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Research Article

Hepatoprotective Activity of Extracts from Stem of *Calycopteris floribunda* Lam. Against Carbon Tetrachloride Induecd Toxicity in Rats

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ABSTRACT
Calycopteris floribunda Lam, *(*Combretaceae) a scandent woody shrub. The present study was conducted to evaluate the Hepatoprotective activity of chloroform fraction and methanol stem extract of *Calycopteris floribunda* (CF) against carbon tetrachloride (CCl_4) induced liver damage in Wistar albino rats. Chloroform fraction and methanolic extract of CF (100mg/kg, 200mg/kg .p.o.), were administerd respectively, Silymarin (25 mg/kg.p.o.) was given as reference standard. The stem extracts were effective in protecting the liver against the injury induced by CCl_4 in animals. This was evident from significant reduction in serum enzyme, Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), Alkaline phosphatase (ALP) and Total bilirubin (TB).Various pathological changes like centribular necrosis and vacuolization were observed in CCL_4 treated rats, which were significant protective activity in groups treated with CF and silymarin. It was concluded from the study that chloroform fraction and methanolic extract of CF possess hepatoprotective activity against CCl_4 induced hepatotoxicity in rats**.**

Keywords: hepatoxicity, *Calycopteris floribunda*, carbontetrachloride induced.

INTRODUCTION

Calycopteris floribunda Lam, a scandent woody shrub with slender brown streaked diffuse branches occasionally twining around supports and storing water abundantly. Commonly known as kokkarai in Hindi, Minnarakoti in Tamil, Adivijama, in Telugu. The plant is also grown in central and southern parts of India[1], where the leaves are reported to have medicinal uses as a laxative and anthelmintic medicine, while the juice derived from the young twigs is used for the treatment of diarrhoea, dysentery and malaria[2]. Fruits are used for jaundice; flowers are reported as anti-tumour agent.Previous phytochemical studies have reported on the isolation of the flavonoids calycoptrin, quercetin[3-4] and five biflavonoids[5] from the leaves and flowers. The evaluation of the stem of *Calycopteris floribunda* in the treatment of liver disease has not been reported in the laboratory animals. The present studies were performed to assess the hepatoprotective activity in rats against carbon tetrachloride as hepatotoxin to prove its claim in the folklore practices against liver disorders.

MATERIALS AND METHODS

Plant material

The stem of *Calycopteris floribunda* Lam., were collected from Bhubaneswar, Orrisa state, India, and authenticated by Dr.M.Venkaiah, Associate professor, Dept of Botany, Andhra University. A voucher specimen (TSNDOP08) was deposited in the herbarium of our department.

Preparation of extract

Freshly collected plant material was shade dried at room temperature and coarsely powdered in Wiely mill. The powdered stem (1kg) was extracted with methanol by the process of continuous extraction (soxhletion).The crude extract was evaporated to dryness in a rotary film evaporator (Roteava,Equitron, Medica instrument, India). 60 gm of methanolic extract was obtained. The methanolic extract was fractionated with Hexane (10×250 ml) and chloroform (10×250ml). All solubles were concentrated and the percentage yield of hexane soluble fraction was 1.6gm and chloroform soluble fraction was 18.5 gms and remainig methanol soluble extract was 28.5 gm. Preliminary phytochemical studies of chloroform fraction revealed presence of alkaloids and the methanolic soluble extract revealed presence of flavonoids, saponins and tannins. The methanolic extract and chloroform fractions were subjected to hepatoprotective activity in rats. Silymarin was used as positive control at dose of 25mg/kg.p.o. All the test substances were suspended in vehicle i.e. 5% acacia mucilage. The extracts were tested for activity at doses of 100mg/kg and 200mg/kg.p.o

Drug and Chemicals

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Table 1: Effect of *Calycoptris floribunda* stem on carbon tetrachloride -induced toxicity in rats.

