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ANALGESIC ACTIVITY OF AQUEOUS EXTRACT OF *ERYNGIUM FOETIDUM* LINN.

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ABSTRACT

Analgesic activity of Aqueous extract of *Eryngium foetidum* (AEEF) was evaluated in a.) Acetic acid induced writhing model in albino mice b.) Tail-flick method in albino rats. Animals were divided into 5 groups of 6 animals each. Group I (control), group II (standard), group III, IV & V (AEEF 200 mg/kg, 400 mg/kg & 800 mg/kg p.o). In acetic acid induced writhing model, with Aspirin 100 mg/kg as standard, 15min after drug administration, writhing was induced by giving 3% acetic acid i.p. Total no. of writhes were counted for 20 minutes and percentage protection calculated. In Tail-flick method, with Pethidine (5mg/kg i.p.) as standard, reaction time of the animals was assessed by analgesiometer at 30, 60, 120 minutes. AEEF significantly reduced ($p<0.05$) acetic acid induced writhing. In tail flick model, AEEF produced significant increase in reaction time ($p<0.05$).

KEYWORDS: Writhing, Percentage protection, Tail flick, Reaction time

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INTRODUCTION

Pain is an unpleasant sensation described as a destructive process or emotional reaction that is often accompanied by anxiety. Therefore it is of dual nature, having both sensation and emotion.[1] Commonly prescribed medications for management of pain such as NSAID's, opoids and steroids have several adverse effects which limits its usage on long term basis. It is estimated that about 90% of people have tried medicinal plants at least once in their life time and some still depend on it for treating ailments. North East region is a genetic treasure house of plant resources and is one of the 12 mega biodiversity rich zones of the world.[2] *Eryngium foetidum* L. (Family: Apiaceae) is a herb cultivated in paddy fields or in wild. It is found extensively throughout Manipur and also known as spicy coriander. It has been used in traditional medicine in the form of raw herb, decoction, paste, tea, etc to treat several ailments such as epilepsy, stomach pain, colic, constipation, to relieve inflammation, flu, diabetes, infertility, hypertension, arthritis, malaria, snakebites, worms, etc. [3] *Eryngium foetidum* has been used for hepatic problem among Chothe tribe of Bishnupur district, Manipur.[4] Present study was conducted to evaluate analgesic activity of Aqueous extract of *Eryngium foetidum* (AEEF) in experimental animal models.

MATERIALS AND METHODS

A. Plant materials

Fresh leaves of *Eryngium foetidum* L. were collected during the month of August-September, 2012. The plant was identified and authenticated by Dr. P. Kumar Singh, Department of Life sciences, Manipur University (Batch No. 000213).

B. Preparation of plant extract

Leaves of the plant were cleaned, dried under shade, powdered by mixer grinder. Preparation of aqueous extract was done by the method described by Verma SCL and Agrawal SL. 50gm of powdered leaves of *Eryngium foetidum* L. were subjected to extraction using soxhlet apparatus. The greenish brown extract obtained was filtered, evaporated, shade-dried, scraped out, weighed and stored in glazed porcelain jar for future use. The yield was 30%.

C. Animals used in experiment

Healthy albino mice weighing 25-30 g and albino rats weighing 100-200 g of either sex were obtained from the central animal house, RIMS, Imphal. Animals were acclimatized for 7 days under laboratory conditions before the experiment. The animals were fed a standard pellet diet and water *ad libitum* and maintained at 24-28^{0}C temperature and 12 hours day and night cycle.

D. Acute Toxicity testing

Acute toxicity testing was done according to OECD guidelines 423. No mortality was observed at a dose of 2000mg/kg. The present study was approved by the Institutional Ethics Committee (Reg No: 1596/GO/a/12/CPCSE.Analgesic activity of aqueous extract of *Eryngium foetidum* L. was tested by following methods.

