Research Article

EVALUATION OF ANALGESIC AND ANTIDIARRHOEAL PROPERTIES OF THE ETHANOLIC EXTRACT OF *CRATAEVA NURVALA* BUCH. HAM (CAPPARIDACEAE) LEAVES

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ABSTRACT

The leaves of medicinal plant *Crataeva nurvala* Buch. Ham (Capparidaceae) was extracted in ethanol to evaluate the peripherally acting analgesic potential using acetic acid induced writhing and antidiarrhoeal activity using intestinal motility test both in mice. The crude extract showed significant (P<0.01) analgesic activity at oral doses of 200 and 400mg/kg body weight with an inhibition of writhing 68.4% and 76.3% compared to 67% for the positive control. In the motility test, the crude extract at same oral doses showed 31.16% and 35.31% inhibition of intestinal propulsion of charcoal marker where as positive control group exhibited 36.25% inhibition of propulsion of charcoal through the intestine.

Keywords: *Crataeva nurvala*, Analgesic activity, Antidiarrhoeal activity.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of these isolations were based on the uses of the agents in traditional medicines[1]. Plants are the good sources of drugs in traditional system of medicine have the advantage of little or no side effect[2]. About 80% of the world population relies on the use of traditional medicine which is predominantly based on plant material[3]. *Crataeva nurvala* Buch. Ham (Bengali name: Borun, Bonna, Pithagola; English name: Three leaved caper) is a moderate sized evergreen tree with their rounded hard fruits, grows on the banks of canals and rivers throughout Bangladesh[4]. It is a deciduous and much branched tree, with trifoliate, glabrous and ovate leaflets[5]. The plant's leaves, barks and fruits have medicinal values. Traditional uses of the investigated species are reported as contraceptive, oxitocic, urinary complaints, laxative and lithotropic, febrifuge and as tonic. It is also useful in the treatment of kidney stone, bladder stone, vomiting, gastric irritation and rheumatic fever and diarrhea[4-7]. The major component isolated from this plant is lupeol, which is used to treat hypercrystalluria, hyperoxaluria and hypercalciuria[8]. The compound also decreases elevated concentration of oxalate, phosphorus and magnesium in renal tissue[9]. It is the evident from the literature survey that plants of the Capparidaceae family are important source of biologically important compounds. Only a few chemical works have been carried out on the plant. No pharmacological work has been reported yet on leaf part of this plant. So as a part of continuous research on medicinal plants available in Bangladesh, the present study has designed to investigate the analgesic and antidiarrhoeal effect of ethanolic extract of leaves of the plant on mice.

MATERIALS AND METHODS

Plant materials

The leaves of *Crataeva nurvala* Buch. Ham (Capparidaceae) were collected from Shreepur, Gazipur, Bangladesh. The plant was identified at Bangladesh National Herbarium, Dhaka, Bangladesh and a voucher specimen (Accession no. DACB 35292) was deposited for future reference.

Preparation of extract and positive control

The sun dried and powdered leaves of *Crataeva nurvala* Buch. Ham (500g) was subjected to cold extraction with ethanol 95% v/v (3L) for 7 days. The filtrate was collected and concentrated by evaporation under reduced pressure at 40°C and the yield was about 9.5gm. Suspension of the extract of *C. nurvala* Buch. Ham was prepared with the incorporation of 0.1 ml 1% Tween 80 and diluted with normal saline and triturated thoroughly. Diclofenac Sodium and Loperamide were used as positive control respectively for analgesic and antidiarrhoeal tests.

Animals

Swiss-albino mice (*Mus musculus*) of either sex, aged 4-5 weeks, weighing 20-25gm obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B) were used for the studies. They were kept in standard environmental condition and had free access to feed and water *ad libitum*. Experiments on mice were performed strictly in accordance with the guidelines provided by the Ethical Review Committee, Faculty of Biological Sciences, University of Dhaka.

Acetic acid induced writhing test

The analgesic activity of the crude ethanolic extract was assessed by the acetic acid induced writhing method[10-14] using Swiss albino mice. Experimental mice were randomly selected and divided into four groups of six mice in each group. The negative control group, was treated orally with only saline with 1% tween 80. The positive control group, received Diclofenac Sodium (10 mg/kg body wt P.O.). Third and fourth groups of six experimental mice each were treated with ethanolic extract of *C. nurvala* (200 and 400 mg/kg body weight, orally). After 40 minutes of administration of the above treatment, each group was treated with 0.7% acetic acid at a dose of 0.1ml/10g body weight intraperitoneally to induce pain sensation. Intraperitoneal administration creates pain sensation to the experimental animals and they squirm their bodies at a regular interval termed as "writhing". After 5 minutes interval, the number of writhe (i.e. abdominal contractions and stretches) were counted and recorded for 10 minutes. Analgesic agents reduce the pain sensation which could be observed from reduced number of writhing compared to control group. As the negative control group (normal saline group) contains no know compounds with analgesic properties (only saline and tween 80 present), the group's response (writhing) was considered as maximum and the percentage of writhing for other group were calculated based on the following formula.

$$\% \text{ of Writhing} = \frac{(\text{Mean writhing of the control group} - \text{Mean wrtihing of the test group})}{\text{Mean writhing of the control group}} \times 100\%$$

The antinociceptive effect of the test extracts was compared with that of the positive control group.

