ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 12, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

STUDY OF THE ANXIOLYTIC ACTIVITY OF ETHANOLIC EXTRACT OF ROOT OF *ACORUS CALAMUS* IN ALBINO MICE

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Received: 27 August 2020, Revised and Accepted: 29 September 2020

ABSTRACT

Objective: Anxiety is classified as a form of sub-acute or chronic fear. Root of *Acorus calamus* has been traditionally used as an anxiolytic. The aim of the study is to assess the anxiolytic activity of ethanolic extract of *A. calamus* (EEAC) by elevated plus-maze test on Albino mice.

Methods: Albino mice of either sex were taken and divided into five groups, each consisting of 5 mice. One group was used as control, one as standard (diazepam), and three as test groups treated with 100, 200, and 400 mg/kg of EEAC. The drugs, that is, 10 ml/kg of normal saline for control, diazepam 2 mg/kg (standard), and 100, 200, and 400 mg/kg EEAC (test groups) were injected intraperitoneally (i.p.), 30 min before placing them in the center of the maze. The preferences of the animal to open/enclosed arm, average time spent in open arm, and numbers of entries in open arm were compared in each group. Data were statistically analyzed by one-way analysis of variance followed by multiple Dunnett's test.

Results: The number of entries in open arm and the average time spent in the open arm by the mice is increased by EEAC in a dose-dependent manner.

Conclusion: EEAC has anxiolytic activity.

Keywords: Anxiolytic, Elevated plus maze, Diazepam, *Acorus calamus*.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i12.39557

INTRODUCTION

Anxiety disorders, the most prevalent psychiatric illness in the general community, are present in 15–20% of medical clinic patients. Anxiety, defined as a subjective sense of unease, dread, or foreboding, can indicate a primary psychiatric condition, or can be a component of, or reaction to, a primary medical disease [1]. Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. Indeed, it is a universal human emotion, closely allied with appropriate fear and presumably serving psychobiological adaptive purposes [2]. The word anxiety is derived from the Latin "anxietas" (to choke, throttle, trouble, and upset) and encompasses behavioral, affective, and cognitive responses to the perception of danger. Anxiety is a normal human emotion. In moderation, anxiety stimulates an anticipatory and adaptive response to challenging or stressful events. In excess, anxiety destabilizes the individual and dysfunctional state results. Anxiety is considered excessive or pathological when it arises in the absence of challenge or stress when it is out of proportion to the challenge or stress in duration or severity, when it results in significant distress, and when it results in psychological, social, occupational, biological, and other impairment [3]. The principal components of anxiety are psychological (tension, fears, difficulty in concentration, and apprehension) and somatic (tachycardia, hyperventilation, palpitation, tremor, and sweating). Fatigue and sleep disturbances are common. Sympathomimetic symptoms of anxiety are both a response to a central nervous system state and reinforcement of further anxiety [4].

Benzodiazepines, being a major class of compounds used for the treatment of anxiety, present a narrow margin of safety between the anxiolytic effect and unwanted side effects, have prompted researchers to evaluate new compounds, especially plant-based drugs having less undesirable effects [5]. The importance of traditional system of medicine and of certain medicinal practices has now been recognized all over the world. Today it is required to have an intelligent and pragmatic approach to evaluate selective drugs of herbal origin [6]. *Acorus calamus* is commonly known as a sweet flag in English and Vasa bach in Hindi. *A. calamus* is a source of essential oil, which is responsible for the medicinal and insecticidal properties. Extractives of different parts of *A. calamus* and calamus oil are widely in traditional systems of medicines for a number of ailments such as antimicrobial, anti-itching, anticonvulsant, antianxiety, antiviral, antiulcer, antispasmodic, anti-inflammatory, anticancer, nootropic, antischizophrenia, insecticide, tranquilizer, and anti-asthmatic [7]. Different parts of the plant showed the presence of large number of phenylpropanoids, sesquiterpenes, and monoterpenes, as well as xanthone glycosides, flavones, lignin, lignans, steroids, and inorganic constituents. Alcoholic extracts of the triploid *A. calamus* were characterized by a higher percentage of b-asarone (11%), which was the main compound [8]. In spite of traditional use, pharmacology of its different parts has not yet been explored scientifically. As such, the present investigation was carried out to evaluate the anxiolytic activity of the ethanolic extract of the root of *A. calamus* (EEAC) in experimental animal models.

METHODS

Plant

Root of *A. calamus* was collected and authenticated by Dr. M. Islam, Professor, Department of Life Science, Dibrugarh University. A voucher specimen (No. DU/LS/219) was deposited at Dibrugarh University.

Preparation of plant extract

The required amount of roots of *A. calamus* was collected and dried at room temperature. The dried roots were ground into powder separately. A sufficient amount of powdered roots was moistened with 95% ethyl alcohol and allowed to remain for 6 h in a percolator. When the liquid began to drop from the percolator, the orifice was closed and the content was allowed to macerate for 24 h. After 24 h, it was allowed to percolate slowly, a rate not exceeding 1 ml/min and the solution was collected in Petri dishes. Alcohol was allowed to evaporate at room

temperature. When the extract got completely dried, it was scrapped out, weighed and stored and the yield at the end of extraction was found to be 80 g [9].

