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Analgesic and Neuropharmacological activity of methanolic extract of *Calamus tenuis* Roxb. Fruits

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Abstract

The present study was carried out for pharmacological investigations on a methanolic extract of *Calamus tenuis* Roxb. fruits. The analgesic activity was investigated for its central and peripheral pharmacological actions using the Tail Immersion Test and acetic acid-induced Writhing Test respectively. Its CNS depressant activity was evaluated by using Hole Cross and Open Field Tests. Acetic acid induced writhing test revealed that the extract at the lower dose inhibited 65.27% writhing and at the higher dose produced a maximum of 93.11% inhibition of writhing which are comparable to the reference drug Diclofenac-Na whose writhing inhibition was 67.06% at 50mg/Kg body weight. On the other hand, the result of the Tail Immersion Test was also impressive. The results of CNS depressant activity showed that the extract decreased the dose dependent motor activity and exploratory behavior of mice in Hole Cross and Open Field Test. The number of fields crossed in open field test and holes crossed in hole-cross test decreased as time passed. These results suggest that the extract possesses analgesic, CNS depressant effect activity.

Keywords: *Calamus tenuis* Roxb, Neuropharmacological, Analgesic activity, Open Field Test, Tail Immersion Test.

Introduction

Medicinal plants play a key role in the human health care. According to a report of World Health Organization, more than 80% of the world's populations depend on traditional medicine for their primary health care needs[1], which is predominantly based on plant material. Plants have started serving as the principal source of drugs from the very first day when the primitive man realized that he could get relief from the sufferings of disease and soothe aches and pains by using plant parts or their products. Medicinal plants have been used in traditional medicine for hundreds of years with a reputation as efficacious remedies although there may not be sufficient scientific data to substantiate their efficacy. [2] Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. A rich heritage of knowledge to preventive and curative medicines was available in ancient scholastic works included in the Atharva Veda, Charaka, Sushruta etc. Over 50% of all modern clinical drugs are of natural product origin[3] and natural products play an important role in drug development programs in the pharmaceutical industry.[4] Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness.

All know it that nature is the greatest sources of medicinal substances. Although synthetic drugs have high profile curative properties, they all are accused to produces,

they all are accused to produce massive side effects. In comparison to these synthetics, the substances obtained from the nature are said to have very less or no side effect. They are also very much cheaper and available as well. *Calamus tenuis* is also a very common plant in Bangladesh. This is used mainly for making furniture. But the fruits of this plant have been being used as an uncommon fruit in this country for a long time. The objectives of this research are to find out whether there are any medicinal (Analgesic and CNS depressant/stimulating) properties present in this plant. If they are, we will go for further studies to find out the specific substances for which they are producing such effects and thus we will be able to develop some analgesic, CNS depressant/stimulating and even anticancer drugs from this plant.

Materials and methods

Plant materials

The fruits of *Calamus tenuis* were collected from the fruit market of New Super Market, Dhaka and were identified by Bangladesh National Herbarium, Mirpur, Dhaka. A sample representing the collection has been deposited in the Bangladesh National Herbarium (Accession Number 33734).

Extraction of plant Materials

Calamus tenuis fruits were dried by shade drying for 15 days. The dried samples were ground to a coarse powder with a mechanical grinder and extracted with methanol by using a Soxhlet extraction method. The extract was filtered. The filtrate was dried at 50^0C to 60^0C and the yielded percentage was calculated

Analgesic screening

Acetic Acid Induced Writhing Method-

The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. The test consists of injecting the 0.7% acetic acid solution intra-peritoneally and then observing the animal for specific contraction of the body referred as writhing. A comparison of writhing was made between positive control (Diclofenac), control and test sample given orally 30 minutes prior to acetic acid injection. If the sample possesses analgesic activity, the animal that received the sample, will give a lower number of writhing than the control, i.e. the sample having analgesic activity will inhibit writhing. Diclofenac-Na is used as a reference standard drug.[5]

Tail Immersion Test-

Immersion of an animal's tail in hot water provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. Again, it is the reaction time that is the time to flick the tail from hot water which is monitored. If a sample contains any analgesic principle it increases the ability of the mice to retain its tail in the hot water which is reflected in the increase in the tail flicking time.[6]

Neuro-pharmacological Screening

Open Field Test-

When the extracts of the *Calamus tenuis* were administered at the 0 min, there were no effects of the test animals. Within 30 min it was observed that the mice began to sleep and therefore little movement was observed. Even after 120 min of administration of the extract, they were still sleeping and there was no significant movement due to sleep.[7]

Hole Cross Test-

Spontaneous movement of the animals through the hole from one chamber to the other was counted for 5 minutes in this test. The observations were made on 0, 30, 60, 90 and 120 minutes after intra-peritoneally injection of the fruit extract of the *Calamus tenuis*. There were no effects of the test animals at 0 min. After 30 min observed that the mice began to sleep and therefore very little movement was observed. Even after 120 min of administration of the extract they were still sleeping.[8]

Results

Results of Analgesic screening

Acetic acid-induced Writhing Test-

Table1 shows the effects of the extract of on acetic induced writhing in mice. The oral administration of both doses of the *Calamus tenuis* extract significantly ($p<0.001$) inhibited writhing response induced by acetic acid in a dose dependent manner.

