

Original Article

PROTECTIVE EFFECT OF ACORUS CALAMUS RHIZOME IN PARACETAMOL EXPOSURE INDUCED HEPATOTOXICITY IN RATS: BIOCHEMICAL AND HISTOPATHOLOGICAL STUDY

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ABSTRACT

Objective: To study the hepatoprotective activity of an aqueous and alcoholic extract of *Acorus calamus* rhizomes against the paracetamol induced hepatotoxicity in rats.

Methods: Hepatotoxicity was induced by oral administration of paracetamol and chemical parameters such as Glutathione peroxidase, Glutathione reductase, Glutathione, Catalase, lipid peroxidation and histopathological changes in liver was studied by comparing with Silymarin, a standard hepatoprotective drug.

Results: Treatment of rats with aqueous and alcoholic extract of *Acorus calamus* rhizome after paracetamol administration normalized the altered levels of above parameters which may comparable with Silymarin and Vit-E. The hepatoprotective activity was confirmed by histopathological examination of the liver tissue of control and treated animals.

Conclusion: Based on the result it can be concluded that *Acorus calamus* rhizome possesses hepatoprotective effect against paracetamol-induced hepatotoxicity in rats.

Keywords: *Acorus calamus* rhizome, Paracetamol, Silymarin, Vit-E, Hepatoprotection

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INTRODUCTION

Hepatotoxicity is one of the major adverse effects produced by many of the drugs. Hepatic disorders are the most common and serious health problems in animals and human beings. Drugs accounted for 50% of the liver failure out of which 39% is due to acetaminophen [1].

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 a 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 [2].

The rhizomes of *A. calamus* possess spasmolytic [3], ectoparasiticide, insect repellent [4], anti-secretagogue, antiulcer and cytoprotective [5], antidiarrheal [3], hypolipidemic [6], anthelmintic and antibacterial [7], neuroprotective [8], larvicidal [9], antioxidant [10], bio pesticide [11], antiproliferative and immunosuppressive [12] anticonvulsant [13] and antifungal [14], properties.

However, a perusal of available literature revealed no reports on the antihepatotoxic potential of *Acorus calamus* extract of the rhizome. Hence the present study is designed to screen the ethanolic and aqueous extract of the rhizome of *Acorus calamus* for its hepatoprotective effect in male rats.

MATERIALS AND METHODS

Paracetamol IP was procured from M/S Granules India as gift sample, Silymarin was procured from Microlabs Bangalore as gratis. All other chemicals used in the study were of analytical grade and procured from a standard supplier.

Collection and identification 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 soxhlet extractor for 18-20 h. 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.

Table 1: Experimental design

Group I	0.5% Carboxymethylcellulose <i>p. o</i> for ten days
Group II	Paracetamol @ 2g/kg <i>p. o</i> on Day one+0.5% Carboxymethylcellulose <i>p. o</i> for ten days
Group III	Paracetamol @ 2g/kg <i>p. o</i> on Day one+Ethanolic extract of <i>Acorus calamus</i> rhizome(600 mg/kg) <i>p. o</i> for ten days
Group IV	Paracetamol @ 2g/kg <i>p. o</i> on Day one+Aqueous extract of <i>Acorus calamus</i> rhizome (600 mg/kg) <i>p. o</i> for ten days
Group V	Paracetamol @ 2g/kg <i>p. o</i> on Day one+Silymarin @ 25 mg/kg <i>p. o</i> for ten days
Group VI	Paracetamol@ 2g/kg <i>p. o</i> on Day one+Vitamin E(30 mg/kg) <i>p. o</i> for ten days

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.

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. Blood samples were used for the estimation of RBC enzyme profile viz Glutathione Peroxidase (GPx) Activity: [15], Glutathione Reductase activity: Spectrophotometric method-[16], Erythrocytic Catalase activity: [17], Lipid peroxidation activity from hepatic tissue homogenate Thiobarbituric acid reactive substances [18]The liver specimens obtained from control and treated groups were fixed in 10% formalin ad stained with H and E stain.

Statistical analysis:

The data were subjected to statistical analysis by applying one way ANOVA as per the standard methods of Snedecor and Cochran [19]. Differences between means were tested using Duncan's multiple comparison tests and significance was set at $P < 0.05$ and $P < 0.01$.

