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HEPATOPROTECTIVE ACTIVITY OF SAMANEA SAMAN (JACQ) MERR BARK AGAINST CCL₄ INDUCED HEPATIC DAMAGE IN ALBINO RATS

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ABSTRACT

The methanol extracts of Samanea saman (Jacq) Merr bark belonging to the family Mimosaceae was studied for hepatoprotective activity against Swiss albino rats with liver damage induced by carbon tetrachloride (CCl₄). It was found that the methanol extract of Samanea saman (Jacq) Merr at a dose of 400 mg/kg body weight reduces the serum level of ALT, AST, and cholesterol upto 52.08%, 57.37%, and 52.90% respectively. Since results of biochemical studies of blood samples of carbon tetrachloride treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by CCl₄ and blood samples from the animals treated with the methanol extracts of Samanea saman (Jacq) Merr showed significant decrease in the levels of serum markers, indicating the protection of hepatic cells. The extract could afford significant dose-dependent protection against CCl₄ induced hepatocellular injury.

INTRODUCTION

Samanea saman (Jacq) Merr is a large umbraculiform tree growing over 20 meters height with a stout trunk about 1.5 m in diameter and large spreading canopy providing shade. Branches are widespread more or less deciduous. Bark is rough and furrowed. It is valuable as a shade tree in pastures, stimulating grass growth. The leaves fold together on the approach of rain hence named as RAIN TREE. Saponin-like alkaloid pithecolobin has been isolated from the bark and the seed. Alkaloids are said to be abundant in the bark, stems, leaves, and seeds. Leaves and stems have saponin and tannin; gum is present in the trunk. Additionally steroids, cardiac glycosides, terpenoids are also present in the plant. The plant is used in acute bacillary dysentery, enteritis, diarrhea, colds, sore throat and headache. A decoction of the inner bark or fresh cambium and leaves are used to treat anaphylactic dermatitis, eczema, skin pruritus. Latex used as gum arabic for gluing. In Venezuela, rain tree is a traditional remedy for colds, diarrhea, headache, intestinal ailments and stomach ache. Root decoction is used in hot baths for stomach cancer. In the West Indies, the leaf infusion is used as a laxative and seeds are chewed for sore throat. The alcoholic extract of the leaves are used to treat tuberculosis.

OBJECTIVE:

Liver, an important organ actively involved in metabolic functions, is a frequent target of a number of toxicants (1). Liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. The liver is often abused by environmental toxins, poor eating habits, alcohol and prescription and over-the-counter drug use, which can damage and weaken the liver and eventually lead to hepatitis, cirrhosis and alcoholic liver disease [2, 3]. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. But there are not much drugs available for the treatment of liver disorders [4,5]. The objective of the present study was framed to determine the effect of methanolic bark extracts of *Samanea saman* (Jacq) Merr on circulating liver enzyme levels, serum bilirubin and protein at liver injury, during the earliest phases of implantation in rats induced with CCl₄ hepatotoxicity.

Collection of plant

The fresh barks of *Samanea saman*(Jacq) Merr were collected in the month of July from Ambattur, Chennai, Tamil Nadu state, India, and authenticated by Prof. P Jayaraman, Ph.D.,

Plant Anatomy Research Centre, Chennai, Tamil Nadu.(Reg.No: PARC/2010/567). The voucher specimen was deposited at the department for future reference.

Extraction of plant material

About 400g of air dried powdered bark was taken in 1000ml soxhlet apparatus and extracted with petroleum ether for 2 days. At the end of second day the powder was taken out and it was dried. After drying it was again packed and extracted by using methanol as solvent, till colour disappeared. The temperature was maintained at 55°C-65°C. After that, the extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness and dry residue was obtained.

Animal

Male Albino rats, weighing 125-150g were used in the present study. All the rats were kept at room temperature (24°C±2) in the animal house. All the animals were housed and treated as per the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were fed with standard food and were acclimatized to laboratory conditions. All the experimental procedures were performed on animals after approval from the ethics committee and in accordance with the recommendations for the proper care and use of laboratory animals.

