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Short Communication

Effects of the crude ethanolic extract of *Ruellia tuberosa* on analgesia in mice

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Abstract

The analgesic effects of organic extracts of *Ruellia tuberosa* using animal model like mice were studied. Four groups of mice (n=7) were taken in this study and test samples (250 mg/kg & 500 mg/kg), control (tween+water) and diclofenac-Na (25 mg/kg) were given orally by means of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (3%) was administered intra peritonially to each of the animal of four groups. After an interval of fifteen minutes, which was given for absorption of squirms (writhing) was counted for first 20 minutes. Ethanolic extract of *Ruellia tuberosa* has demonstrated significant reduction of writhing both at higher dose (500 mg/kg) and lower dose (250 mg/kg) comparative to that of a standard analgesic diclofenac- Na. Thus the crude ethanolic extract of *Ruellia tuberosa* has moderate antinociceptive effect comparative to that of a standard analgesic Diclofenac-Na.

Keywords: Algesia, Analgesic, *Ruellia tuberosa*, Diclofenac-Na, Writhing

Introduction

Ruellia tuberosa (Bengali- Patpatey) is a perennial herb with tuberous rootstock, leaves are opposite and entire. The inflorescence is few flowered, rather contracted. The tubular bell shaped flower is purple to pale grey purple, bloom almost in all year round. Fruit is a cylindrical capsule, when a dry one is put on water burst with popping sound (Ahmed, 1997). Grows all over the country on higher land, mostly on non-flooded land. This plant is also found in the humid region of Burma, Thailand and Laos (Ahmed, 1997). Traditional uses of the investigated species are reported as emetics, and used in the treatment of stone in bladder. Decoctions of the leaves are used for chronic bronchitis (Ahmed, 1997). It is also used as an anthelmentic, against joint pains and strained muscles (www.tropilab.com). Synthesis of glucuronides in the flavonoid-series 3. Isolation of apigenin-7- -D-glucuronide from *Ruellia tuberosa* L has been reported (Wagner *et al.*, 1971). To establish and search for bioactive secondary metabolites (Madulid, 1995) from local medicinal plants an investigation were carried out with an ethanolic extract of *Ruellia tuberosa* for potential analgesic activity. A preliminary Hippocratic screen (Molone, 1962) revealed that the crude extract markedly decreased the frequency of acetic acid induced writhing at higher doses. The current study was therefore conducted to screen the crude extract for their effect on mice. The isolated compounds were not tested because of the scarcity of samples.

Materials and Methods

Analgesic activity was determined by measuring the writhing effect which was produced by administration of the acetic acid and the inhibition of writhing effect produced by the test drug i.e. crude extracts in comparison with a standard drug might prove efficacy of the analgesic action. (Amour *et al.*, 1941).

Collection of plant materials

Ruellia tuberosa was collected from Khulna, Bangladesh in December 2003. The plant was identified at Bangladesh National Herbarium.

Extraction

The air dried and powdered stem berks (400 gm) was soaked in 600 ml of 95% of ethanol in a glass container for six days. The extract was separated from the plant debris by filtration through filter paper (Whitman filter paper). The extract was concentrated by evaporation and dried to solid in an oven (106.8 gm, yield 26.7%). It rendered a gummy concentrate of grey color.

Selection of animal

Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20 and 25 gm were used for in-vivo pharmacological screening. The mice were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They were housed in four groups in plastic cages having dimension of (28×22×13) cm. Soft wood shavings were used as bedding of cages. The newly collected mice were acclimatized to the new environment for one week prior to the investigation and were maintained at constant room temperature (24.0±1.0ºC), humidity 55-65% and 12 hrs light/12 hrs dark cycles. Husk and excreta were removed from the cages every day. Pellets of mice food provided by ICDDR, B were given to the mice with fresh water *ad.libitum.* (Chatterjee, 1993).

Preparation of samples

To prepare suspension of the test samples at the doses of 250mg /kg and 500mg/kg body weight 250 mg and 125 mg of samples were measured respectively. The extract was triturated in unidirectional manner by the addition of small amount of tween-80. After proper mixing of extract and tween-80, the distilled water was slowly added. The final volume of the suspension was made 2.5 ml and 5 ml respectively. For the preparation of diclofenac sodium at the dose of 25 mg/kg body weight, 6.25mg of diclofenac sodium was taken and a suspension of 5 ml was made. For the preparation of 3% of acetic acid solution, 3 ml of acetic acid was mixed with 97 ml of distilled water.