Tail Immersion test-

The tail withdrawal reflex time following administration of the extract of *Calamus tenuis* was found to increase with

increasing dose of the sample. The result was statistically significant (p<0.05-0.001) and was comparable to the reference drug Diclofenac-Na (Fig.1).

Table 1: Effects of methanolic extract of *Calamus tenuis* on Acetic acid- induced writhing test in mice.

Administered Substance	SEM	Mean ± SEM	% of Inhibition
Control	1.35	33.4±1.35*	0.00
Positive control	2.15	11.0±2.15*	67.06
Group-I	6.25	30.2±6.25*	9. 58
Group-II	6.48	11.6±6.48*	65.27
Group-III	1.85	2.30±1.85*	93.11

Values are expressed as Mean ± SEM (n=5); * donates p < 0.001. Dunnett test as compared to control. Control: Tween-80 + Water, Positive Control: Diclofenac- (50 mg/kg), Group-I: Extract (100mg/kg), Group-II: Extract (200mg/kg), Group-III: Extract (400mg/kg), SD : Standard deviation, SE : Standard error

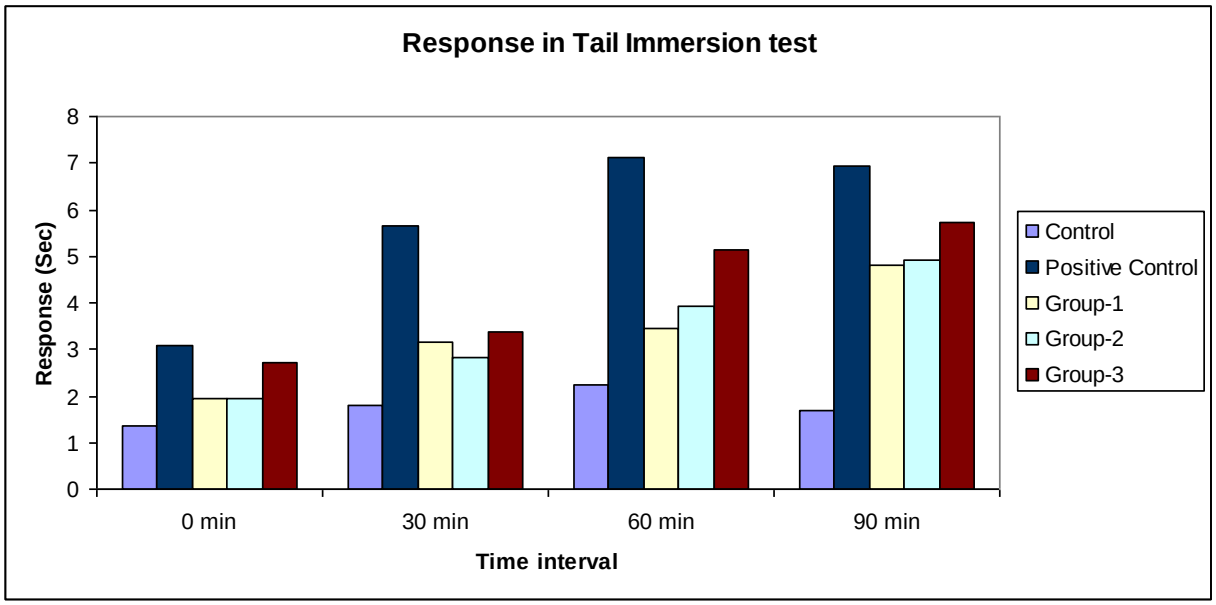

Figure 1: Effect of Methanolic Extract of *Calamus tenuis* on Tail Withdrawal Reflex in Mice

Values are expressed as Mean±SEM (n=5); ** donates p < 0.001. Dunnett test as compared to control. Control: Tween-80 + Water, Positive Control: Diclofenac-Na (50 mg/kg); Group-I: Extract (100mg/kg), Group-II: Extract (200mg/kg) and Group-III: Extract (400mg/kg)

Neuro-pharmacological Screening

Open Field Test-

Historically, open-field defecation and activity have been used to assess the "fearfulness" or "emotional reactivity" of rodents.[9-17] Open field tests showed that the depressing action of the extracts was evident from the second observation period in the test animals at the dose of 100mg/kg body weight. The maximum depressant effect was observed from the second (60 min) to fifth (120 min). The results were dose dependent and statistically significant (p < 0.05-0.001). Results are shown in fig 2.

