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Research Article

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Hepatoprotective Activity of Leaves of *Pongamia pinnata* In CCl_4 Induced Hepatic Model

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ABSTRACT

The liver is an organ of utmost importance, which plays a vital role in the metabolism of drugs entering the body. A variety of drugs and plant extracts were evaluated for their hepatoprotective activity. Present study was undertaken to ascertain the hepatoprotective effect of dried leaves of *Pongamia pinnata*. Chloroform and ethanolic extract of dried leaves of *Pongamia pinnata* was evaluated and subjected for hepatoprotective activity in two doses of 250mg and 500mg in wistar strain of albino rats against CCl_4 induced hepatic damage. SGOT, SGPT, ALP and total Bilirubin were used as biochemical marker for assessment of the activity. The Chloroform 500 mg and ethanolic extract 250 mg has found significant hepatoprotective activity by virtue of lowering the marker enzymes like SGPT, SGOT and ALP.

KEYWORDS *Pongamia pinnata*, Hepatoprotective activity, SGOT, SGPT, ALP, Bilirubin, CCl_4.

Introduction

Today we find a renewed interest in traditional medicines. Human beings are exposed to these compounds through environmental exposure, consumption of contaminated food or during exposure to chemical substances in the polluted environment. In addition, human beings consume a lot of synthetic drugs during diseased conditions, which are essential to treat the diseases but have a variety of side effects and produce a variety of toxic manifestations[1]. Conventional drugs used in the treatment of liver diseases are often inadequate. Modern medicines have little to offer for alleviation of hepatic ailments whereas most important representative is of phytoconstituents. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety [2-3].

Pongamia pinnata (Family: Leguminosae) is a medium sized glabrous semi-evergreen tree growing up to 18 m or higher, with a short bole, spreading crown with greyish green or brown bark. Leaves are imparipinnate, alternate, leaflets 5-7, ovate and opposite. This tree is popularly known as Karanja in Hindi, Indian Beech or *Derris indica* in English, and Hongae in Kannada. *Pongamia pinnata* occurs all over India in the bank of rivers, streams and planted as avenue tree in gardens. The leaves of *Pongamia pinnata* have been used in Ayurvedic medicine as digestive, laxative, anthelmintic, to cure piles, wounds healing, relieving rheumatic pains, for cleaning ulcers in gonorrhea and scrofulous enlargement[4]. Previous studies have demonstrated that *Pongamia pinnata* is rich in flavonoids and related compounds. Seeds and seed oil, flowers and stem bark yield karanjin, pongapin, pongaglabrone, kanugin, desmethoxykanugin and pinnatin[5]. Furanoflavonoid glucosides, pongamosides A-C, flavonol glucoside pongamoside D are also been reported [6].

Materials and Methods

Chemicals

CCl_4 was obtained from BDH and Merck Ltd. Mumbai and was employed as a 1:1 solution in liquid paraffin at a dose of 1.25 ml/kg b.w.p.o. 4% w/v aqueous acacia mucilage (1 ml/kg b.w.p.o.) was used as a vehicle. All biochemical and chemicals used for the experiments were of analytical grade.

Plant material

Leaves of *Pongamia pinnata* were collected from local forest area of sirsi in Western Ghats, Karnataka and authenticated by Prof. G. S. Naik (Botanist) of Department of Botany, G. C. Science and Art College, Ankola. A voucher herbarium specimen number GCSAC/PP/01 was also preserved in the same college. The collected material was chopped into small pieces and powdered to coarse consistency in cutter and grinder mill respectively. The powder passed through.40 # mesh particle size and stored in an airtight container at room temperature.

Animals

Healthy young female albino rats (Sprague-Dawley Strain) of weighing 150 to 250 g were selected for experiment &

were obtained from animal house of KLES College of Pharmacy, Belgaum and ethical clearance was granted by institutional ethical committee in resolution no. 1/18/2007 held on 23rd November 2007 at J N Medical college, Belgaum (Ethical committee IAEC reg. no.:627/02/a/CPCSEA). The animals were fed on a standard pellet diet (Goldmohar rat feed, Mumbai) & water ad libitum. All the protocols were performed in accordance with institutional animal ethical committee as per the direction of the CPCSEA (Committee for the purpose of control and supervision of experiments on animals).