Drugs
Diazepam obtained from Ranbaxy Laboratories, New Delhi.

Animal
Healthy albino mice of either sex were taken from Central Animal House, Assam Medical College (Registration no. 634/02/a/CPCSEA dated 19/05/02). The animals were housed in standard cages and maintained under normal room temperature. The rats were maintained on standard animal diet of Bengal gram, wheat, maize, and carrot in sufficient quantity for the entire period of the experiment. Water was given *ad libitum* during the entire period of the experiment.

Acute oral toxicity studies
Acute oral toxicity test was done according to the Organization for Economic Corporation and Development guidelines 425 [10]. Albino mice of either sex were used. A total of five animals were used. After overnight fasting, they received a single oral dose (2000 mg/kg body weight) of EEAC. Then, food was withheld for a further 3–4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. At the end of the study, the animals were observed for general toxic signs, morphological behavior, and mortality [11].

Preparation of drug doses
1. Vehicle: Normal saline 10 ml/kg was used in the control group
2. Test drugs: 100, 200, and 400 mg/kg of EEAC were prepared with normal saline as the solvent
3. Standard drugs: Diazepam 2 mg/kg prepared with normal saline.

Experimental design
Elevated plus-maze (EPM) test
Procedure
Healthy albino mice of either sex were taken and divided into five groups, each consisting of 5 mice, and treated as follows
1. Control – received normal saline (10 ml/kg) i.p
2. Test 1 – received EEAC 100 mg/kg i.p
3. Test 2 – received EEAC 200 mg/kg i.p
4. Test 3 – received EEAC 400 mg/kg i.p
5. Standard drug – received diazepam 2 mg/kg i.p.

The maze comprised two open arms of 50 cm × 10 cm and two closed arms of 50 cm × 10 cm × 40 cm, extending from a central platform and elevated to a height of 50 cm above the floor. The maze was placed inside a light and sound-attenuated room [12]. The animals were placed individually in the center of the maze, head facing one of the enclosed arms [13] and the stopwatch started and following parameters noted for 5 min:
a. First preference of mouse to open or enclosed arm
b. Number of entries in open and enclosed arms (an arm entry defined as entry of four paws into the arm)
c. Average time each animal spends in an open arm.

The drugs, that is, 10 ml/kg of normal saline for control, 100, 200, and 400 mg/kg EEAC (test groups) and diazepam 2 mg/kg (standard), were injected i.p., 30 min before placing them in the center of the maze. The maze was cleaned after every test to eliminate potential cues such as excreta and urine droplets or spots. The preferences of the animal to open/enclosed arm, average time spent in open arm, and numbers of entries in open arm were compared in each group [14].

Statistical analysis
The statistical significance between groups was analyzed separately using one-way analysis of variance, followed by Dunnett's multiple comparison test. The significance was expressed by "p" values, as mentioned in the tables. $p<0.05$ were considered as statistically significant.

RESULTS

Acute toxicity test
There was no mortality and no sign-symptom of toxicity reported among the animals up to 2000 mg/kg. Hence, the LD_{50} was calculated more than 2000 mg/kg body weight.

EPM model
The number of entries in open arm and the average time spent in the open arm in the three test groups of EEAC are significantly (<0.01) increased when it was compared with control.

DISCUSSION

The present study was undertaken to evaluate the anxiolytic activity of the EEAC in experimental animal models. The experimental design selected was EPM model.

The EPM test is the most popular and valid animal model for measuring anxiety by investigating aspects of physiological and pharmacological behavior [15].

It is the most simple apparatus to study the anxiolytic response of almost all types of antianxiety agents. It is based on a premise where the exposure to an EPM evokes an approach-avoidance conflict that is considerably stronger than evoked by the exposure to an enclosed arm [16]. Rodents have an aversion for high and open space and prefer enclosed arm and, therefore, spend greater amount of time in enclosed arm. When animals enter open arm, they freeze, become immobile, defecate, and show fear-like movements [17].

The EPM test is a behavioral test of anxiety based on the naturalistic tendency of rodents to avoid open and elevated areas [17]. The open arms are more fear-provoking than the closed arms. The ratio of entries, time spent, and rearing behavior in open arms to closed arms reflects the safety of closed arms with relative fearfulness of open arms. The reduction in entry, time spent, rearing in open arms, ratio of open arm to total arm entries, and increased defecation are the indication of high level of fear or anxiety. Anxiolytic drugs increase the propagation of entries, time spent, and rearing in open arms. They also increase the ratio of open arms to total arm entries [18].

In the present study, as shown in Table 1, the number of entries to the open arm of the plus maze for the control Group A was 0.20±0.20. The number of entries to the open arm of the plus maze for the test groups B_1, B_2, and B_3, namely, EEAC 100, 200, and 400 mg/kg and the standard Group C were – 3.17±0.3, 5.8±0.37, 6.2±0.66, and 7.2±0.97, respectively. The average time spent in the open arm for the control group was 1.6±0.74 s for the test groups of EEAC 100, 200, and 400 mg/kg were 38.61±0.74, 40.19±3.4, 41.61±2.95 s, respectively, and for the standard group was 42.92±4.21 s.