Treatment	Serum biochemical parameters			
	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TB (mg/dl)
5% gum acacia suspension (1 ml/kg.p.o.)	123.83±1.70	82.66±1.54	158.33±2.20	1.06±0.08
CCl_4 ($_{0.5}$ ml/kg.i.p.)	997.66±22.74	736.0±17.57	488.48±8.23	4.48±0.17
Silymarin (25mg/kg.p.o.)	223.16±1.40	164.50±1.60	185.16±2.80	1.84±0.16
CECF (100mg/kg.p.o.)	414.33±1.38	326.83±1.35	262.33±1.94	2.46±0.11
CECF (200mg/kg.p.o.)	308.5±1.54	276.50±1.89	241.5±2.32	2.15±0.05
MECF (100mg/kg.p.o.)	512.66±0.88	433.66±2.43	323.33±1.68	2.93±0.14
MECF (200mg/kg.p.o.)	381.0±1.46	348.0±1.15	281.33±2.59	2.71±0.10

CECF – Chloroform extract of *Calycopteris floribunda*, MECF – Methanol extract *Calycopteris floribunda*.Values are expressed in mean ± SEM, n=6, in each group. **Significant increase compared to Control (P≤0.01), ***Significant reduction compared to Control. (P≤0.01),

CCl_4 was obtained from Poona Chemical Laboratory, Pune, India. Silymarin-Microlab, Bangalore, Karnataka,India. Estimation kits-Span Diagnostics, Surat, India. All other chemicals were obtained from local sources (Sai chemicals, Visakhapatnam) and were of analytical grade.

Animals

Wistar albino rats of either sex weighing between 200-250 gm were obtained from M/s. Mahavir Enterprises, Hyderabad. The animals were housed under standard environmental conditions (temperature of 22 ± 1°C with an alternating 12 h light – dark cycle and relative humidity of 60 ± 5%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Ethics Committee and by animal regulatory body of the government (Regd: No: 516/01/A/CPCSEA). They were fed with standard laboratory diet supplied by M/s. Rayans biotechnologies Pvt. Ltd., Hyderabad, and water *ad libitum.*

Table 2:Percentage decrease in levels of biochemical parameters due to treatment with different extracts of of stem of *Calycoptris floribunda.*

Treated with	% Decrease Biochemical Levels			
	SGOT	SGPT	ALP	TB
Silymarin 25mg/kg	83.63	87.47	91.87	81.28
CECF 100mg/kg	66.77	62.62	68.49	59.06
CECF 200mg/kg	78.86	70.33	74.80	68.12
MECF 100mg/kg	55.50	46.27	50.02	45.32
MECF 200mg/kg	68.79	59.38	62.74	51.75

Acute Toxicity Studies

Acute toxicity studies were performed for extracts according to the toxic classic method as per OECD guidelines[6]. Female albino rats were used for the acute toxicity study. The animals were kept fasting overnight providing only water, after which the extracts were administered orally at the dose of 300 mg/kg and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If the mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If the mortality was not observed, the procedure was repeated for further higher dose i.e., 2000 mg/kg, 2500mg/kg, 3000mg/kg.

Carbon tetrachloride-induced hepatotoxicity

The animals were divided into seven groups of six animals each. Group-I served as normal control received 5% acacia mucilage (1 ml/kg.p.o) daily once for 7 days. Group-II served as toxic control and received CCl_4 (0.5 ml/kg i.p) daily once for 7 days[7]. Group-III was treated with the reference drug Silymarin (25 mg/kg .p.o) and followed by CCl_4 (0.5 ml/kg i.p) daily once for 7 days[8]. Groups IV-V were treated with chloroform fraction of *C.floribunda* stem at doses of 100 and 200 mg/kg *p.o.* followed by CCl_4 (0.5 ml/kg i.p) *daily* once for 7 days. Groups VI-VII were treated with methanolic soluble extract of *C.floribunda* stem at doses of 100 and 200 mg/kg, *p.o.* followed by CCl_4 (0.5 ml/kg i.p) *daily* once for 7 days. After completion of treatment blood was collected, serum was separated and used for determination of biochemical parameters.