a. Acetic acid induced writhing test on albino mice as described by Witkin LB et al.[5]

b. Tail-flick method in albino rats by D'Armor and Smith.[6]

a. Acetic acid induced writhing

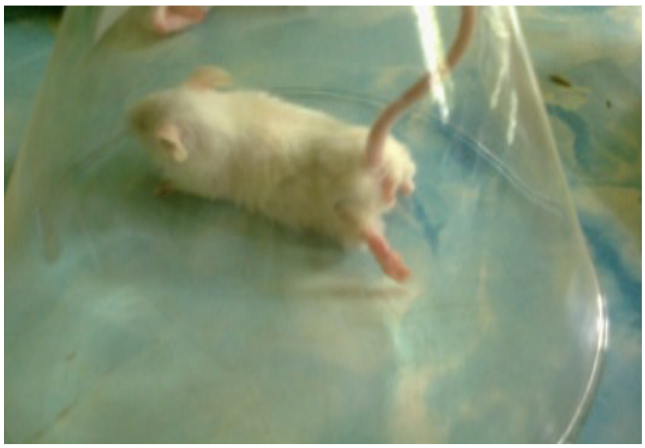
Animals were screened and those which failed to exhibit writhing within 10 minutes were discarded. Screened animals were fasted overnight, but allowed free access to water during the experiment. Animals were divided into five groups with six animals in each group. Drugs were suspended in 2% gum acacia and administered orally. The volume of the medications was kept constant at 10 ml/kg body weight of the animals [Table1]. Writhing was induced 20 minutes later in each mouse by intra-peritoneal injection of 10 ml/kg body weight of 3% acetic acid in distilled water [Picture 1]. The percentage of protection at each dose is calculated as follows

% protection= (1-Experiment/ Control) x 100.

Table 1
Drug administered for Acetic acid induced writhing in mice

Group	Drugs
I (Control)	2% Gum acacia (10 ml/kg bw)
II (Standard)	Aspirin (100 mg/kg)
III (Test 1)	EFL (200 mg/kg)
IV (Test 2)	EFL (400 mg/kg)
V (Test 3)	EFL (800 mg/kg)

Picture 1
Albino mouse showing writhing response



b. Tail flick test

Animals with normal reaction time of 3-4 sec were used in the study and they were divided into five groups of six animals each. Animals were fasted overnight and during the experiment but given water *ad libitum.* Drugs were suspended in 2% gum acacia and administered orally. Standard was administered intraperitoneally with the volume kept constant at 1ml/kg body weight of the animals [Table 2].

Table 2
Drug administered for Tail flick model in rats

Group	Drugs
I (Control)	2% Gum acacia (10 ml/kg)p.o
II (Standard)	Pethidine (5 mg/kg)i.p
III (Test1)	EFL(200 mg/kg)p.o
IV (Test 2)	EFL (400 mg/kg)p.o
V (Test 3)	EFL (800 mg/kg)p.o

Reaction time (Tail flick latencies) of animals was assessed by analgesiometer. A brief current of 6mA was allowed to pass through the naked nichrome wire and it was applied on the proximal tail at a distance of 2.5 cm away from root [Picture 2]. The time taken by the animals to withdraw (flick) tail from the hot wire was taken as reaction time. The cut-off reaction time was fixed at 10 second to avoid any tissue damage. The reaction was noted at 30, 60 and 120 minutes after administration of drug.

Picture 2
Assessment of analgesic activity with analgesiometer

Statistical analysis was done using one way ANOVA followed by Dunnetts't' test for significantdifference between different groups. P value< 0.05 was considered significant.

RESULTS

Acetic acid induced writhing test

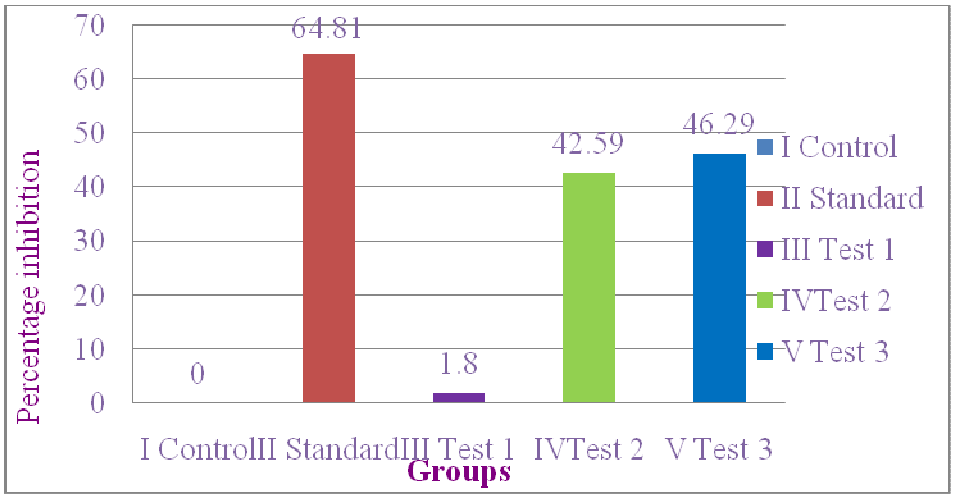
The mean number of writhing movements in Group I (control) was 53.83±0.75, Group II (Standard drug-Aspirin 100 mg/kg) 18.83±0.98, Group III (test drug 200 mg/kg) 53±0.89, Group IV (test drug 400 mg/kg) 31.17±0.75, Group V (test drug 800 mg/kg) 28.83±0.98 respectively. The test drug at the doses of 200 mg/kg, 400 mg/kg, 800 mg/kg produced 1.8%, 42.59%, 46.29% inhibition of writhing movements respectively. The standard drug Aspirin at the dose of 100mg/kg produced 64.81% inhibition of writhing movements [Graph 1]. The number of writhes were significantly reduced in Test 2, Test 3 and standard group as compared to control group [Table 3].

