Evaluation of Antinociceptive, Antidiarrheal and Antimicrobial Activities of Leaf Extracts of *Clerodendrum indicum*

Arindom Pal,[1] Zobaer Al Mahmud,[2]* Nahia Akter,[3] Md. Saiful Islam,[4] Bachar Sitesh C[5]

[1]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
[2]Lecturer, Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
[3]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
[4]Professor, Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
[5]Professor, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.

ABSTRACT

Introduction: The methanolic extracts and its different partitioning fractions of leaves of *Clerodendrum indicum* were evaluated for their anti-nociceptive, anti-diarrheal and *in vitro* antimicrobial activities. **Methods:** The anti-nociceptive activity was evaluated using the acetic acid-induced writhing test in mice; the anti-diarrheal activity was investigated by the effect of extracts on castor oil-induced diarrhea while the *in vitro* antimicrobial activities were examined by the disc diffusion method. *Results:* In the acetic acid-induced writhing test, the methanolic extract at a dose of 200 and 400 mg/kg showed a significant (p<0.001) and dose-dependent reduction in the number of writhes with 62.57% and 70.76% of inhibition, respectively, while the CCL_4 fraction at the same dose showed potent anti-nociceptive activity (p<0.001) with 73.09% of inhibition of writhing which was even higher than that of standard diclofenac sodium (55.56% inhibition). The methanolic extract, CCL_4 and chloroform fraction showed moderate activity against the tested microorganisms in terms of both zones of inhibition (ranged from 9-13 mm, 10-13 mm and 10-13 mm, respectively, at a concentration of 400 µg/disc) and spectrum of activity. In castor oil-induced diarrhea testing, the methanolic extract and chloroform fraction at a dose of 400 mg/kg produced 21.74% and 26.96% inhibition of defecation, respectively, which were found to be comparable to that of standard drug loperamide (37.39% inhibition at 50 mg/kg) with regard to the severity of diarrhea. **Conclusion:** The results of the investigation demonstrated that the methanolic extract and its different fractions of leaves of *Clerodendrum indicum* possess significant anti-nociceptive, antimicrobial and antidiarrheal activities.

Key words: *Clerodendrum indicum*; leaves; anti-nociceptive; antimicrobial; anti-diarrheal; writhing

INTRODUCTION

Since ancient times, medicinal plants have been used for the treatment and management of various health problems. About 80% of the world's population relies on the use of traditional medicine, which is predominantly based on herbal products.[1] To ensure the rational use of herbal medicine, it is imperative to validate the folkloric claim of medicinal plants used in traditional medicine so that the beneficial ones can be deployed as phytomedicines and the bioactive constituents from such beneficial plants could be isolated and used as "leads" in drug discovery process.[2]

Clerodendrum indicum (family: Verbenaceae; vernacular names: Bamunhatti, Nuli gach) is an annual shrub which is found in areas with moderate temperature. The species occurs variably in India, Nepal, Myanmar, Malaya, Indo-China, Indonesia, Java and Bangladesh. Leaves (aerial parts) and roots of *Clerodendrum indicum* are used for various medicinal purposes. In traditional system of remedies, the plant is mainly used in the treatment of asthma, bronchitis, cold fever, intestinal worms, arthritis, epilepsy, febric convulsion, gastric tumor, hematuria, hysteria, impotence, lipoma, nasal polips, painful micturation and rheumatism.[3-5] Paste made out of its leaves is effective in application on wounds for early healing,

***Address for correspondence:**
Zobaer Al Mahmud,
Lecturer, Department of Clinical Pharmacy and Pharmacology,
Faculty of Pharmacy, University of Dhaka
Dhaka-1000, Bangladesh.
Tel: +88-01722597925 (Cell)
+880-2-96619000-ext (8155/8160) (Phone)
Fax: +880-2-8615583
E-mail: zalmahmud@yahoo.com

DOI: 10.5530/pj.2012.30.8

and healing of the infected lymph node (lymphadenopathy). Leaf powder is used in digestive disorders and other GI-related ailments. It purifies blood, improves blood circulation and suppresses all kind of swelling of the body. It acts on the respiratory system thus expelling out the excessive mucus in the tract relieving cough, cold and asthma symptoms.