Table 2: Effects of alcoholic and aqueous extracts of *A. calamus* rhizome on erythrocyte antioxidant system (Glutathione peroxidase (U/ml), Glutathione reductase (U/ml), catalase (moles/sec)

S. No.	Title	Glutathione peroxidase (U ml ⁻¹)	Glutathione reductase (U ml ⁻¹)	Catalase (moles sec ⁻¹)
1.	Group I	72.45±1.75 ^a	40.81±0.77 ^a	2.98±0.02 ^a
2.	Group II	47.58±3.65 ^c	27.35±1.12 ^d	1.50±0.11 ^c
3.	Group III	60.38±1.19 ^b	34.13±1.12 ^b	2.58±0.07 ^b
4.	Group IV	62.50±1.33 ^b	31.50±0.72 ^c	2.63±0.11 ^b
5.	Group V	67.38±1.17 ^{ab}	39.65±0.75 ^a	3.02±0.01 ^a
6.	Group VI	61.81±2.19 ^b	36.95±1.25 ^b	2.49±0.17 ^b

Table 3: Effects of alcoholic and aqueous extracts of *A. calamus* rhizome on glutathione (mg/dl) levels in serum

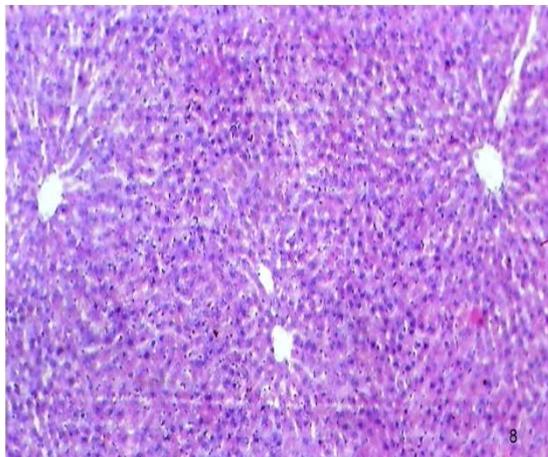
S. No.	Title	Glutathione (mg dl ⁻¹)
1.	Group I	6.40±0.27 ^a
2.	Group II	1.45±0.07 ^d
3.	Group III	5.58±0.15 ^b
4.	Group IV	3.98±0.14 ^c
5.	Group V	5.78±0.09 ^b
6.	Group VI	5.30±0.21 ^b

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

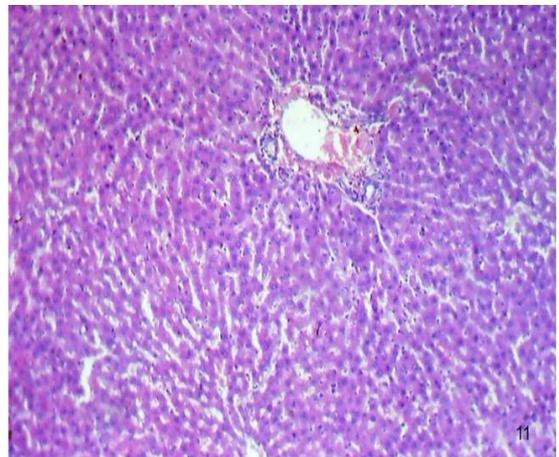
Table 4: Effects of alcoholic and aqueous extracts of *A. calamus* rhizome on lipid peroxidation in hepatic tissue (nM MDA/g tissue)

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}

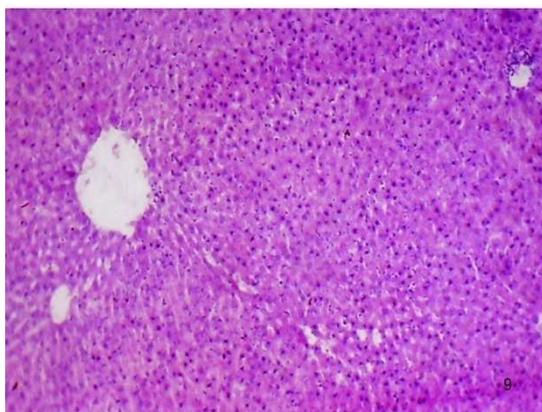
One way ANOVA, the values are mean±SE, n=6, Different superscripts a, b, c, d are statistically significant at $P < 0.001$ and $P < 0.005$



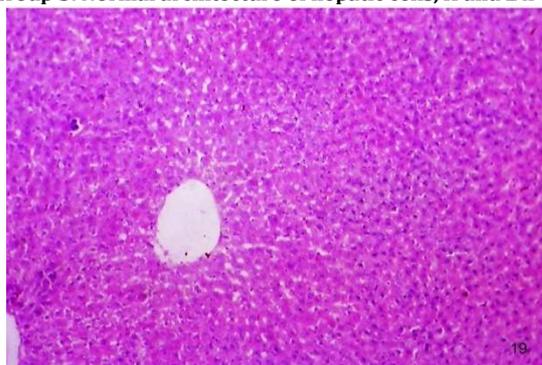
Group 1: Normal architecture of hepatic cells, H and E x 70



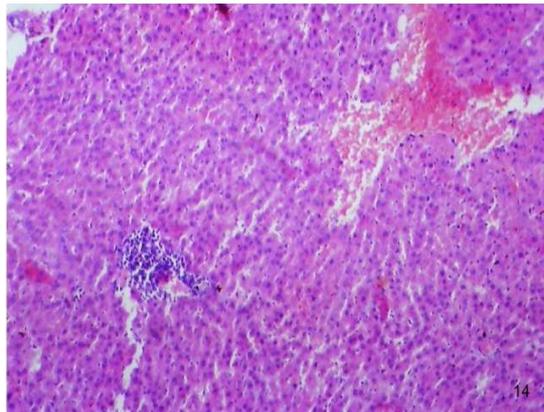
Group 2: Perivascular infiltration of mononuclear cells, H and E x 70