Collection of blood samples

All the animals were sacrificed on 7th day under light ether anesthesia. The blood samples were collected separately in sterilized dry centrifuge tubes by puncture retro-orbital plexes and allowed to coagulate for 30 min at 37 °C. The clear serum was separated at 2500 rpm (Microcentrifuge) for 10min and subjected to biochemical investigation viz.., serum glutamic oxaloacetate transe aminase (SGOT), serum glutamic Pyruvate transe aminase (SGPT), Alkaline phosphatase (ALP) and Total Bilirubin (TB).

Assessment of liver function

The Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated by UV kinetic method in which both SGOT

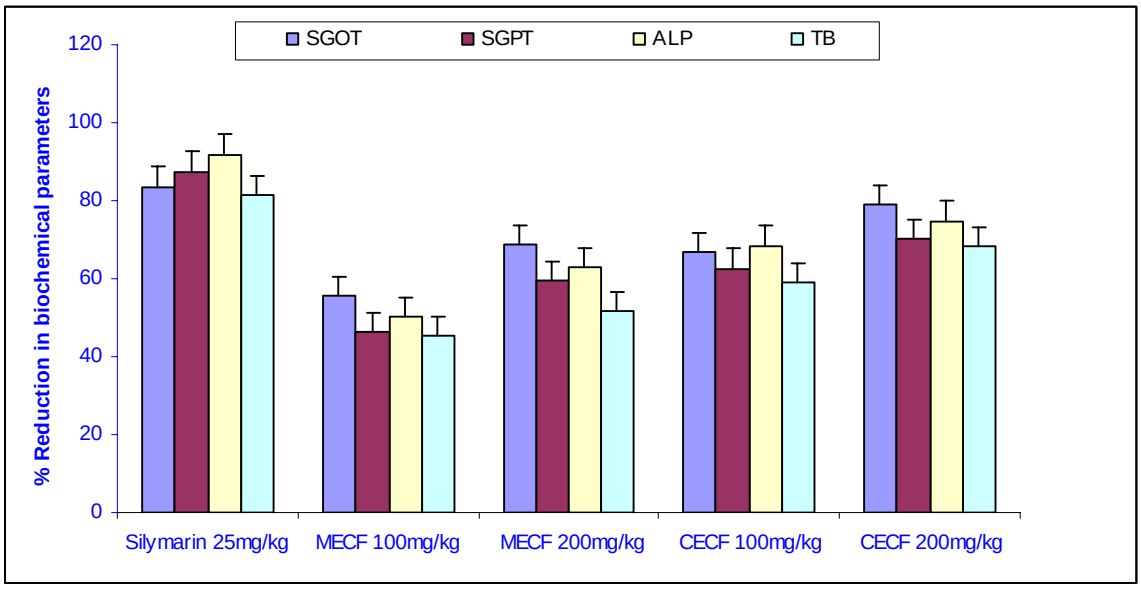

Fig 1: Hepatoprotective activity of chloroform and methanolic extracts of *C.floribunda* stem against CCl_4 induced toxicity in rats

and SGPT were assayed based on enzyme-coupled system; where keto acid formed by the aminotransaminase reacts in a system using NADH. The coenzyme is oxidized to NAD and the decrease in absorbance at 340 nm for SGOT malate dehydrogenase (MDH) reduces to malate with simultaneous oxidation of NADH to NAD. The rate of oxidation of NADH is measured, where as SGPT [9] the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase. Estimation of Alkaline phosphate (ALKP) [10] involves hydrolysis of P-nitrophenyl phosphate by alkaline phosphatase to give P- nitrophenol, which gives yellow color in alkaline solution. The increase in absorbance due to its formation is directly proportional to alkaline phosphate (ALKP) activity. Estimation of total bilirubin (TB) [11] involved the reaction of bilirubin with diazotized sulphanic acid to form an azocompound, the color of which is measured at 546 nm .All the estimations were carried out using standard kits in semi auto analyzer Screen Master 3000.

Histopathological studies

The isolated liver specimens were trimmed to small pieces and preserved in formalin (10%) solution for 24 hrs. The liver specimens were subjected to dehydration with acetone of strength 70, 80, 100 % respectively, each for one hour. The infiltration and impregnation was done by treatment with paraffin wax twice each time for one hour.

