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Research Article

Antinociceptive and Diuretic Activities of *Tagetes erecta* Linn

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ABSTRACT

In the present investigation, the possible antinociceptive and diuretic activities of methanolic extract of *Tagetes erecta* has been tested in animal models. The methanol extract of both aerial part and root of the plant exhibited significant antinociceptive activity at higher dose (400 mg/kg body weight) in Swiss albino mice. The root extract was found to reduce the writhing more effectively than that of aerial part which is comparable to that produced by aminopyrine, used as standard drug. In addition, crude whole plant extract was also showed efficient diuresis at higher dose 400 mg/kg tested. Diuretic activity was proved by the electrolyte loss ratio (Na+/K+ excretion ratio) and we used furosemide as the reference.

Keywords: *Taqetes erecta*; crude extract; writhing; aminopyrine.

INTRODUCTION

Tagetes erecta (Family - Asteraceae) commonly known as African Marigold and locally known as Genda, is an annual and short lived plant distributed widely from America to Asia. This plant is native to Middle America and widespread well known all over the world including Bangladesh and different parts of India as ornamental plant[1,2]. Juices of leaves and flowers are emmenagogue and cure bleeding pile and purifiers blood. Infusion of plant is used in rheumatism, cold and bronchitis. Leaves are used in kidney troubles, muscular pains and applied to boils and carbuncles. Extract of root is also used as laxative[1].

Previous phytochemical studies[3-5] described the isolation and characterization of flavonoids, carotenoids, xanthophylls and polyketides, which are endowed with antimutagenic[6], phytotoric[7],nutritional supplementas[8], as well as anticarcinogenic and ophthalmologic agents[9,10]. *T.erecta* essential oil is used as antioxidant[11], analgesic and volatile compounds generated by *T.erecta* are used as antifungal. The main aim of this study was to evaluate the antinociceptive and diuretic activities of the methanolic crude extract of different parts (aerial part and root) of *T.erecta*.

MATERIALS AND METHODS

Plant material: The plants were collected from Curzon Hall, IER and Mokarram Bhaban of Dhaka University, Bangladesh in April 2009. A voucher specimen for this collection has been deposited in the herbarium of the Department of Botany, University of Dhaka. The collected whole plant parts with root were washed, cut into small pieces and dried in the sun in a three separated parts; whole plant, root and aerial part for about a week. The pieces were then kept in an oven for 24 hours at considerably low temperature to ensure complete drying and effective grinding and were then ground into coarse powder. The powdered whole plant (4kg), aerial part (3kg) and root (1kg) was macerated separately with solvents (10, 9 and 6 liters of methanol) for 7 days and then filtered through a cotton plug followed by Whatman number one filter paper[12]. The filtrate thus obtained was concentrated by using a rotary vacuum evaporator.

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Animals: For pharmacological investigation, Swiss Albino mice of either sex (4 weeks of age, weight: 22-30 gm) were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) animal house, Dhaka, Bangladesh. Animal studies were performed in accordance with the declaration of Helsinki and the European Community guidelines for the ethical handling and the use of laboratory animals and through the clearance of Institutional Animal Ethics Committee (IAEC).The animals were kept at animal house (Faculty of Pharmacy, University of Dhaka) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 - 65%, room temperature 25.0 ± 2.0°C and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. All the experiments were conducted on an isolated condition.

Drugs: Aminopyrine (Sigma, USA), Furosemide (Square Pharmaceuticals Ltd, Bangladesh) and Normal Saline solutions (Beximco Infusions Limited, Bangladesh).

Experimental: In the present study, the evaluation of pharmacological activity was primarily focused on the phenomenon of antinociceptive and diuretic activity, which is manifested through a number of biological events such as algesia (pain) and diuresis.

