

Research Article



Hepatoprotective Activity of *Acacia senegal* Pod against Carbon Tetrachloride-Induced Hepatotoxicity in Rats

Rishi Pal¹, Mangal Sain Hooda^{1,2*}, Chain Singh Bias¹, Janardhan Singh³

¹Jodhpur National University, Jodhpur, 342003. Rajasthan, India.

²Janta College of Pharmacy, Butana, (Sonapat), 131302. Haryana, India.

³Pt. B.D. Sharma PGIMS, Rohtak, 124001. Haryana, India.

*Corresponding author's E-mail: mhooda1968@gmail.com

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ABSTRACT

Many synthetic components show toxic and/or mutagenic effects. Hence attention has been given to naturally occurring hepatoprotective components. Identification of effective hepatoprotective components from plants origin is an ideal strategy for new drug development. *Acacia Senegal* Willd (Leguminosae), known as Arabic gum tree and traditionally used for several medicinal purposes. The *Acacia Senegal* pod extract was investigated for the reduction of the risk on CCl₄-induced acute hepatotoxicity in rats. The toxicant CCl₄ was used to induce hepatotoxicity at a dose of 1 ml/kg as 1:1 mixture with olive oil. Pod hydro alcoholic extract of *Acacia senegal* was administered in the doses of 400 and 800 mg/kg/day orally for 7 days. Silymarin (100 mg/kg) was used as standard drug. The hepatoprotective effect of these extracts was evaluated by the assessment of biochemical parameters such as serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin and histopathological studies of the liver. Treatment of animals with Pod hydro alcoholic extract significantly reduced the liver damage and the symptoms of liver injury by restoration of architecture of liver as indicated by lower levels of serum bilirubin as compared with the normal and silymarin-treated groups. Histology of the liver sections confirmed that the extract prevented hepatic damage induced by CCl₄ showing the presence of normal hepatic cords, absence of necrosis, and fatty infiltration. The pod hydro alcoholic extract of *Acacia senegal* showed significant hepatoprotective activity against CCl₄-induced liver injury model.

Keywords: *Acacia senegal*, hepatoprotective effect, histology, CCl₄.

INTRODUCTION

The liver is the second largest organ in the body, and is often seen as the most important one. The liver receives a dual blood supply with about 20% of blood coming from the hepatic artery and 80% from the portal circulation. The blood flow to the liver is around 20 to 25% of the total cardiac output. Toxins, infectious agents, medications, and serum inflammatory mediators may result in a diverse range of disease processes, leading to loss of normal histological architecture, reduced cell mass, and loss of blood flow. Consequently, functional liver capacity may be lost. Efforts have been made to search for effective hepatoprotective agents. However, no effective hepatoprotective therapies are available until now. Therefore, the prevention of liver diseases has a great significance both in theory and in practice¹. Herbal drugs play a major role in the treatment of hepatic disorders. In the absence of reliable hepatoprotective drugs in modern medicine, a number of medicinal plants and their formulations are used to cure hepatic disorders in traditional systems of medicine in India².

Several plants containing flavonoids, rutin, silymarin, and quercetin have been reported to possess a hepatoprotective potential³⁻⁵. Hence, it was decided to evaluate whether this activity could be related to this family. In this study, pods hydro alcoholic extract (AS Pod-HA) of *Acacia Senegal Willd* was investigated for the hepatoprotective activity against carbon tetrachloride (CCl₄)-induced toxicity.

Acacia Senegal willd (Leguminosae) also known as Arabic gum tree, locally it is called Kumbat in Sind⁶ Rajasthan and Lasbella it is called Kher^{7,8}, distributed mainly in tropical and sub tropical region of southern part of West Pakistan and India in Jaipur and Jodhpur, the species grows to 2-15m tall with a flat or rounded crown⁹. *Acacia Senegal* (AS) leaves are small, grey-green, alternate cream colored flowers occur on 2-12cm long spikes, pods are dehiscent (open by splitting at maturity) and seeds greenish-brown¹⁰. The tree is highly valued for centuries for gum arabic production, which is used in food, pharmaceuticals and other industries in the USA and Europe¹¹. Gum Arabic is approved for use as food additives by the US Food and Drug Administration and in the list of substances that are generally recognized as safe¹². Folk medicine, demulcent, emollient, the gum is used in inflammation of intestinal mucosa, and externally to cover inflamed surfaces, as burns and nodular leprosy. It is also said to be used for antitussive, astringent, cold coughs, diarrhea, dysentery, expectorant gonorrhoea, sore throat and urinary track¹³. Various chemical studies on *Acacia Senegal* gum contain neutral sugars (rhamnose, arabinose and galactose), acids (glucuronic acid and 4-methoxyglucuronic acid) calcium, magnesium, potassium and sodium¹⁴. Phytoconstituents, reported in the literature from plant AS (L.) Willd: Flavone, catechin, polyphenols, tannins, chalcones, alkaloids and flavonoids¹⁵. Various Pharmacological activities on *Acacia senegal* gum exudates offers protection against cyclophosphamide induced urinary bladder cytotoxicity¹⁶.



