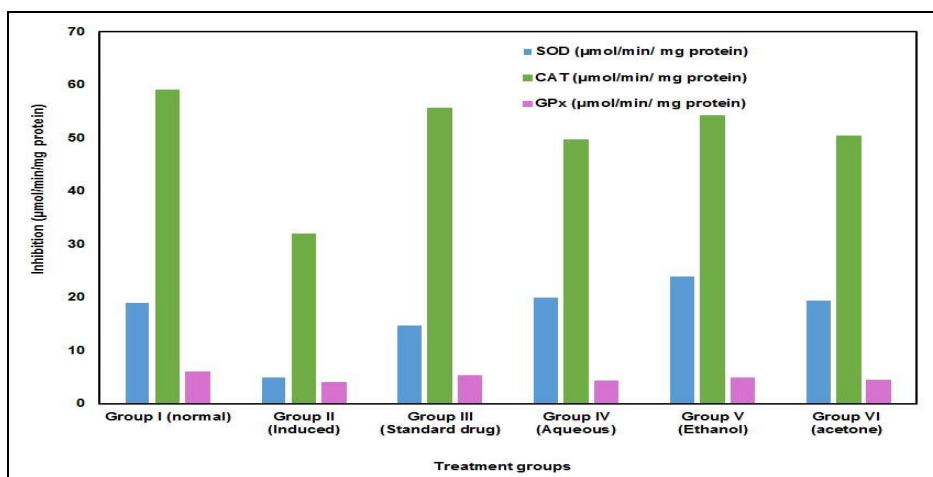


FIG. 4: ANTIOXIDANT LEVELS IN CARBON TETRA CHLORIDE INDUCED HEPATOTOXIC MODEL SHOWS CHANGES IN THE LEVELS OF SOD, CAT AND GPx

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).

The extract at a dose of 300 mg/kg body wt. exhibited orally, the 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 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 **Table 3, Fig. 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 show significant activity near to the normal group of animals.

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 CCl_4 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 CCl_4 treated animals, reflecting liver injury.

In the extracts treated animals, there was a decrease in serum levels of the markers and a significant increase in total protein, indicating the recovery of hepatic cells. A strong conclusion can be drawn that, extract of *Sesbania grandiflora* possess the 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

the treatment of liver disorders and hepatoprotective activity.

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CONFLICT OF INTEREST: Nil

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