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Original Article

Anti-Lipid Peroxidation Activity of *Acorus Calamus* against Paracetemol induced Hepatotoxicity in Rats

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Abstract

Lipid Peroxidation is an Indicator of Oxidative Damage in the Liver. Upon exposure to the free radicals, the ratio of natural antioxidants in the liver and reactive oxygen species gets altered which results in the production of various lipid peroxyl radicals and hydro peroxides. The extent of lipid peroxidation can be estimated by elevated levels of Malondialdehyde (MDA) in Liver tissue. The present study was designed to screen the anti-lipid peroxidation activity of *Acorus calamus* rhizome extract in paracetamol induced hepatotoxic rat model. Paracetamol at the dose rate of 200 g/kg B.wt was given orally to induce hepatotoxicity and observed the rise in MDA levels. Forty eight hours after paracetamol administration the rats were treated with alcoholic and aqueous extracts of Acorus calamus rhizome for a period of ten days and the liver homogenate levels of MDA was estimated. The treatment of *Acorus calamus* rhizome extract in paracetamol induced hepatotoxic rats normalized the altered MDA levels in liver homogenate which are comparable with Silymarin, a standard hepato-protective drug.

Keywords: Acorus calamus; Lipidperoxidation; Paracetomol; Silymarin; Vitamin-E

Introduction

Lipid peroxidation can be described generally as a process under which oxidants such as free radicals or non-radical species attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (PUFAs) that involve hydrogen abstraction from a carbon, with oxygen insertion resulting in lipid peroxyl radicals and hydro peroxides (Yin *et al.*, 2011*)*.

Amongst of all the products produced by lipid peroxidation MDA is one of the most popular and reliable markers that determine oxidative stress in clinical situations (Giera *et al*., 2012).Most of the drugs have the common side effect of producing the hepatic damage through lipid peroxidation. In spite of tremendous strides in modern medicine there is a lack of ideal hepatoprotective drug hence the phytotherapeutic approach to modern drug development has provided numerous plants and poly herbal preparations in the treatment of liver disorders. This is prompting many scientists in medical research to explore the rich flora of India to come out with effective hepatoprotectants.

So the present study was designed to counter the lipid peroxidation produced by the Paracetamol in rats.

Acorus calamus is a semiaquatic perennial aromatic herb with creeping rhizomes belonging to the family Araceae. It is commonly called as vacha, vasa, sweet flag etc. It is commonly used to protect the children from kapha disorders and for the improvement of the intelligence and as memory enhancer. The rhizome powder of *A. calamus* is used in the training

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of talking birds. The medicated oil of calamus roots is used externally for massages to relieve vata and kapha disorders (Kulkarni, 1998).

The rhizomes of *A.calamus* possess spasmolytic (Gilani *et al.*,2006), ectoparasiticide, insect repellant (Ghosh *et al.*, 2011), anti-secretagogue, antiulcer and cytoprotective (Rafatullah *et al.*, 1994), antidiarrheal (Gilani *et al.*, 2006), hypolipidemic (Parab and Mengi, 2002), anthelmintic and antibacterial (Gaw *et al.*, 2002), neuroprotective (Pradeep *et al.*, 2002), antioxidant (Ulyana et al., 2002), larvicidal (Suryadevara and Khanam, 2002), bio pesticide (Rani et al., 2003), antiproliferative and immunosuppressive (Mehrotra et al., 2003), anticonvulsant (Yende et al., 2009) and antifungal (Jaripa Begum *et al.*, 2004), properties.

It is also used as an ingredient in polyherbal preparations like Asthamania gritha, Canadian bitters, brahmarasayan etc.

Objectives of the present study

1. To screen the ethanolic and aqueous extracts of rhizome of Acorus calamus for its anti-lipid peroxidation activity.
2. To compare the anti-lipid peroxidation activity of *Acorus calamus* with a standard hepatoprotective drug Silymarin and antioxidant vitamin E.

Materials and Methods

Drugs and Chemicals

Paracetamol complying with the specifications of IP was procured from M/s Granules India Limited, Hyderabad as gratis. Sylimarin was procured from M/s Micro labs, Bangalore as gratis. Vitamin E was procured from Himedia Laboratories Pvt. Limited, Mumbai. Analytical grade chemicals from SD Fine Chemicals Ltd. and SRL Pvt. Ltd., were used in the study. Paracetamol suspension was prepared with 0.5% Carboxy Methyl Cellulose (CMC) in water. Sylimarin was suspended in 0.5% CMC.

Collection of Plant Material

Whole plant of *Acorus calamus* was collected from the local market and surrounding areas of Tirupati, Andhra Pradesh, India. The plant was identified and authenticated by the herbarium specialist, Department of Botany, S.V.University, Tirupati.