Experimental Procedure

Swiss albino rats weighing between 125 and 150 gm were used in this evaluation. The rats were divided randomly into six groups of six rats each. The hepatoprotective activity of the plant extract was tested using CCl₄ model.

Group I- (normal control) received neither the plant extract nor CCl₄.

Group II - (induction control) received 0.2ml/100g of CCl₄ orally.

Group III - received 100mg/kg of methanol extract of *Samanea saman* (Jacq) Merr.

Group IV - received 200mg/kg of methanol extract of *Samanea saman* (Jacq) Merr.

Group V - received 400mg/kg of methanol extract of *Samanea saman* (Jacq) Merr.

Group VI - received silymarin (2.5 mg/100 g).

The suspensions of test samples were administered to rats 1hr, 24 hrs and 48 hrs after CCl₄ injection. After 72 hours of drug treatment, the animals were dissected under ether anesthesia. Blood from each rat was withdrawn from carotid artery at the neck and collected in previously labelled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was

separated by centrifugation at 3000rpm for 15 minutes. The separated serum were used for the estimation of some biochemical parameters like Alanine aminotransferase(ALT/SGPT), Aspartate aminotransferase (AST/SGOT), cholesterol, bilirubin and glucose. The substrate and the buffer solution used in the measurement of serum levels were supplied by Randox, UK. The present research had used the chemical analyzer apparatus, named RA-50, chemical analyzer, manufactured by Technocon, United kingdom which was an automatic machine to measure the amount of the required enzymes.

Statistical analysis

All the values are expressed as mean \pm S.E.M for groups of six animals each. Analyzed by one way ANOVA and compared by using Tukey- Kramer multiple comparison test. The values are statistically significant at three levels, *** $p < 0.001$. ** $p < 0.01$. * $p < 0.05$. But ns if $p > 0.05$.

RESULTS AND DISCUSSION:

The results of hepatoprotective activity of crude methanol extracts of the plant at doses of 100 mg/kg, 200mg/kg and 400mg/kg b.wt. on rats intoxicated with carbon tetrachloride were illustrated in the table 1. The table shows the comparison of effects among the untreated (normal control) and carbon tetrachloride treated (induction control or standard) group with the drug treated group of rats. When rats were treated with carbon tetrachloride, it induces hepatotoxicity by metabolic activation, therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. The rise in serum levels of AST, ALT and cholesterol has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages. [6] Carbon tetrachloride is metabolically activated by the cytochrome P-450 which combined with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation [7] [8] [9]. These result in changes of structures of the endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose -6-phosphatase activation, leading to liver injury [10,11,12,13]. Treatment with methanol extract of *Samanea saman* (Jacq) Merr recovered the injured liver to normal after 72 hrs at a dose of 400 mg/kg b.wt. which indicate that the plant has antihepatotoxic effect. In addition, the possible antihepatotoxic mechanism of *Samanea saman* (Jacq) Merr have not been reported yet. It is assumed that the effect of *Samanea saman* (Jacq) Merr extract on liver protection is related to glutathione-mediated detoxification as well as free radical suppressing activity.

GROUP	TOTAL BILIRUBIN (mg/dl)	AST (IU/l)	ALT (IU/l)	GLUCOSE (mg/dl)	CHOLESTEROL (mg/dl)
Control	0.47±0.03	49.54±2.39	11.97±1.53	87.44±2.98	98.84±8.38
CCl ₄ induced	1.41±0.31**	81.09±2.91***	24.76±2.60***	86.87±3.21	201.75±14.4***
MESS (100mg/kg)	1.39±0.09	70.70±1.86*	22.17±2.11	87.25±1.69	171.61±3.1
MESS (200mg/kg)	1.22±0.12	67.29±2.05***	19.99±1.58	87.89±1.45	163.18±5.63*
MESS (400mg/kg)	0.93±0.08	62.01±1.77***	16.38±1.09*	88.90±2.32	149.09±9.79**
Silymarin(25mg/kg)	0.62±0.07**	58.66±0.43***	13.42±1.35**	88.77±1.88	107.34±2.28***

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