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Preliminary antinociceptive, antioxidant and cytotoxic activities of *Leucas aspera* root

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Abstract

The ethanolic extract of *Leucas aspera* root was subjected to acetic acid induced writhing inhibition, 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assay and brine shrimp lethality bioassay for screening of antinociceptive, antioxidant and cytotoxic activity, respectively. The extract produced significant writhing inhibition in acetic acid induced writhing in mice at the doses of 250 and 500 mg/kg. The extract showed a significant free radical scavenging activity with an IC_{50} of 8 μg/ml. The extract showed significant lethality to brine shrimp with an LC_{50} value.

Keywords: *Leucas aspera*; Antinociceptive activity; Antioxidant activity; Cytotoxic activity

1. Introduction

Leucas aspera is a common aromatic herb (Darkolos or Dandokolos in Bangladesh) of waste places grows in Dhaka, Comilla, Chittagong and Hill tracts [1]. Traditionally the decoction of the whole plant is taken orally for analgesic–antipyretic, antirheumatic, antiinflammatory and antibacterial treatment and its paste is applied topically to inflamed areas [2]. Glucosides, tannins, saponins, sterols, oleic, linoleic, palmitic, stearic, oleanolic and ursolic acids have been isolated from the leaves of this plant [3–6]. Leaves are useful in chronic rheumatism, psoriasis, scabies, chronic skin eruptions and their juice is antibacterial. Chloroform and ether extracts possess antifungal activity [7]. Roots are specially used traditionally for different ailments of human being in different parts of Bangladesh and no scientific report on biological activity of this part of the plant has been reported. The present study was aimed to investigate the antinociceptive, antioxidant and cytotoxic activities.

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2. Experimental

2.1. Plant

L. aspera Link (Labiatae), collected from Pabna district of Bangladesh in February, 2005 was identified by Forestry and Wood Technology Discipline, Khulna University, Khulna. A specimen sample was preserved in the Phytochemistry Laboratory of Khulna University (No. PL-81).

2.2. Extraction

Roots dried and ground were macerated in 80% EtOH, filtered and evaporated to give the dried extract (yield 9.5%).

2.3. Preliminary phytochemical analysis

The extract was subjected to preliminary phytochemical screening for the detection of major chemical groups [8]. In each test 10% (w/v) solution the extract was taken unless otherwise mentioned in individual test.

2.4. Animals

Male and female mice *Swiss-webstar* strain, 20–25 g, bred in the animal house of the Department of Pharmacy, Khulna University, were collected from animal resources branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) and used for the experiments. The animals were kept at animal house under standard laboratory conditions (relative humidity 55–65%, r.t. 25.0±2 °C and 12 h light/dark cycle) and fed with standard diets (ICDDR, B formulated) with free access to tap water.

2.5. Acute toxicity test

Test mice were divided into different groups containing six animals in each. The groups received the extract orally at the doses of 62.5, 125, 250, 500, 1000, 2000 and 4000 mg/kg b.w. whereas the control group received distilled water. General signs and symptoms of toxicity and mortality were recorded for 24 h [9].

2.6. Antinociceptive activity

Antinociceptive activity of the extract was tested using the model of acetic acid induced writhing in mice [10,11]. The experimental animals were randomly divided into four groups, each consisting of eight animals. Control group I received 1% (v/v) of Tween-80 solution in water, group II, as positive standard, was given diclofenac sodium at dose of 25 mg/kg, test groups III and IV were treated with the extract at 250 and 500 mg/kg, respectively. Control vehicle, standard drug and extracts were administered orally 30 min prior to acetic acid (0.7 %) injection. after 15 min, the numbers of writhes were counted for 5 min.

2.7. Free radical scavenging activity

The free radical scavenging activity of the extract was determined on the basis of its ability to scavenge the stable DPPH free radical.

The test was performed on the basis of the modified method of Gupta et al. [12]. Stock solution (10 mg/ml) of the extract was prepared in EtOH from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 μg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% EtOH solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm for each concentration and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against respective concentrations used and from the graph, IC_{50} was calculated. The experiment was performed in duplicate and average absorbance was noted for each concentration. Ascorbic acid was used as positive control.