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Therapeutic efficacy of antihepatotoxic and antioxidant activities of *Acorus calamus* on acetaminophen-induced toxicity in rat

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Abstract
Acorus calamus (AC) is a traditional medicinal plant that is commonly used for treating central nervous system abnormalities. In ayurvedic medicine, it is used for the treatment of insomnia, melancholia, epilepsy, hysteria, loss of memory remittent fevers and neurosis. This plant extract is mainly used for various pharmacological activities like antidiabetic, antiproliferative and immunosuppressive, antidiarrhoeal, hypolipidemic activities. The main constituents of AC were found belonging to monoterpene, sesquiterpene, phenylpropanoid, flavonoid and quinone. The present study is aimed to evaluate the antihepatotoxic and antioxidant activities of ethanolic extract of *Acorus calamus* at two dose level of 250mg/kg & 500 mg/kg B/W on acetaminophen-induced hepatotoxicity in rats. It observed that the ethanol extract of AC confers hepatoprotective and antioxidant activities by histopathological and biochemical observations against acetaminophen induced liver injury in rats. The activity of ethanol extract of AC (500 mg/kg B/W) is comparable to the standard drug silymarin (25mg/kg B/W).

Keywords: *Acorus calamus*, antihepatotoxic, antioxidant, Acetaminophen, silymarin.

INTRODUCTION

Acorus calamus also known as sweet flag is a native plant of India. It is commonly known as Bach or Ugragandha in north India. It is a semi aquatic, perennial, aromatic herb with creeping rhizomes. It exhibits polyploidy. This plant belongs to Araceae family and has been used in the Indian and Chinese system of medicine for hundreds of years to cure disease especially the CNS abnormalities (Lai *et al.*, 2002; Shukla *et al.*, 2006; Koo *et al.*, 2003; Mukherjee *et al.*, 2007). Ethanolic extract of this plant traditionally used for antidiabetes (Cesspooch 2005; Letitia *et al.*, 2002) antiproliferative and immunosuppressive (Mehrotra *et al.*, 2003), antidiarrhoeal (Shoba, 2001) and hypolipidemic (Parab 2002) activities. It is reportedly useful in clearing speech in children (Ignacimuthu *et al.*,2006, Chellaiah Muthu1 *et al.*, 2006) and has allopathic (Nawamaki *et al.*, 1996) properties. In ayurvedic medicine, it is used for the treatment of insomnia, melancholia, epilepsy, hysteria, loss of memory remittent fevers (Agarwal *et al.*, 1956) and neurosis (Shukla *et al.*, 2001). Recently, *Acorus calamus* has been reported to possess high antioxidant activity (Acuna *et al.*, 2002, Shahin *et al.*, 2008). The main constituents of AC were found belonging to monoterpene, sesquiterpene, phenylpropanoid, flavonoid and quinone (Patra and Mitra, 1979). Reports also suggest that the rhizome contains active ingredients possessing insecticidal (Singh *et al.*, 1993, Schmidt *et al.*, 1994, Perrett *et al.*, 1995, Sugimoto *et al.*, 1995, Paneru *et al.*, 1997, Rham *et al.*, 1999, Raina *et al.*, 2003, Lahlou *et al.*, 2004), antifungal (Lee *et al.*, 2004), antibacterial (McGraw *et al.*, 2002), and mitogenic activities towards human lymphocytes (Jagmohan Singh Bainsa *et al.*, 2005). An earlier study showed that the essential oil from this plant is b-asarone that possesses anti-carcinogenic(Hu and Ji 1986; Taylor *et al.*, 1967), anti-proliferative, and immunosuppressive activity (Mehrotra *et al.*, 2003), besides sedative and hypothermic effects (Zanoli *et al.*, 1998).

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However, to the best of our knowledge, the antihepatotoxic effects of this plant extract have not been reported till date. The present study is aimed at addressing this shortfall and evaluates the anti-hepatotoxic and anti-oxidant activities of ethanolic extract of *Acorus calamus* against APAP induced toxicity in rats.

MATERIALS AND METHODS

Plant material

Aerial part of *Acorus calamus* (Araceae) was collected from Tirunelveli district, Tamil Nadu, India in the month of March. The plant material was taxonomically identified and authenticated by V. Chelladurai (Research Officer) Botany (CCRAS) Government of India. Voucher specimen (AECBT-07/2007-2008) has been retained in the Anna bioresearch foundation, Arunai engineering college, Tiruvannamalai, Tamilnadu, India.

