

# Protective effect of Methanolic extract of Rhizome *Calamus Rotang* Linn on Carbon Tetra Chloride induced Hepatotoxicity in Rat

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## ABSTRACT

The methanol extract of the Rhizome of *Calamus Rotang* Linn. (Arecaceae) was investigated for hepatoprotective activity in rats with liver damage induced by carbon tetrachloride. The extract at an oral dose of 500 mg/kg exhibited a significant protective effect by lowering the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP), total serum bilirubin, total cholesterol and triglycerides. These biochemical observations were supplemented by histopathological examination of liver sections. The activity of extract was also comparable to that of Silymarin, standard known hepato protective reference drug

**Key words:** Calamus Rotang, Rhizome, Hepatoprotective, Carbon tetrachloride

## INTRODUCTION

Liver, one of the most important organs of body, plays a pivotal role in regulating various physiological process. It is involved in several vital functions such as metabolism, secretion, storage and excretion of many endogenous and exogenous compounds causing its injury or impairment. It has great capacity to detoxify toxic substance and synthesize of useful material. Its typical positions and function make it not only the most essential organ but also prone to number of toxicant – targets leading to liver diseases (Ghosh, 2013). Liver diseases remain one of the serious health problems and our Indian traditional system of medicine, particularly Ayurveda have put forward a number of medicinal plants and their formulations for liver disorders. In this modern age it is very important to provide scientific proof to justify the various medicinal uses of herbs. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost (Valiathan, 1998). In south Asia, number of medicinal plants and their formulations are used as medicine for serious liver disease; most of them speed up the natural healing process of liver. Therefore the research for the effective hepatoprotective drug is still continued (Kumar, 2012).

*Calamus Rotang* (Tamil:Pirambu) is also known as vetra. It is a thorny slender climbing shrub occurring in central and southern parts of India. It is also cultivated in gardens. It bears contorted pieces of horizontally growing, woody rhizome, 5 to 6cm in length, 2.5 to 3.5 cm width, surface is rough, longitudinally striated, encircled by prominent, closely arranged leaf scars, and well developed tortuous roots, at lower sides; roots are cylindrical, branched, smooth or faintly longitudinally striated, exfoliated, externally dirty earthy brown in colour, internally, pinkish greyish, fracture is hard, fibrous, odour not characteristic, taste astringent and slightly sour in taste. The rhizome of the plant are used in various ailments viz. It is used to cure piles, burning sensation cough, leprosy, and bleeding disorder and in treatment of inflammations. Earlier chemical examination of this plant have shown the presence of Afzelechin, bergapten, umbelliferon,  $\beta$ -sitosterol, campesterol, stigmasterol and fucosterol, glucosyl- $\beta$ -sitosterol, calotropenyl acetate, and lupeol acetate. Previously it was reported that the methanolic extracts of *Acorus calamus* possess significant hepatoprotective activity against carbon tetrachloride-induced hepatotoxicity. In view of the reported hepatoprotective activity of other *Calamus species* (monocots) and traditional claims, the methanolic of *Calamus Rotang* was evaluated against carbon tetrachloride induced hepatic damage in rats with the aim of developing a natural hepatoprotective drug

## MATERIALS AND METHODS

**Plant material:** *Calamus Rotang rhizomes* were collected from Kodad, Andhra Pradesh, India and authenticated by Dr. Vishnuvardhan, Taxonomist, Botany department, ANU University, GUNTUR. A voucher specimen (UCPSC/ANU/27) is deposited in the laboratory of Pharmacognosy.

**Preparation of extract:** Dried Rhizome powder (200 g) was extracted with methanol by maceration for five days. The concentrated methanolic extract (16.6 g) was tested for qualitative phytoconstituents and indicated the presence of steroids/triterpenoids and their glycosides.

**Animals:** Male Wistar rats (200-220 g) procured from the Mahaveer enterprises, Hyderabad. The animals were

maintained under standard environmental conditions (relative humidity 55-65%, room temperature  $23.0 \pm 2.0^\circ\text{C}$ ). Animals had free access to feed (Hindustan Liver, Bangalore) and tap water *ad libitum* during the quarantine period. All procedures compiled with the norms of the Institutional animal ethics committee (IAEC) of our college.

