

**Short communication**

**ANTINOCICEPTIVE EFFECT OF THE CRUDE ETHANOLIC EXTRACT OF CRATAEVA NURVALA. BUCH. ON MICE**

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**ABSTRACT**

In order to scientifically appraise some of the anecdotal, folkloric, ethnomedical uses of (Bengali name - borun), the present study was undertaken to investigate the antinociceptive properties of the ethanolic extracts of *Crataeva nurvala* on mice in the Phytochemistry and Pharmacology Laboratory, Pharmacy Discipline, Khulna University, Khulna, during the period from December 2003 to February 2004. The antinociceptive effect of crude ethanolic extracts was evaluated by 'acetic acid' analgesic test method in mice. Crude ethanolic extracts of *Crataeva nurvala* (250–500 mg/kg PO) produced dose-dependent, significant ( $p < 0.05$ – $0.001$ ) antinociceptive effect against chemically induced nociceptive pain stimuli in mice. The results obtained in this study suggest that the antinociceptive effect of the extracts of *Crataeva nurvala* are peripherally and centrally-mediated. The findings of this experimental animal study indicate that crude ethanolic extracts of *Crataeva nurvala* possesses antinociceptive properties and thus lend pharmacological support to folkloric, anecdotal uses of 'borun' in the treatment and/or management of painful, arthritic inflammatory conditions.

**Key Words:** Antinociceptive, *Crataeva nurvala*, diclofenac sodium, writhing

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**INTRODUCTION**

*Crataeva nurvala*. Buch. (Bengali- Borun, Bonna, pithagola) is a moderate sized evergreen tree with their rounded hard fruits, grows on the banks of canals and rivers throughout Bangladesh (Ghani, 1998). Traditional uses of the investigated species are reported as contraceptive, oxicotic, urinary complaints, laxative, and litho tropic, febrifuge and as tonic (Ghani, 1998). It is also useful in the treatment of kidney stone, bladder stone, vomiting, gastric irritation and rheumatic fever (Ghani, 1998).

Lupeol, a pentacyclic triterpene was isolated from *C. nurvala* stem bark and its ester lupeol linoleate was synthesised. These triterpenes were tested for their anti-inflammatory activity in complete Freund's adjuvant induced arthritis rats (Geetha *et al.*, 1998). There was a significant increase in lipid peroxide level in plasma of arthritic rats but it was found to be decreased in the liver. Blood glutathione was decreased in arthritis. The effect of lupeol linoleate was found to be better in this respect when compared with lupeol (Geetha *et al.*, 1998). The effects of lupeol and lupeol linoleate on the development of complement in adjuvant arthritis in rats were studied and compared with those of indomethacin. The effect of lupeol linoleate in reducing the foot-pad thickness and complement activity in arthritic rats was even greater than that of unesterified lupeol and indomethacin (Geetha and Varalakshmi, 1999). Lupeol, isolated from *Crataeva nurvala* stem bark in doses 40 and 80 mg/kg body weight, PO, for 10 days, decreased the concentration of blood urea nitrogen, creatinine and lipid peroxidation and increased glutathione and catalase activities in cisplatin (5 mg/kg body weight, IP) induced nephrotoxicity in rats. The increased glutathione and catalase activities are indicative of antioxidant properties of lupeol (Shirwaikar *et al.*, 2004).

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To establish and search for bioactive secondary metabolites from local medicinal plants an investigation was carried out with an ethanolic extract of *Crataeva nurvala* for potential antinociceptive activity. A preliminary Hippocratic screen (Malone and Robichaud, 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 antinociceptive effect on mice. The isolated compounds were not tested because of the scarcity of samples.

## MATERIALS AND METHODS

The present study was undertaken to investigate the antinociceptive properties of the ethanolic extracts of *Crataeva nurvala* on mice in the Phytochemistry and Pharmacology Laboratory, Pharmacy Discipline, Khulna University, Khulna, during the period from December 2003 to February 2004.

### *Plant materials*

*Crataeva nurvala* was collected from Pabna, Bangladesh in December 2003. The plant was identified at Bangladesh National Herbarium.