Scavenging of nitric oxide by gum arabic has been reported to limit the acetaminophen-induced hepatotoxicity in mice¹⁷. Other studies have documented the antioxidant properties of gum arabic in a variety of animal model system¹⁸. Antioxidant potential and free radical scavenging activity by pod extracts of *Acacia senegal*¹⁹ and Antidiabetic activity of *Acacia senegal* pod extract in streptozotocin-induced diabetic rats²⁰. Bark of AS are made into a poultice to treat bedsores and wounds. The investigation was initiated to identify wound-healing plants and lesser known edible plant in Oman that have high antioxidant capacity¹⁵. In present study we aimed to explore the hepatoprotective activity of hydro alcoholic extracts of *Acacia senegal* pod against CCl₄-induced acute hepatotoxicity in rats.

MATERIALS AND METHODS

Identification and authentication of plant material

The matured pod with seeds of *Acacia Senegal Willd* was purchased from Central Arid Zone Research Institute, Jodhpur, Rajasthan. (India), the plant material was authenticated by Dr. Ashok Kumar Sharma, M.D. (Dravyaguna Vigyan), Prof. & Head of Department Shri Baba Mastnath Ayurvedic Degree College, Asthal Bohar, Rohtak. (Haryana) India.

Extraction of *Acacia senegal* pod

Acacia senegal shed dried plant material (matured pods with seeds) were grinded and powdered material (100 g) was used for extraction. The hydro-alcoholic and aqueous extracts were prepared by hot continuous percolation method in a Soxhlet apparatus. Both extracts were collected separately and dried in vacuum system. Hydro-alcoholic extract with 70% ethanol (Pod-HA, yield: 12.45%) and Water extract with water (Pod-W, yield: 12.95%). Both extracts were condensed by re-distillation and dried in vacuum desiccators to obtain a final extract residue.

Drugs and chemicals

Serum was separated and analyzed for SGOT, SGPT, ALP and Total bilirubin using commercial kit of Aspen Lab, Span diagnostic LTD, and Merck India diagnostic respectively. Analysis was done using Rapid Bioautoanalyser (Star 21). Silymarin, CCl₄, olive oil, petroleum ether, carboxymethylcellulose (CMC) and all chemicals were of analytical grade.

Animals for the study

Albino wistar rats weighing 200 ± 25 gm were used for all experiments in present study. Animals were collected at random from Animal house of PBRI, Bhopal. All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (Reg No. - 1283/c/09/CPCSEA). Animals were housed in a group of four in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water ad libitum.

Animals were kept at 12:12, light: dark cycle. Animals were further divided in six groups with six animals in each group.

Acute toxicity study of the extract

Acute toxicity was determined in fasting mice. Animals were divided into groups of six each and the extract was administered orally with 1% CMC or 10, 30, 100, 300, 1000 and 1200mg/kg body weight of AS Pod-HA. The mice were observed continuously for 2nd, 4th, 6th, 12th, 24th, 48th and up to 72 hours²¹.

Experimental design

All animals were divided randomly into six groups with six animals in each group. Group I, received normal saline p.o. only and served as vehicle control, Group II, III, IV, V and VI were treated with carbon tetrachloride (CCl₄). Group II received saline, group III received silymerine, 100 mg/kg per day p.o, group IV received extract (200 mg/kg), group V received extract (400 mg/kg) and group VI received extract (800 mg/kg). All dosing of test samples was done p.o. for 7 days.

