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Evaluation of Analgesic, Antipyretic and Ulcerogenic Activities of *Acorus Calamus* Rhizome Extract in Swiss *Albino* Mice.

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ABSTRACT

Acorus calamus L. commonly known as sweat flag, a traditional Ayurvedic medicinal plant used in India**.** Number of bioactive constituents were identified and characterized from the rhizomes and their essential oils of *Acorus calamus*. Major chemical constituents identified are alpha and beta asarone which is responsible for therapeutic and medicinal properties of *Acorus* species. The current study was carried out to evaluate the analgesic, antipyretic and ulcerogenic activities of ethyl acetate extract from *Acorus calamus* rhizome on Swiss albino mice. The activity of the extract was compared with the standard drug paracetamol. The result illustrated very strong analgesic, antipyretic effect, with the absence of gastric damage by the ethyl acetate rhizome extract.

Keywords: sweat flag, beta asarone, antioxidant, anti-inflammatory.

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INTRODUCTION

Inflammatory diseases are major health problems in mankind. The available anti-inflammatory drugs cannot be used continuously for long term as they cause ulcer as side effect. In spite of modern medicine, rural population of India depends on traditional medicines made from plants. Plants are natural resources of medicine. In the current study we investigated the Analgesic, Antipyretic and Ulcerogenic activities of rhizome extract of *Acorus calamus* on Swiss albino mice. *Acorus calamus* (L.) family of Araceae is a well known plant in Indian traditional medicines for centuries [1]. The rhizomes, roots and essential oils are reported to posses the active ingredients responsible for several important biological activities including antifungal [2], antimicrobial [3], allelopathic [4], anticellular and immunosuppressive [1]. Our previous study proved antimicrobial, antioxidant and antihelmintic activity of *Acorus calamus* [5-7].This promoted us to evaluate the analgesic, antipyretic and ulcerogenic activities of ethyl acetate extract from *Acorus calamus* rhizome.

MATERIALS AND METHODS

Plant materials and extract preparation

Plants were collected from Horticultural Research Station, Yercaud in Tamil Nadu, India and grown in the Herbal Garden, Vellore Institute of Technology University, Vellore, India. The fresh rhizomes were collected, washed thoroughly, weighed and kept for drying. The dried rhizomes were powdered in mixer grinder. A known amount of rhizome powder (4 g each) was extracted with ethyl acetate as per the procedure published previously [1]. Extract thus prepared were weighed and stored at 4° C.

Animals

Group of Swiss *albino* mice was chosen for the study. Swiss *albino* mice were issued from the VIT University animal house. Experiments were conducted in male and female weighing 25-30 g. Different groups of mice were maintained in different cages with food and water *ad libitum* condition. All the animals were maintained in the animal house at 24 ± 1°C with a 12-h light-dark cycle. During the time period of experiments mice were kept free from any pathogenic infection.

Analgesic activity

Analgesic activity was evaluated in *albino* mice using acetic acid writhing assay [8]. Mice (25-30 gms) were injected with 0.2 ml of 0.6% acetic acid and observed for stretching response for 30 minutes. Number of writhes produced by each mouse was counted during this period. Mice consisting of six in each group were given orally the ethyl acetate extract of rhizome (200 and 400 mg/kg body weight) and one group with paracetamol (100 mg/kg body weight) 15 minutes prior to acetic acid and writhing was again counted for each mouse.

Hot plate reaction in mice

Three groups of mice were given ethyl acetate rhizome extract of *Acorus calamus* (200-400 mg/kg body weight) and paracetamol (100 mg/kg body weight) 30 minutes prior to the experiment. Mice were placed on hot plate maintained at 55 ± 1° C in a glass beaker. The pain threshold is considered to be reached when the animals lift their paws or jump out of the beaker. A control mouse was used giving equal volume of saline and experiment was repeated [9].

Antipyretic activity

The antipyretic activity of rhizome extract was evaluated using Brewer's yeast [10]. Temperature was induced to the animals by injecting 10 ml/kg of 15% aqueous suspension of Brewer's yeast. The rectal temperature was measured with clinical thermometer immediately and 18 hours after the Brewer's injection. Mice with 36-37° C after 18 hours were given the rhizome extract of *Acorus calamus* (200 and 400 mg/kg body weight) and temperature was measured for 5 hours at one hour intervals. Paracetamol (100 mg/ kg bt) was used as standard drug for comparing the antipyretic activity of rhizome extract.

