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Research Article

A STUDY ON HEPATOPROTECTIVE ACTIVITY OF *CALOTROPIS GIGANTEA* LEAVES EXTRACT

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ABSTRACT

Calotropis gigantea L, belonging to family: Asclepiadaceae, also known as Sweat akand, is used in traditional medicine for treatment of various ailments. Leaf extracts of Calotropis gigantea were prepared by using various solvents like Petroleum ether, Acetone, Chloroform and Methanol in increasing polarity. Hepatoprotective activity was studied by Carbon tetrachloride induced hepatotoxicity. Animals in different groups were treated with 450mg/kg body weight of different extracts through oral route. Silymarin at a dose of 100mg/Kg body wt. was administered as standard. Since no such work has been investigated and reported in detail earlier, therefore an effort has been made to explore the hepatoprotective activity in this plant.

Keywords: Calotropis gigantea, Silymarin, Carbon tetrachloride, Hepatoprotective.

INTRODUCTION

Calotropis gigantea L, belonging to family: Asclepiadaceae, also known as sweat akand is found throughout plains and lower hills of India usually near water found growing upto an altitude of 900m throughout India including Andamans [1, 2].

Various chemical constituents have been reported from different parts of the plant[3]. Flowers contain waxy matter which has esters of resinols, α-, β-calotropeol, β -amyrin, stigmasterol, giganteol, calotropin, a triterpenoid flavonoid, flavonoid glycoside, wax, acids and alcohols [1, 4]. Seeds are rich in aminoacids, major being phenylalanine, lysine and histidine. The leaf contains ascorbic acid, ortho-pyrocatechic acid and also contains β -amyrin, taxasterol, tarasterol and beta-sitosterol[1]. Shoot and leaf extracts possess antibacterial activity. Tender fresh leaves have been reported to cure fits and convulsions in children. Extracts of leaf with oil and rock salt warmed are poured into ear for earache[1]. Fresh warmed leaves or poultice is bandaged on painful rheumatic [1]. Plant is purgative, antihelmintic, antitumor and has been used in diseases of spleen and liver [2]. Leaves have been used in enlargement of liver and flowers are also good for liver Paracetamol induced hepatic damage in rats has been reported[5]. Aerial parts were collected from medicinal garden of BBDNITM and authenticated by pharmacognostic, phytochemical and other studies while voucher (sample No. N.B.R.I/CIF/Re/08/2008/32) was deposited in taxonomy lab, Ethnopharmacology division, NBRI, Lucknow for future reference. Healthy male wistar rats each weighing 150-200 g were used for study. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25 ±3^{0}C and 35-60% humidity). Standard pelletized feed and tap water were provided *ad libitum.*

Pharmacological screening [6]

Determination of acute toxicity and LD_{50} values

Wistar rats weighing 150-175 g of either sex, maintained under standard husbandry conditions, were used for all sets of experiments in groups of six animals. Animals were allowed to take standard laboratory feed and tap water. The relevant extracts were administered to respective groups of rats in doses ranging from 100-5000 mg/kg. There was no lethality in any of the groups. One tenth of the maximum dose of the extracts, tested for acute toxicity, was selected for evaluation of hepatoprotective activity (10), i.e., 2500 mg/kg. The experiments were performed as per protocols approved by Institutional Animal Ethics Committee (CPCSEA approval no. BBDNITM/IAEC/Clear/8/2008).

Effect of extracts on carbon tetra chloride induced liver toxicity in rats [7, 8]

The extracts of *C. gigantea* leaves of different concentrations and Silymarin at a dose of 100 mg/kg body wt. were administered orally to rats of the respective groups three times at intervals of 12 hrs.

Group I- Normal control given only saline 0.5ml through oral route for seven days.

Group II- Disease control-Treated with Carbon tetrachloride for seven days.

Group III- Reference control–Diseased animals treated with Reference drug, Silymarin, through oral route (100mg/Kg).

Group IV- Diseased animals treated with Chloroform Extract through oral route (50 mg/kg).

Group V- Diseased animals treated with Methanol Extract through oral route (450 mg/kg).