Hole-Cross Test-

Neuro-pharmacological property of crude extract was carried out by Hole cross test. The extract Significantly (p < 0.05-0.001) displayed a dose dependent suppression of motor activity and exploratory behavior in this test. The locomotor activity lowering effect was evident in the third observation (60 min) and continued up to a fifth observation period (120 min); results are shown in fig 3.

Table 2: Effect of Methanolic extract of *Calamus tenuis* on Open Field Test

Group	Route of Admin	Observation				
		0 min	**30 min**	**60 min**	**90 min**	**120 min**
Control	Oral	113 ±3.60	106.6±1.8	91.2 ±1.71	87.4 ±1.82	74.2±1.59**
Positive Control	Oral	85.4±3.6	50.4±7.64*	37±7.16**	26.4±2.9*	13±5.37**
Group-I	Oral	70±8.81	51 ±5.0.8*	39.6±7.30*	28±10.99*	20.8±9.04**
Group-II	Oral	84.4± 10.52	38.6±9.27*	25.4±10.36**	21.80±9.0*	21.8±2.81**
Group-III	Oral	58.6±9.15	17.4±5.15*	9±2.06**	4.4±2.3**	2.8±0.82**

Values are expressed as Mean±SEM (n=5); ** and * donate $p < 0.001$ and $p < 0.05$ respectively. Dunnett test as compared to control. Control: Tween-80 + Water, Positive Control: Diazepum (3 mg/kg); Group-I: Extract (100mg/kg), Group-II: Extract (200mg/kg) and Group-III: Extract (400mg/kg)

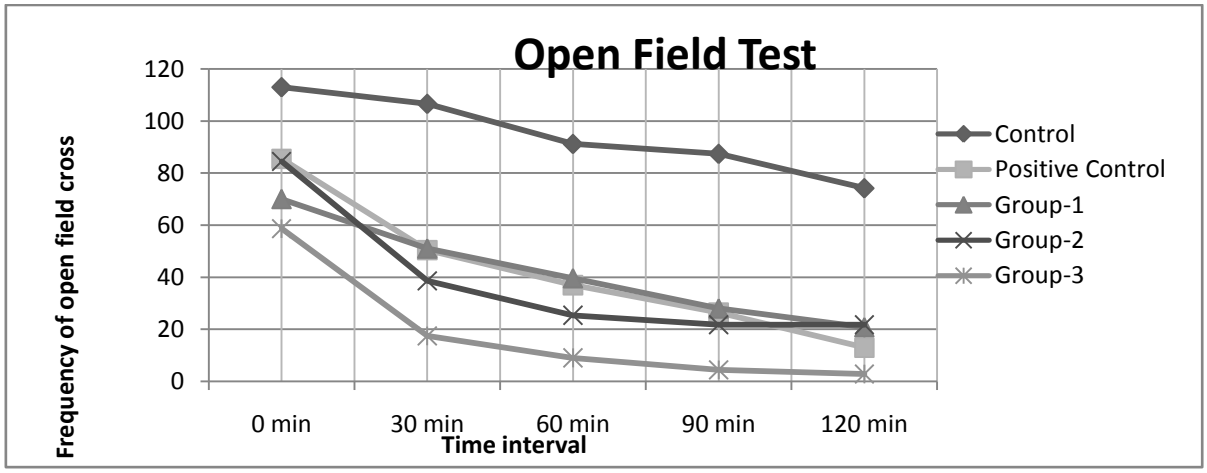

Figure 2: The graphical representation of Methanolic Extract of *Calamus tenuis* on Open Field Test

Values are expressed as Mean ±SEM (n=5); ** and * donate $p < 0.001$ and $p < 0.05$ respectively. Dunnett test as compared to control. Control: Tween-80 + Water, Positive Control: Diazepam (3 mg/kg); Group-I: Extract (100mg/kg), Group-II: Extract (200mg/kg) and Group-III: Extract (400mg/kg)

