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Short Communication

PHARMACOLOGICAL EVALUATION OF RHIZOMES OF *ACORUS CALAMUS* FOR ANALGESIC ACTIVITY

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ABSTRACT

Objective: To evaluate the analgesic activity of aqueous extract of rhizomes of *Acorus calamus*in mice.

Methods: Analgesic activity of aqueous extracts of *A. calamus* at different doses were studied using the hot plate method. The study was carried out at three different dose levels (5, 25 and 50 mg/kg) of *A. calamus*, given subcutaneously to mice.

Results: The different dose of aqueous extracts of *A. calamus* showed dose and time dependent analgesic effect and maximum effect was observed at 50 mg/kg dose of extract after 90 min of administration, which was comparable to diclofenac sodium, used as standard drug in the study.

Conclusion: The aqueous extract of rhizomes of *A. calamus* was found to be effective analgesic in mice using the hot plate method. This study merits further detailed pharmacological investigation of this extract as well as isolation and characterization of its bioactive chemical constituents (s).

Keywords: *Acorus calamus*, Analgesic activity, Hot plate method.

International Association for the Study of Paindefined pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [1, 2]. Pain of any type is the most common reason for physician consultation of patients to seek medical care annually. It interferes with a person's quality of life and general functioning. Its diagnosis is based on characterizing pain in various ways, according to duration, intensity, type (dull, burning or stabbing), source, or location in body. According to Woolf, pain is classified into three type's namely nociceptive pain, inflammatory pain and pathological pain. Inflammatory pain is associated with tissue damage and the infiltration of immune cells while pathological pain is associated with damage to the nervous system (neuropathic pain) or abnormal function of the brain (dysfunctional pain) [1, 4, 5]. Acute pain usually stops without any treatment and responds to measures such as resting or taking an analgesic. But it may also become intractable and develop into a condition called chronic pain, in which pain is no longer considered a symptom,but an illness by itself [6, 7]. The study of pain has in recent years attracted many different fields such as pharmacology, neurobiology, nursing, dentistry, physiotherapy, and psychology. Pain medicine is a separate subspecialty figuring under some medical specialties like anesthesiology, neurology, and psychiatry.

Pain is part of the body's defense system, triggering a reflex reaction to retract from a painful stimulus, and helps adjust behavior to increase avoidance of that particular harmful situation in the future. Given its significance, physical pain is also linked to various cultural, religious, philosophical, or social issues [1, 8].

Acorus calamus has long been known for its medicinal value [9]. It originated in Europe, but has been extensively used in Ayurveda, particularly to enhance memory. It has been an important herb in the Ayurvedic medicine and indigenous medical system for over 100 years. *A. calamus* rhizomes have been used as a single drug or as a component of certain compound drug preparations in the Indian Ayurvedic system of medicine for psychoneurosis, insomnia, hysteria, epilepsy and loss of memory [10, 11]. Various parts of *A. calamus* possess many biological activities like antispasmodic, carminative, antiepileptic, mental ailments, insecticidal, antifungal, antibacterial, tranquilizing, antidiarrheal, anti-dyslipidemic, neuroprotective, antioxidant, spasmolytic, vascular modulator activities [12-14]. The different extract forms possess the antispasmodic, anthelmintic, antifungal, anti-bacterial, fish toxin, insecticidal, anti-diabetic, anti-proliferative, immunosuppressant, antidiarrheal, antioxidant and hypolipidemic activities [15]. It is also used in the treatment of cough, fever, bronchitis, inflammation, depression and other mental disorders, tumors, hemorrhoids, skin diseases, numbness and general debility [16], stimulant, emetic, carminative, stomachic, as antidotes for several poisoning [11]. Vacha powder mixed with ghee is given ritually in India to newborn babies on the seventh day to improve the intellect and speech development. In China it is used in a similar way, improves speech and said recovery from stroke. The powder is sometimes blown into the nose of a patient in a coma to help regain consciousness. There are several polyploid varieties to be found, some of which do not contain toxic constituent, and these are preferable for medicinal use [17].

