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IN-VIVO INVESTIGATION OF ANALGESIC, ANTIPYRETIC ANTI-DIARRHEAL AND ANXIOLYTIC ACTIVITY OF *BLUMEA DENSIFLORA* DC.

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ABSTRACT

The present study was conducted based on the traditional uses of *Blumea densiflora* and to evaluate *in-vivo* analgesic, antipyretic, antidiarrheal and anxiolytic activities. Analgesic effect was assessed using Eddy's hot plate models and antipyretic activity was determined by Brewer's yeast-induced pyrexia in mice. The ethanol extract of *B. densiflora* (400mg/Kg, b.wt.) significantly ($P<0.05$) decreased latency time in the hot plate method and it also reduced rectal body temperature in Brewer's yeast-induced pyrexia at the dose of 500mg/Kg body weight of plant extract. The antidiarrheal effect was studied in mice against castor oil induced diarrhea at the dose of 500mg/Kg body weight. The ethanolic extract of *Blumea densiflora* (EEBD) reduces the number of the faces. The anxiolytic activity was evaluated by open field test, hole cross test and swing test. The result showed that EEBD (500mg/Kg b.wt.) decreases the number of field cross, hole cross and swing which was statistically significant ($P<0.05$). Altogether these results showed that the EEBD may have analgesic, antipyretic, antidiarrheal and anxiolytic activities which provide a scientific evidence for its traditional claim.

KEYWORDS: *In vivo*, Plant extract, analgesic, antipyretic, antidiarrheal and anxiolytic.

INTRODUCTION

The demand of the natural product based medicinal plant is increasing whole world especially in the developing countries such as Bangladesh, India, Pakistan, China and the Middle East.[1] Bangladesh is a subtropical country and good sources of repository of plants in Chittagong Hill tracts. There are many molecules which present in medicinal plants has been used to remedy many complicated diseases. At present, natural product based scientists have focused to isolate bioactive molecule and novel compound from medicinal plants. The plant species *Blumea densiflora* (*BD*) belong to family Asteraceae is mainly grows in sub tropical regions. Juice of fresh leaves of *BD* used as insecticidal, mosquito repellant. In addition, this plant yields an essential oil which contains campor. Aerial part it contains sequiterpene, lactones, tagitinin A, tiroludin ethyl ether and iso-alantolactone derivatives has been reported.[2] The leaf extracts *BD* had significant anthelmintic activity.[3] This plant has been used for rheumatism and analgesia.[4] The plant of *Blumea* of another species like *B. balsamifera* (*BM*) had passes many pharmacological activities such as anti-obesity[5], plasmin- inhibitory[6], liver-protective[7] and anti-cancer effects.[8] The leaves of the *BM* are also found antifungal, antibacterial, antifebrile, anodyne, coryza, fever, influenza, cough and dyspepsia properties.[9,10] The *Blumea lacera* has evaluated phytochemical screening, antimicrobial, alpha-amylase, anxiolytic anti-inflammatory and analgesic acivity.[11] There is no research work has been reported *in-vivo* of analgesic, antipyretic, anti-diarrheal and anxiolytic activity of extract of BD.

MATERIALS AND METHODS

THE PLANT SELECTION AND IDENTIFICATION

The plant named as *Blumea densiflora* DC was selected based on its medicinal uses. The traditional practitioners called as "kabiraj" were the main source of reliable information about the traditional uses of this plant. Taxonomical identification of this plant was made by the expert of Bangladesh Forest Research Institute (BFRI) Herbarium, Chittagong. The herbarium sheet was prepared following the standard procedure and specification suggested by the expert of the herbarium.

COLLECTION AND GARBLING

Whole plant parts were collected from Chittagong hill tracts of Bandarban, Bangladesh in the month of August. The extraneous, undesired substances from the plant material were removed at two stages of processing of it. At first the rotten leaves, stems etc were removed by hands immediately after collection of the leaves. Again, the soil was removed by sieving through a net aided by a flow of air from an electric fan before the plant materials are dried.