Screening for analgesic activity

The 'acetic acid' test method used in this study was adopted from those described in detail earlier by Koster *et al.* (1959), Williamson *et al.* (1996), Zakaria *et al.* (2001) and Silva *et al.* (2003). Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group-IV, consisting of 8 mice in each group. Each group received a particular treatment i.e. control, positive control and the two doses of the extract. Group-I is served as the control and received only distilled water and tween-80. Group-II was received diclofenac–Na (100 mg/kg, i.p), the standard drug for comparison of potencies. The last two groups i.e. Group-III & Group-IV were administered orally with the crude extract suspensions. Thirty minutes interval was given to ensure proper absorption of the administered substances. Then each group was treated with intraperitoneally administered 0.2 ml of a 3% acetic acid solution (Koster, *et al.* 1959). The number of writhes (i.e., abdominal contractions and stretches) that occurred within the first 20 min following acetic acid administration were counted and recorded. The recorded numbers of acetic acid-induced writhes that occurred in the positive control and test group i.e. crude extracts treated mice were compared with those in the control group mice (Table 1).

Statistical analysis

Experimental values were expressed as Mean ± SEM. Independent Sample t-test was done for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

Results and Discussion

From the result of analgesic activity of ethanolic extracts of *Ruellia tuberosa* shown in Table 1 and Fig-1 & 2 were found to exhibit significant analgesic activity both at 500 mg/kg and 250 mg/kg dose level and were statistically significant. The ethanolic extract of whole plant produced 50.01% and 63.21% writhing inhibition at oral doses of 250-mg/kg and 500mg/kg-body weight of mice whereas the standard drug diclofenac sodium produced 66.98% of writhing inhibition.

Table 1. Effects of the ethanolic extract of *Ruellia tuberosa at* the doses of 250 and 500 mg/kg-body weight on acetic acid induced writhing of mice

Group	Mean writhing ± S.E.M.	% Writhing	% Inhibition
Group-I	15.145 ± 1.511	100	0
Group-II	5.0 ± 0.554	33.020 ± 3.66	66.98 3.916 (<0.001)
Group-III	7.57 ± 0.945	49.99 ± 6.241	50.01 2.384 (<0.02)
Group-IV	5.57 ± 1.035	36.79 ± 6.835	63.21 2.853 (<0.02)

S.D. = Standard Deviation; S.E.M. = Standard Error of Mean; p< 0.05 vs. control

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function (Tripathi, 1999). Pain differs from other sensory modalities in that it does not exist out side the animal world, as do, for example, light, sound and mechanical energy. Pain may result from damage to tissue by trauma or disease or from potentially damaging stimuli. This is called nocigenic pain. And analgesic means a drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. So, analgesic activity means capacity of a substance to neutralize the pain sensation (Rang *et al*, 1993). Acetic acid is a pain stimulus. Intraperitoneal administration of acetic acid (3%) causes localized inflammation. (Koster *et al.*, 1959) Such pain stimulus causes the release of free arachidonic acid from tissue phospholipids by the action of phospholipase A_2 and other acyl hydrolases. There are three major pathways in the synthesis of the eicosanoids from arachidonic acid. All the eicosanoids with ring structures, which is the prostaglandins, thromboxanes and prostacyclines, are synthesized via the cyclooxygenase pathway. The leucotrienes, HETE

(hydroxy eicosatetraenoic acids) and HPETE (hydroperoxy eicosatetraenoic acids) are hydroxylated derivatives of straight-chain fatty acids and are synthesized via the lipooxygenase pathway (Ranolds *et al.*, 1982). The released prostaglandins, mainly prostacyclines (PGI_2) and prostaglandin-E have been reported to be responsible for pain sensation by exciting the Aδ-fibers. Activity in the A δ-fibers cause a sensation of sharp well localized pain (Rang *et al.*, 1993). Any agent that lowers the number of writhing will demonstrate analgesia by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. This hypothesis is in consonance with those authors (Koster *et al.*, 1959; Williamson *et al.*, 1996; and Silva *et al.*, 2003) who postulated that acetic acid-induced writhing test methods are useful techniques for the evaluation of peripherally- and centrally-acting analgesic drugs, respectively. Crude ethanolic extract of *Ruellia tuberosa* decreased the frequency of acetic acid induced writhing in mice at higher doses. These observations tend to suggest that crude extracts possess centrally and peripherally-mediated analgesic properties. Diclofenac used as the positive control in this method acts by inhibition of prostaglandin synthesis. Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis and/or production (Small, 1989; Todd and Sorkin, 1988; Skoutakis *et al.*, 1988). The drug also affects polymorphonuclear leukocytes function in vitro, thereby reducing chemotaxis, superoxide toxic radical formation, oxygen-derived free radical generation, and neutral protease production (Mahgoub, *et al.*, 2002; Freeman, *et al.*, 1986). Diclofenac has also been reported to suppress inflammation induced by various phlogistic agents in experimental animal models (Freeman, *et al.*, 1986; Menasse *et al.* 1978; Al-Tuwaijri et al., 1992). Although the present experimental findings are inconclusive, the results obtained tend to suggest that Crude ethanolic extracts of *Ruellia tuberosa* probably exerts its peripheral antinociceptive effects by inhibiting the release, synthesis and/or production of inflammatory cytokines and mediators, including: prostaglandins, histamine, polypeptide kinins, and so on. In the present study, the reduction of the antinociceptive process obtained within the first hour is probably related to reduction in the release of preformed inflammatory agents, rather than to a reduced synthesis of the inflammatory mediators by inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators). The present study was carried out to establish the rationality of traditional approach of using the plant parts as medication and to develop newer lead for better and safer analgesic agents. The precise mechanism of analgesia remains uncertain. Yet no more relevant research work has been found for the comparison of our findings. Taking this as reference one further studies is needed to establish the exact mechanism of action. All of the above finding suggests an analgesic action of the ethanolic extracts. It is of further value to those interested in "deciphering" the actual value of folkloric remedies.