Intestinal motility test

Antidiarrhoeal activity of the crude ethanolic extract of *Crataeva nurvala* was assessed by the charcoal plug method slightly modified procedure[15,16] previously described by Janssen & Jageneau[17] and Wong & Way[18]. Experimental mice were randomly selected and divided into four groups of six mice in each. Each group received a particular treatment. The ethanolic extract of *C. nurvala* (200 and 400 mg/kg body weight) were administered orally to the two experimental groups of six mice each, while negative control received 1% Tween 80 in normal saline and positive control group was treated with the standard drug Loperamide (5mg/kg body weight) orally. Ninety minutes after the administration of the extracts, suitably prepared 0.3 ml of a 5% charcoal suspension was administered to each mouse orally. The mice were sacrificed 45 minutes later (sacrifice was carried out by putting mice in a CO_2 saturated chamber) and abdomen was opened by surgery. The percent distance of the small intestine (from pylorus to the cecum) traveled by the charcoal plug was determined. In this method, charcoal plug was used as a marker to investigate the constipating action. The distance traveled by the charcoal marker is the indicator to measure the effect of crude extracts on G.I. motility. The motility inhibition of test samples and control groups (both negative and positive control) were measured using the following formula and was compared with positive control group.

$$\% \text{ of inhibition of motility of a group} = \frac{(A-B)}{A} \times 100\%$$

Here,

A = Mean distance traveled by charcoal plug in negative control groups

B = Mean distance travelled by charcoal plug of experimental groups

As the negative control group doesn't have any known antidiarrhoeal agents, the inhibition of motility was considered to be nil. In general, in this experiment if a group has antidiarrhoeal property, it will show more inhibition of motility.

Statistical analysis

Results were expressed as mean ± SEM. The significance of the results was calculated using one way analysis of variance (ANOVA) followed by Dunnett's test. P-values less than 0.05 were considered significant.

RESULTS

The ethanol soaked dried powder of leaves of *Crataeva nurvala* Buch. Ham (Capparidaceae) was filtered and test samples (200 and 400mg/kg body weight) were prepared from 9.5 gm solid residue to assess the analgesic and anti-diarrhoeal properties by well established and validated experimental methods using mice model.

Acetic acid-induced writhing test

The ethanolic extract showed statistically significant ($P<0.01$) analgesic activity in a dose dependent manner (Table 1, Figure 1). The test samples exhibited 68.4% and 76.3% writhing inhibition at a dose of 200 and 400 mg/kg body weight which are comparable to Diclofenac Sodium at dose of 10mg/kg body weight showed 67% writhing inhibition.

Table 1: Analgesic effect of ethanolic extracts on mice by acetic acid induced writhing

Group	Dose (mg/kg)	Mean Writhing	Percent of Writhing Inhibition
Control	-	35.4 ± 1.72	-
Diclofenac Sodium	10	11.7 ± 1.04*	67.0
Ethanolic Extract	200	11.2 ± 0.60*	68.4
Ethanolic Extract	400	8.4 ± 0.53*	76.3

Each value represents the mean ± SEM., N= 6. *P<0.01 compared with control. One way ANOVA: F = 34.4, df = 23 (3, 20), P< 0.0001.

Intestinal motility test

Ethanolic extract of *Crataeva nurvala* leaves showed a significant ($P<0.05$) reduction in distance traveled by the charcoal marker in the intestine. Loperamide at a dose of 5mg/kg body weight significantly ($P<0.01$) reduced the distance traveled by the marker and showed 36.25% inhibition of intestinal propulsion. Test samples at doses

of 200 and 400mg/kg body weight exhibited 31.16% and 35.31% inhibition of intestinal propulsion of charcoal meal respectively (Table 2, Figure 2).

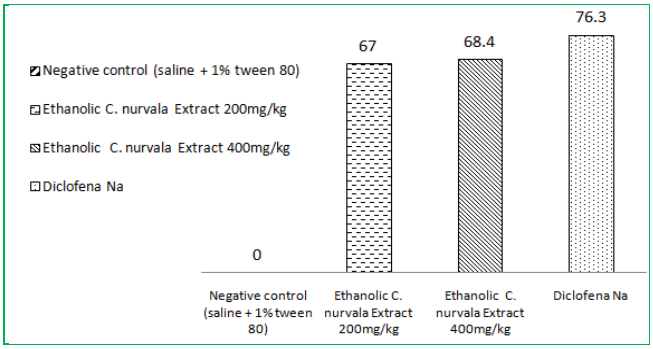

Figure 1: Comparison between "the percent of inhibition of writhing" of negative and positive control and different dose groups of the ethanolic extract of the *C. nurvala.*