Specimens were cut into sections of 4-6 µm thickness and were stained with haemotoxylin and eosin (H-E) and later the microscopic slides of the liver were photographed in light microscope (Axiostar plus).

Statistical analysis

Results of biochemical estimation were reported as mean ±SEM for determination of significant inter group difference was analysed separately and one-way analysis of variance (ANOVA) was carried out[12].Dunnet's test was used for indidual comparisons[13].

RESULTS

The LD_{50} of chloroform fraction and methanol soluble extracts were found to be 2000 mg/kg .b.w. $1/10^{th}$ and $1/20^{th}$ of these doses (200 mg/kg. b.w, and 100mg/kg. b.w) were selected for the evaluation of hepatoprotective activity.

The effect of chloroform fraction and methanol extracts of stem of *Calycopteris floribunda* on CCl_4 induced liver damage in rats with reference to biochemical changes in serum is shown in table.(1). Percentage decrease or increase was calculated by considering the enzyme level difference between hepatotoxin treated and control rats as 100% of level of reduction and recorded in (Table- 2).The comparative efficacy of the extracts and silymarin tested for their hepatoprotective activity were depicted in the form of a bar diagram fig(1).

Histopathology of liver tissues (a) Group I — section shows central vein surrounded by hepatic cord of cells (normal architecture). (b) Group II—section shows patches of liver cell necrosis with inflammatory collections, around central vein. (c) Group III—almost near normal. (d) Group IV—inflammatory collections around central vein and focal necrosis with sinusoidal dilatation. (e) Group V—less inflammatory cells around central vein, absence of necrosis. (f) Group VI—less inflammation around dilated central vein. (g) Group VII—minimal inflammatory cellular infiltration. Almost near normal liver architecture. Regeneration of hepatocytes around central vein.

DISCUSSION AND CONCLUSION

The carbon tetrachloride mechanism begins with the trichloromethyl radical ($\cdot CCl_3$) by the action of the mixed function of cytochrome P-450 oxygenase