Extraction

The aerial part of *Acorus calamus* was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in soxhlet apparatus at 60^{o}C (Chattopadhyay, 2003). The solvent was completely removed by rotary vacuum evaporator. The residue was dissolved in distilled water and filtered. The filtrate was evaporated to dryness. The dried mass was diluted with normal saline and used in experiments.

Animals

Studies were carried out using Wistar albino male rats (150-200g), obtained from Indian Veterinary Preventive medicine (IVPM), Ranipet, Tamilnadu, India. The animals were grouped and housed in polyacrylic cages (38x23x10cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2^{o}C) with dark and light cycle (12/12h). The animals were fed with standard pellet diet supplied by Poultry Research Station, Nandhanam, India and fresh water *ad libitum*. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Drugs and Chemicals

Silymarin was purchased from Micro labs, Tamilnadu. India. Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin and total protein kits were procured from Span Diagnostics, Surat, India, and all the other chemicals used were of analytical grade and were obtained from Ranbaxy research laboratory, Hyderabad, India.

Experimental treatments

Animals were divided into five groups of six animals each. Group I treated with vehicle (distilled water) was kept as normal. Group II treated with a single dose of acetaminophen (APAP) of 750mg/kg body weight was kept as toxin control. Group III and IV were treated with ethanol extract of *Acorus calamus* at two different doses of 250 and 500 mg/kg body wt plus APAP. Group V were fed with standard drug silymarin 25mg/kg body wt. daily for seven days. The extract was administered by oral gavages 1h before APAP administration (Deepak *et al.*, 2007).

Preparation of serum from blood

After 24h, animals were sacrificed by chloroform anesthesia. Blood was collected by heart puncture. The blood samples of each animal were taken and allowed to clot for 45min at room temperature. Serum was separated by centrifugation at 600×*g* for 15min and analyzed for various biochemical parameters including serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (King and Armstrong, 1934), bilirubin (Malloy and Evelyn 1937) and total protein (Gornall *et al.*, 1949).

Preparation of liver homogenate

Hepatic tissues were homogenized in KCl (10mM) phosphate buffer (1.15%) with ethylene-diamine tetra acetic acid (EDTA; pH 7.4) and centrifuged at 12,000×g for 60min. The supernatant was used for assay of the marker enzymes (glutathione peroxidase, glutathione-s-transferase, superoxide dismutase and catalase), reduced glutathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

Biochemical estimation of markers of oxidative stress

MDA content was Measured according to the earlier method reported (Yoshioka *et al.*, 1979). SOD activity was determined according to previous report (Rai *et al.*, 2006). CAT activity was determined from the rate of decomposition of H_2O_2 by the reported method (Bergmeyer *et al.*, 1974). GPX activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and

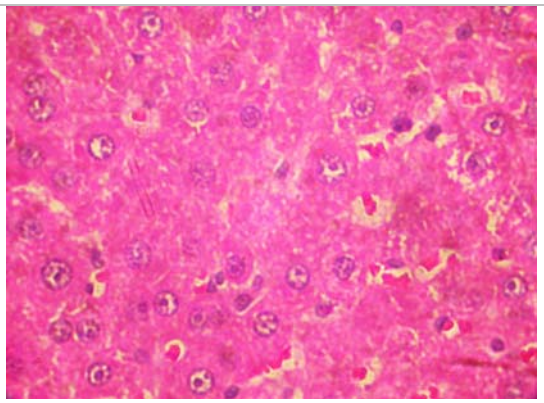

Figure 5a: Normal photomicrograph of liver tissue of control rat showing normal hepatic cells with central vein and sinusoidal dilation. (H and E 100X)

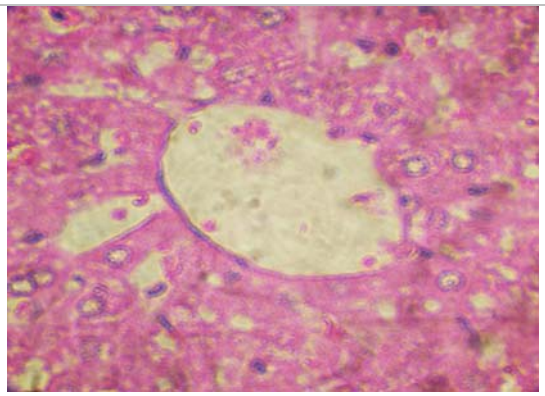

Figure 5b: Liver section of rat showing disarrangement and degeneration of normal hepatic cells with centrilobular necrosis extending to mid zone and sinusoidal hemorrhages and dilation. (H and E 100X)