DRYING AND GRINDING

The plants were then subjected for shade drying at normal room temperature. Then they were ground into coarse powder with the help of a grinder. The powder were stored in an airtight container and kept in a cool, dark and dry place until extraction was commenced.

EXTRACTION OF THE PLANT POWDER BY SOXHLET EXTRACTOR

About 150 gm of plant powder of BD was subjected with 800 mL of Ethanol (97.7%) in a Soxhlet Apparatus (Quickfit, England). The obtained extract was collected, filtered and evaporated with a rotary evaporator (Heidolp, Germany) under reduce temperature and pressure to provide a gummy residue (yield 25.46%). The crude extract is collected properly with packed of it and kept for further studies.

CHEMICALS

In our experiment, all chemicals and solvent used analytical grade and purchased from Merck, Germany. All standard drugs such as diclofenac sodium, paracetamol, loperamide hydrochloride and diazepam collected from Square Pharmaceuticals Ltd. as gifted samples.

EXPERIMENTAL ANIMALS

Swiss albino mice of either sex, 6-7 weeks age, weighing between 20-25g were purchased from Bangladesh Council of Scientific and Industrial Research (BCSIR) in Chittagong for our experiment. The mice were maintained under standard environmental conditions of temperature: (26.0±1.0ºC), relative humidity: 55-65% and 12h light/12hr dark cycle and had free access to BCSIR formulated diet and water ad *libitum*. Appropriate measures were taken to minimize the pain or discomfort of animals and the mice were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.[12]

ANALGESIC ACTIVITY TEST

The central analgesic activity of EEBD was assessed in Swiss albino mice, as per the method described by Eddy and Leimbach.[13] Overnight fasted mice were placed individually on a thermostatically controlled heated beaker and the reaction time of each mouse was recorded. The temperature of the hot plate was maintained at 45 ± 1°C. The reaction time was considered as the time elapsed between placing of the mouse on the hot plate and appearance of signs of acute discomfort, characterized by flicking or licking of the hind paw, fore paw or jumping in an attempt to escape from the pain. The mice showing initial reaction time of 11 sec or less were selected for this study and were divided into 3 groups (3 in each group). After regrouping, animals in Groups 2 were orally administered with the test substance, at the dose rate 400 mg/kg body weight. Group 3 received the standard drug Diclofenac at the dose rate of 9 mg/kg body weight intraperitoneally and the control group (Group 1) received a comparable volume of vehicle. Thereafter, the initial latent reaction time of each mouse in heated beaker was recorded on "0" minute (just after treating with respective substances) and finally 30 minutes later the latent reaction time of each mouse was recorded (Table 1).

ANTIPYRETIC ACTIVITY TEST

This study was conducted by slightly modifying the method described by Adams.[14] In this test, antipyretic activity of EEBD was evaluated on Swiss albino mice (25-30g) of either sex. The animals were divided into three groups, each group containing three mice. The normal body temperature of each mouse was recorded using digital thermometer and then pyrexia was induced in all mice by injecting 10% aqueous suspension of Brewer's yeast (10 ml/kg b.wt.). All groups were fasted overnight but free accesses to drinking water were provided. At the 18hrs after yeast injection, the vehicle, standard drug and extracts were administered into three different groups. Distilled water at dose of 10 mL/kg body weight was administered orally to the control groups of animals and Paracetamol at dose of 150 mg/kg body weight was administered orally to standard group of animals. The EEBD plant was administered orally at a dose of 500 mg/kg of body weight. Rectal temperature was recorded by digital thermometer at 0, 1, 2 and 3 hours after drug administration and tabulated in the table 2.

ANTI-DIARRHEAL ACTIVITY TEST

The method described earlier by Shoba and Thomas was followed for this study.[15] The animals were divided into control, positive control and three test groups containing five mice in each. Control group received 1% tween-80 (10 ml/kg). The positive control group received loperamide (3 mg/kg b.wt.); test groups received the EEBD (500 mg/kg b.wt.) orally. After 30 mins animals were all screened by giving 0.4 ml of castor oil. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. During the observation period (4 hr), the total latency periods (first diarrhoeal stool after the administration of sample) and the number of diarrheic faeces excreted by the animals were recorded. A numerical score based on stool consistency was assigned as follows: normal stool =1 and watery stool = 2.