Assessment of hepatotoxicity

After overnight fasting (deprived of food for 16 h had been allowed free access to water), On the 1st day hepatotoxicity was induced in rats by CCl₄ suspension in olive oil (1:1 v/v) at a dose of 1 ml/kg body weight orally by oral gavage tube. On day 7, blood was collected by cardiac puncture under mild ether anesthesia. Serum was separated and analyzed for SGOT, SGPT, ALP and Total bilirubin²² using commercial kit of Aspen Lab, Span diagnostic LTD, and Merck India diagnostic respectively. Analysis was done using Rapid Bioautoanalyser (Star 21).

Histopathological studies

After draining the blood, liver samples were excised, washed with normal saline, and processed separately for histological observations. Initially, the material was fixed in 10% buffered neutral formalin for 48 hours. The sections were cut in 5-mm thickness, stained with hematoxylin and eosin, (H & E) and examined microscopically for histopathological changes.

Statistical analysis

All data were analysed by One way ANOVA followed by Bonferroni test. P <0.05 was considered as level of significance. All data are presented in mean ± SEM.

RESULTS

Acute toxicity studies

Acute toxicity studies revealed the nontoxic nature of the Pod-HA extract of *Acacia senegal*. There were no lethality or toxic reactions found at any of the doses selected until the end of the study period. All the animals were alive, healthy and active during the observation period.



Liver function test

The administration of CCl_4 to the animals resulted in a marked increase ($P < 0.05$) in TB, SGOT, SGPT and serum ALP. when compared with Group I (vehicle control) [Table-1]. The oral administration of pod hydro alcoholic

extract of *Acacia senegal* and silymarin significantly reduced the CCl_4 -induced increase in the SGOT, SGPT, ALP and TB levels ($P < 0.05$). when compared with Group II (CCl_4 -treated group).

Table 1: Effect of *Acacia senegal* pod-HA on CCl_4 -induced hepatotoxicity in rats

S. No.	Treatment	SGPT (IU/dl)	SGOT (IU/dl)	ALP (IU/dl)	Total Bilirubin (mg/dl)
1	Vehicle control	44.02±1.303	56.94±1.917	104.36±2.606	0.51±0.0188
2	CCl_4 control	96.56±1.876	111.80±2.761	206.98±3.018	1.28±0.0288
3	Silymerin-100mg/kg	56.74±2.130*	64.90±2.110*	117.56±2.981*	0.57±0.0206*
4	Pod-HA-200mg/kg	80.66±1.847*	89.14±2.499*	168.08±3.966*	1.11±0.0250*
5	Pod-HA-400mg/kg	67.64±1.683*	75.96±2.234*	141.78±3.144*	0.71±0.0210*
6	Pod-HA-800mg/kg	64.74±2.013*	70.26±1.559*	133.92±1.850*	0.69±0.0264*

Values are mean ± SEM; N = 6; * $P < 0.05$ Compared with CCl_4 Control Group 2; Silymerin and pod-HA; 200, 400, 800 mg/kg; Serum glutamate pyruvate transaminase (SGPT); Serum glutamate oxaloacetate transaminase (SGOT); Serum alkaline phosphatase (ALP); Total bilirubin (TB).

The toxic effect of CCl_4 was controlled in the animals treated with the extracts (Pod-HA-800mg/kg) by way of restoration of the levels of the liver function biochemistry nearly to that of the standard drug silymarin. Among the extract-treated groups, significant hepatoprotective activity was observed.

In the histopathological studies, the liver sections of rats treated with vehicle showed normal hepatic architecture [Figure-1], whereas that of CCl_4 -treated group showed total loss of hepatic architecture with intense peripheral central vein necrosis, fatty changes, congestion of sinusoids, Kupffer cell hyperplasia, crowding of the central vein, and apoptosis [Figure-2]. In case of rats treated with silymarin [Figure-3] and *Acacia senegal* pod hydro alcoholic extract 400 mg/kg [Figure-4] and 800 mg/kg [Figure-5], respectively, a normal hepatic architecture was seen with only moderate accumulation of fatty lobules and mild degree of cell necrosis, clearly indicating the protection offered by standard drug silymarin and the plant extract.