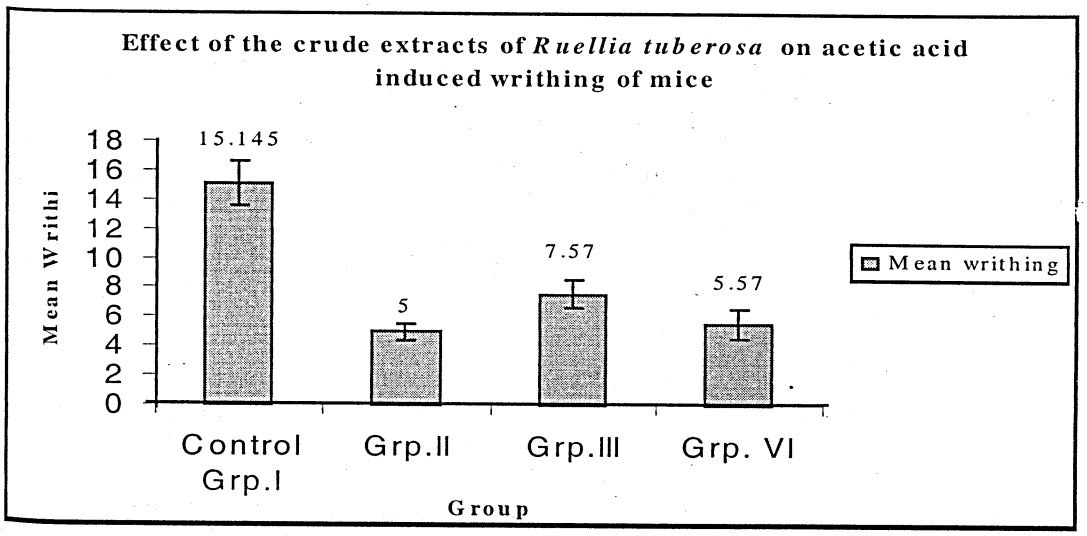

Fig 1. Effect of crude extract of *Ruellia tuberosa* on acetic acid induced writhing of mice. Each bar represents mean writhing

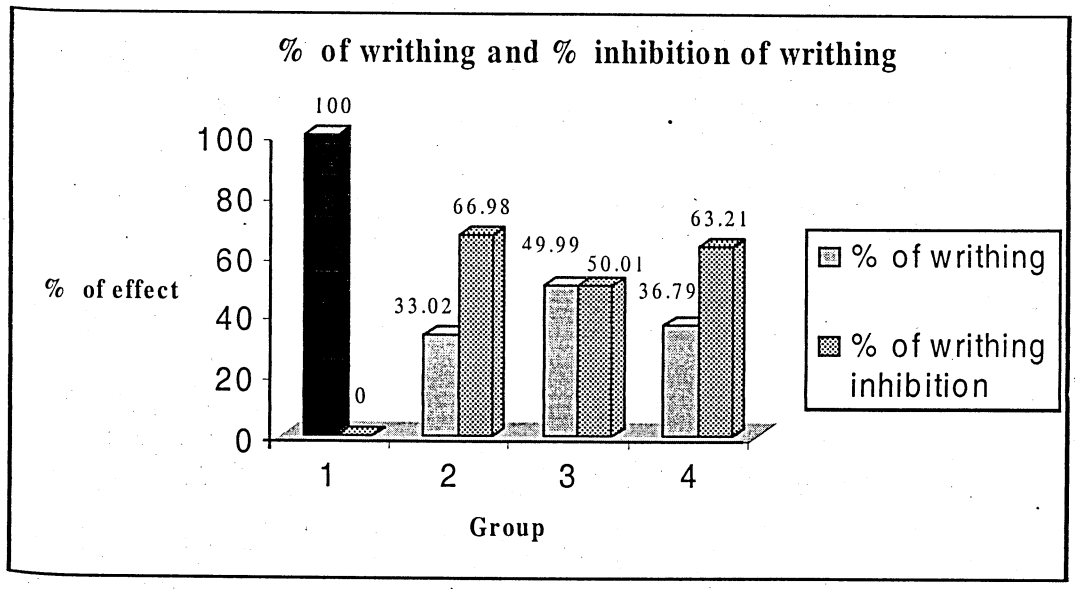

Fig 2. Effect of crude extract of *Ruellia tuberosa* on acetic acid induced writhing of mice. Each bar represents % effect of writhing and % inhibition of writhing

