ANALGESIC AND NEUROPHARMACOLOGICAL EFFECT ON ETHYL ACETATE EXTRACT OF *IPOMOEA PES-TIGRIDIS* IN ALBINO MICE

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Abstract

The present study was performed to investigate the probable analgesic and neuropharmacological activities of the ethyl acetate extract of *Ipomoea pes-tigridis*Linn. We designed our study to search new analgesic drug from plants which may be harmless to humans as available drugs for the management of pains, fever have many known adverse effects. In phytochemical screening, it was also found the presence of flavonoids, glycosides, alkaloids, saponins, carbohydrates and tannins which have been reported to be responsible for the analgesic activity in many studies. In addition, comprehensive studies have not been performed yet to justify potential CNS responses of the selected plant. In analgesic studies, the extract of *Ipomoea pes-tigridis* showed significant analgesic activity($p< 0.05$-0.000)both acetic acid induced writhing test and hot plate method in mice. In acetic acid induced writhing model, the extract showed 16.5 6% and 33.125% of inhibition of writhing response at 100mg/kg and 200mg/kg respectively. 100mg/kg dose exhibit maximum nociception inhibition at 30 min and 200 mg/kg exhibit highest nociception inhibition also at 30 min. 200 mg/kg extract exhibit basal reaction time 14 and 100 mg/kg extract exhibit basal reaction time 13.8, ,where the positive control shows basal reaction time 12.8 at 30 min. In case of neuropharmacological effect, the extract didn't display any significant dose dependent depression of motor activity as

well as exploratory behavior in both hole cross method and open field test. The results of this present study suggest that the extract possesses analgesic effect but it doesn't have any CNS depressant activities. The plant extract might contain CNS stimuli effect and further investigation is required for the confirmation.

Keywords: *Ipomoeapes-tigridis*Linn.,Analgesic and Neuropharmacological effect.

Introduction

*Ipomoea pes-tigridis*is an annual herbaceous vine with spreading hispid axial parts. This plant belongs to the family Convolvulaceae and is commonly known as "Tiger Foot Morning Glory" in English and locally known as 'Langulilala' in Bengali. The plant was investigated for its morphological and microanatomocal characters (1, 2). It can be found flowering throughout the year when sufficient water is available. Its geographical distribution includes the Sahel zone from Senegal to Niger and North Nigeria, and dispersed across tropical Africa and throughout India, Bangladesh Pakistan, Ceylon, Burma, Malaya, China, Polynesia etc. (1,3). It usually found in bush land, riverside, waste places, cultivated ground and sandy soil. Different parts of this plant are claimed as diversified uses. Leaves are used as purgative and antidote (4). Leaves are also useful to treat poulticing sores and pimples, headaches, swellings, and poisonous string (5). Traditionally extracts of this plant is used against snake bites in Indian sub-continent (6). Moreover, medicinal properties of this herb have also been well documented as inflammation, skin disease, boils, gout, ulcer, arthritis, rheumatism, dropsy and burning sensation (7, 8). Leaf powder is smoked to get relief from bronchial spasm and roots are also reported to treat urinary retention, constipation and gynecological disorders (9, 10). In phytochemical screening, it was also found the presence of flavonoids, glycosides, alkaloids, saponins, carbohydrates and tannins (1). Besides these multitude biological uses, we performed our study to explore analgesic and neuropharmacological activities of this plant.

Materials and methods

Chemicals and drugs

Ethyl acetate used as solvent for the extraction and acetic acid used in writhing test were purchased from Merck, Germany. Aspirin, pentazocain, diazepam and normal saline were collected from Square Pharmaceuticals Ltd. Bangladesh.

Plant material:

The leaves of *Ipomeapes-tigridis*were collected from Chittagong University's area in (Date: 14/03/2013 to 22/03/2013) and its identification was verified by Bangladesh Forest Research Institute (BFRI), Chittagong. The leaves of the plant was cut in to small pieces and ground into fine powder with the help of grinder. Then the powder of the plant stored in air tight container and placed in a cool, dry dark place.