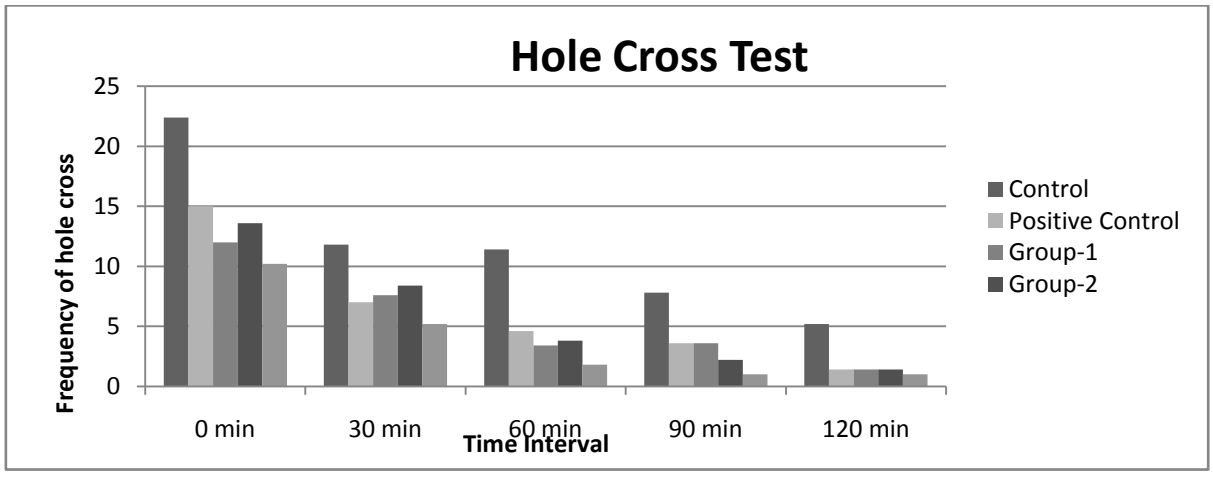

Figure 3: Graphical representation of effect of Methanolic Extract of *Calamus tenuis* on Hole Cross Test

Values are expressed as Mean ±SEM (n=5); ** and * donate $p < 0.001$ and $p < 0.05$ respectively. Dunnett test as compared to control. Control: Tween-80 + Water, Positive Control: Diazepam (3 mg/kg); Group-I: Extract (100mg/kg), Group-II: Extract (200mg/kg) and Group-III: Extract (400mg/kg)

Discussion

The tail flick test and acetic acid induced writhing reflex are useful techniques for the evaluation of central and peripheral anti-nociceptive effects of extracts respectively.[18] The methanolic extract of *Calamus tenuis* displayed a significant and dose dependent analgesic activity against acetic acid induced writhing test in mice. The results were found to be better to that of the standard.

The mean number of abdominal constriction after I.P. Injection of acetic acid was 33.4 in vehicle treated control animals. Diclofenac sodium (50 mg/kg body weight) treatment produced 67.06% inhibition of writhing response. A dose dependent reduction in the number of abdominal constriction was observed in animals treated with different concentration of methanolic extract of *Calamus tenuis*. At the dose of 100mg/kg 200mg/kg and 400mg/kg body weight, inhibition of writhing response was observed 9.58%, 65.27% and 93.11% respectively.

The tail withdrawal reflex time following administration of the extract of *Calamus tenuis* was found to increase with increasing dose of the sample. The mean reflex time of positive control was 3.1 Sec, 5.66 Sec, 7.12 Sec and 6.94 Sec at 0, 30, 60, 90 minutes respectively. Withdrawal reflex time of group-1 (100 mg/kg) was found 1.96 Sec, 3.15 Sec, 3.44 Sec, and 4.80 Sec at 0,30,60,90 minutes respectively whereas it was found 1.95 Sec, 2.81 Sec, 3.92 Sec and 4.92 seconds in group-2 (200mg/kg body weight) and 2.70 Sec, 3.39 Sec, 5.13 Sec and 5.71 seconds in group-3 (400mg/kg body weight).

The most important step in evaluating drug action on the CNS is to observe its effect on locomotors activity of the animal. The activity is a measure of the level of excitability of the CNS 19 and this decrease may be closely related to sedation resulting from depression of the central nervous system. The extract significantly decreased the locomotor activity as shown by the results of the open field and hole-cross tests (Table 2 and Fig 3). The locomotor activity lowering effect was evident in the third observation (60 min) and continued up to a fifth observation period (120 min). Moreover, the validation of anxiety was carried out by measuring external signs, through hole-cross tests.

Open field test showed that the depressing action of the extracts was evident from the second observation period in the test animals at the dose of 200 mg / kg body weight. The maximum depressant effect was observed from a third (60 min) to fifth (120 min) observation period at the dose of 400 mg/kg. Therefore, the results were strongly dose dependent.

Conclusion

Based on the results of the present study, we concluded that the plant extract possesses strong analgesic and CNS depressant activity. In addition, positive results in analgesic and Neuro-pharmacological tests led us to the inference that the plant extract may contain bioactive compounds that may aid ongoing analgesic and CNS depressant drug discovery from florist sources. Thus, further extensive investigations are necessary to prove its clinical efficacy as an analgesic, CNS depressant.

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