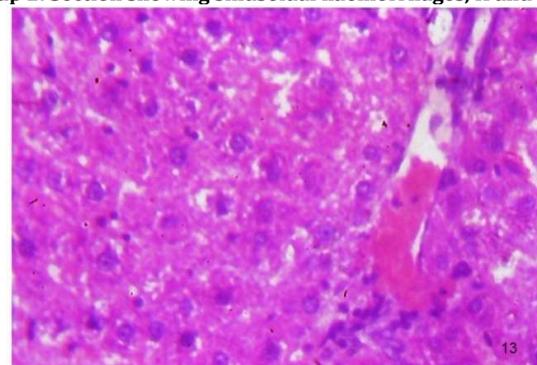
Group 5: Normal architecture of hepatic cells, H and E x 70



Group 6: Mild degenerative changes in liver parenchyma, H and E x 70



Group 2: Section showing sinusoidal haemorrhages, H and E x 70



Group 3 and 4: Mild proliferation of bile duct, H and E x 70,

Fig. 1: Histoarchitecture of the liver in different treatment groups

DISCUSSION

In the present study, the levels of glutathione peroxidase were reduced in group 2 when compared to group 1 [20, 21]. Silymarin prevented the reduction of glutathione peroxidase by protecting the glutathione depletion. *Acoruscalamus* prevented the altered glutathione peroxidase levels due to its antioxidant activity [22].

Glutathione reductase activity was reduced in group 2 which are in accordance with the other research findings on hepatotoxicity studies [23]. Vitamin E antagonized the glutathione reductase levels due to its antioxidant activity [24]. *Acoruscalamus* attenuated the altered glutathione reductase level due to its antioxidant activity [25].

Catalase, primarily antioxidant defence component is a tetrameric hemoprotein present in peroxisomes and catalyzes the dismutation of H₂O₂ reduces methyl and ethyl hydroperoxides. The liver, kidney and RBC possess relatively high levels of catalase [26].

The catalase activity in the paracetamol treated group was reduced significantly ($P < 0.01$) when compared to control group which is in correlation with the reported data [27, 28]. Silymarin normalized the altered catalase levels [29]. Vitamin E increased catalase levels due to its antioxidant property [30]. *Acoruscalamus* prevented the reduction in catalase activity. In the present study glutathione levels were reduced significantly ($P < 0.01$) in paracetamol toxic group when compared to the control group [20, 27]. Silymarin antagonized the depletion of glutathione due to its free radical scavenging activity. Alcoholic extracts of *Acoruscalamus* showed marked protective effect than aqueous extract in glutathione depletion. Some of the studies reported that concurrent administration of *Acoruscalamus* extract with acrylamide significantly increased the glutathione levels in corpus striatum of rats [8]. The protective action of Vitamin E may be due to its antioxidant activity and also due to its increased red cells level of reduced glutathione [24].

Lipid peroxidation serves as a marker of oxidative stress. Lipid peroxidation is oxidative deterioration of polyunsaturated lipids and

it involves ROS and transition metal ions. It is a molecular mechanism of cell injury leading to the 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. A substantial increase in hepatic lipid peroxidation was evident by elevated MDA level in liver homogenate of the paracetamol administered group [20, 21]. There is a direct correlation between glutathione depletion and lipid peroxidation. The elevated levels of MDA were suppressed by Silymarin. Silymarin reduced the MDA levels in tissue due to its antioxidant activity. Vit. E inhibits the lipid peroxidation due to its antioxidant activity. *Acoruscalamus* reduced the paracetamol-induced lipid peroxidation.

The results of the present study revealed that treatment with aqueous and alcoholic extracts of *Acoruscalamus* normalized the activity of lipid peroxidation in liver tissue. The present study revealed the anti-lipid peroxidation activity of *Acoruscalamus* against paracetamol-induced lipid peroxidation in rats. Hence, the *Acoruscalamus* rhizomes can be used in the treatment of liver disorders after a detailed investigation of active compounds and the exact mechanism involved in the hepatoprotective activity.

In the present study, histopathological findings such as degeneration of hepatic cells sinusoidal haemorrhages and bile duct proliferation were noticed in group II [20, 31]. Treatment with silymarin (group V), Vit. E (group VI) and *Acoruscalamus* (Group III and IV) preserved the structural integrity of the hepatic cellular architecture.

The present study demonstrates the antioxidative capacity of *Acoruscalamus*. The antioxidative effect in turn could have protected the decrease of GSH and other enzymes restoring the free radical scavenger's enzymatic activity. Thus, the use of *Acoruscalamus* may be recommended for lifestyle-related diseases including hepatic diseases and also to improve the general health condition in animals including human beings. Extensive studies in target species should be preceded before the clinical application of *Acoruscalamus* as an antioxidant agent.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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