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Ameliorative Effect of *Acorus Calamus* in Drug Induced Hepatotoxicity in Rats

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Abstract:
Drug induced hepatotoxicity is most common in humans as the liver is the major organ for biotransformation of various xenobiotics including drugs. Medicinal plants often proved to be effective in preventing hepatotoxicity induced by drugs or xenobiotics. Acorus calamus is a semiaquatic perennial aromatic herb with creeping rhizomes and possesses various medicinal properties such as antioxidant, antidiarrheal, cyto-protective, neuroprotective etc. However the available literature suggests that there are no reports on hepatoprotective effect of Acorus calamus rhizome extract. The present study was designed to screen the hepatoprotective activity of Acorus calamus rhizome extract in paracetamol induced hepatotoxic rat model. Paracetamol at the dose rate of 200 g/k.g B.wt was given orally to induce hepatotoxicity and the serum levels of AST, ALT, ALP and total proteins were estimated to assess the liver damage. 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 serum levels of liver enzymes and total proteins were estimated. The hepatoprotective activity of Acorus calamus was confirmed by estimating the liver marker enzymes and total proteins. The treatment of Acorus calamus rhizome extract in paracetamol induced hepatotoxic rats normalized the altered serum levels of AST, ALT, ALP and total proteins which are comparable with Silymarin, a standard hepatoprotective drug.

Keywords*: Acorus calamus, Hepatotoxicity, Paracetomol, Silymarin, Vitamin-E*

1. Introduction

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 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) and antifungal (Jaripa Begum et al., 2004), properties.

The methanolic extract of A.calamus potentiated the anticonvulsant activity of phenytoin in mice (Yende et al., 2009). It is also used as an ingredient in polyherbal preparations like Asthamania gritha, Canadian bitters, brahmarasayan etc.

However, a perusal of available literature revealed no reports on antihepatotoxic potential of Acorus calamus.

The present study is designed in paracetamol induced hepatotoxic rat model with the following objectives.

i. To screen the ethanolic and aqueous extract of rhizome of Acorus calamus for its antihepatotoxic potency
ii. To compare the antihepatotoxic potential of Acorus calamus with a standard hepatoprotective drug.

2. Materials and Methods

2.1. 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^{o}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 was 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.

2.2. 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 biosafety committee. The experiment was conducted in Department of Pharmacology and Toxicology, College of Veterinary Science, Tirupati.

3. 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 a 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 blood was collected from all the experimental animals by retrobulbar plexus puncture under ether anesthesia and whole livers were collected after sacrificing the animals by decapitation.

4. 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 $P<0.01$.

5. Results

The results pertaining to the present study revealed that paracetamol at 2g/kg B.wtp.o. causes significant ($P<0.01$) rise in serum AST, ALT and ALP and reduction in serum total proteins. In Group III, IV, V and VI treatment with ethanolic and aqueous extracts of A. calamus rhizome, Sylimarin and vit. E reduced the altered serum marker enzyme and increased serum total proteins. The estimated values have been depicted in table mentioned.

Effects of alcoholic and aqueous extracts of A.calamus rhizome on serum marker enzymes in hepatic damage AST (IU/L), ALT (IU/L) and ALP (KA Units)

S. No.	Title	AST (IU l^{-1})	ALT (IU l^{-1})	ALP (KA units)	Total protein (g dl^{-1})
1.	Group I	66.50 ± 2.43^{c}	28.00 ± 2.08^{c}	54.91 ± 1.28^{cd}	7.68 ± 0.10^{b}
2.	Group II	205.33 ± 5.13^{a}	54.33 ± 1.58^{a}	70.68 ± 1.39^{a}	5.17 ± 0.21^{d}
3.	Group III	56.33 ± 0.76^{d}	32.66 ± 1.33^{bc}	53.43 ± 3.12^{cd}	6.76 ± 0.28^{c}
4.	Group IV	54.66 ± 0.99^{d}	31.00 ± 1.53^{bc}	58.11 ± 3.06^{bc}	7.46 ± 0.19^{bc}
5.	Group V	74.66 ± 2.06^{b}	27.00 ± 1.93^{c}	62.18 ± 1.98^{b}	8.61 ± 0.31^{a}
6.	Group VI	73.00 ± 1.53^{b}	33.50 ± 1.36^{b}	49.03 ± 2.35^{d}	6.63 ± 0.37^{c}

Table 1

One way ANOVA, the values are mean±SE, n=6

Different superscripts a, b, c, d are statistically significant at $P < 0.001$ and $P<0.005$

6. 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 the 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).

The present study reports the antihepatotoxic potential of A.calamus rhizome extract against paracetamol induced hepatic damage. The hepatic damage produced by paracetamol is estimated by rise in serum AST, ALT and ALP. Silymarin protected the acetaminophen induced rise in serum levels of these enzymes (Suresh Kumar; et al., 2006). Vit E reduced the elevated AST and ALT levels due to the free radical scavenging activity. (Seneur et al., 2003). Aqueous and alcoholic extracts of A.calamus rhizome antagonized the rise in AST, ALT and ALP levels in serum, which might be attributable due to its antioxidant activity and free radical scavenging property (Acuna et al., 2002 and Subathra et al., 2012. This property could have protected cellular damage and prevented the leakage of enzymes. Serum total proteins in paracetamol treated group is markedly reduced and treatment with Silymarin, Vit E and A.calamus rhizome extracts restored the levels of total proteins to normal level. The aqueous extract of A.calamus rhizome showed more significant results as compared to the alcoholic extract. In conclusion the result of this study demonstrates that A.calamus has the protective effect against the paracetamol induced hepatic damage in rats. The present, thus justifies the A.calamus rhizome can be used as a hepatoprotective agent after detailed investigation of the active components and detailed mechanisms involved in these effects.

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