Table 1: Effect of extract on biochemical parameters

GROUP	DOSE (mg/kg)	S.G.P.T (IU/L)	S.G.O.T (IU/L)	A.L.P (IU/L)	Bilirubin (mg/dl)
Group-I	_	61.8±.629	129±.483	138±.382	1.13±.125
Group-II	_	692±.583	824±.424	437±.529	3.35±.112
Group-III	100mg/kg	64.8±.382***	153±.31***	163±.417***	.73±.086***
Group-1V	450mg/kg	289±.527***	563±.330**	266±.443***	2±.0217*
Group-V	450mg/kg	213±.707***	291±.441***	233±.629***	1.66±.56**

Values are expressed as Mean ±SEM (n=6), p* < 0.05, p** < 0.01, p*** < 0.001c

Carbon tetrachloride diluted with olive oil (1:1) was administered in a dose of 1 ml/kg body wt. for 2 days to all animal groups except for control. Animals of the diseased group received only CCl_4, to assist assessing the severity of toxicity produced by carbon tetrachloride administration. After 36 hr of carbon tetrachloride treatment, blood was collected from all groups of rats by puncturing the corneal plexus. Serum was separated by centrifugation at 2500 rpm at 37^{0}C for 15 min and analyzed for various biochemical parameters namely

S.G.P.T, S.G.O.T, A.L.P and Bilirubin [9]. Silymarin, chloroform extract, methanol extract, caused very significant reduction in SGPT level ($p<0.001$). The increase in Carbon tetrachloride induced SGOT level was decreased significantly by methanolic extract, Silymarin ($P<0.001$).The Chloroform extract showed only moderately significant ($p<0.01$) reduction in SGOT level. The ALP level was reduced significantly ($p<0.001$) by Silymarin, chloroform, methanol extract. The Carbon tetrachloride induced increase in the Bilirubin was reduced significantly ($p<0.001$) by Silymarin but less with chloroform ($p<0.05$) and methanol extract ($p<0.01$).

Histopathological studies

One animal from the treated groups showing maximal activity as indicated by improved biochemical parameters from each test, positive control, hepatotoxin and control groups were utilized for this purpose. The animals were sacrificed, and the abdomen was cut open to remove the liver. Then 5 mm thick pieces of the liver were fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 hr and then embedded in paraffin, using conventional methods [10], and cut into 5 mm thick sections and stained, using haematoxylin-eosin dye, and finally mounted in diphenylxylene. Then the sections were observed under microscope for histopathological changes in liver architecture, and their photomicrographs were taken.

Histopathological study of the sections of liver of animals treated with various extracts of leaves of *Calotropis gigantea*

In histopathological studies, section of liver of normal control animals shows normal sinusoidal cellular (Fig. 1) structure with minimum fatty changes and regeneration of hepatocytes. The liver histological section in animals with carbon tetrachloride induced hepatic damage shows damaged sinusoidal architecture with broad patches of hepatic cellular necrosis, centrilobular fatty degeneration and swelling (Fig. 2). In case of liver histology of animals treated with Silymarin normalization of hepatic cells, central vein, and portal triad was observed with minimum or no necrotic patches (Fig.3). The chloroform (Fig. 4) extract and Methanolic (Fig 5) extract treated animals showed liver histology improved as compared to diseased animals with few necrotic patches here and there with very little fatty change.

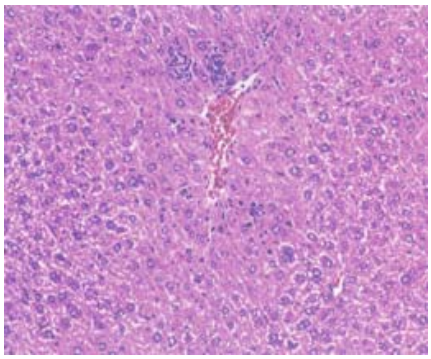

Fig. 1: Histological Section of Rat Liver (Normal control)

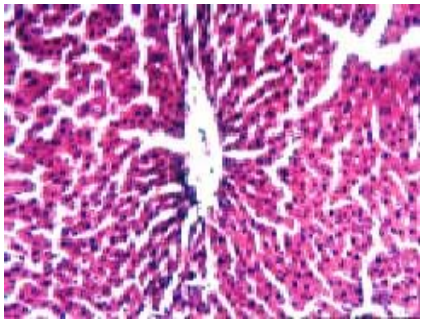

Fig. 2: Histological section of rat liver from animals treated with carbon tetrachloride

