International Journal of Phytomedicine 8 (2016) 294-299
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Hepatoprotective activity of aqueous extract of *Sesbania grandiflora* Linn leaves against carbon tetrachloride induced hepatotoxicity in Albino rats

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Abstract

The present study was conducted to evaluate the hepatoprotective activity of aqueous extract of leaves of *Sesbania grandiflora* Linn (AESG) at the dose of 250 and 500 mg/kg body weight per oral using Carbon tetrachloride induced liver damage in wistar albino rats. Aqueous extract showed significant ($p<0.05$) hepatoprotective effect by lowering the serum levels of various biochemical parameters such as serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvates transaminase (SGPT), alkaline phospatase (ALP), total bilirubin (TBL), total cholesterol (CHL) and by increasing the levels of total protein (TPTN) and albumin (ALB), in the selected model. These biochemical observations were inturn confirmed by histopathological examinations of liver sections and were comparable with the standard hepatoprotective drug Silymarin (100 mg/kg body weight i.p.) which served as a reference control. It was concluded from the result that the aqueous extract of *Sesbania grandiflora* L. possesses hepatoprotective activity against Carbon tetrachloride induced hepatotoxicity in rats.

Keywords: Hepatoprotective effect, Histopathological Studies, Biochemical Parameter, Carbon Tetrachloride, *Sesbania grandiflora*

Introduction

Liver disease is a collective term for a whole group of problems that afflict the tissues, structures and cells of the human liver. The liver performs a multitude of important functions, so there's plenty of opportunity for something to go wrong. One of the most common causes of liver disease is inflammation, which often results from abuse of alcohol, poor diet or even malnutrition [1]. Drug induced liver injury or liver dysfunction is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. According to the United States Acute Liver Failure Study Group, drug induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by over dose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs [2]. Liver-cell injury caused by various toxic chemicals (certain antibiotic, chemotherapeutic agents, carbon tetrachloride (CCl_4), thioacetamide (TAA) etc.), excessive alcohol consumption and microbes [3]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

Sesbania grandiflora (family: Fabaceae) is known as agati or the hummingbird tree (or scarlet wisteria), a small tree believed to have originated either in India or Southeast Asia and grows primarily in hot and humid tropical areas in the world. A native to Asian countries such as India, Malasia, Indonesia and the Philippines where it is commonly seen growing on the dikes between rice paddies, along roadsides and in backyards vegetable gardens [4]. The whole plant contains Grandifloral, arginine, cystine, histidine, isolucine, phenylalanine, tryptophan, valine, threonine, alanine, aspargine, aspartic acid and a saponin yielding oleanolic acid, galactose, rhamnose and glucuronic acid [5-7] and it also contains flavonol glycoside, kaempfrol [8]. The root-bark of the red-flowered variety is useful in vitiated condition of *vata* and arthralgia. The bark is astringent, cooling, bitter, tonic, anthelmintic and febrifuge. The pounded bark is externally applied to cure scabies. The juice of the bark is good for dyspepsia, diarrhea and gastralgia [9]. The leaves are acrid, bitter, sweet, cooling, aperient, tonic and diuretic and contain a non-poisonous saponine like substance. The leaf juice is used is nasal catarrh [10], nyctalopia and cephalagia. Leaves are chewed to disinfect mouth and throat and are useful in stomatalgia [6]. The flowers are cooling, bitter, astringent, acrid and antipyretic.

The juice of the flowers is applied to the eyes for nyctalopia and is used for intermittent fevers. The fruits are sweet, bitter, laxative and alexiteric and are useful in flatulent-colic, astringent, cooling, bitter, tonic, anthelmintic, febrifuge, cure scabies, dyspepsia, diarrhea and gastralgia, astringent, antipyretic, for nyctalopia naemia, emaciation and vitated conditions of *tridosa*[A]. Ethanol extract of *Sesbania grandiflora* of both leaves and flowers showed anticancer activity in Swiss albino mice against Ehrlich Ascites Carcinoma cell line at the doses of 100 and 200 mg/kg i.p [11]. Antioxidant and Cardioprotective effect was evaluated in rats with the dose of 1000 mg/kg bw of *Sesbania grandiflora* aqueous suspension [12,13]. The leaf juice of *S. grandiflora* showed significant antiurolithiatic activity against calcium oxalate-type stones in rats [14]. The ethanol leaves extract showed significant protective effect against erythromycin estolate-induced hepatotoxicity [15]. The anticonvulsive activity of *S. grandiflora* leaves was evaluated using a variety of animal models of convulsions [16]. Wound healing activity of methanol extract of bark of *Sesbania grandiflora* (L.) Poir had been evaluated by using excision wound model in Wistar albino rats [17]. Seed oils of *Sesbania grandiflora* were investigated for their anthelmintic property against Pheritima pasthuma [18].