Preparation of the Extract:

200 grams of dried powder was cold macerated in 700 ml ethyl acetate as well as in n hexane for 15 days with occasional shaking and stirring. The whole mixture was filtered through cotton wool and the filtrate was concentrated by evaporation and dried in oven. For ethyl acetate extract percent of yield was 2.4 gm.

Test animal

Albino mice (25–35 g) were collected from Jahangirnagar University, Dhaka. They were housed in plastic cages having dimension of (28×22×13cm). Soft wood shavings were used as bedding of cages. Animals were maintained under standard environmental conditions (temperature: (24.0±1.0°C), relative humidity: 55-65% and 12 hrs light/12 hrs dark cycle) (11). The animals had free access to food and water. The institutional animal ethical committee approved the protocol of this study (USTC/DP/13/516, Date: 22/2/2013).

Analgesic activity

Acetic acid induced writhing in mice

The mice were divided into five groups comprising of five mice per group. Writhings were induced by the method of Koster et al.(12). The test groups were administered 100 and 200 mg/kg of *ipomoea pes-tigridis* intra peritoneally while the control group received 0.3ml normal saline. The reference group received 20 mg/kg aspirin intra peritoneally. The animals were fasted for 16 hr prior to the treatments. One hour after treatment, the mice were injected intra peritoneally with 0.3 ml of 0.6% acetic acid solution to induce the writhing. The number of abdominal writhing and stretching with a jerk of the hind limb was counted between 20 to 30 minutes after acetic acid injection. The response of the extract and aspirin treated groups were compared with those of the animals in the control group (0.3ml saline) percentage protection against writhing movement (%inhibition of writhing) was taken as an index of analgesia and it was calculated as follows:

Percent inhibition = Wt(control) – Wt(test group)/ Wt(control)

Where, Wt = mean number of writhing.

Hot plate method in mice

There were five mice in each groups and groups of mice were treated with pentazocain(17.5mg/kg,i.p) and ethyl acetate extract of *Ipomoea pes-tigridis* (100 mg/kg and 200mg/kg). They were placed on a hot plate maintained at a temperature of 55^0c (13). The latency to lick the paw or jump from the hot plate was noted as the reaction time. The reaction time was noted at 0, 15, 30, 45, 60, 90, 120 min. The cut off time was considered as 15s. The cut off time is determined by taking the average reaction time plus 3 times the standard deviation of the combined latencies of the control mice at all time periods.

Neuro Pharmacological Investigation

Hole cross test

The paws of mice are very sensitive to heat at temperatures which are not responsible for skin damage. The responses are jumping, withdrawal of the paws and licking of the paws. The method was carried out as described by Takagi et al. (14). A wood partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the centre of the cage. The animals were divided into control, positive control, and test groups containing five mice each. The test groups received extracts at the doses of 100 and 200 mg/kg body weight orally the vehicle control and positive control groups received vehicle (1% Tween 80 in water) and the standard drug was diazepam (1 mg/kg b.w.), respectively. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drugs and the standard.

Open field test

In open field test, the animals were divided into control, positive control, and test groups containing five mice each. The test groups received extracts at the doses of 100 and 200 mg/kg body weighorally whereas the control group received vehicle (1% Tween 80 in water). Like hole cross test, animals in positive control group received diazepam (1 mg/kg b.w.). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, and 120 min after oral administration of the test drugs and the standard. The method was carried out as described by Gupta et al., 1971(15).

Statistical analysis: The results of statistical analysis for animal experiment were expressed as mean ± SEM and were evaluated by ANOVA

followed by Dennett's multiple comparisons. The obtained results were compared with the vehicle control group. The $p<0.05$, 0.001 were considered to be statistically significant.

Results and Discussion

In analgesic studies, the extract showed significant analgesic activity both acetic acid-induced writhing test and hot plate method in mice at 100mg/kg and 200mg/kg dose levels. The analgesic studies revealed that the ethyl acetate extract of *Ipomoea pes-tigridis* exhibited potent analgesic (central analgesic activity) effect against thermal noxious stimuli.