ANXIOLYIC ACTIVITY TEST

The anxiolytic activity of EEBD was examined by using the hole cross test (HCT), open field test (OFT) and swing test (ST). The animals were divided in to three groups, with each group consisting of three mice. First group received normal saline, second group received diazepam (1mg/kg b.wt.), while the third groups received EEBD at 500mg/kg body weight respectively.

The Hole Cross Test

The HCT, described by Takagi et al was adopted for this test.[16] The test was performed after a slight modification of the previously proposed method. The aim of this study was to characterize the emotional behavior of mice using the HCT. The hole board is a white painted wooden board (30cm×20cm×14cm) with 16 holes (each of diameter 3cm) evenly distributed on the base of box. The Swiss mice were grouped into three distinct groups with three mice in each namely for EEBD dose (500 mg/kg b.wt.), vehicle (Distilled Water 10 ml/kg b.wt.) and for standard Diazepam (4 mg/kg, b.wt.) was administered to the experimental animal intraperitonally. A 15 minutes interval was taken after drug administration then the mice were allowed to move through the hole from one chamber to the other chamber. The numbers of hole crossed by the mice were counted for 5 minutes.

Open field test

The Open Field Test (OFT), described by Cícero Francisco *et al* was adopted for this test.[17] The proposed method was performed after a slight modification. The OFT is used to observe general motor activity, exploratory behavior and measures of anxiety. The open field area was made of plain wood and consisted of a square area (45cm×45cm×20cm). The floor had a square sheet of wood (45cm×45cm) with the surface divided into sixteen small squares. The Swiss mice were grouped into three distinct groups with three mice in each namely for extract dose (500 mg/kg b.wt.), vehicle (DW 10 mL/kg b.wt.) and for standard Diazepam (4 mg/kg b.wt.) was administered to the experimental animal intraperitonally. A 15 minutes interval was taken after drug administration then the numbers of square crossed by the mice were counted for 5 minutes. Then the samples data were compared with normal and standard group for possible action of the extract. Number of field cross was calculated manually and converted to the final result.

Swing test

Swing Test (ST) described by Islam M et al was adopted for the evaluation of activity of the crude extractives on Swiss albino mice.[18] The aim of this study was to characterize the emotional behavior of mice using the swing test. The Swiss mice were grouped into distinct groups with 3 mice in each namely for extract dose (500 mg/kg b.wt.), vehicle (DW 10 ml/kg b.wt.) and for standard Diazepam (4 mg/kg b.wt.) was administered to the experimental animal intraperitonally. A 15 minutes interval was taken after administration of the controls, standard and samples under investigation were placed inside the swing box.

Statistical Analysis

Results are evaluated as the mean ± SEM. The results obtained from analgesic activity test were expressed as the mean ± SEM. The results were analyzed using one-way ANOVA followed by using Dunnett's t-test. The statistical analysis was carried out with SPSS software version 15 (Windows). A difference was considered significant at $p<0.05$.

RESULTS AND DISCUSSION

EXTRACTION

The ethanolic extract of *B. densiflora* is prepared from bark.

ANALGESIC ACTIVITY

The EEBD showed significant analgesic activity as depicted in Table 1. After 30 minutes of administration of standard drug Diclofenac (9 mg/kg b.wt.) mean latency was found to be 22.05 seconds while the extract at concentration of 400 mg/kg revealed mean latency of 14.11 seconds respectively which is of statistically significant ($P<0.05$).