Materials and methods

Plant material

The leaves of Sesbania grandiflora were collected from Tripura, India in the month of October 2012. The plant was authenticated by Dr. A.P. Singh, Principal Investigator, Weed Control, Dept. of Agronomy, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India. A voucher specimen of the plant was preserved in the herbarium for further reference (Agro/WC/13/252).

Preparation of the extract

Leaves of Sesbania grandiflora were washed under running tap water and dried in shade for seven days. Dried leaves were mechanically reduced to a coarse powder and then sieved and stored in an air tight container at room temperature. The extraction method was based on the presence of active constituents in the drug, using various solvents ranging from non-polar to polar. Dried powder (500 g) was extracted sequentially with hexane, dichloromethane, ethanol and distilled water by using soxhlation method. The extracts were concentrated to dryness by distilling the solvent at low temperature using rotary evaporator. The extracts were preserved in refrigerator at 4OC.

Preliminary phytochemical analysis

The aqueous extract was then subjected to preliminary phytochemical analysis19,20 to assess the presence of various phytoconstituents, it revealed the presence of flavonoids, alkaloids, phenolic compounds, terpinoids and saponins.

Animals

Wistar albino rats weighing 150 to 200 g of either sex maintained under standard husbandary conditions (temp 23±2oC, relative humidity 55±10% and 12 hours light dark cycle) were used for the screening. Animals were fed with standard laboratory food and ad libitum during the study period. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee.

Toxicity studies

Acute toxicity study was performed for aqueous extract of Sesbania grandiflora leaves (AESG) according to method of OECD guidelines21. Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract was administered orally at the dose of 300 mg/kg and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 500, 1000 & 5000 mg/kg body weight. The animals were observed for toxic symptoms for 72 h.

Carbon tetrachloride induced hepatotoxicity22

Albino wistar rats of either sex weighing between (150-200gms) were divided into five groups of six animals each. Animals were treated for a period of 7days. Group I received distill water for 7 days. Group II received CCl4 (30% in liquid paraffin 1 ml/ kg body weight, i.p.) once in every 36 hr. Group III were received daily oral dose of Silymarin (25 mg/kg p.o.) once in a day along with CCl4. Group IV and V were received aqueous extract of Sesbania grandiflora (Linn) leaves with dose of 250 and 500 mg/kg p.o. respectively. In this study the role of Silymarin was used as a positive control, as well as the hepatoprotective potential of different doses of Sesbania grandiflora (Linn) leaves was compared with the effect of silymarin. On 8th day, animal were sacrifed and blood was collected by puncturing the retro-orbital plexus and serum was separated and used for determination biochemical parameters.

Assessment of liver function

Blood was collected from all the groups by puncturing the retro-orbital plexus and was allowed to clot at room temperature and

serum was separated by centrifugation at 2500 rpm for 10 min. The serum was used for estimation of biochemical parameters to determine the functional state of the liver. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by a UV kinetic method based on the reference method of International federation of Clinical Chemistry in which both SGOT and SGPT were assayed based on enzyme-coupled system; where keto acid formed by the aminotransferase reacts in a system using NADH. The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm is measured. For SGOT malated dehydrogenase is used to reduce oxaloacetate to malate where as for SGPT the pyruvated formed in the reaction is converted to lactate by lactate dehydrogenase. Alkaline phosphatase (ALP) was estimated by method described by23 involving hydrolysis of p-nitrophenol which gives strong yellow colour in alkaline solution. The increase in absorbance due to its formation is directly proportional to ALP activity; while total bilirubin (TBL) by24 which ivolves the reaction of bilirubin with diazotized sulphanilic acid to form an azo compound, the colour of which is measured at 546 nm. Total cholesterol (CHL) was determined by CHOD-PAP Method25 in which the free cholesterol is hydrolysed by cholesterol oxidase to cholestenone-4-en-3-one and hydrogen peroxide. Hydrogen peroxide by the action of peroxidase liberates oxygen which reacts with 4- amino antipyrine and phenol to form red coloured compound which is measured at 500nm. Total protein (TPTN) was estimated by Biuret method26 where proteins produce a violet colour complex with copper ions in an alkaline solution. The absorbance of the colour complex is directly proportional to the protein in the sample, while the albumin (ALB) was estimated by BCG27 involving formation of blue-green complex with bromocresol green at slightly acidic pH which is measured photometrically.

