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Analgesic, Antidiarrheal and CNS-depressant Activities of *Flemingia macrophylla* (Willd.)

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Abstract

Plants are the priceless sources of bioactive natural compounds. Medicinal plants have been used since time immemorial in both developing and developed countries. *Flemingia macrophylla* (Willd.) is widely used as a hereditary medicines. The current study was designed to observe the analgesic, antidiarrheal and CNS depressant activities of methanol extract of the leaves of *F. macrophylla* (MEFM) and its petroleum ether (PEFM), dichloromethane (DFM), chloroform (CFM) and aqueous (AFM) fractions. The analgesic activities was assessed by acetic acid induced writhing method at doses 200- and 400-mg/kg body weight. The CNS-depressant effect was assayed by phenobarbitone sodium-induced sleeping time test. The anti-diarrheal activity of the extract was evaluated using castor oil-induced diarrhea in mice. The crude extract displayed significant peripheral analgesic activity at both test doses with 56.72- to 59.70-% inhibition of writhing responses, respectively. In CNS-depressant test, the extract revealed its activity in a dose dependent manner. In screening for antidiarrheal activity, the extract exhibited 20.83 and 41.67 % inhibition of defecation at 200- and 400-mg/kg bw, respectively whereas the standard loperamide (50 mg/kg bw) displayed 70.83 % inhibition of defecation.

Key words: *Flemingia macrophylla*, Analgesic, Antidiarrheal, CNS-depressant.

Introduction

Plants have been the fundamental resources of many noble medicines in the world and ready to provide any kind of new remedies (Samuelsson, 2004). Various types of bioactive natural compounds are attained from medicinal herbs and they serve as raw materials for noble drug discovery (Ramawat *et al.*, 2009). The interconnection between man and drugs for disease is proportional. The use of drugs and dietary supplements accessed from plants have raised in recent years. Scientists such as pharmacologists, microbiologists, botanists, and phytochemists are working with phytochemicals for developing medicines for various diseases. This study was therefore based on electronic database (Google Scholar, SciFinder, PubMed, etc.)

Flemingia macrophylla is a 1-4 meter long woody, perennial, deep rooting and tussock farming shrub, which belongs to the family Fabaceae. It is the third largest flowering plant. The roots of this plant have been used in folk medicines for the treatment of trauma, arthritis, rheumatism and influenza. Previous studies with *F. macrophylla* with have reported the neuroprotective (Shiao *et al*., 2005), analgesic and anti-inflammatory activities (Ko *et al*., 2010). It was also found to be effective in the treatment of osteoporosis (Ho *et al*., 2011). The decoction of root of *F. macrophylla* are used as an external application

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to ulcers and swellings, mainly of the neck (Kirtikar *et al.*, 1993).

Taking into account of its medicinal value to the traditional healers, the leaves of *F. macrophylla* were subjected to different bioassays to determine its analgesic, anti-diarrheal and CNS-depressant activities with the goal to execute the pharmacological basis for its folkloric use in different disorders.

Materials and Methods

Collection of plant material: The leaves of *F. macrophylla* (Willd.) were collected from Jahangirnagar University Campus, Savar, Bangladesh and identified by an expert of Bangladesh National Herbarium, Dhaka, where a voucher specimen has also been retained.

Drying and grinding: The collected plant materials were cleaned with water and subjected to shed-drying for one week followed by oven drying below 40^{o}C and then ground to a coarse powder with a grinding machine (Wuhu motor factory, China). Finally, the powder material was stored in an airtight container and kept in a dark, cool and dry place until further processing.

Cold extraction: The powered plant material (500 gm) was taken in a clean glass container and soaked in 2500 ml of methanol for 15 days with occasional shaking and stirring. The mixture was then filtered by Whatman filter paper number 1. The filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure. About 5 gm of the concentrated extract of *F. macrophylla* was subjected to solvent-solvent partitioning following the modified Kupchan partitioning protocol (Van Wagenen *et al.*, 1993) into petroleum ether (PEFM), dichloromethane (DFM), chloroform (CFM) and aqueous (AFM) soluble fractions. Then the crude extract and its Kupchan fractions were separately evaluated for biological activities.

Drugs and reagents: Methanol, Tween-80, loperamide (Square Pharmaceuticals Ltd.), normal saline (Opsonin Pharmaceuticals Ltd.) and phenobarbitone-Na (Gonoshasthaya Pharmaceuticals Ltd.), acetyl salicylic acid (Aspirin) (Essential Drugs Company Ltd.) and castor oil were collected as indicated. All other chemicals and reagents were of analytical grade.

Experimental animals: Swiss-albino mice of either sex (18-22 g) were obtained from the Animal Resources Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (icddr,b). They were harbored in standard polypropylene cages at a constant temperature of 24 ± 2^{o}C and relative humidity 60-70% with 12 hrs light-dark cycle for 1 week at least before the experiment. They were fed with food and water ad libitum. Animals used in this study were housed and handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The placebo groups were administered orally with 0.1 ml/10 **gm** bw saline. All tests were conducted under the guidelines of the International Association for the Study of Pain (Young *et al.*, 2005).