Antinociceptive activity: The crude methanolic extract of both aerial part and root of *T. erecta* was subjected to screening for antinociceptive activity by well recognized acetic acid induced writhing method in mice[13-15]. The writhing inhibition of positive control (NSAID, Aminopyrine) was taken as standard and compared with test samples and control (1% Tween80 in saline). In this experiment, the animals were divided into four groups denoted as control, positive control (standard) and two test groups (both aerial part and root) with six mice in each group. Crude extract test samples, standard drug and control vehicle were administered orally 30 minutes before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 minutes, mice were observed for specific contraction of body referred to as 'writhing' for 5 minutes. Each group received a particular treatment i.e. control, positive control and four doses of extracts at the dose of 400 and 200 mg/kg body weight for both aerial part and root. Aminopyrine (standard drug) was administered to positive control group at the dose of 50 mg/kg of body weight and vehicle control group was treated with 1% Tween 80 in normal saline at the dose of 0.1 ml/10 gm of body weight.

Diuretic activity: Diuretic activity of the extract was investigated using metabolic case method[16]. The test animals were randomly chosen and divided into five groups containing ten mice in each group. Group I or the control group was provided only with normal saline solution and 1% Tween 80 at a dose of 10 ml/kg body weight orally. Group II was given with urea solution at a dose of 500 mg/kg. Group III was treated with standard diuretic drug furosemide at a dose of 0.5 mg/kg. Group IV and group V, the test groups were received methanolic extract of whole plant *T.eracta* at a dose of 200 and 400 mg/kg respectively. Twenty-four hours prior to the experiment, the test animals were placed in to metabolic cages with the withdrawal of food and water. After oral administration of test samples, the urinary of each group was recorded after five hours from the graduated urine chamber of metabolic cage. Sodium and potassium ions are determined by using digital flame photometer (model CL 22D) after centrifuging the total urinary output. In addition, chloride ion was also estimated[17] by applying modified method by Godkar.

Statistical analysis: Results are analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's Multiple Value of t-test using SPSS software. Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

RESULTS

Antinociceptive activity: Table 1 showed the effect of the methanolic extract of *T.erecta* on acetic acid induced writhing in mice. The experiment showed (Table 1) that the methanol root extract of *T.erecta* exhibited 41.42% and 23.57% inhibition of writhing reflexes at doses of 400 and 200 mg/kg body weight respectively. On the other hand, at the doses of 400 and 200 mg/kg body weight the aerial part of plant extract produced 32.14% and 16.42% inhibition of writhing accordingly which was found less effective than the root extract. The results were statistically significant ($P<0.01$) and were comparable to the standard drug aminopyrine, which showed 58.57% writhing inhibition at the dose of 50 mg/kg.

Diuretic activity: The effect of the methanolic extract of *T.erecta* on the urination of mice was observed for 5 h which revealed that the extract has a marked diuretic effect in test animals. This was comparable to that of standard drug furosemide and

diuretic agent urea. For methanolic extract of whole plant at the doses of 400 and 200 mg/kg, Na+/K+ excretion ratio (1.41 and 1.36, respectively) was found similar to the ratio as that of the loop diuretic furosemide (1.37) (Table 2).

DISCUSSION

As the plant *Tagetes erecta* Linn (Asteraceae) is traditionally used in pain and as diuretics, the whole phytochemical investigation of the plant was guided by methods developed for evaluation of antinociceptive and diuretic activity. Since, plants possesses different kind of polar and non polar constituents in nature, so for preparing crude extract of the plant we used methanol as a solvent because of it's wide range of solubility in both polar and non polar media. However, the solvent was evaporated completely to dryness to avoid from any unusual solvent effect on experimental animals. To observe the antinociceptive effect of methanolic crude extract on pain perception of mice in a peripheral model of analgesia, we applied well accepted acetic acid induced writhing method. In this method acetic acid is administered intra-peritoneal to the experimental animals to create pain sensation. That means intra-peritoneal administration of acetic acid (0.7%) causes localized inflammation in mice[18]. Following inflammation, there is biogenesis of prostaglandin (from cyclooxygenase pathway) and leukotrienes (lipooxygenase pathway). The released prostaglandin, mainly prostacyclin (PGI_2) and prostaglandin – E (PGE_2) have been reported responsible for pain sensation[19] . Depending on the result by following this method, it can be stated that *T.erecta* crude extract might possesses antinociceptive activity. However, we also studied that activity or strength of the plant can be differ depending on using different parts (aerial and root part) as well as different doses. Thereby, we found that root part showed maximum activity at higher dose than the aerial part.