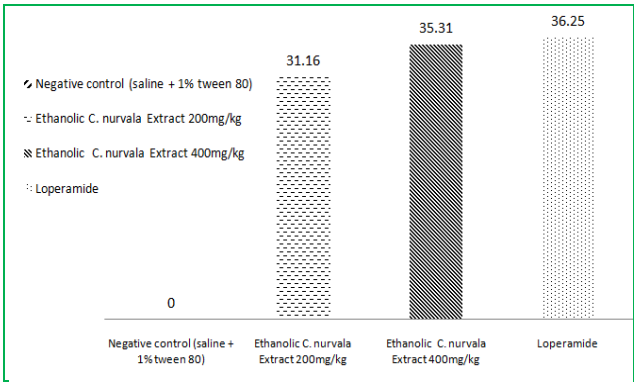

Figure 2: Comparison between "the percent of inhibition of motility" of negative and positive control and different dose groups of the ethanolic extract of the *C. nurvala.*

DISCUSSION

Pain is an alarming signal indicating impending damage to an organ or tissue[19]. Intraperitoneal administration of acetic acid produces localized inflammation by releasing the free arachidonic acid from tissue phospholipids through the action of phospholipase A_2 and other acyl hydrolases[10]. Ecosanoids (prostaglandins, thromboxanes, and prostacyclines etc.) are synthesized via cyclooxygenase pathway and the hydroxyl derivatives of fatty acids (Leucotrienes, HETE – hydroxy ecosatetraenoic acids, HPETE – hydroperoxy ecosatetraenoic acids etc.) are synthesized via lipooxygenase pathway. The released prostaglandins, mainly prostacyclines (PGI_2) and prostaglandin-E_2 have been reported to be responsible for pain sensation by exciting the Aδ-fibers. Aδ-fibers cause a sensation of sharp well localized pain[20]. So it can be assumed that any agent having inhibitory function on cyclooxygenase pathway may reduce the production of free arachidonic acid from phospholipids or may inhibit the enzyme system responsible for the synthesis of prostaglandins, while the central analgesic action may be mediated through inhibition of central pain receptors. This hypothesis is in consonance with those of Koster *et al.*[10], Whittle BA[11], Williamson *et al.*[21], Zakaria *et al.*[22] and Silva *et al.*[13] who postulated that acetic acid induced writhing method is a useful techniques for the evaluation of peripherally acting analgesic drugs. So, the observed analgesic activity of the crude extract of the plant might be due to its possible interference in the biosynthesis of prostaglandins and some other autacoids.

Table 2: Antidiarrhoeal effect of Ethanolic extracts on mice by Intestinal motility test

Group	Dose (mg/kg)	Mean Intestinal Length	Mean distance traveled by Charcoal plug	Intestinal Transit %	Inhibition %
Control	-	61.00 ± 0.73	49.67 ± 1.82	81.43	-
Ethanolic Extract	200	59.33 ± 0.67	29.83 ± 2.34*	50.27	31.16
	400	58.17 ± 1.05	26.83 ± 1.76*	46.12	35.31
Loperamide	5	56.67 ± 0.80	25.83 ± 1.01**	45.18	36.25

Each value represents the mean ± SEM., N= 6. *P<0.05, **P<0.01 compared with control

Diarrhoea refers to an increase in the frequency, fluidity and/or volume of faeces greater than normal for an individual resulting from an imbalance between the secretory and absorptive forces in the intestine[23]. Antidiarrhoeal activity would be related to the motility of gastrointestinal tract[24]. Loperamide, a piperidine butyramide derivative with μ receptor activity[25], is a commonly used opioid antidiarrhoeal agent which acts by increasing colonic phasic segmenting activities through inhibition of presynaptic cholinergic nerves in the submucosal and myenteric plexuses. These effects result in increased colonic transit time and feacal water absorption thus reducing the frequency of defeacation[26]. In addition, Loperamide has anti-secretory activity against cholera toxin and some forms of toxin[25]. Loperamide also decreases colonic mass movements and suppress the gastrocolic reflex[27]. The extract appears to act on all parts of the intestine and has similar activity as Loperamide. At dose of 400mg/kg body weight, extract showed almost similar activity to that of Loperamide (5mg/kg body weight). The result of the experiment indicated that ethanolic extract of *Crataeva nurvala* possess significant antidiarrhoeal activity due to its inhibitory effect on gastrointestinal propulsion.

CONCLUSION

Pain and diarrhea have been recognized as the most two important health problems in the developing countries. The result of experiments has suggested that the leaf of *Crataeva nurvala* Buch. Ham may be used as folk medicine for having analgesic and antidiarrhoeal properties that will significantly reduce the causes of disability and mortality with a cost effective way.

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