### *Extraction*

The air dried and powdered stem berks (400 g) was extracted with 95% of ethanol in a Soxhlet apparatus at an elevated temperature. The extract was separated from the plant debris by filtration through fresh cotton beds. The extract was concentrated by evaporation under reduced pressure at 40°C using Buchi rotary evaporator to have gummy concentrate of reddish black color.

### *Experimental animals*

Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 g 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±1.0 °C), humidity 55-65% and 12 h light/12 h dark cycle. 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*

The crude extracts of *Crataeva nurvala* were dispersed in distilled water using small amount of tween-80 as suspending agent and the final volume were adjusted to 5 ml with distilled water. The control group was treated with distilled water and tween-80. Diclofenac sodium at the dose of 100 mg/kg body weight was prepared from available market preparation of diclofenac sodium 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. Each mouse was weighed and the doses of the test samples and control materials were adjusted accordingly.

### *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 and 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 was served as the control and received only distilled water and tween-80. Group-II was received diclofenac sodium (100 mg/kg, IP), the standard drug for comparison of potencies. The last two groups i.e. group-III and 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.

## RESULTS AND DISCUSSION

Ethanollic extracts of *Crataeva nurvala* were found to exhibit moderate analgesic activity both at 500 mg/kg and 250mg/kg dose level and were statistically significant. The ethanollic extract of whole plant produced 26.88% and 43.55% writhing inhibition at oral doses of 250 mg/kg and 500 mg/kg body weight of mice (Table 1).

Table 1. Effects of the ethanollic extract of *Crataeva nurvala* at the doses of 250 and 500 mg/kg body weight on acetic acid induced writhing of mice

Group	Mean writhing $\pm$ SEM	% Writhing	% Inhibition
Group-I	18.6 $\pm$ 0.4656	100	–
Group-II	6.8 $\pm$ 0.2174	36.55	63.45 9.005 ( $<$ 0.001)
Group-III	13.6 $\pm$ 1.3797	73.12	26.88 5.699 ( $<$ 0.001)
Group-IV	10.5 $\pm$ 0.8100	56.45	43.55 6.805 ( $<$ 0.001)

SEM = Standard Error of Mean;  $p < 0.001$  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). Crude ethanollic extracts of *Crataeva nurvala* (250 and 500 mg/kg orally) significantly ( $p < 0.05$ – $0.001$ ) inhibited acetic acid-induced writhes in mice. Similarly, diclofenac sodium (100 mg/kg IP) markedly reduced acetic acid-induced writhes in the animals. These observations tend to suggest that crude ethanollic extracts may possess centrally- and peripherally-mediated analgesic properties. The peripheral analgesic effect of the plant's extract may be mediated via inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators), while the central analgesic action of the extract may be mediated through inhibition of central pain receptors. This hypothesis is in consonance with those of Eddy and Leimback (1953), Koster *et al.* (1959) and Williamson *et al.* (1996) who postulated that acetic acid-induced writhing and hot-plate test methods are useful techniques for the evaluation of peripherally- and centrally-acting analgesic drugs, respectively. Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), is commonly employed in the treatment and/or management of rheumatoid arthritis, osteo-arthritis and ankylosing spondylitis (Siraux, 1977; Brooks *et al.*, 1980), and for its anti-inflammatory and analgesic effects (Small, 1989). Diclofenac reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis and/or production (Todd and Sorkin, 1988; Skoutakis *et al.*, 1988; Mahgoub, 2002). 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 (Freeman *et al.*, 1986; Mahgoub, 2002). Diclofenac has also been reported to suppress inflammation induced by various phlogistic agents in experimental animal models (Menasse *et al.*, 1978; Al- Tuwajri and Mustafa, 1992; Mahgoub, 2002). Although the present experimental findings are inconclusive, the results obtained tend to suggest that crude ethanollic extracts of *Crataeva nurvala* probably exerts its anti-inflammatory and 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 anti-inflammatory 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 cyclo-oxygenases and/or lipoxygenases (and other inflammatory mediators).

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