These relevant pharmacological activities reported for this extract gave us the rationale to evaluate it for analgesicactivity using mice. This aqueous extract of rhizomes of *A. calamus* was evaluated first time for analgesic activity using hot plate apparatus.

Swiss Albino mice of either sex (3–4 months old) weighing 18-22 g were obtained from Sanjay Biological laboratory, Amritsar. The animals were housed in standard cages and maintained at room temperature with natural day and night cycles. Animals were allowed free access to food (standard laboratory rodent chow) and water during the study. The experiments were performed between 09:00-16:00 hAnimals were acclimatized to the laboratory conditions by handling them at least once a day during this period. All procedures were conducted as per guidelines of the committee for the purpose of control and supervision of experimental animals and the protocol for the use of animals for this study was approved by the Institutional Animal Ethics Committee.

Diclofenac Sodium was procured from Jackson laboratory, Amritsar, Punjab. Dried Rhizomes of *A. calamus* were collected from Herbotech Pharmaceuticals, Pandori, Amritsar, Punjab.

The dried rhizomes of *A. calamus* were crushed to powder with the help of the grinder and then the powder was subjected to extraction procedure. The powdered rhizomes were successfully extracted by using maceration procedure. 100 g of powder rhizomes were placed in a

beaker containing 800 ml of water and it was kept for 7 dayswith occasional shaking. The extract obtained was filtered and concentrated to fine powder.

Phytochemical Screening was performed to detect the presence of various phytoconstituents like carbohydrates, glycosides, phenolic compounds, monosaccharides, reducing sugar and alkaloids in the aqueous extract of *A. calamus*.

Determination of petroleum ether soluble extractive was carried out. 5g of the air dried drug coarsely powdered was macerated with 100 ml of pet. Ether of the specified strength in a closed flask for 24 h shaking frequently during 6 h and allowed to stand for 18 h Evaporate 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish, dry at 1050, and weigh. Calculate the percentage of pet. Ether-soluble extractive with reference to the air dried drug [18].

Animals were randomly allocated into five different groups (n=6 per group). Animals of Group I served as Control and was administered with water for injection (1 ml/kg, s.c.) as a vehicle. Group II animals served as positive control and were administered Diclofenac sodium at a dose of 20 mg/kg (s.c.). The groups III, IV and V served as drug treated control groups of AEAC and received the plant extracts at the doses of 5, 25 and mg/kg (s.c.) respectively. The latency was recorded before and after 20, 60 and 90 min following subcutaneous administration of the standard and Extract using Eddy's hot plate apparatus.

The hot plate, which is commercially available, consists of an electrically heated surface. The temperature was controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals were placed on the hot plate and the time until either licking or jumping occurs was recorded by a stopwatch.

The prolongation of the latency times comparing the values before and after administration of the extract or the values of the control with the experimental groups were used for statistical comparison [19].

All the results were expressed as mean±Standard Error (SEM). Data was analyzed using Student's t-test or one-way ANOVA followed by Tukey or Dunnett's t-test (Sigma Stat Software, 2.03). P-values<0.05 were considered as statistically significant.

Phytochemical screening revealed the presence of saponins, monosaccharide, carbohydrates and reducing sugar in the aqueous extract of rhizomes of *A. calamus* whereas phenolic compounds and alkaloids comes out to be absent in the extract (table 1).

Table 1: Results of qualitative chemical tests

S. No.	Chemical tests	Result
1.	Test for Glycosides:	
i.	Test for saponinglycoside (Foam test)	+
2.	Test for Phenolic compounds	
i.	Test with $FeCL_3$ solution	–
ii.	Lead acetate test	–
3.	Test for Carbohydrates	
i.	Molish's test	+
4.	Tests for Reducing sugars	
i.	Fehling's test	+
ii.	Benedict's test	+
5.	Test for Monosaccharides	
i.	Barfoed's test	+
6.	Tests for Alkaloids	
i.	Mayer's test	–
ii.	Hager's test	–
iii.	Wagner's test	–

Note: '-' = Absent; '+' = Present

Standardization of the aqueous extract of rhizome of *A. calamus* plant gives the 10% w/w pet, ether soluble extractive.

