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# Hepatoprotective potential of ethanolic and aqueous extract of flowers of *Sesbania grandiflora* (Linn) induced by $CCl_4$

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ABSTRACT

**Objective:** To investigate the hepatoprotective activity of ethanolic and aqueous extract of *Sesbania grandiflora* (Linn) flower in $CCl_4$ induced hepatotoxicity models in rats. **Methods:** The ethanolic and aqueous extract of *Sesbania grandiflora* (Linn) flower are screened for its hepatoprotective activity in $CCl_4$ (0.5 ml/kg, i.p) induced liver damage in Swiss albino rats at a dose of 200 mg/kg bw. **Results:** The ethanolic and aqueous extract of *Sesbania grandiflora* (Linn) flower significantly ($P<0.001$) decreases the biochemical parameters (SGOT, SGPT, ALP, TP, and TB). Silymarin (25 mg/kg), a known hepatoprotective drug used for comparison exhibited significant activity ($P<0.001$). The extract did not shown any mortality up to a dose of 2000 g/kg bw. These findings suggest that the ethanolic and aqueous extract of *Sesbania grandiflora* (Linn) flower (500mg/kg) was effective in bringing about functional improvement of hepatocytes. The healing effect of this extract was also confirmed by histological observations. **Conclusions:** The ethanolic extract at doses of 250 and 500 mg/kg, p.o. and aqueous extract at a dose of 500 mg/kg, p.o. of *Sesbania grandiflora* (Linn) flower have significant effect on the liver of $CCl_4$ induced hepatotoxicity animal models.

## 1. Introduction

Liver is one of the important organs of the body which plays a major role in the metabolism of proteins, carbohydrates, lipids. It is also having wide range of functions including detoxification, storage of glycogen, vitamin A, D and B12, production of several coagulation factors, growth factors (IGF-1), hormones (angiotensinogen) and biochemicals necessary for digestion (bile). Hepatic damage occurs due to its multi dimensional functions, various xenobiotics and oxidative stress leading to distortion of all of its functions [1]. Liver disease is still a worldwide health problem. Jaundice and hepatitis are two major hepatic disorders that account for high death rate [2]. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometime can have serious side effect

*Sesbania grandiflora* (family Leguminosae) commonly called as Agati in Hindi. The literature survey revealed that libido [3], night blindness [4], anti-ulcer [5], antioxidant [6-7], cardioprotective [8], emollient, astringent,analgesic, antipyretic [9], anti-obesity [10], ozoena, dim vision [11],gout and bronchitis [12], anxiolytic, anticonvulsant [13], hepatoprotective [14] and anthelmintic [15] activity of *Sesbania grandiflora* flower has been already reported. The active ingredients of S. grandiflora are leucocyanidin and cyanidin present in seeds, oleanolic acid and its methyl ester and kaemferol-3-rutinoside which are present in flower. The bark contains tannins and gum. Saponin isolated from the seeds. Sesbanimide isolated from seeds [16]. Based on its diversified pharmacological properties and its used in liver disease, the objective of the present studywas to evaluate the hepatoprotective potential of ethanolic and aqueous extract of flowers of *Sesbania grandiflora* (Linn)

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induced by $CCl_4$.

## 2. Material and method

### 2.1 Plant collection and Preparation of extract

*Sesbania grandiflora* flowers was collected from the surrounding fields of Harapanahalli, Davengere, Karnataka, india in the month of July 2009. The plant material was identified and authenticated by Professor K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli, and voucher specimen of the collected sample was deposited in the departmental herbarium for further reference. The flowers were washed with doubled distilled waternto eliminate dirt, then shade dried. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powered material was extracted by using soxhlet column and extracted with 70% ethanol (60 - 800) and distilled water for 24 hrs. The extract were filtered, pooled and concentrated at 5oC on a rotatory flash evaporator and then dried on hot water bath (70 - 800). The dried extract was stored in airtight container in refrigerator below 100C. Further dilutions were made to obtain different concentration and were used pharmacological investigation.

### 2.2 Experimental animal

The male albino rat wistar stain 150 - 200 g and albino mice 20 - 30 g were procured from Venkateshwara Associates, Bangalore, Karnataka, and kept in conventional cages with free access to water ad libitum and standard rat feed with rodent pellet diet (Gold mohr, Lipton India Ltd.,) at 27 ± 30C, humidity 65 ± 10%, 12 hrs light/dark cycle, respectivily. All the experiments were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgaated by the Institutional Animal Ethics Committee (IAEC) [17].

### 2.3 Determination of acute toxicity (LD50)

After acclimization of the animals for 4-5 days, study was carried out on healthy, young adult Albino swiss female mice (20-25 g), nulliporous and non pregnant were used for this study. Food, but not water was with held for 3-4 hr and further 1-2 hr post administration of smple under study. Fixed dose level of 5, 50, 100, 250, 500 mg/kg were initially chosen as dose level that would be expected to allow the identification of dose producing evident toxicity. During the validation procedure, a fixed dose of 2000 mg/kg was added to provide more information on substance of low acute toxicity.

After the administration of extract of formulation, animals were observed individually during the first 30 min and periodically during 24 hr with special attention during the first hours and daily thereafter for a period of 14 days. Once daily animals were observed principally in relation to change in skin, fur, eyes and mucous membrane (nasal) and also autonomic symptoms like sedation, lacrimation, perspiration, pilorection, urinary incontinence and control nervous system (ptosis, drawsiness, gait tremors and convulsions). Fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies.

### 2.4 Experimental design (Malaya gupta et al., was followed [18])

Animals were divided into seven groups of six rats each. Animals were treated for a period of 10 days. Group I received a single daily dose of CMC (1ml of 1% w/v) for 10 days. Group II received CCl4 (30% in liquid paraffin 1 ml/kg body weight, i.p.) once in every 72 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 received ethanolic extract (250 and 500 mg/kg p.o.) and group VI and VII were received aqueous extract of *Sesbania grandiflora* (Linn) flower 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) flower was compared with the effect of silymarin. On 11th day, animal were sacrifed and blood was collected by carotid bleeding for biochemical analysis.

The liver tissue was dissected out for histological investigation and fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xyline, and embedded in paraffin. Section were prepared and then stained with hematoxylin and eosin (H-E) dye for photomicroscopic observation, including cell necrosis, fatty changes hyaline generation, ballooning generation. All samples were observed and photographed with microscope.

### 2.5 Determination of biochemical parameters

The collected blood was allowed to clot and centrifuged at 3000 rpm for 15 min to obtain the serum. The biochemical parameters like serum glutamate-pyruate (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP) [19-21] were estimated using respected assay kits according to the methods described by the manufacturers.

### 2.6 GSH estimation in CCl4 induced hepatotoxicity

Tissue samples were homogenized in ice cold Trichloroacetic acid (500mg tissue plus 5 ml 10% TCA) in an ultra turrax tissue homogenizer. Glutathione measurements were performed using a modification of the Ellamn procedure (Aykae, et. al.,) [22]. Briefly, after centrifugation at 3000 rpm for 10 minutes, 0.5 ml supernatant was added