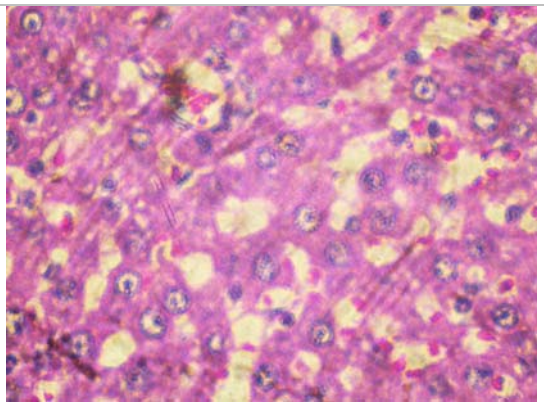

Figure 5c: Histology of liver from rat which received *Acorus calamus* ethanol extract at 250 mg/kg(Group III) showing mild degenerative changes and absence of centrilobular necrosis. (H and E 100X)

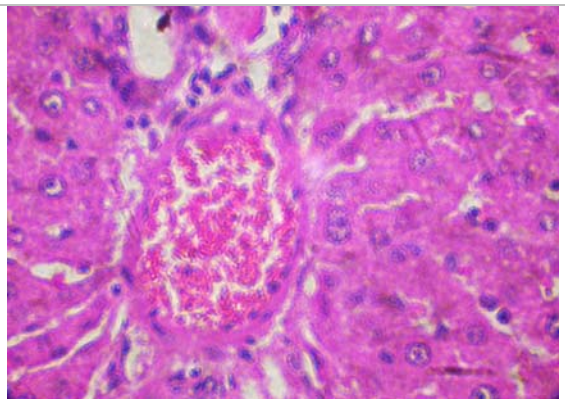

Figure 5d: Histology of liver from rat which received *Acorus calamus* ethanol extract at 500mg/kg(Group IV) showing normal hepatocytes with mild inflammation .(H and E 100X)

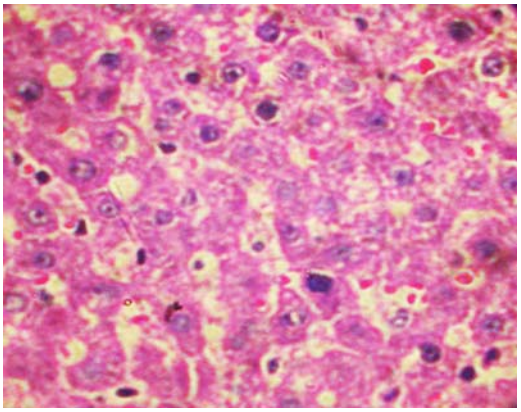

Figure 5e: Liver section of rat treated with silymarin at 25 mg/kg showed less vacule formation reduced sinusoidal dilation, less disarrangements and degeneration of hepatocytes. (H and E 100X)

NaN3 (Hafemann *et al.*, 1974). Glutathione reductase activity was assayed according to previous reports (Carlberg and Mannervik, 1975; Mohandas *et al.*, 1984). Protein content in the tissue was determined by according to earlier reports (Lowry *et al.*, 1951), using bovine serum albumin (BSA) as the standard.

Histopathological study

On completion the regimen animals were sacrificed and the liver was dissected out. Paraffin sections were prepared for histological examination following standard procedure (Galighor Kozloff, 1976). Hematoxylin-eosin stained sections were observed.

Statistical analysis

The obtained results were analyzed for statistical significance using one way ANOVA followed by Dunnet test using the graph pad statistical software for comparison with control group and acetaminophen treated group. $P < 0.05$ was considered as significant.

RESULTS

The effect of ethanol extract of *Acorus calamus* on serum marker enzymes is presented in Fig 1-4. The

serum levels of GOT, GPT, ALP and total bilirubin were significantly ($p< 0.01$) elevated (Fig. 1 and Fig. 2) and that of protein levels were significantly ($p< 0.01$) (Fig. 2) decreased in acetaminophen treated animals, indicating liver damage. Administration of ethanol extract of *Acorus calamus* at the doses of 250 and 500 mg/kg significantly ($p< 0.05$; $p< 0.01$) prevented hepatotoxicity induced by acetaminophen.