Table 3
Analgesic activity of aqueous extract of Eryngium foetidum L. on acetic acid writhing in albino mice.

Group	Drug dose	No. of writhing in 20 mins Mean±SD	% Protection
I(control)	10 ml/kg	53.83±0.75	
II(Standard)	100 mg/kg	18.83±0.98*	64.81%
III(Test 1)	200 mg/kg	53±0.89	1.8%
IV(Test 2)	400 mg/kg	31.17±0.75*	42.59%
V (Test3)	800 mg/kg	28.83±0.98*	46.29%

One way ANOVA F=28.46, df=(4, 25), *p≤ 0.05 when compared to control, standard, test n=6 in each group

Graph 1



Tail flick test

There was no significant difference between the mean pre-drug reaction time of different groups. After 30 mins of administering of various drugs there was significant increase in reaction time for the test and standard drugs when compared to pre-drug reaction time. The mean reaction time in seconds after 30 mins, 60mins,120mins of administering test drug at the dose of 200 mg/kg were 6.00±0.89, 6.17±0.75, 7.00±0.89 respectively. With the test drug at the dose of 400 mg/kg, the mean reaction time in seconds after 30, 60,120 mins were 6.17±0.98, 7.67±1.0, 7.83±0.75 respectively. The mean reaction time in seconds for test drug at the dose of 800 mg/kg after 30, 60, 120 mins of administration were 7.83±0.75, 8.50±1.04, 8.83±0.75 respectively. The reaction time in seconds for standard drug Pethidine 5 mg/kg i.p after 30, 60, 120mins were 8.83±1.16, 9.17±0.75, 9.27±0.75 respectively [Graph 2]. The test drug at the dose of 200 mg/kg, 400 mg/kg, 800 mg/kg produced significant increase in reaction time at various hours of observation [Table 4].