Although the leaves of the plant have been traditionally used in the treatment of various painful and anti-inflammatory conditions, gastrointestinal disorders and infectious disease, there is no extensive anti-nociceptive, antimicrobial and anti-diarrheal study of this valuable medicinal plant. Only Raihan et al.[6] reported the analgesic activity of crude ethanolic extract of leaves and Rahman et al.[7] reported the *in vitro* antibacterial activity of root and stem of the plant previously. To prove the ethno-medical claims, the present study was designed to evaluate the anti-nociceptive and anti-diarrheal activities of the methanolic extracts and its different partitioning fractions of leaves of *Clerodendrum indicum* in mice model. The *in vitro* antimicrobial activities of the methanolic extracts and its different partitioning fractions of leaves were also investigated.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used were: acetic acid (Merck, Germany), castor oil (Sigma Chemicals, USA), diclofenac sodium and loperamide (Square Pharmaceuticals Ltd; Dhaka, Bangladesh), normal saline solution (0.9% NaCl; Orion Infusion Ltd, Bangladesh). Dimethylsulfoxide and Tween-80 were from Sigma–Aldrich and rests of the chemicals used were of BDH and E-Merck analytical grade.

Preparation of plant sample

Leaves of *Clerodendrum indicum* were collected from Modhupur, Tangail, Bangladesh, in November 2009 and authentification of the sample was confirmed by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. A voucher specimen no. has been deposited (accession No: DACB 34556) in the Herbarium for further reference. The leaves were sun dried for several days. After complete drying, the dried leaves were then ground to a coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy, University of Dhaka. The coarse powder was then stored in an air-tight container marked for identification and kept in a cool, dark and dry place for future use.

Extraction and partitioning of the plant material and sample preparation

About 900 gm of powdered leave material was taken in a clean, round bottomed flask (5 liters) and macerated at room temperature in 3 liters of methanol for 10 days with occasional shaking for better extraction. The whole mixture was then filtered through cotton followed by Whatman's No. 1 filter paper. After filtration, the filtrate was concentrated at 40 °C with a Heidolph rotary evaporator. The concentrated extract was then air dried to a solid residue. The weight of the crude methanolic extract of leaves obtained was 56 gm. Fractionation of the methanolic extracts was carried out by using solvent-solvent partitioning using the protocol designed by Kupchan[8] and modified version by Wagenen et al.[9] The crude extract (35 gm) was dissolved in 10% aqueous methanol which was subsequently extracted with petroleum ether, carbon tetrachloride and chloroform. All the three partitioning (pet ether fraction, carbontetrachloride fraction and chloroform fraction) fractions were evaporated to dryness by using rotary evaporator and kept in airtight containers for further analysis. The extracts and standard drug (diclofenac sodium, loperamide) were suspended in normal saline using 0.1% Tween-80.

Experimental animals

Swiss-albino mice (*Mus musculus*) of either sex, aged 4-5 weeks, obtained from the Animal Resource Branch of the International Center for Diarrheal Diseases and Research, Bangladesh (ICDDR, B) were used for the experiment. They were housed in polypropylene cages (30x20x13 cm) and kept in standard environmental conditions (temperature 23 ± 2 °C, relative humidity 55 ± 10% and 12 hours light/dark cycle). The animals were fed with standard rat food (ICDDR, B formulated) and water *ad libitum*. As these animals are very sensitive to environmental changes, they were kept in the environment where the experiment would take place 7 days before the test. The design and performance of research study involving mice was approved by the Ethical Review Committee, Faculty of Biological Science, University of Dhaka through the submission of a research protocol before the study.

Experimental procedures

Acetic acid-induced writhing response in mice

The methanolic crude extract and the different fractions of the methanolic extract of the leaves of *Clerodendrum indicum* were subjected to a screening for analgesic activity by acetic acid-induced writhing inhibition method.[10] Initially, the Swiss albino mice were divided into five groups (n=5). Subsequently, vehicle (1% Tween-80 solution in normal saline, 10 ml/kg, as control group), diclofenac sodium (50 mg/kg, as standard), methanolic crude extract (200 and 400 mg/kg) and CCl_4 fraction of methanolic extract (200 mg/kg) were administered orally by means of a long needle with a ball-shaped end. After 40 minutes, acetic acid (0.7%, 0.1 mL/10 g) was administered intra-peritoneally to each of the animals of all the groups to induce pain. A forty-minute interval between the oral administration