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References

Ahmed, N. 1997. Wild flowers of Bangladesh, The University Press Ltd. p- 40.

Al-Tuwaijri, A.S., Mustafa, A.A. 1992. Verapamil enhances the inhibitory effect of diclofenac on the chemiluminescence of human polymorphonuclear leukocytes and carrageenan-induced rat's paw oedema. International Journal of Immunopharmacology. 14: 83–91.

Amour F.E. and Smith D.L. 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72:74-79.

Chatterjee, T. K. 1993. Handbook on Laboratory Mice and Rats, Department of Pharmaceutical Technology, Jadavpur University, 1st edition: pp-157.

Freeman, C., Johnston, C., Chew, C., Davis, P. 1986. Effect of diclofenac sodium, tolfenamic acid and indomethacin on the production of superoxide induced by *N*-formyl-methionyl-leucyl-phenylalanine in normal human polymorphonuclear leukocytes. Scandinavian Journal of Rheumatology. 15: 41–46.

Koster, R., Anderson, M., De-Beer, E.J. 1959. Acetic acid for analgesic screening. Federation Proceedings, 18: 412–418.

Madulid, D. R. and Tyler, V. E. 1995. Natural product drug discovery and development: New perspectives on international collaboration. 58:1325.

Mahgoub, A.A. 2002. Grapefruit juice potentiates the anti-inflammatory effects of diclofenac on carrageenan-induced rat's paw oedema. Pharmacological Research 45: 1–4.

Menasse, R., Medwall, P.R., Kractz, T., Pericin, C., Riesterer, L., Sallmann, A., Ziel, R. 1978. Pharmacological properties of diclofenac sodium and its metabolites. Scandinavian Journal of Rheumatology, 22: 5–16.

Molone, M.H. and Robichaud, R.C. 1962. A Hippocratic screen for pure or crude drug. *Lloydia,* 25:320-32.

Rang, H.P. and Dale, M.M. 1993. Pharmacology, Second edition, Churchill Livingstone, UK, p. 706-711.

Ranolds, James E.F and Martindale, 1982. The Extra Pharmacopoeia, 18th edition, The Pharmaceutical Press, p. 254.

Silva, J., Abebe, W., Sousa, S.M., Duarte, V.G., Machado, M.I., Matos, F.J. 2003. Analgesic and anti-inflammatory effects of essential oils of *eucalyptus*. Journal of Ethnopharmacology 89: 277–283.

Skoutakis, V.A., Carter, C.A., Mickle, T.R., Smith, V.H., Arkin, C.R., Alissandratos, J., Pretty, D.E. 1988. Review of diclofenac and evaluation of its place in therapy as a non-steroidal anti-inflammatory agent. Drug Intelligence and Clinical Pharmacy 22: 805–859.

Small, R.E. 1989. Drug reviews: diclofenac sodium. Clinical Pharmacy 8: 545–558.

Todd, P.A., Sorkin, E.M. 1988. Diclofenac sodium: a reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy. Drugs 35: 244–285.

Tripathi, K.D. 1999. Essentials of Medical Pharmacology. 4th ed. Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, India. p-432

Wagner, H., Danninger, H., Iyengar, M.A., Seligmann, O., Farkas, L., Subramanian, S.S and Nair, A.G. 1971. Synthesis of glucuronides in the flavonoid-series. 3. Isolation of apigenin-7- -D-glucuronide from Ruellia tuberosa L. and its synthesis. Chem Ber.; 104(9):2681-7.

Williamson, E.M., Okpako, D.T., Evans, F.J., 1996. Pharmacological methods in phytotherapy research. In: Selection, Preparation and Pharmacological Evaluation of Plant Materials, vol. 1. John Wiley, Chichester, pp. 184–186.

Zakaria, M.N., Islam, M.W., Radhakrishnan, R., Chen, H.B., Kamil, M., Al-Gifri, A.N. and Chan-Attas, A., 2001. Antinociceptive and anti-inflammatory properties of *Caralluma arabi*. Journal of Ethnopharmacology 76: 155–158.