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Antiulcer activity

Mice consisting of three groups were left in fasting for 16 hours and rhizome extract (200-400 mg/kg body weight) and paracetamol (100 mg/kg body weight) were given to each group respectively. After 5 hours the animals were sacrificed and stomach of each mouse was opened through great curvature and examined for lesions or bleedings. The lesions were scored using the following scale: 0 = no lesion, 0.5 = hyperaemia, 1 = one or two lesions, 2 = severe lesions, 3 = very severe lesions, 4 = mucosa full of lesions [11]. Control mice were used giving equal volume of saline.

STATISTICAL ANALYSIS

Results are expressed as mean ± SEM. Statistical significance was determined by using the one way ANOVA followed by Dunnett's multiple comparison tests. P values less than 0.05 were considered as significant.

RESULTS

Analgesic activity

Rhizome extract treatment significantly reduced the number of writhings similar to paracetamol when compared to control. The ethyl acetate extract of *Acorus calamus* rhizome reduced the number of writhing responses in a dose-dependent manner within 15 min of injection of acetic acid. The writhing number of the mice received 200 and 400 mg/kg of rhizome extract was even lower than that of the mice received paracetamol (Fig.1). In hot plate method the reaction time of rhizome extract was significantly (400 mg/kg) high then that of standard drug paracetamol (Fig.2).

Antipyretic activity

The oral administration of brewer's yeast suspension markedly elevated the rectal temperature after 18 h. Treatment with rhizome extract (200 and 400mg/ kg bt) reduced the rectal temperature in dose dependent manner (Fig.3). The result obtained from both rhizome extract and paracetamol treated mice were compared with control mice and a significant reduction in rectal temperature was observed.

Antiulcer activity

The mice treated with rhizome extracted (200 and 400 mg/ kg bt) of *Acorus calamus* showed to possess the highest protective effect against ulcerogenesis. When compared with paracetamol treatment, stomachs of animals treated with rhizome extract were completely protected from any visible damage. Hence gastric tolerance towards the rhizome extract was better than that of paracetamol.

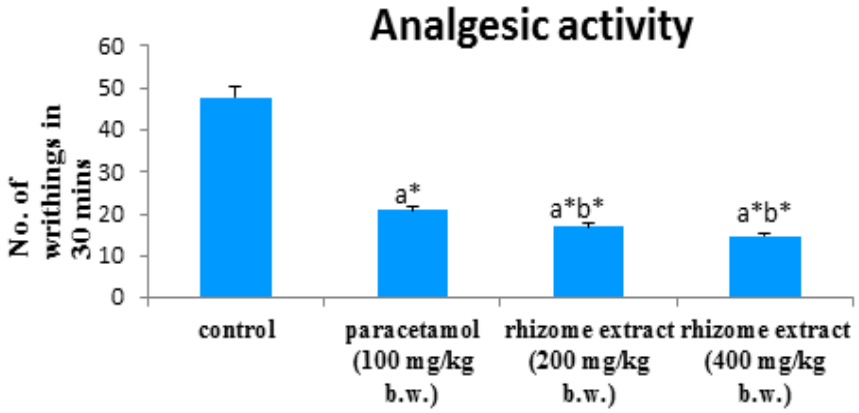

Figure 1: Effect of rhizome extract of *Acorus calamus* and paracetamol on acetic acid induced writhing response in mice. Values represent the mean ± S.E.M. Number of animals used (n=6). a*- significance versus control, b* significance versus paracetamol.

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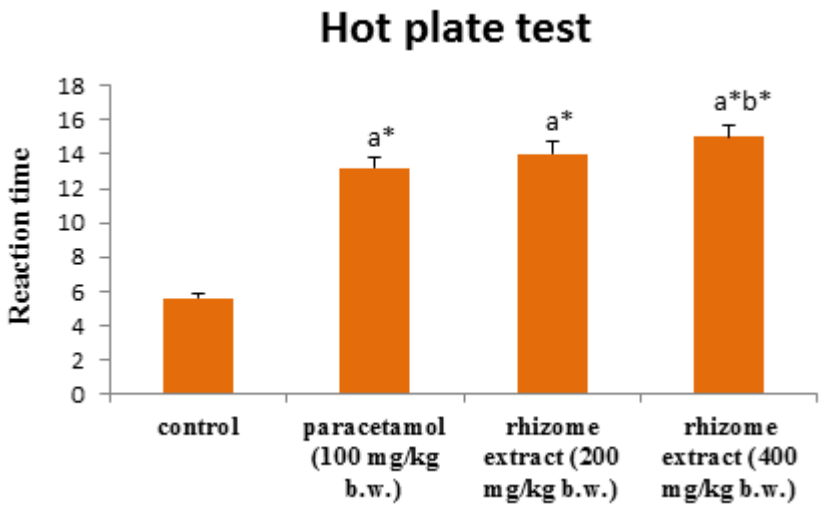

Figure 2: Effect of rhizome extract of *Acorus calamus* and paracetamol on hot plate reaction time in mice. Values represent the mean ± S.E.M. Number of animals used (n=6). a*- significance versus control, b* significance versus paracetamol.