Table 1: Effect of EEAC on number of open arm entries and average time spent in open arm of EPM in albino mice

Treatment (mg/Kg IP)	Number of animals	Number of open arm entries (Sec±SEM)	Average time spent in open Arm (Sec±SEM)
10 ml/kg normal saline	5	0.20±0.20	1.6±0.74
EEAC 100	5	3.17±0.3[a]	38.61±4.1[a]
EEAC 200	5	5.8±0.37[a]	40.19±3.4[a]
EEAC 400	5	6.2±0.66[a]	41.61±2.95[a]
Diazepam 2 mg/kg	5	7.2±0.97[a]	42.92±4.21[a]
One way ANOVA	F	31.61	35.36
	df	4,16	4,16
	P	<0.01	<0.01

EEAC: Ethanolic extract of root of *A. calamus, A. calamus: Acorus calamus*, EPM: Elevated plus maze, *n=5 in each group; all values were expressed in mean±SEM – [a]p<0.01 is significant when compared to control (ANOVA followed by Dunnett's multiple comparison test). ANOVA: Analysis of variance

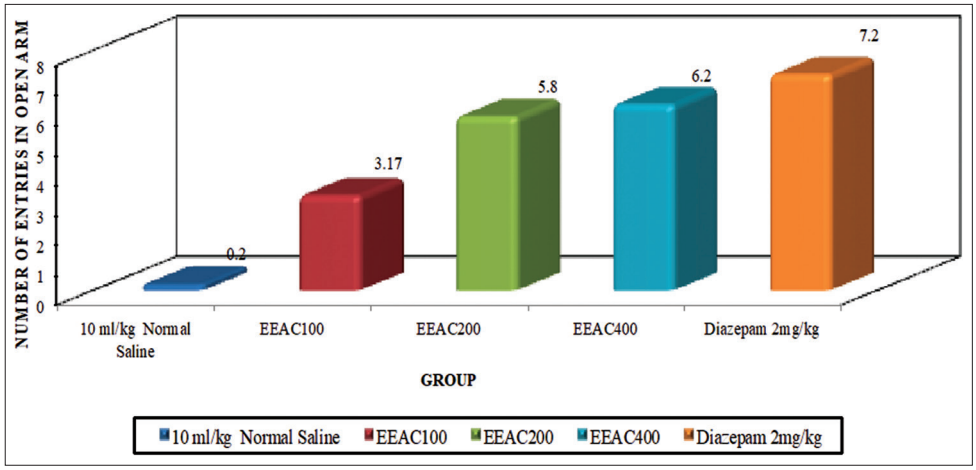

Fig. 1: Effect of ethanolic extract of *Acorus calamus* on No. of entries to the open arm in elevated plus-maze test

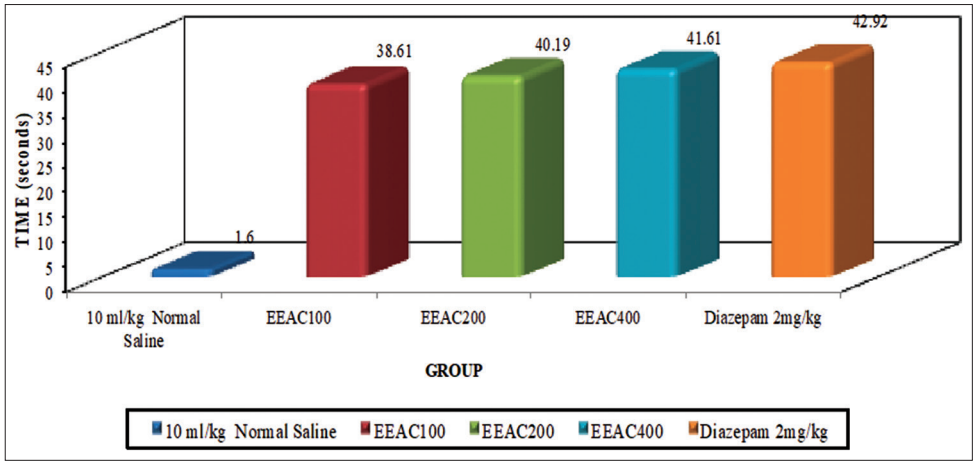

Fig. 2: Effect of ethanolic extract of *Acorus calamus* on the average duration of time spent in the open ARM in the elevated plus-maze test

It can be seen from Figs. 1 and 2, that EEAC increases the number of open arm entries and average duration of time spent in the open arm by the mice. Thus, it can be said that the EEAC has got anxiolytic activity in a dose-dependent manner.

ACKNOWLEDGMENT

We are thankful to Dr. M. Islam, Professor, Life Science, Dibrugarh University, for his help in taxonomical identification.

AUTHORS' CONTRIBUTIONS

Dr. Shipra Kaushik contributed in the drafting of protocol, conducting the experiment, collection, and analysis of data, drafting of the manuscript. Both authors read and approve the final manuscript.

CONFLICTS OF INTEREST

Nil.

AUTHORS' FUNDING

Nil.

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