In acetic acid-induced writhing test ethyl acetate extract of *Ipomoea pes-tigridis* produced a significant inhibition of writhing response (Table 1) in a dose dependent manner but maximum inhibition (33.125%) of writhing was found at 200mg/kg dose and the results were comparable to standard drug aspirin (20mg/kg). The extract showed 16.5 6% inhibition of writhing response at 100mg/kg and 50.652% of inhibition in aspirin. Inhibition result is better in 200mg/kg dose than 100mg/kg dose (16.5625).

It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, such as PGE2 (prostaglandin E2) and PGE2α in peritoneal fluids, as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs (16, 17). Therefore, the results of the acetic acid- induced writhing strongly suggests that the mechanism of this extract may be linked partly to the inhibition of lipooxygenase and/or cyclooxygenase in the peripheral tissues, thereby reducing PGE2 synthesis and interfering with the mechanism of transduction in the primary afferent nociceptor.

Table 1: Effect of ethyl acetate extract of *ipomoea pes-tigridis* on acetic acid-induced writhing in mice.

`No. of mice	**No. of writhing in mice**			
	*Ipomoea pes-tigridis*EtOAc extract		Control	Standard
	Dose(100mg/kg)	Dose(200mg/kg)		
M1	**52**	**41**	**70**	**33**
M2	**54**	**48**	**65**	**35**
M3	**49**	**42**	**60**	**30**
M4	**57**	**38**	**58**	**32**
M5	**55**	**45**	**67**	**28**
Average	**53.4**	**42.8**	**64**	**31.6**
% of inhibition	**16.5625**	**33.125**		**50.625**
SD	**3.0495**	**3.8340**	**4.9497**	**2.7018**
SE	**0.3811**	**0.4792**	**0.6187**	**0.3377**

Figure 1: Graphical representation:

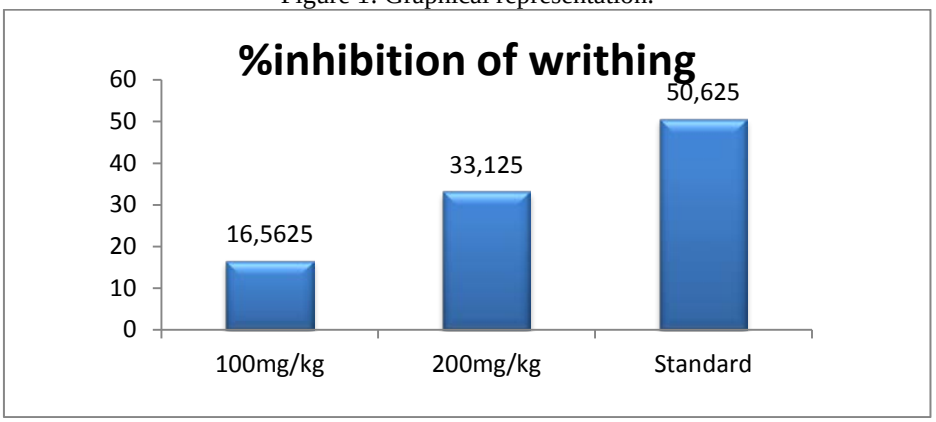


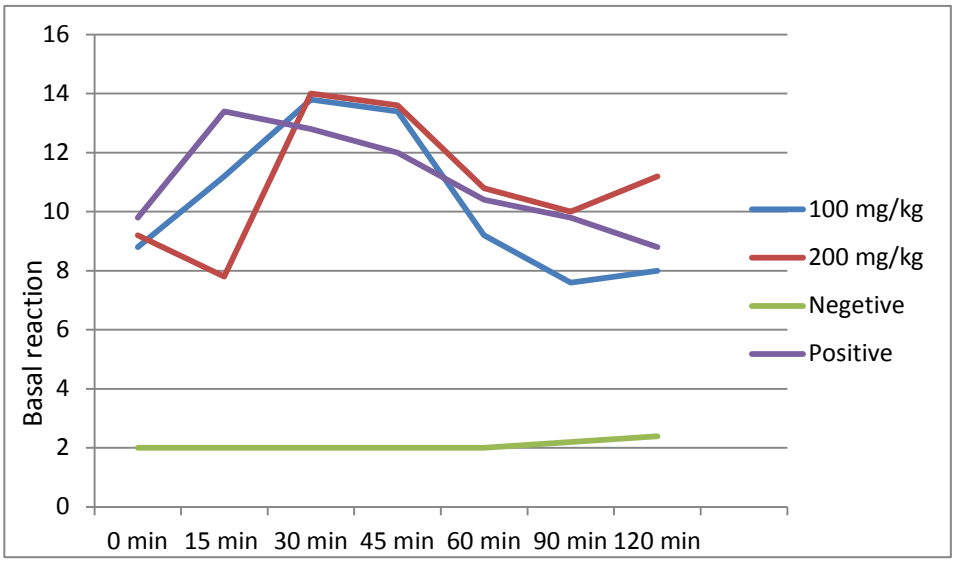
Figure 1: Comparison of % of inhibition of ethyl acetate extract of *ipomoea pes-tigridis* (100mg/kg and 200mg/k g) with standard drug.