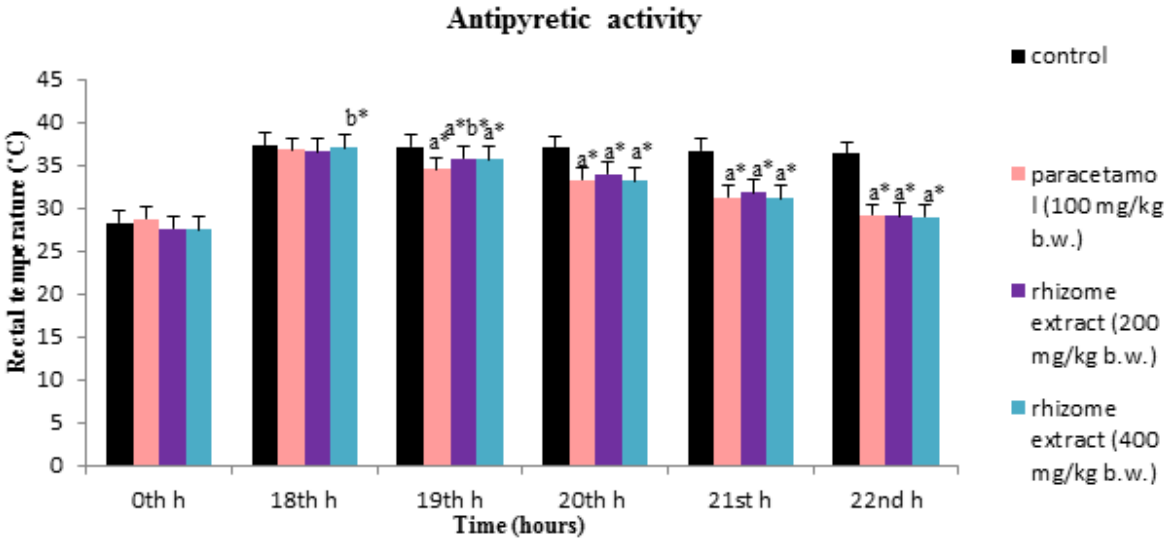

Figure 3: Effect of rhizome extract of *Acorus calamus* and paracetamol on heat induced hyperthermia in mice. Values represent the mean ± S.E.M. Number of animals used (n=6). a*- significance versus control, b* significance versus paracetamol.

DISCUSSION

Acorus calamus is previously reported to exhibit various beneficial pharmacological effects like epilepsy, memory deficits, rheumatic pain and neuralgia [12]. It also inhibits the expression of tumor necrosis factor-alpha [1, 13], and the drugs which inhibit TNF-alpha synthesis and receptor are found to ameliorate neuropathic pain in animals [14] as well as human beings [15]. The ethanolic extract of *Acorus calamus* exhibited potential analgesic, anti-oxidative, anti-inflammatory as well as neuroprotective actions [16]. Our previous study on rhizome extract of *Acorus calamus* proved to have antimicrobial, antioxidant and anthelmintic activity [5-7]. Therefore, with support from literature and data in hand it seems quite evident that *Acorus calamus* and secondary metabolities derived from it exerts beneficial effects in acetic acid induced pain and reduce body temperature in yeast treatment. Our result exhibited significant reduction in the writhing induced by acetic acid treatment by rhizome extract and significantly reduced yeast induced fever.

The reduction in elevating body temperature might be antipyretic activity of *Acorus calamus*. The rhizome extract also exhibited very strong antiulcerogenic effect which might be due to antioxidant compound present in the extract [17]. These compounds capable of settling on the membrane and counteracting with lipid peroxide formation. Our study strongly suggests that active constituents of *Acorus calamus* have enough potential to be used as an analgesic, antipyretic and anti-ulcer drug with excessively limiting side effects.

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