Preparation of plant extract

The dried leaves were defatted with Petroleum ether (60-80^{0}C) for 72 hours and extracted with Chloroform and 95% ethanol in a Soxhlet assembly for 48-hours to get their extracts. These extracts were filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites [7]. Preliminary phytochemical analysis was carried out by different methods of phytochemical analysis [8]. A known volume of extract was suspended in distilled water and was orally administered to the animals by gastric intubation using a force feeding needle during the experimental period.

Acute oral toxicity studies [9]

The acute oral toxicity studies of extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted on 17th December 2001 received from CPCSEA, Ministry of social justice and empowerment, Govt. of India. Administration of the stepwise doses of ethanolic extract of *Pongamia pinnata* from 50 mg/kg b.w. up to the dose 5000 mg/kg b.w. caused no considerable signs of toxicity in the tested animals.

Table 1: Effect of *Pongamia pinnata* leaves extract on CCl_4 induced hepatic toxicity (After 3 days)

Groups	SGPT (IU/ml)	AST (IU/ml)	ALP (IU/ml)
Group I (Normal)	42.2 ± 2.4	68.4 ± 2.3	206.5 ± 5.6
Group II (Toxicant)	270.3 ± 2.1	334.7 ± 5.2	1026.1 ± 4.7
Group III (Toxicant + Chloroform extract of 250 mg/kg b.w.p.o.)	180.5 ± 3.6	196.2 ± 5.6	681.0 ± 7.2
Group IV (Toxicant + Chloroform extract of 500 mg/kg b.w.p.o.)	168.1 ± 4.1	201.8 ± 4.6	587.3 ± 9.1
Group V(Toxicant + Ethanolic extract of 250 mg/kg b.w.p.o.)	203.2 ± 3.6	248.2 ± 7.1	729.6 ± 8.2
Group VI(Toxicant + Ethanolic extract of 500 mg/kg b.w.p.o.)	217.1 ± 3.9	219.5 ± 6.9	692.1 ± 6.2

All values are expressed as mean ± SE (n = 6)

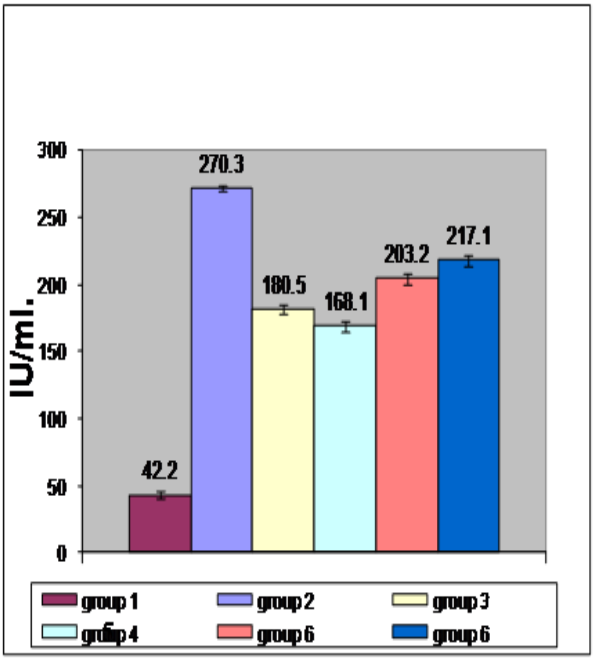

Fig. 1: Effect of *Pongamia pinnata* leaves extract on SGPT levels on rats

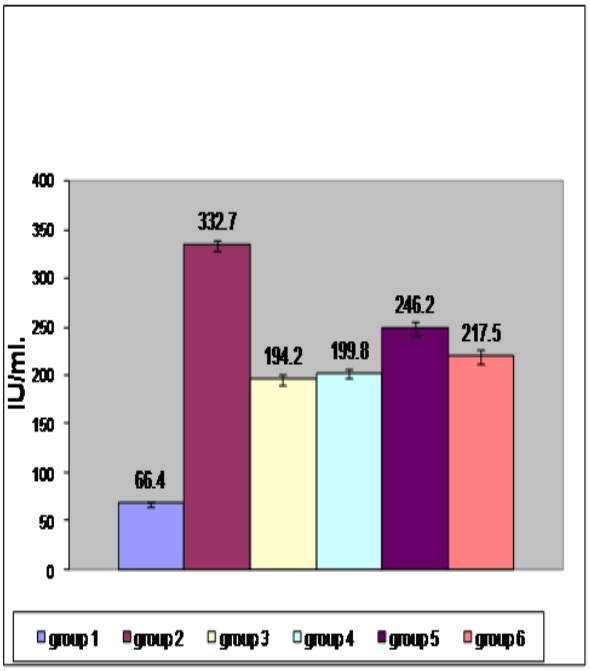

Fig. 2: Effect of *Pongamia pinnata* leaves extract on AST levels on rats

Experimental design and assessment of Hepatoprotective activity
Assessment of Hepatoprotective activity[10] was carried out on male albino rats. The animals were segregated into 6 groups of six rats.