There was significant (P<0.05) dose dependent increase in reaction time in mice at 5, 25 and 50 mg/kg dose of Aqueous Extract of *A. calamus* (Group III, IV, V) after 30, 60 and 90 min (fig. 1)of s.c. injection showing maximum effect at 50 mg/kg dose (Group V) after 90 min as compared to control group (Group 1). Standard group (Group 2) also showed significant (P<0.05) increase in reaction time as compared to control group which shows its analgesic activity and this effect is comparable to the effect of 50 mg/kg dose of extract after 90 min (fig. 1). These results showed that different doses of extract have analgesic properties.

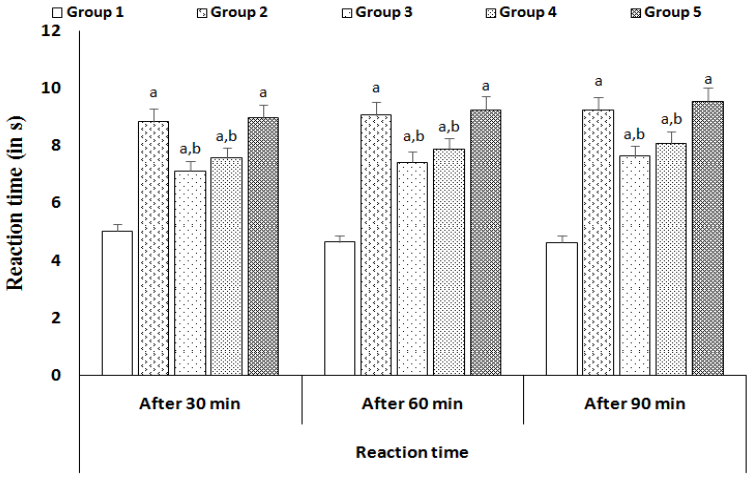

Fig. 1: Effect of different doses aqueous extract of *A. calamus* on reaction time in mice after 30, 60 and 90 min.[a]. P<0.05 as compare to vehicle control, [b] P<0.05 as compare to standard group

The use of traditional medicine in the form of extracts is widespread and plants still present a large source of novel phytoconstituents that might serve as leads for the development of novel drugs [20]. The present investigation was carried out to evaluate the analgesic activity of aqueous extract of *A. calamus* rhizomes. The results showed that extracts from the rhizomes of *A. calamus* have an analgesic effect against the hot plate induced thermal stimuli. Subcutaneous injection of diclofenac sodium produced a significant analgesic effect in this method by inhibition of COX 2 [21]. Since *A. calamus* rhizome extract was active in this type of pain, it may belong to such class of analgesics. Phytochemical screening of the aqueous extract of *A. calamus* rhizomes showed the presence of saponins, carbohydrates, glycosides, monosaccharides and reducing sugar, which might be partly responsible for the analgesic activity reported in the current investigation. Analgesic activity of the extract was evaluated using hot plate mice model. The extract

exhibited significant and marked analgesic actions in the test. The hot plate test measures the response to a brief, noxious stimulus thus bears a closer resemblance to clinical pain [22]. The increase in reaction time in the hot plate test suggests the analgesic effect of *A. calamus*. The ability of *A. calamus* in analgesic activity may be due to the involvement of endogenous prostaglandins. The analgesic activity of extract can be attributed to its phytoconstituents present in the extract.

The above study thus suggests that the aqueous extract of rhizomes of *A. calamus* at different doses shows dose dependent analgesic effect showing maximum effect at 50 mg/kg dose after 90 min which is comparable to diclofenac sodium.

CONFLICT OF INTERESTS

Declared none

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