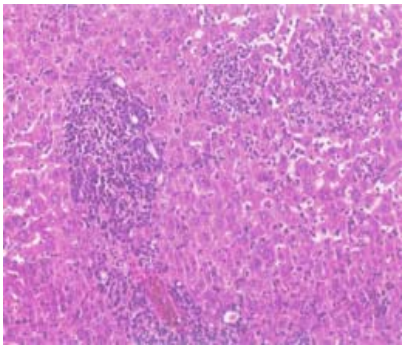

Fig. 3: Histological section of rat liver treated with silymarin

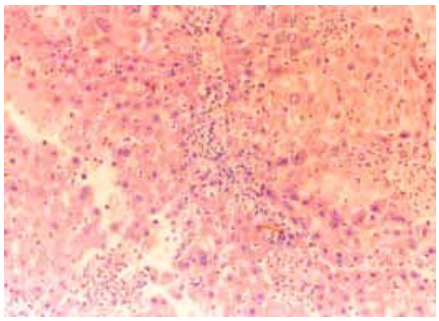

Fig. 4: Histological section of rat treated with with methanolic extract (Leaf)

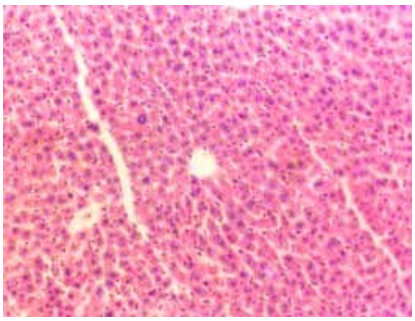

Fig. 5: Histological section of rat treated chloroform extract (leaf)

CONCLUSION

Carbon tetrachloride produces peroxidation of lipids, denaturation of proteins or other chemical changes in the liver which lead to their disruption. These changes begin immediately after the administration of the toxin, the culmination of which is hepatic necrosis, steatosis or both. Hepatic injury precedes the alteration of DNA or RNA, impaired synthesis of proteins or depression of the levels of ATP or glutathione. The direct injurious effect involves a physiochemical alteration of the membrane integrity as a result of protein denaturation, peroxidation of the unsaturated fatty acids or by other destructive chemical changes.

The mechanism of Carbon tetrachloride induced hepatic injury seems to be mediated by a reactive metabolite trichloromethyl free radical formed by the hemolytic cleavage of carbon tetrachloride.

$$CCl_4 \longrightarrow CCl_3 + Cl^-$$

Trichloromethyl peroxy free radical is more reactive species. The toxicity produced by Carbontetrachloride is thought to be due to the reaction of free radicals with lipid and protein. These free radicals cause the peroxidation of the polyenoic lipids of the endoplasmic reticulum and the generation of secondary free radicals derived from these lipds starts a chain reaction.

SGPT- Silymarin, chloroform extract, methanol extract caused very significant reduction in SGPT level ($p<0.00$)

SGOT – The increase in Carbon tetrachloride induced SGOT level was decreased significantly by methanolic extract and Silymarin ($P<0.001$).The Chloroform extract showed only moderately significant ($p<0.01$) reduction in SGOT level.

ALP- The ALP level was reduced significantly ($p<0.001$) by Silymarin, chloroform and methanol extract.

Bilirubin-The Carbon tetrachloride induced increase in the Bilirubin was reduced significantly ($p<0.001$) by Silymarin.

In histopathological studies section of liver of normal control animals shows normal sinusoidal cellular structure with minimum fatty changes and regeneration of hepatocytes.

The liver histological section in animals with carbon tetrachloride induced hepatic damage shows damaged sinusoidal architecture with broad patches of hepatic cellular necrosis, centrilobular fatty degeneration and swelling.

In case of liver histology of animals treated with Silymarin normalization of hepatic cells, central vein, portal triad was observed with minimum or no necrotic patches.

The methanolic extract and chloroform extract treated animals showed liver histology improved as compared to diseased animals with few necrotic patches here and there with very little fatty change.

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