Group I served as control receiving 1ml. of vehicle per kg b.w.p.o. (4% w/v aqueous acacia mucilage)
Group II Received toxicant 1.25 ml/kg b.w.p.o. i.p. Carbon tetrachloride.
Group III Received toxicant + Chloroform extract of 250 mg/kg b.w.p.o.
Group IV Received toxicant + Chloroform extract of 500 mg/kg b.w.p.o.
Group V Received toxicant + Ethanolic extract of 250 mg/kg b.w.p.o.
Group VI Received toxicant + Ethanolic extract of 500 mg/kg b.w.p.o.

The leaves extract were administered orally three times at 12 hr interval. A single dose of CCl_4 was administered intra peritonialy (1.25 ml/kg b.w.p.o.) and reversal of the toxicity was assessed by measuring Serum Glutamate Pyruvate Transaminase (SGPT) and Aspartate transaminase (AST) and serum alkaline phosphatase (ALP). After 3 days CCl_4 administration 1.0 ml. of blood was collected by puncturing the retro-orbital plexus in a clean and dry test tube and was allowed to clot at room temperature for 30 minutes. Serum was then separate by centrifuging at 2500 rpm for 10 minutes. The level of SGPT, AST [11] and ALP [12] was measured.

Statistical Analysis
The results of biochemical estimations have been reported as Mean SEM. The Mean value and SEM was calculated for each parameter. The student's t test was performed to calculate level of significance[13].

Results and Discussion

The result of the reversal of the hepatotoxicity in terms of increased level of SGPT, AST and ALP were as follows **(Table 1)**. The degree of hepatotoxicity developed can be known by elevated level of liver enzyme activity which is attributed to generation of CCl_3 free radical generation during metabolism by hepatic microsomes which in turn cause peroxidation of lipids of cellular membrane. From the table 1, it is clear that when CCl_4 was used to induce hepatotoxicity there is marked increase in the level of SGOT, AST and ALP **(Figure 1-3)**. From the results it is seen that the leaves extracts of *pongamina pinnata* of chloroform 500 mg and ethanol 250mg are found to be effective significantly by decreased level of SGPT, AST and ALP activity, but chloroform 500mg extract was found more effective as compared to the ethanolic extract. The Hepatoprotective activity of this drug might be due to its antioxidant property, which leads to inhibit the lipid peroxidation and free radical generation.

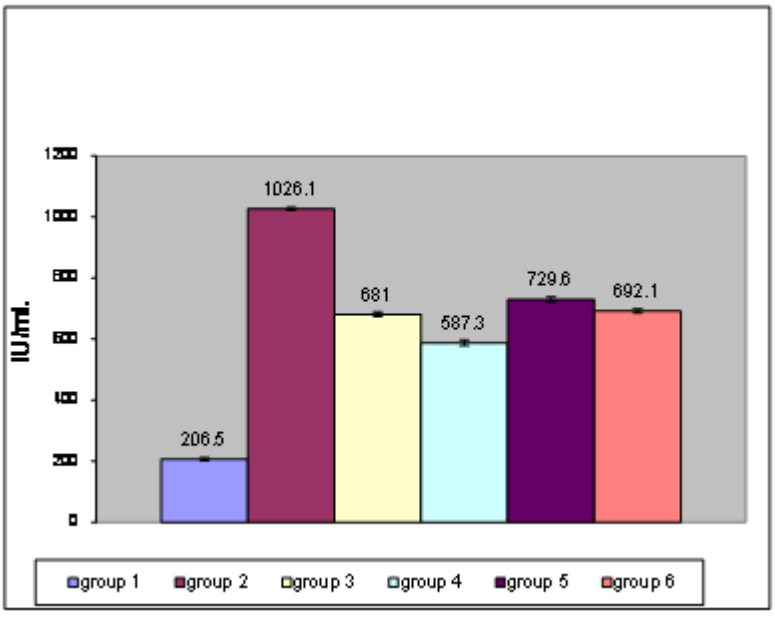

Fig. 3: Effect of *Pongamia pinnata* leaves extract on ALP levels on rats

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