Again, the effect of methanol plant extract on urination was also investigated in mice since the plant *T.erecta* Linn has traditional use as diuretic. Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, cirrhosis of liver. Furosemide, (the loop or high-ceiling diuretic) used as the standard drug in this experiment act by inhibiting Na+/ K+/Cl- co-transport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing Na+ and Cl- from the body. The extract was able to increase the volume of urine with statistical significance along with a considerable Na+ and Cl- load which was comparable to that of furosemide. The actual mode of action of the extract, may be due to its effect either loop permeability or reduction of antidiuretic hormone (ADH) secretion or inhibition of carbonic anhydrase enzyme[20], is not clear from the test results. The exact mechanism of diuretic activity exhibited by the extract can only be established after extensive phytochemical investigation of the extract and screening of diuretic activity of isolated pure constituents in a wide range of experimental models.

CONCLUSION

In conclusion, it can be stated that investigations of the crude extract of the plant *Tagetes erecta* was found very crucial in the perspective of the entire work and the findings of the preliminary study (antinociceptive and diuretic activity) was to direct the next step of the work. In addition, this would also help to rationalize the folklore use of the plant concerned.

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Table1. Effect of methanolic extract of *T.erecta* on acetic acid induced writhing in mice (n=6)

***Animal Group*/ Treatment**	**[a]Number of writhes (% writhing)**	**Inhibition (%)**
Control 1% tween-80 solution 10 ml/kg, p.o.	23.3 ± 2.01 (100)	—
Positive control (Standard) Aminopyrine 50 mg/kg, p.o.	9.67 ± 0.88* (41.42)	58.57
Test group-1		
Me. Extract (root) 400mg/kg, p.o.	13.7 ± 1.62* (58.57)	41.42
Me. Extract (root) 200mg/kg, p.o.	17.8 ± 1.24* (76.42)	23.57
Test group-2		
Me. Extract (aerial part) 400mg/kg, p.o.	15.8 ± 1.32* (67.85)	32.14
Me. Extract (aerial part) 200mg/kg, p.o.	19.5 ± 1.94*(83.57)	16.42

[a]Values are expressed as mean ± SEM; Me.: Methanolic: * indicates *P* <0.01 vs. control; n: Number of mice; p.o.: per oral.

Table 2. Effect of methanolic extract of whole plant (*T. erecta)* on urinary excretion in mice

Treatment	**Dose (mg/kg; P.O)**	**Volume of urine (ml) [b]**	**Concentration of ions (m.eq.l $^{-1}$)**			
			Na+	**K+**	**Cl -**	**Na /K+**
Group-I (Control)	-	2.43 ± 0.07	76.67 ± 1.24	48.75 ± 1.18*	76.55 ± 1.24	1.42
Group-II (Urea)	500	3.74 ± 0.08	113.66±1.35**	76.56±1.27**	85.46±1.67**	1.38
Group-III (Furosemide)	0.5	4.15±0.14	122.87±1.74*	85.46±1.67**	92.36±1.49*	1.37
Group-IV Me. extract of whole plant	200	4.24±0.08	116.23±1.19**	79.95±1.86**	91.74±1.68*	1.36
Group-V Me. extract of whole plant	400	4.86±0.04	132.74±1.62**	92.24±1.69**	97.60±1.86*	1.41

ME: Methanolic extract of *T. erecta* ; Values are expressed as mean ± SEM (Number of animals, n = 10); *indicates P<0.01, **indicates P<0.001 vs. control; [b] Collected for 5 hours after treatment.