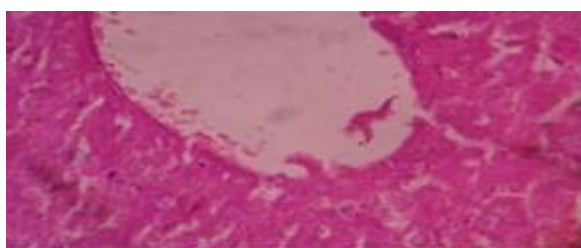


Figure 1: Liver cells of control group

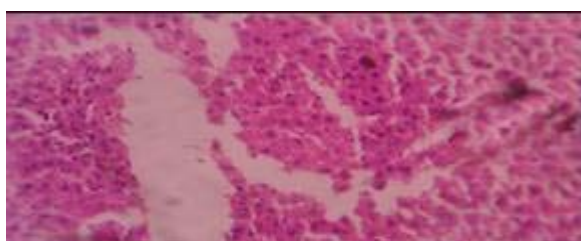


Figure 2: Liver treated with CCl_4

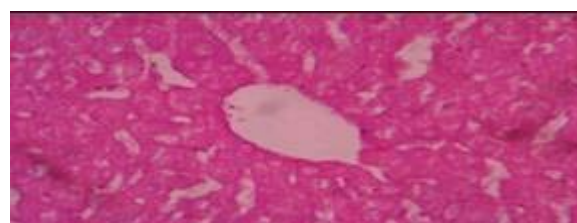


Figure 3: Liver treated with silymarin

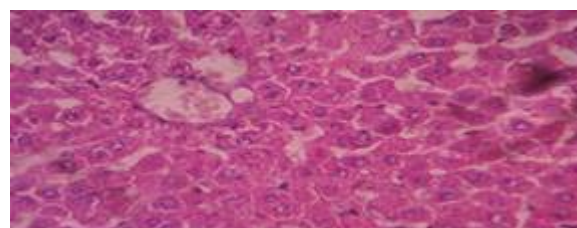


Figure 4: Liver treated with 400 mg/kg Pod-HA

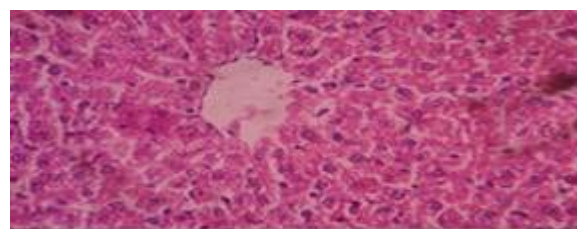


Figure 5: Liver treated with 800 mg/kg Pod-HA

DISCUSSION

Carbon tetrachloride is commonly used for induction of experimental liver toxicity. This toxic chemical causes per oxidative degradation in the adipose tissue, resulting in fatty infiltration of the hepatocytes. Its metabolites such as trichloromethyl radical (CCl_3) and trichloromethyl peroxy radical (CCl_3O_2) are involved in the pathogenesis of liver. As shown in Figure-2, CCl_4 causes change around the central vein in the liver and other oxidative damages with the leakage of marker enzymes like SGOT, SGPT, and ALP in the serum, increase in serum TB levels^{23,24}.

Administration of pod-HA extracts of *Acacia senegal* showed significant hepatoprotective activity, which was

comparable with the standard drug silymarin. The effect was more pronounced with pod-HA 800mg/kg extract. The quantitative phytochemical investigations on the hydro alcoholic extract of *Acacia senegal* also showed positive for flavonoids by ferric chloride, alkaline reagent, and Shinoda tests. Furthermore, it has been reported that the flavonoid constituents of plant possess antioxidant properties²⁵ and was found to be useful in the treatment of liver damage. Antioxidant potential and free radical scavenging activity by pod extracts of *Acacia senegal* as *in-vitro* basis¹⁹. Earlier works have reported the presence of flavone, catechin, polyphenols, tannins, chalcones, alkaloids and flavonoids in the hydro alcoholic extract. The results indicate that the hydro alcoholic extract of *Acacia senegal* has significant hepatoprotective activity. This may be probably due to the higher content of flavonoids. The earlier investigators have screened the hepatoprotective activity of the flavonoids, which is also claimed to have free radical scavenging property and it inhibits the lipid per-oxidation against CCl₄-induced hepatic toxicity. Antioxidant potential, free radical scavenging and excellent total antioxidant capacity of *Acacia senegal* pod-HA *in-vitro* system and screening of the pharmacological action against the liver damage is being investigated.

CONCLUSION

The results of the present study suggest that hydro alcoholic extract of *Acacia senegal* pod has significant hepatoprotective activity against CCl₄-induced liver injury model.

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