In case of Hot plate method, there were five subjected animals for both 100mg/kg and 200 mg/kg doses as well as for negative and positive control. 100mg/kg dose of *ipomoea pes-tigridis* extract exhibit maximum nociception inhibition at 30 min and 200 mg/kg exhibit highest nociception inhibition also at 30 min. 200 mg/kg extract exhibit basal reaction time 14 and 100 mg/kg extract exhibit basal reaction time 13.8 ,where the positive control shows basal reaction time 12.8 at 30 minute (Table 2).

Effect of ethyl acetate extract (100 and 200 mg/kg) of *ipomoea pes-tigridis* on hot plate method in mice.

Dose (mg/kg)	Basal reaction time (sec)						
	0 min	15min	30min	45min	60min	90min	120min
100	8.8±0.42	11.2±0.42	13.8±0.41	13.4±0.44	9.2±0.42	7.6±0.57	8±0.35
200	9.2±0.42	7.8±1.19	14±0.5	13.6±0.27	10.8±0.65	10±0.61	11.2±0.65
Negative	2	2	2	2	2	2.2	2.4
Positive	9.8	13.4	12.8	12	10.4	9.8	8.8

Figure 2: Graphical representation:
Figure 2: Comparison of basal reaction time (sec) of ethyl acetate extract of *Ipomoea pes-tigridis*(100mg/kg and 200mg/kg) with standard drug.



To obtain CNS depressant effect on ethyl acetate extracts of *Ipomoea pes-tigridis*, a number of methods namely open field and hole cross were adopted. The most important step in evaluating drug action on the CNS is to observe the behavior of the test animals. Substances that have CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both. Another important step in evaluating drug action on CNS is to observe its effect on locomotors activity of the animal. Decrease in locomotors activity of mice may be closely related to sedation resulting from depression of the central nervous system. The rate of locomotors activity of albino mice, after applying *Ipomoea pes-tigridis* ethyl acetate extracts, is moreover gradually increased in consideration of depressing agent Diazepam on 1st, 2nd, 3rd, 4th, and 5th observation data of mice (Tables 3, 4). The observation is similar in both hole cross and open field method. In Hole cross method, the average passage of a mouse through the hole from one chamber were 11.20 for 100 mg/kg dose and 11.20 for 200 mg/kg dose at the initial stage. After two hour, 100 mg/kg dose treated mice passed 4.60 times and 200 mg/kg treated mice passed 4.00 times. The value was more than positive control and differences was maximum at 60 min. On the other hand, in case of open field method the numbers of squares visited by the animals were more in our plant extract for both 100 mg/kg and 200 mg/kg dose in consideration of diazepam. So, this extract doesn't have any

CNS depressant activity on mice. It might have anti-depressant activity which needs further investigation for the confirmation.

Table 3: Effect of 100mg/kg and 200 mg/kg dose of etheyl acetate extract of *Ipomoea pes-tigridis*forHole cross.