Acetic acid-induced writhing test: The analgesic activity of the sample was carried out in mice by using acetic acid-induced writhing method (Biswas *et al*., 2009; Hossain *et al*., 2016). Mice were divided into 4 groups of 5 mice in each group. The control group received 1% Tween-80 in normal saline (10 ml/kg bw), the standard group received Aspirin (50 mg/kg bw) and the experimental groups received crude extract of two different doses of 200 and 400 mg/kg bw. After 30 minutes, each mouse was injected with 1% acetic acid at a dose of 10 ml/kg bw. The number of muscular contractions was counted over a period of 5 min after acetic acid injection. The total number of writhing was observed and counted for 10 min and the percentage of inhibition was calculated by using the following formula:

$$\text{Percentage inhibition of writing} = \frac{\text{Mean number of writhing (control)} - \text{Mean number of writhing (drug)}}{\text{Mean number of writhing (control)}}$$

Evaluation of antidiarrheal activity: To evaluate castor oil-induced antidiarrheal activity of the extract, the experimental mice were also divided into four groups: two test groups, control and standard consisting of 5 mice in each group. The negative and positive control groups received Tween-80 (1% in water) and the standard drug, loperamide (50 mg/kg bw) whereas the test groups of mice received the extractives at 200 and 400 mg/kg bw. After 60 minutes, 0.5 ml of castor oil was administered to each mouse of all groups through the oral route. In order to count the number of faces all animals were then kept separately in transparent cage having white blotting paper. The blotting paper was changed in every hour and it was observed for a period of 5 hours. The mean number of feces (dry and wet diarrheal droppings) was determined and compared with the negative control group (Rahman *et al.*, 2010; Hasan *et al.*, 2017; Islam *et al*., 2013). By using the following formula the percent inhibition of defecation was calculated:

Percent inhibition = $(D_0\text{-}D_1/D_0) \times 100\%$

Where, D_0 is the number of defecation of the control group, and D_1 is the number of defecation of the test or standard group.

CNS-depressant activity: For assaying the CNS-depressant activity, the crude methanol extract of *F. macrophylla* was subjected to phenobarbitone-Na induced sleeping time test (Rahman *et al*., 2015). Here, the test groups were orally administered with test samples prepared in normal saline water and Tween-80 at doses of 200 and 400 mg/kg bw, while the positive control group was treated with diazepam (1 mg/kg bw) and the negative control group with normal saline water containing 1% Tween-80. After 30 min, phenobarbitone-Na (40 mg/kg bw) was administered intraperitoneally to each mouse to induce sleep. The animals were observed for the latent period for time of onset of sleep (i.e., time between phenobarbitone-Na injection and loss of righting reflex) and duration of sleep (i.e., time between the loss and recovery of righting reflex).

Results and Discussion

Acetic acid-induced writhing test: The results of the test showed that MEFA exhibit significant ($p < 0.01$) inhibition of writhing reflex by 56.72 and 59.70% at the doses of 200 and 400 mg/kg, respectively while the standard (Aspirin, 50 mg/kg bw) drug was found to inhibit the writing response by 80.60% (Table 1). The result was statistically evaluated and the t-test and p values were determined. The test materials exhibited significant peripheral analgesic activity at both the doses while the methanolic extract at 400 mg/kg dose exhibited maximum inhibition of writhing 59.70%. Statistical evaluation of the data confirmed that the crude extract showed significant peripheral analgesic activity at both 200 and 400 mg/kg doses with percent inhibition of writhing within the range of 56.72 to 59.70%, respectively.

Table 1. Analgesic activity of methanol extract of *F. macrophylla* leaves in acetic acid-induced pain in mice.

Treatment (n=5)	Dose (mg/kg)	No of writhes	% Inhibition
Control	1% Tween-80	22.33	---
Aspirin	50	4.33	80.60
MEFA	200	9.67	56.72
MEFA	400	9.00	59.70

Evaluation of antidiarrheal activity: In the castor oil-induced diarrhea, administration of MEFA significantly ($p<0.01$) decreased the total number of faeces in mice after at the doses of 200 and 400 mg/kg bw as well as delayed the onset of diarrhea in a dose dependent manner. The methanolic extract of leaves of *F. macrophylla* exhibited statistically significant anti-diarrheal activity with a 20.83% and 41.67% reduction of diarrhea at dose of 200- and 400-mg/kg bw compared to the standard drug, loperamide (70.83% inhibition) (Table 2).

Table 2. Effects of methanol extract of *F. macrophylla* leaves on castor oil-induced diarrhea in mice.

Group	Number of stools after 4 hrs (mean)	Inhibition of defecation (%)
Control (1% Tween-80 in water)	8.00	---
Standard (Loperamide 50 mg/kg bw)	2.33	70.83
MEFA 200 mg/kg bw	6.33	20.83
MEFA 400 mg/kg bw	4.67	41.67

Evaluation of CNS-depressant activity: The methanol extract of leaves of *F. macrophylla* slightly increased the phenobarbitone sodium-induced sleeping time in a dose dependent manner (Table 3). In the control group of mice, the time of onset of sleep was 150.33 min whereas in experimental group it was 62.33 min and 110.67 min at 400 and 200 mg/kg body weight, respectively. The total sleeping time was about 177.67 min and 129.33 min at the doses of 400 and 200mg/kg body weight, respectively while in control group it was 89.67min. This experimental finding from the study showed that the methanol extract of leaves of *F. macrophylla* have mild CNS-depressant activity in mice.

Table 3. CNS-depressant activity of crude extract of *F. macrophylla* in Swiss Albino mice.

Test groups	Time of onset of sleep (min.)	Total sleeping time (min)
Control	150.33	89.67
Diazepam (1 mg/kg b.w.)	58.33	181.67
MEFA (200 mg/kg b.w.)	110.67	129.33
MEFA (400 mg/kg b.w.)	62.33	177.67

Conclusion

The current study with the rude methanolic extract of *F. macrophylla* demonstrated significant analgesic and antidiarrheal activities but mild CNS-depressant activities However, further studies are necessary to isolate the bioactive compounds and explain the probable mechanisms related to these bioactivities.

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