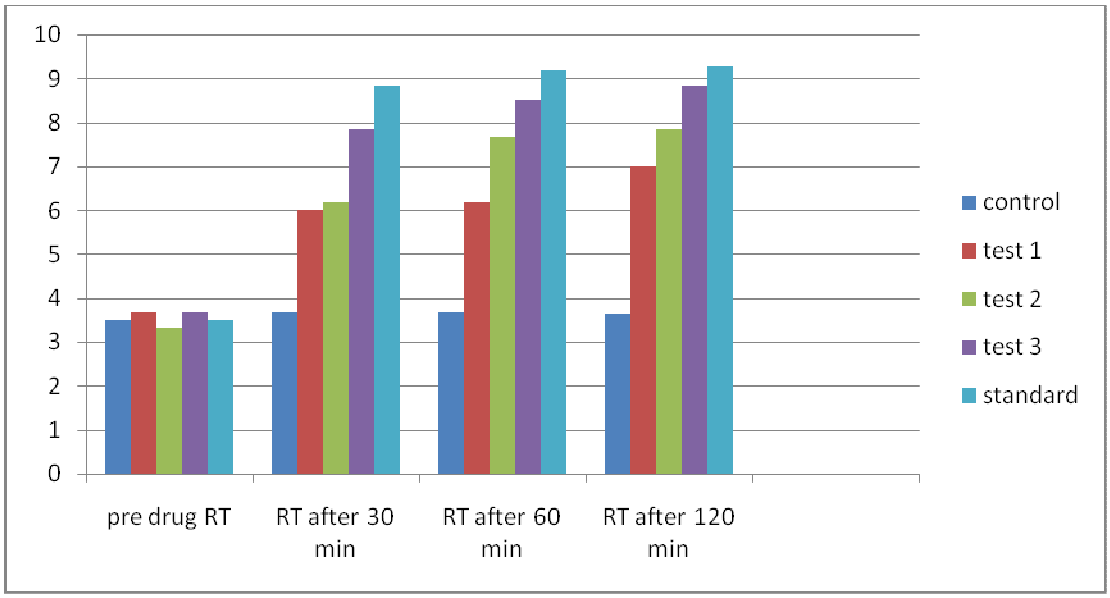
Table 4

Analgesic activity of aqueous extract of Eryngium foetidum L. on tail flick test in albino rats.

Group	Drug dose	Pre-drug reaction time in secs. MEAN±SD	REACTION TIME IN SECS AFTER TREATMENT		
			30 MINS	60 MINS	120 MINS
I(Control)	10 ml/kg p.o	3.50±0.54	3.67±0.81	3.67±0.81	3.67±0.51
II(Standard)	5 mg/kg i.p	3.50±0.83	8.83±1.16*	9.17±0.75*	9.27±0.75*
III(Test 1)	200 mg/kg p.o	3.37±0.51	6.00±0.89**	6.17±0.75**	7.00±0.89**
IV(Test2)	400 mg/kg p.o	3.33±0.81	6.17±0.98**	7.67±1.0*	7.83±0.75*
V(Test3)	800 mg/kg p.o	3.67±0.81	7.87±0.75*	8.50±1.04*	8.83±0.75*
ONE WAY ANOVA df n=6	F	4 0.224	4 26.81	4 36.28	4 45.97

**P<0.05, when compare to the control. ** p<0.05 when compare to control, standard and test 3, n=6 in each group.*

Graph 2



DISCUSSION

Tail flick test is used for detecting centrally acting analgesics whereas acetic acid induced writhing test is used for detecting both centrally and peripherally acting analgesics. AEEF at doses 200, 400 and 800 mg/kg when given orally exhibited a decrease in writhing movement by 1.8%, 42.59% and 46.29% respectively, while the standard drug Aspirin had inhibition of 64.81%. The number of writhing movements during 20 minutes observation in the control group was 53.83±0.75 which corresponds to the finding of Kallappa M Hosamani et al.[7] The intraperitoneal injection of acetic acid produces abdominal writhing due to sensitization of chemosensitive nociceptors by PGs. Increased level of prostanoids particularly PGE2 and PGF [8] as well as LOX products have been found in the peritoneal fluid after intraperitoneal injection of acetic acid. The results strongly suggested that the mechanism of action of the AEEF may be linked partly to inhibition of local peritoneal receptors.Tail flick method is a standard model for evaluating analgesic activity of a drug in albino rats and the reaction time was recorded at 30, 60 and 120 minutes after drug administration. Pethidine (5 mg/kg) was used as standard drug in this study, which was also used as a standard drug by Pankaj Pradhan et al. [9] There was no significant difference between the pre-drug reaction time of the different groups. The animals which showed normal reaction time of 3-4 sec were used for the study. The test drug at doses of 200, 400 and 800 mg/kg produced significant increase ($p<0.05$) in the pain threshold after 60, 90 and 120 minutes of administration. The standard drug Pethidine (5mg/kg), increased the pain threshold significantly ($p<0.05$) at 60, 90 and 120 minutes. The results in control and standard groups were comparable to that of Chakraborty A et al.[10] The increase in the pain threshold in the tail flick method may be due to the possible partial opioid agonistic effect of the aqueous extract of the leaves of *Eryngium foetidum.* AEEF increased the stress tolerance capacity of the animals and hence also indicates involvement of a higher centre.[11] The standard drug Pethidine exerts its action through the μ receptors indicating narcotic involvement.[12]

CONCLUSION

Aqueous extract of EF at doses 400 mg/kg and 800 mg/kg increased the pain threshold significantly on acetic acid induced writhing test in albino mice. At the doses of 200 mg/kg, 400 mg/kg and 800 mg/kg AEFL produced significant increase in the mean reaction time in the tail flick test in albino rats in various hours of observation. The number of writhes was significantly reduced by 400 mg/kg and 800 mg/kg of the aqueous extract. The findings of the present study show that the aqueous extract of the leaves of *Eryngium foetidum* Linn. has significant analgesic

activity. Further studies on the active constituents present in EF is necessary to understand the mechanism of action.

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