**Hepatoprotective effect against CCl<sub>4</sub>-induced hepatotoxicity in rats:** Animals were divided into four groups of six rats each. Group I and II served as normal and intoxicated control, respectively and received only the vehicle (5% gum acacia; 1 ml/kg; p.o). Group III animals were treated with standard Silymarin at an oral dose of 100 mg/kg. Group IV received the methanolic extract of *Calamus Rotang* at an oral dose of 500 mg/kg, with a fine suspension of 5% aqueous gum acacia. The treatment was continued for 7 days, once daily. On the day of 7 for groups II-IV, 30 min post-dose of extract administration animals received CCl<sub>4</sub> at the dose of 1.5 ml/kg (1:1 of CCl<sub>4</sub> in olive oil) orally. The animals were sacrificed after 36 hour administration of acute dose of CCl<sub>4</sub>. The blood was collected by carotid artery. The serum was separated out and used for estimation of aspartate aminotransferase (AST) (Reitman, 1957), alanine aminotransferase (ALT) (7), alkaline phosphatase (ALP) (Ohkawa, 1979) and total serum bilirubin using Span diagnostic kits. The liver was immediately removed and a section of liver was processed for histological studies.

**Histopathological studies:** Immediately after the sacrifice, a portion of liver were fixed in 10% formalin, then washed, dehydrated in ascending grades of alcohol and finally rinsed with xylene. The tissue was then embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin and histological observations were made under light microscope (Galighor, 1976; Luna, 1968).

**Statistical analysis:** The results are expressed as means  $\pm$  S.D. The difference between experimental groups were compared by one way ANOVA (toxic control versus treatment, followed by post hoc Dunnet S test using Statistical Package for Social science (SPSS software, Version 18.0). Values with  $p < 0.05$  were considered as statistically significant

## RESULTS AND DISCUSSION

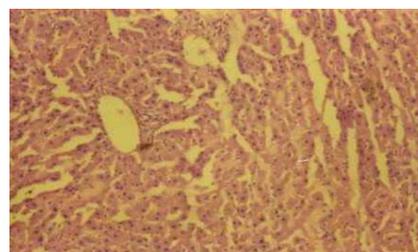
**Hepatoprotective effects:** The animals treated with toxic doses of carbon tetrachloride had markedly elevated values of the serum ALT, AST, ALP and total bilirubin compared to normal rats, indicating acute hepato-cellular damage (Table-1). Serum enzyme values in the animals pretreated with methanolic extract of *Calamus Rotang* (500 mg/kg; p.o) were significantly ( $p < 0.001$ ) lower than those of toxic control values and except for ALP. ALT, AST, total bilirubin serum enzyme values in treated animals were similar to the normal control values. The effects of the methanolic extract of *Calamus Rotang* were comparable to that of standard silymarin activity. Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig.1). Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed in CCl<sub>4</sub> intoxicated animals (Fig. 2). The liver sections of the rats treated with methanolic extract of *Calamus Rotang* and silymarin followed by CCl<sub>4</sub> intoxication showed a sign of protection as it was evident by the less disarrangement and degeneration of hepatocytes (Fig. 3 and 4).

**Table.1. Effect of pretreatment with methanolic extract of *Calamus Rotang* on ccl<sub>4</sub>-induced rats**

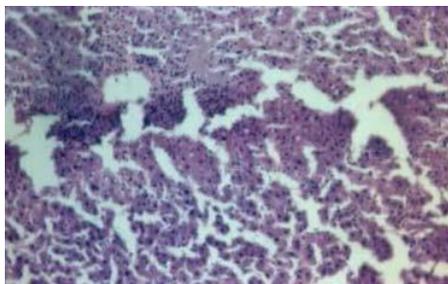
Group	Treatment, p.o	Biochemical parameters			
		ALT(U/L)	AST(U/L)	ALP(U/L)	Total Bilirubin (mg/dl)
1	Control	21 $\pm$ 2.4	120.7 $\pm$ 4.2	115.4 $\pm$ 7.6	0.40 $\pm$ 0.04
2	CCl <sub>4</sub> -treated	37.6 $\pm$ 1.7	126.5 $\pm$ 3.9	196.5 $\pm$ 6.8	12.80 $\pm$ 1.7
3	Silymarin+ CCl <sub>4</sub>	21.2 $\pm$ 1.2	115 $\pm$ 2.8	186.6 $\pm$ 3.6	0.54 $\pm$ 0.2
4	Test extract + CCl <sub>4</sub>	22.4 $\pm$ 3.3	117.6 $\pm$ 3.2	189 $\pm$ 4.4	0.6 $\pm$ 1.5



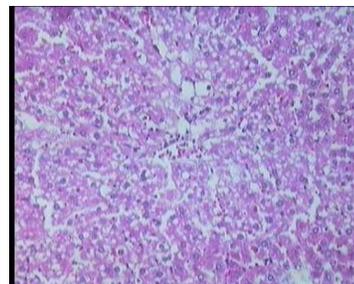
**Figure.1. Microphotograph of normal rat liver section H&E staining  $\times 200$**



