Research Article

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EVALUATION OF ANALGESIC ACTIVITY OF *BULBOPHYLLUM NEILGHERRENSE* WIGHT (ORCHIDACEAE)

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ABSTRACT

Background: *Bulbophyllum neilgherrense* is considered a medicinal orchid in Bangladesh and south India, the pseudobulb being used to treat leucoderma. However, the plant has not been studied scientifically to any large extent. It was the objective of the present study to evaluate the analgesic activity of methanol extract of the whole plant. Materials and Methods: Analgesic activity was studied by the acetic acid-induced writhing method in Swiss albino mice. Results: Methanolic extract of *B. neilgherrense* (MEBN), when administered to mice in acetic acid-induced writhing tests dose-dependently reduced the number of writhings (constrictions). At doses of 50, 100, 200 and 400 mg per kg body weight MEBN reduced the number of writhings, respectively, by 23.5, 41.2, 50.0 and 67.6%. By comparison, a standard analgesic drug, aspirin reduced the number of writhings by 41.2 and 67.6%, respectively at doses of 200 and 400 mg per kg body weight. Conclusion: The results indicate that *B. neilgherrense* methanolic extract can effectively reduce pain in a manner comparable to that of aspirin. The results further suggest that the plant may prove to be a good source of pain-alleviating compound(s).

KEY WORDS: *Bulbophyllum neilgherrense*; analgesic; writhing; pain; Orchidaceae.

INTRODUCTION

Bulbophyllum neilgherrense Wight (Orchidaceae) is found in the northeastern parts of Bangladesh. The orchid is also endemic to South India and is used in traditional medicines to cure leucoderma[1]. In Bangladesh, it is known as 'ek pata ek fol' in the Bengali or Bangla vernacular. Although orchids form an integral part in the ethnomedicine of many communities, few reports have come out on ethnic uses of this plant. The Kaadar tribes of Sholayar forest in Thrissur district, Kerala, India take whole plants as treatment for scabies[2]. Fruits of the plant are taken to increase sexual strength, decrease anger and reduce gastric disorders by folk medicinal practitioners residing around the Rema-Kalenga Wildlife Sanctuary in Habiganj district, Bangladesh[3].

Pharmacological activity studies on the plant are also few. Antibacterial activity of ethanolic and chloroform soluble extracts of the leaves and pseudobulb has been reported[4]. Methanolic extract of aerial parts of the plant have been shown to give synergistic effects in oral glucose tolerance tests in mice[5].

A large segment of the population of the world suffers from pain on a daily basis and takes over-the-counter (OTC) drugs like aspirin or acetaminophen. Aspirin can cause gastric and cerebral hemorrhage[6]. Acetaminophen reportedly can cause cutaneous and hepatic disorders[7]. As a result, new analgesic drugs are necessary towards alleviating pain in a safe manner.

Since pain is something universal and more or less all people suffer from acute or chronic pain sometimes in their lives, it is of interest to screen new sources of analgesic drugs. Plants have always been an excellent source for lead compounds and novel drugs. The objective of the present study was to evaluate the analgesic activity of methanolic extract of whole plants of *B. neilgherrense* in acetic acid-induced gastric writing pain model in mice. Interestingly, chrysin, a flavonoid has been reported to be present in the methanolic extract of the pseudobulb of the plant. Anti-inflammatory activity has been attributed to chrysin[8].

MATERIALS AND METHODS

Collection of plant material

Whole plants of *B. neilgherrense* were collected during December 2016 from Rema-Kalenga Wildlife Sanctuary in Habiganj district, Bangladesh. The plant was identified at the Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. 43775) and sample specimens deposited.

Preparation of the test samples

Whole plants were cut into small pieces and air-dried in the shade and pulverized into a fine powder. 70g of the powder was mixed with 350 ml methanol. After 48 hours with occasional stirring, the mixture was filtered and filtrate collected. Filtrate was evaporated to dryness at 50°C. The weight of the final extract (MEBN) was 2.56g. The extract was stored in small aliquots and suspended in 1% Tween 80 in water prior to administration.

Animals

The present study was conducted with Swiss albino mice (male), weighing 15-20g and obtained from ICDDR,B (International Centre for Diarrhoeal Disease and Research, Bangladesh). The study was approved by the Institutional Animal Ethical

Committee of the University of Development Alternative, Dhaka, Bangladesh (Institutional Animal Ethical Clearance Number: UODA/2018/19-2) and followed international guidelines for prevention of cruelty to experimental animals[9].