Preparation of Alcoholic extract of Acorus calamus rhizome

Acorus calamus rhizomes were dried in shade, later they were powdered and extracted (1.5 kg) successively with 30 liters of 60% alcohol in a soxhalet extractor for 18-20 hours. The extract was distilled and concentrated to dryness under reduced pressure and controlled temperature (40-50°c) and finally freeze-dried. The ethanolic extract yielded a weight of 150 g (10% w/w).

Preparation of Aqueous extract of Acorus calamus rhizome

The dried rhizomes of *Acorus calamus* were powdered and the powdered material was taken in a round bottom flask and was extracted with water for 48 h at room temperature. After 48 h, the solution was concentrated in a rotatory evaporator. Aqueous and alcoholic extract of *Acorus calamus* was suspended in 0.5% CMC.

Collection of organs

Liver pieces were taken and minced into fine pieces and 10% homogenate was prepared with 0.1M Tris–HCl buffer, pH 7.4.The samples were centrifuged in a R-8C centrifuge at 7000 rpm for 15 min and the supernatant was stored at –20°C for further analysis.

Assay of Thiobarbituric acid reactive substance (TBARS) (Yagi, 1984)

To 0.5ml of homogenate, 1ml of 20%TCA and 2 ml of 46 mM TBA reagent were added, mixed well and kept in boiling water bath for 30 min. The test tubes were then cooled and centrifuged at 3000 rpm for 3 min. The supernatant was taken and the color formed was read at 532 nm. Standards of different concentrations (0.1 – 0.5 n moles of MDA) were run simultaneously for getting standard curve. The concentration of test samples was obtained using the standard curve.

Experimental animals

Male albino rats of *wistar* strain weighing 150-200g were obtained from Department of Laboratory Animal Medicine, TANUVAS, Madhavaram milk colony, Chennai. The animals were maintained under standard laboratory conditions with food and water adlibitum. Approval of the experimental

protocol was obtained prior to the conduct of the experiment from the institutional animal ethics and bio-safety committee. The experiment was conducted in Department of Pharmacology and Toxicology, College of Veterinary Science, Tirupati.

Experimental Design

Forty eight rats were assigned randomly to six groups each containing eight rats. Group I received 0.5% Carboxy methyl cellulose *p.o.* for ten days. Animals of Group II to VI received single oral dose of paracetamol @ 2g/kg on Day one. Group III and IV received ethanolic and aqueous extract of *Acorus calamus* rhizome orally forty eight hours post administration with paracetamol for ten days respectively. Group V received Sylimarin @ 25mg/kg orally forty eight hours post administration with paracetamol for ten days. Group VI received vitamin E @ 30 mg/kg orally forty eight hours post administration with paracetamol for ten days.

Twenty four hours after the last day of treatment under ether anesthesia and whole livers were collected after sacrificing the animals by decapitation.

Statistical analysis

The data was subjected to statistical analysis by applying one way ANOVA as per the standard methods of Snedecor and Cochran (1994). Differences between means were tested using Duncan's multiple comparison test and significance was set at $P<0.05$ and $P<0.01$.

Results

The mean values of lipid peroxidation in various groups of the present study was presented in Tab.1 and Fig.1

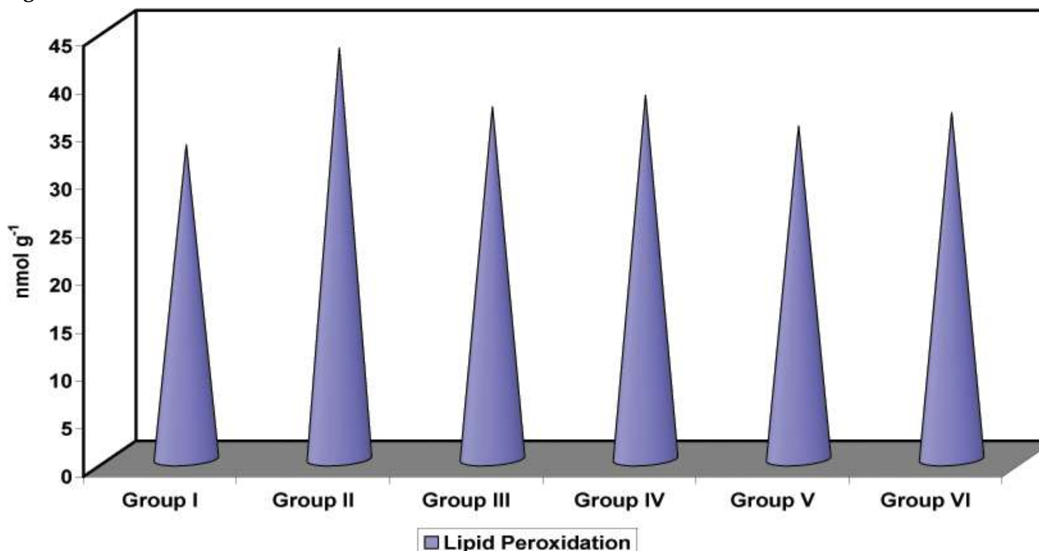
There was significant ($P<0.01$) elevation of MDA levels in group II to 42.95±0.869 when compared to group I 32.8±0.357. There is no significant differences between group III (36.78±0.41), IV (38.00±0.44) and VI (36.18±0.75) at $P<0.01$. The values of group III and VI are on par with each other. The values of group I (32.80±0.35) and group V (34.78±0.59) are similar with each other.