**Figure.2. Microphotograph of rat liver section treated with ccl<sub>4</sub> H&E staining ( $\times 200$ )**



**Figure.3. Microphotograph of rat liver section treated with silymarin and ccl4 treated rat H&E staining (x200)**



**Figure.4. Microphotograph of rat liver section treated with methanolic extract and ccl4 treated rat H&E staining (x200)**

## DISCUSSION

The present studies were performed to assess the hepatoprotective activity in rats, against Carbon tetra chloride as hepatotoxic to prove its claim in folklore practice against liver disorder. The changes associated with Carbon tetrachloride induced liver damage of the present study appeared similar to the acute viral hepatitis (Venukumar, 2002). Animals of Group II (received Carbon tetrachloride) significantly lost their body weight and showed reduced food consumption as compared to control group. Animals of Group III and IV (received Carbon tetrachloride plus Standard drug 100mg/kg body weight of silymarin and test extract 500mg/kg body weight) showed a significant increase in body weight and food consumption when compared to Carbon tetra chloride group animals. These findings suggested the extract administered has significantly neutralized the toxic effects of Carbon tetra chloride and helped in regeneration of hepatocytes (Farooq, 1997).

Estimating the activities of serum marker enzymes, like ALT, AST, ALP can make the assessment of liver function when liver cell plasma membrane is damaged, a variety of enzyme normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepato cellular damage (Mc Comb, 1972). The tendency of these enzymes to return to near normally in extract administered group is a clear manifestation of anti-hepatotoxic effects of the extract. Reduction in ALP levels with concurrent depletion of raised bilirubin levels suggests the stability of the biliary function during injury with Carbon tetra chloride. The protein albumin levels were also raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. This hepato protective effect exhibited by the methanolic extract of Calamus Rotang at the dose level of 500mg/kg body weight was comparable with the standard drug, Silymarin. Histopathological liver sections also revealed that the normal liver architecture was disturbed by hepatotoxin in Carbon tetra chloride group, whereas in the liver sections of the rat treated with the methanolic extract and intoxicated with Carbon tetra chloride the normal cellular architecture was retained and it in comparable with the standard Silymarin group, hence confirming the significant hepato protective effect of extract of calamus Rotang at the dose of 500mg/kg body weight. In accordance with these results, it may be confirmed due to the presence of phytoconstituents such as flavonoids, alkaloids and glycosides which are present in the methanolic extract could be considered as, responsible for the significant hepatoprotective activity. In conclusion, it can be said that the methanolic extract of Calamus Rotang exhibited a hepato protective effect against Carbon 6tetrachloride induced hepatotoxicity. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hepatoprotective efficacy of this valuable medicinal plant

## ACKNOWLEDGEMENT

The authors wish to thank The Secretary, Nimra Group of Institutions for providing the research facilities. The authors also wish to thank Department of Pharmacy, Nimra College of Pharmacy for their valuable help and suggestions during the course work.

## REFERENCES

- Afzelechlin from the rhizome of Calamus Rotang linn, Indian Drugs, December, 49(12), 2012.
- Farooq S, Ahmed I, Pathak GK, In protective role of koflet (an Ayurvedic preparation) against cellular toxicity caused by Carbon tetrachloride and flyash, J Ethnopharmacol, 53, 1997, 109-116.

Galighor AE, Kozloff EN. In: Essentials of practical Micro Technique. 2nd ed. New York: Lea and Febiger, 1976.

Ghosh D, Firdaus Sb, Mitra E, Dey M, Chattopadhyay A, Pattari Sk, Hepatoprotective activity of aqueous leaf extract of *Murraya koenigii* against lead-induced hepatotoxicity in male wistar rat. International Journal of Pharmacy and Pharmaceutical Sciences, 5, 2013, 431.

Kumar A, A review on hepatoprotective herbal drugs, Int J Res Pharm Chem, 2(1), 2012, 96-102.

Luna GLHT, Manual of Histologic and special staining methods of the Armed Forces Institute of Pathology, 3rd ed. New York: Mc Graw Hill, 1968.

Mc Comb RB, Bowers GN, Study of optimum buffer conditions for measuring alkaline phosphatase activity in human serum, Clin Chem, 18(2), 1972, 97-104.

Ohkawa H, Ohishi N, Yagi K, Assay of lipid peroxide in animal tissues by thiobarbituric acid reaction, Anal Biochem, 95, 1979, 351-358.

Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, Amer J Clin Pathol, 28, 1957, 56-63.

The Ayurvedic pharmacopoeia of India, Government of India, ministry of health and family Welfare, Department of AYUSH, New Delhi, 1(1), 2008, 225-226.

Valiathan MS, Healing plants, Curr Science, 75, 1998, 1122-1127.

Venukumar MR, Latha, M.S, Hepato protective effect of the methanolic extract of orchids in Carbontetrachloride treated male rats, J. Pharmacol, 2002, 2, 34.