Histopathological studies

The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Boucin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12h, then embeded in paraffin using conventional methods28 and cut into 5 m thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Statistical analysis

The values Mean±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analyzed separately and one-way analysis of variance (Gennaro, 1995) was carried out and the individual comparisons of the group mean values were done using Dunnet's test29.

Results

Acute toxicity studies

Aqueous extract of Sesbania grandiflora (Linn) leaves did not produce any toxic symptoms or mortality up to the dose level of 5000 mg/kg body weight in rats, and hence the extract was considered to be safe and non-toxic for further pharmacological screening.

Hepatoprotective activity

The results of Carbon tetrachloride induced hepato-toxicity were shown in Table1. In the Carbon tetrachloride control group, the significant acute hepato cellular damage, and biliary obstruction was indicated by the elevated level of SGPT, SGOT, ALP, TBL and CHL and decreased levels of TPTN and ALB. But the group which received the test drug of aqueous extract at the dose of 250 and 500 mg/kg body weight p.o showed a significant decrease in the elevated levels of SGPT, SGOT, ALP, TBL and CHL and significant increase in the reduced levels of TPTN and ALB and these biochemical parameters are comparable with the standard silymarin hepatoprotective drug. Therefore, the silymarin and the aqueous extract restored the altered level of enzymes significantly ($P<0.01$).

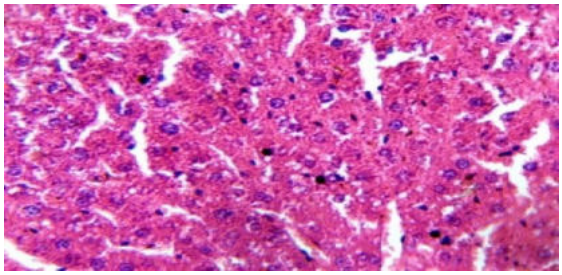
Table1 Effect of *Sesbania grandiflora* Linn leaves extract in CCl_4 induced hepatotoxicity in rats on various biochemical parameters.

Gr No	Treatment	Does (mg/kg b.w.)	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)	TBL (mg/dl)	CHL (mg/dl)	TPTN (gm/dl)	ALB (gm/dl)
I	Normal Control	10 ml/kg Distilled water	36.35± 0.92	41.49±1.50	37.17±1.84	0.22± 0.01	6.95± 0.05	16.40±0.16	4.1± 0.14
II	Toxicant Control	CCl_4 1 ml/kg	132.2± 2.30	182.9±1.35	80.98±2.37	1.39± 0.08	13.44±0.66	6.25± 0.65	1.23± 0.27
III	Silymarin	100 mg/kg + CCl_4	49.68± 0.66**	83.33±0.75**	38.47±2.50**	0.45± 0.05**	5.55± 0.22**	14.88± 055**	4.5± 0.21**
IV	AESG	250 mg/kg + CCl_4	87.3± 3.21*	125.4±1.65	60.2± 0.69*	1.08± 0.07	4.10± 055**	11.05±0.31*	3.22± 0.53*
V	AESG	500 mg/kg + CCl_4	72.14±0. 87**	102.77± 0.98**	35.47±0.77***	0.71± 0.05**	5.01± 0.08**	13.12±0.78**	3.95± 0.36**

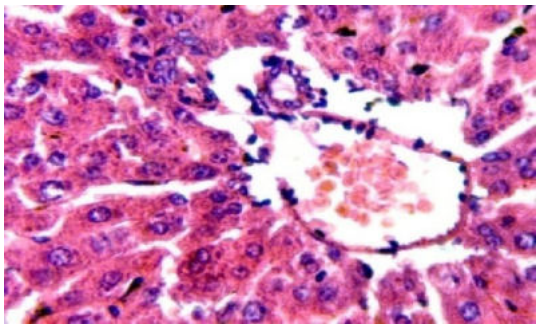
*: P<0.05, **: P< 0.01 as compared to control group

Histopathological studies

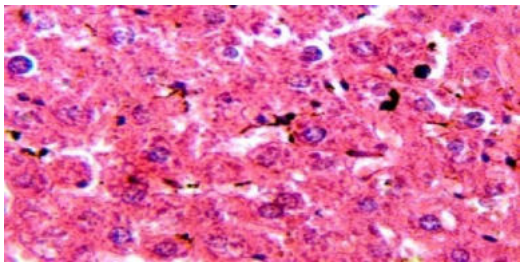
Histopathological liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein (Fig 1a). Disarrangement of normal hepatic cells with necrosis and vacuolization are observed in Carbon tetrachloride intoxicated liver (Fig 1b). The liver sections of the rat treated with 250 and 500 mg/kg body weight p.o of aqueous extract of Sesbania grandiflora (Linn) leaves followed by Carbon tetrachloride intoxication (Fig 1d and 1e) showed less vacuole formation and absence of necrosis and overall less visible changes observed were comparable with standard Silymarin (Fig 1c), supplementing the protective effect of the test drug and the standard hepatoprotective drug.