Table 1: Effects of alcoholic and aqueous extracts of *A. calamus* rhizome on Lipid peroxidation in hepatic tissue (nM MDA/g tissue)

S.No.	Title	Lipid peroxidation (nmol g^{-1})
1.	Group I	$32.80±0.35^{d}$
2.	Group II	$42.95±0.86^{a}$
3.	Group III	$36.78±0.41^{b}$
4.	Group IV	$38.00±0.44^{b}$
5.	Group V	$34.78±0.59^{c}$
6.	Group VI	$36.18±0.75^{bc}$

One way ANOVA, the values are mean±SE, n=6
Different superscripts a, b, c, d are statistically significant at $P < 0.001$ and $P<0.005$

Fig.1



Discussion

Paracetamol (acetaminophen) induced hepatotoxicity is thought to be caused by N-acetyl p benzoquinoneimine (NAPQI), a cytochrome P_{450} mediated intermediate metabolite. NAPQI react with -SH group such as glutathione and protein thiols. The covalent binding of NAPQI to cell proteins is the initial step for cell necrosis (Trimenstein and Nelson, 1990).

Paracetamol, primary cellular targets have been postulated to be mitochondrial proteins with resulting loss of energy production as well as proteins involved in cellular ion control (Nelson, 1990).Oxidative stress is another mechanism postulated to be important in the development of paracetamol toxicity (Bhattacharya *et al.*, 2003 and Kumar *et al.*, 2005).Lipid peroxidation serves as a marker of oxidative stress. Lipid peroxidation is oxidative deterioration of poly unsaturated lipids and it involves ROS and transition metal ions.It is a molecular mechanism of cell injury leading to generation of peroxides and lipid peroxides, which can be decomposed to yield a wide range of cytotoxin products, most of which are aldehydes such as MDA (Sangeeta *et al.*, 2004).

A substantial increase in hepatic lipid peroxidation was evident by elevated MDA level in liver homogenate of paracetamol administered group which was similar to the findings of Kapur *et al.* (1994), Ahmed and Khater (2001) and Kumar *et al.* (2005). There is a direct correlation between glutathione depletion and lipid peroxidation (Neha and Rawal, 2000). The elevated levels of MDA were suppressed by Silymarin (Bhattacharya *et al.*, 2003).Silymarin reduced the MDA levels in tissue due to its antioxidant activity (Shenoy *et al.*, 2002). Vit.E inhibits the lipid perodixation due to its antioxidant activity (Ayla *et al.*, 2003). *Acorus calamus* reduced the paracetamol induced lipid peroxidation. Manikandan et al. (2005) reported Acorus calamus reduced lipid peroxidation levels in rats exposed to noise induced stress.

In normal cell, a balance exists between oxidative products and antioxidant protection. The intracellular antioxidants GSH, vit. E and vit. C are interrelated with each other and they can be recycled. Recycling of tocopheroxyl radicals to tocopherol is achieved by reaction with ascorbic acid, a major mechanism for maintenance of tissue tocopherol levels (Arivazhagan *et al.*, 2000).

Vit. C deficiency results in depletion of tissue vit. E level vit. C itself acts as a powerful scavenger of superoxide induced lipid peroxidation. Endogenous vit.C levels have been reported to decline under stress conditions (Acharya and Acharya, 1997). The levels of Vit.C and E were increased in the animals treated with á-asarone, one of the active principles present in *Acorus calamus*, which prevented the protein oxidation and enhanced the GSH levels in stress induced rats (Manikandan and Sheeladevi, 2005).

The results of the present study revealed that treatment with aqueous and alcoholic extracts of *Acorus calamus* normalized the activity of lipid peroxidation in liver tissue.

The present study revealed the antilipid peroxidation activity of *Acorus calamus* against paracetamol induced lipid peroxidation in rats. Hence, the *Acorus calamus* rhizomes can be used in treatment of liver disorders after detailed investigationof active compounds and the exact mechanism involved in the hepatoprotective activity. The usage of the herbal hepato-protectectives in the therapy reduces the cost of the treatment of the animal which will be economical to the farmer.

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