Preliminary phytochemical screening

Preliminary phytochemical analysis of MEBN for presence of steroids, alkaloids, flavonoids and other phytochemicals were conducted as described before[10].

Analgesic activity test

Analgesic activity of the methanol extract was examined through acetic acid-induced writhing tests in mice using previously described procedures[11]. Mice were divided into seven groups of five mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard analgesic drug aspirin at a dose of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MEBN at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or MEBN, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 5 minutes was given to each animal to ensure bioavailability and onset of chemically induced irritation of acetic acid[12], following which period, the number of abdominal constrictions (writhings) was counted for 10 min. The percent inhibitions of abdominal constrictions were calculated according to the formula given below.

$$\text{Percent inhibition} = (1 - W_e/W_c) \text{ X } 100$$

where W_e and W_c represents the number of writhings in aspirin or MEBN administered mice (Groups 2-7), and control mice (Group 1), respectively.

Acute toxicity test

Acute toxicity test was conducted as previously described[13].

Statistical analysis

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases[14].

RESULTS

Preliminary screening of phytochemicals

Steroids, alkaloids, and flavonoids were found to be present in MEBN as determined through the various tests for phytochemical screening.

Toxicity evaluation

The crude extract was non-toxic to mice at all the tested doses.

Analgesic activity evaluation results

MEBN caused dose-dependent reductions in acetic acid-induced abdominal constrictions. MEBN reduced the number of constrictions, respectively, by 23.5, 41.2, 50.0, and 67.6% at the four doses tested of 50, 100, 200 and 400 mg per kg body weight. A standard analgesic drug, aspirin, reduced the number of abdominal constrictions by 41.2 and 67.6%, respectively, when administered to experimental animals at doses of 200 and 400 mg per kg body weight. MEBN showed better analgesic activity than 200 mg per kg aspirin and equivalent activity to 400 mg per kg aspirin at a dose of 400 mg per kg. The results are shown in Table 1. Taken together, the results suggest that the extract has powerful analgesic properties. To our knowledge, this is the first report on the analgesic properties of *B. neilgherrense*.

Table 1: Analgesic effect of crude methanol extract of *Bulbophyllum neilgherrense* whole plant in acetic acid-induced gastric pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of writhings	% inhibition
Control	10 ml	6.8 ± 058	-
Aspirin	200 mg	4.0 ± 0.45	41.2*
Aspirin	400 mg	2.2 ± 0.37	67.6*
MEBN	50 mg	5.2 ± 0.37	23.5*
MEBN	100 mg	4.0 ± 0.44	41.2*
MEBN	200 mg	3.4 ± 0.51	50.0*
MEBN	400 mg	2.2 ± 0.37	67.6*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); *$P < 0.05$; significant compared to control.

DISCUSSION

Sensation of pain occurs through excitation of Adelta-nerve fibers, which in turn is caused by production of prostaglandins [mainly prostacyclins (PGI_2) and prostaglandin- (PG-E)][15,16]. The analgesic activity exhibited by MEBN may be due to the extract's ability to inhibit prostaglandin synthesis. This inhibition may occur through inhibition of cyclooxygenase along with lipooxygenase activities.

Steroids, alkaloids and flavonoids have been implicated in analgesic activities demonstrated by a number of plant extracts. Such compounds have been implicated behind the analgesic activity of *Jacquemontia tamnifolia*[17], *Telfairia occidentalis*[18], and *Moringa oleifera*[19]. Alkaloids and flavonoids have also been implicated in analgesic and anti-inflammatory activities of extracts from stem wood of *Pterocarpus marsupium*[20]. An orofacial antinociceptive effect in zebra fish (*Danio rerio*) has been observed with kaempferol-3-*O*-rutinoside, isolated from the plant *Ouratea fieldingiana*[21]. Methanolic extract of barks of *Himalrandia tetrasperma* and *Wendlandia exserta* reportedly showed analgesic effects in hot plate analgesic assays in mice; the extracts contained alkaloids and flavonoids[22]. It is to be noted that these classes of phytochemicals were present in the extract, MEBN. However, more studies are necessary to isolate and identify the responsible bio-active analgesic component(s) and elucidate their mechanism of action.

CONCLUSION

Methanolic extract of *B. neilgherrense* demonstrated analgesic activity in acetic acid-induced writhing tests in mice suggesting

that the extract can be a potential source of lead compounds or new drugs effective against pain.

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