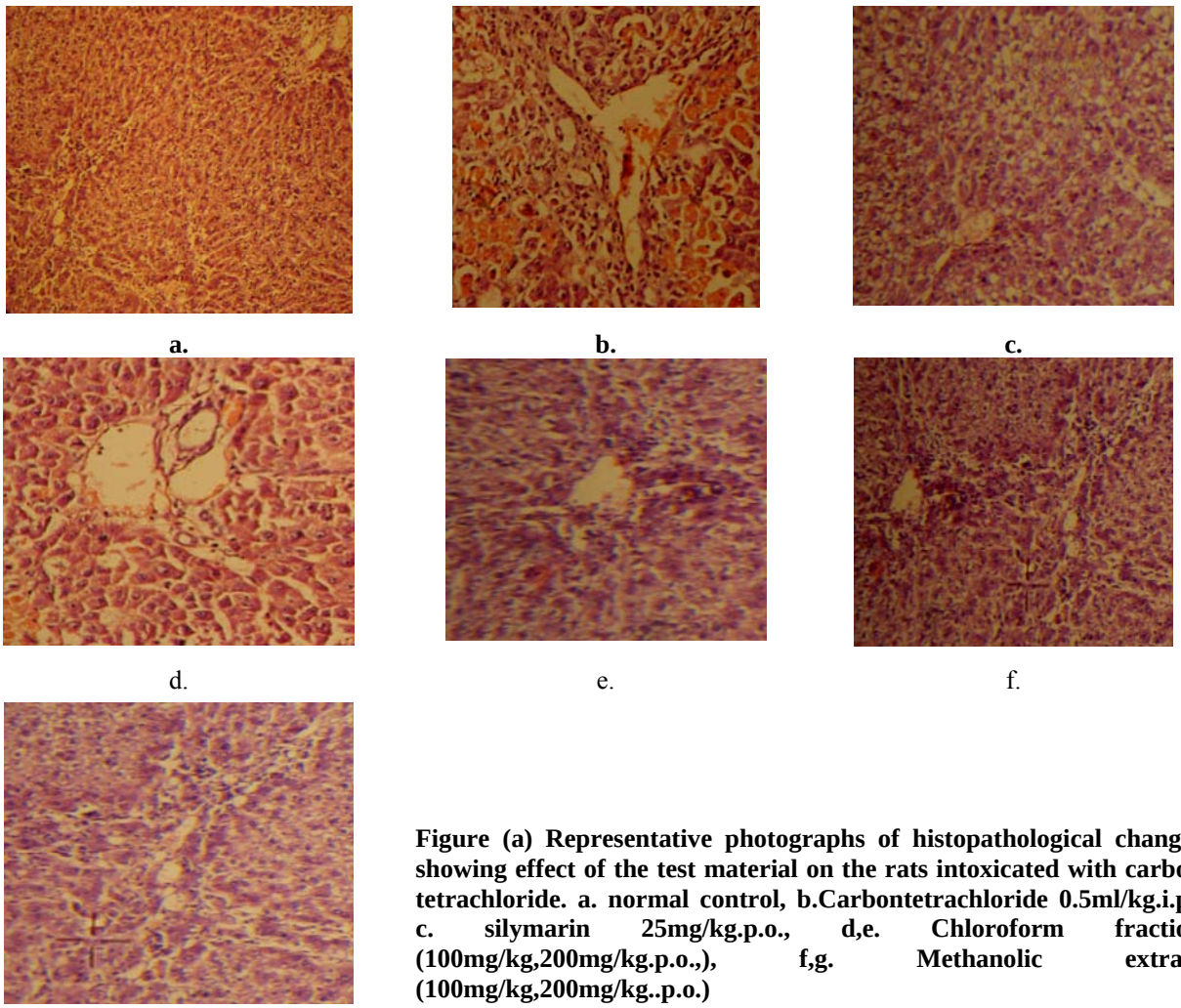

Figure (a) Representative photographs of histopathological changes showing effect of the test material on the rats intoxicated with carbon tetrachloride. a. normal control, b.Carbontetrachloride 0.5ml/kg.i.p., c. silymarin 25mg/kg.p.o., d,e. Chloroform fraction (100mg/kg,200mg/kg.p.o.,), f,g. Methanolic extract (100mg/kg,200mg/kg..p.o.)

system. This free radical, which is initially formed as unreactive, reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical ($\cdot OOCCl_3$). Both radicals are capable of binding to proteins, lipids or abstracting a hydrogen atom an unsaturated lipid, thus initiating lipid peroxydation. This proceses of lipid peroxidation can significantly damage hepatic plasma membranes[14].The increased levels of SGOT, SGPT, ALP and TB are conventional indicators of liver injury[15].The ability of hepatoprotective drug to reduce the injurious effect or to preserve the normal hepatic physiological mechanisms, that have been disturbed by a hepatotoxin, is the index of its protective effect[16]. Hepatocellular necrosis leads to evaluation of the serum marker enzymes, which are released from the liver in blood[17].The present study revealed a significant increase in the activities of SGOT,SGPT,ALP and TB levels on exposure to CCl_4 indicating considerable hepatocellular injury. Administration of both chloroform fraction and methanol extracts at two different dose levels attenuated the increased levels of the serum enzymes, produced by CCl_4 and caused a subsequent recovery towards normalization almost like that of silymarin treatment.

The hepatoprotective effect of the drugs was further concluded by the histopathological examinations of the liver sections which reveal that the normal liver architecture was distrurbed by hepatotoxin intoxication. In the liver sections of the rats treated with chloroform fraction and methnolic extract and intoxicated with CCl_4 the normal cellular architecture was retained as compared to silymarin, thereby confirming the protective effect of the extracts of C.floribunda.

Accordance with these results, chloroform fraction and methanol extract at different dose levels offer hepatoprotection dose dependent activity. But Group VII (methanol extract 200mg/kg.b.w.p.o) is more effective than all other groups and it may be hypothesized that rich content of flvonoids may be responsible. The hepatoprotective activity of these drugs might be due to stabilization of the membrane inhibiting effect on lipid peroxidation or due to their stimulatory effects on hepatic regeneration. The protective action may be due to scavenging effect of free radicles. In conclusion this

study confirms the therapeutic potential of stem of *Calycopteris floribunda*.

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