Minutes	Control	Diazepam	*Ipomoea pes-tigridis* extract	
			100 mg/kg	200mg/kg
0	18.60	12.80	11.20±1.34	11.20±1.92
30	9.20	6.60	9.40±1.03	9.60±1.15
60	5.40	4.40	10.40±1.09	10.20±1.64
90	5.40	4.60	7.00±2.06	7.20±2.06
120	4.60	3.80	4.60±1.09	4.00±1.95

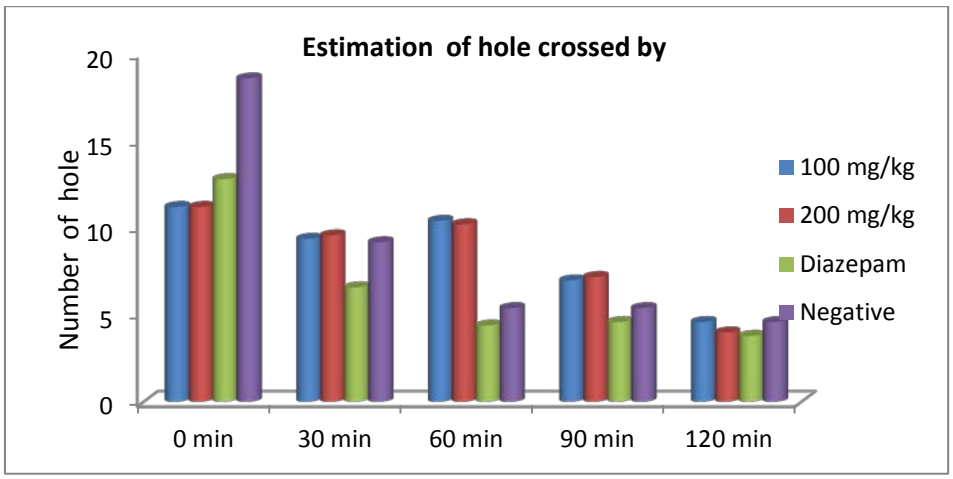


Figure 3: Comparison between 100mg and 200 mg/kg dose with standard drug (diazepam).

Table 4: Effect of ethyl acetate extract of *ipomoea pes-tigridis*(100 and 200mg/kg)foropen field method.

Minutes	Control	Diazepam	***Ipomoeapes-tigridis* extract**	
			100 mg/kg	200 mg/kg
0	104.00	104.40	79.00±5.74	71.20±13.23
30	57.80	60.60	92.20±7.71	63.60±2.53
60	35.40	41.80	57.00±3.79	85.00±10.62
90	29.40	21.80	36.00±2.12	61.20±10.57
120	28.80	24.80	24.20±2.63	38.40±8.49

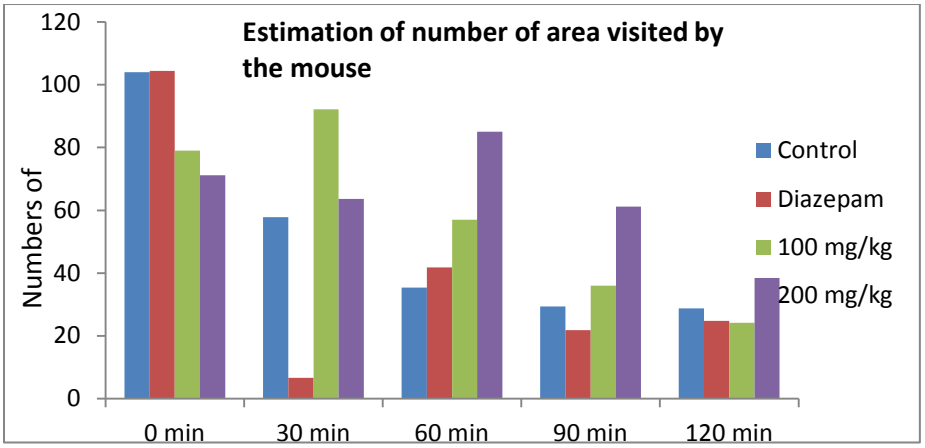


Figure 4: Effect of ethyl acetate extact of (100mg/kg and 200mg/kg) *Ipomoea pes-tigridis* for open field method.

Conclusion

Based on the results of the present study, it can be concluded that ethyl acetate extracts of *Ipomoea pes-tigridis*possesses remarkable analgesic potential but it does not possess any CNS depressant activity in animal behavioral model. Hence, further studies are suggested for the conformation of its CNS stimulation activity as well as to be undertaken to pinpoint the exact compounds and to better understand the mechanism of such actions.

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