Analysis of MDA levels by thiobarbituric acid reaction showed a significant ($P<0.01$) increase in the acetaminophen treated rats. Treatment with ethanol extract of *Acorus calamus* (250 mg/kg & 500 mg/kg) significantly ($P<0.01$; $P<0.01$) prevented the increase in MDA level which was brought to near normal (Fig 1). Acetaminophen treatment caused a decrease in the level of SOD, catalase, GPX and GST in liver tissue when compared with control group ($P<0.01$). The treatment of ethanol extract of *Acorus calamus* at the doses of 250 and 500 mg/kg resulted in a significant ($P<0.05$; $P<0.01$) increase of SOD, catalase, GPX and GST when compared to Group II (Fig 2 & Fig. 3). The standard drug, silymarin treated animals also showed a significant ($P<0.01$) increase in antioxidant enzymes levels compared to Group II.

Morphological observations showed an increased size and enlargement of the liver in acetaminophen treated groups. These changes were reversed by treatment with silymarin and also ethanol extract of *Acorus calamus* at the two different doses tested groups. Histopathological profile of the normal animal showed normal hepatocytes with well preserved cytoplasm and there was no sign of inflammation, which has been illustrated in Fig. 5a. The acetaminophen treated animals showed severe centrilobular necrosis and fatty infiltration (Fig 5 b). Treatment with different doses of ethanol extract of *Acorus calamus* and silymarin produced mild degenerative changes and absence of centrilobular necrosis when compared with control (Fig. 5c, 5d, 5e). All these results indicate a hepatoprotective potential by the ethanol extract of *Acorus calamus*.

DISCUSSION

Acetaminophen (*N*-acetyl-*p*-aminophenol, Paracetamol), a widely used analgesic and antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses (Prescott *et al.*, 1971; Mitchell, 1998; Kuma and Rex 1991; Eriksson *et al.*, 1992; Thompsen *et al.*, 1995). The laboratory features of hepatotoxicity induced by APAP resemble other kinds of acute inflammatory liver disease with prominent increase in levels of of GOT, GPT, and ALP (Davidson and Eastham, 1996).

In the present study, the serum level of hepatic enzymes GOT, GPT, ALP and total bilirubin levels were increased and reflected the hepatocellular damage in the APAP-induced hepatotoxicity animal model. This is indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978). However the total protein level was decreased. There was a significant ($P<0.01$) restoration of these enzyme levels on administration of the ethanol extract of *Acorus calamus* in a dose dependent manner and also by silymarin at a dose of 25mg/kg. The reversal of increased serum enzymes in acetaminophen induced liver damage by the ethanol extract of *Acorus calamus* may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew and Joice, 1987; Maiti *et al.*, 2005). Effective control of ALP, bilirubin and total protein levels suggests towards an early improvement in the secretary mechanism of the hepatic cells, as well as repair of hepatic tissue damage caused by APAP. This indicates the anti-lipid peroxidation and/or adaptive nature of the systems as brought about by ethanol extract of *Acorus calamus* against the damaging effects of free radical produced by APAP.

Previous studies have demonstrated that oxidative stress is a major mechanism in the development of APAP-induced hepatotoxicity (Lin *et al.*, 1998; Ahmed and Khater, 2001; Shanmugasundaram *et al.*, 2006). In the present study, the data suggested that high dosage of APAP in the liver could lead to decreased levels of antioxidant enzymes (SOD, CAT, GPx) and present a significant level of hepatotoxicity in the course of the treatment. However, the ethanol extract of *Acorus calamus* could raise the levels of SOD, CAT, and GPX against the APAP-induced oxidative stress mediated by ROS and RNS. Both reductions of GST and GSH activity in APAP-treated rats as observed in this study indicate the damage to the hepatic cells. Administration of ethanol extract of *Acorus calamus* promoted the reactivation of hepatic glutathione reductase enzyme in APAP-treated rats. The restoration of GSH level to that of APAP treated rats may be due to the protective effect after the administration of ethanol extract of *Acorus calamus*.

Furthermore, the level of MDA was increased in the group receiving APAP administration, but treatment with the ethanol extract of *Acorus calamus* reduced the amount of MDA. This result indicated that decreasing the formation of lipid peroxidation is also one of the events in preventing the oxidative toxicity by APAP.

CONCLUSION

In conclusion, ethanol extract of *Acorus calamus* significantly protects against liver injuries as well as oxidative stress, resulting in improved serum biochemical parameters such as SGOT, SGPT and SALP. The reduced levels of SOD, CAT, GSH, GPX, and GST in acetaminophen-treated rats were significantly increased by treatment with ethanol extract of *Acorus calamus*. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

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