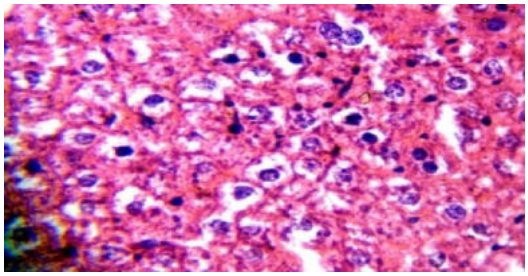
1a – Control



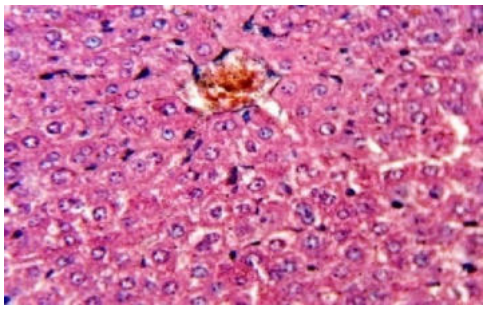
1b – CCl_4 treated group



1c – Standard Silymarin group



1d – Test extract (250 mg/kg)



1e – Test extract (500 mg/kg)

Discussion

The present studies were performed to assess the hepatoprotective activity in rats, against Carbon tetrachloride as hepatotoxin to prove its hepatoprotective effect against liver disorder. The changes associated with Carbon tetrachloride induced liver damage of the present study appeared similar to the acute viral hepatitis30. Carbon tetrachloride is a widely used experimental hepatotoxicant, is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca2+ haemostasis and finally result in cell death31.

Animals of Group II (received Carbon tetrachloride) significantly lost their body weight and showed reduced food consumption as compared to control group. Animals of Group III, IV and V(received Carbon tetrachloride plus 250 and 500 mg/kg body weight of test extract and standard drug Silymarin 100mg/kg body weight) showed a significant increase in body weight and food consumption when compared to Carbon tetrachloride group animals. These findings suggested the extract administered has significantly neutralized the toxic effects of Carbon tetrachloride and helped in regeneration of hepatocytes32.

Estimating the activities of serum marker enzymes, like SGPT, SGOT, ALP can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepato cellular damage33. The tendency of these enzymes to return to near normally in extract administered group is a clear manifestation of antihepatotoxic effects of the extract.

The levels of total protein and albumin were reduced due to the Carbon tetrachloride-induced hepatotoxicity. The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulam which result in the loss of P-450 leading to fatty liver. Inhibiting of bile acids synthesis from cholesterol which is synthesis in liver or derived from plasma lipids, leading to increase in cholesterol levels were also resulted due to Carbon tetrachloride intoxication suppression of cholesterol levels by the extract suggest the bile acid synthesis inhibition was reversed. Reduction in the levels of SGPT and SGOT towards the normal value is an indication of regeneration process. Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stability of the biliary function during injury with Carbon tetrachloride. The protein albumin levels were also raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. This hepatoprotective effect exhibited by the aqueous extract of Sesbania grandiflora (Linn) leaves at the dose level of 250 and 500 mg/kg body weight was comparable with the standard drug, A Silymarin.

Histopathological liver sections also revealed that the normal liver architecture was disturbed by hepatotoxin in Carbon tetrachloride group, whereas in the liver sections of the rat treated with the aqueous extract and intoxicated with Carbon tetrachloride the normal cellular architecture was retained and it in comparable with the standard Silymarin group, hence confirming the significant hepato protective effect of extract of Sesbania grandiflora (Linn) leaves at the dose of 250 and 500 mg/kg body weight.

In accordance with these results, it may be confirmed due to the presence of phytoconstituents such as flavonoids and alkaloids which are present in the aqueous extract could be considered as, responisible for the significant hepatoprotective activity. In conclusion, it can be said that the aqueous extract of Sesbania grandiflora (Linn) leaves exhibited a hepato protective effect against Carbon tetrachloride induced hepatotoxicity. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hepatoprotective efficacy of this valuable medicinal plant.

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