Table No: 1 Effect of *B. densiflora* on hot plate reaction time in mice

Group	Design of treatment	Mean Latent (Sec)	
		Initial	After 30 min
1	Control (1% v/v tween 80, 1 mL/100 g)	4.50 ± 0.408	5.23 ± 0.204
2	*B. densiflora* (400 mg/kg b.wt, *p.o*)	4.76 ± 0.498	14.11 ± 0.523*
3	Diclofenac (9 mg/kg i.p)	4.87 ± 0.176	22.05 ± 0.491*

Values are expressed as mean ± SEM; EEBD = Ethanol Extract of *B. densiflora*; n=3; *p < 0.05

ANTIPYRETIC ACTIVITY

The effect of EEBD on mice is presented in Table 2. In this test, the extract at a dose of 500 mg/kg b.wt significantly attenuated hyperthermia in mice up to 3 hours. Throughout the experiment, the extract reduced temperature from 101.35°F to 99.57°F ($p<0.05$), 98.24°F ($p<0.05$) and 98.0°F ($p<0.05$) in 1st 2nd and 3rd hour respectively and caused maximum reduction of temperature in 1st hour.

Table No: 2 Antipyretic effect of ethanol extracts of *B. densiflora* on Swiss albino mice

Groups	Oral dose	Rectal temperature in °F at different hours				
		-18 hr	0 hr	1 hr	2 hr	3 hr
Control (DDW)	10 mL/kg	98.28 ± 0.24	101.25 ± 0.15	101.18 ± 0.07	101.38 ± 0.47	101.55 ± 0.27
P'tamol	150 mg/kg	98.51 ± 0.15	104.38 ± 0.21	98.53 ± 0.29*	98.07 ± 0.23*	98.00 ± 0.18*
EEBD	500 mg/kg	98.62 ± 0.43	101.35 ±0.53	99.57± 0.25*	98.24 ± 0.16*	98.00 ± 0.3*

Values are expressed as mean ± SEM (n = 3); P'tamol= paracetamol; *p < 0.05.

ANTI-DIARRHEAL ACTIVITY

In the castor oil induced diarrheal mice, the EEBD at the dose of 500 mg/kg showed total 35.30% inhibition of defecation compared to standard, loperamide (3 mg/kg) with total 58.87% inhibition of defecation.

Table No: 3 Inhibition of defecation

No. of Faeces			SD	SEM	% of inhibition
Negative control	11	11.33	0.4714	0.33	00
	12				
	11				
Positive control	5	4.66	0.4714	0.33	58.87±0.33
	5				
	4				
Extract	9	7.33	1.247	0.88	35.30±0.88
	7				
	6				

ANXIOLYTIC ACTIVITY

During the OFT, squares crossed by the group receiving standard drug of diazepam were 43.33. The EEBD showed depressing activity on the experimental animals at the dose of 500 mg/kg (85.00 squares crossed). In HCT, hole crossed by the group receiving standard Diazepam and control (vehicle) were 6.00 and 14.67 respectively. The EEBD showed depressing activity of the experimental animals at the dose of 500 mg/kg (8.00 holes crossed). During the ST, number of swings observed after administration of Diazepam was 4.00 and for vehicle the number was 18.33. The EEBD at the dose of 500mg/kg was showed depressant activity on the experimental animals (Table No. 4).

Table No: 4 Anxiolytic activities

Treatment groups	Field Cross ± SD	Hole Cross ± SD	Swing ± SD
Vehicle (DW; 10mL/kg)	132.00±0.17	14.67±9.29	18.33±2.12
Diazepam (4mg/kg, i.p)	43.33±6.24*	6.00±2.65*	4.00±1.53*
EEBD (500mg/kg)	85.00±8.19*	8.00±1.73*	14.33±2.52*
DW: Distilled water; EEBD Ethanol extract of *B. densiflora*; *p < 0.05			

DISCUSSION

The result of the current research proposed that the EEBD has considerable analgesic, antipyretic, antidiarrheal and anxiolytic effects.

In the analgesic activity test, hot plate method has been used on experimental animals which can be a useful tool for elucidating mechanism of pain and analgesia since it measures the response of clinical pain. In our current investigation EEBD exhibited moderate analgesic activity in the hot plate models of analgesia which reveals that the analgesic activity of EEBD is of the type which may be produced non narcotic analgesics.

The antipyretic activity test, pyrexia is occurred by brewer's yeast induces through subcutaneous injection which increased the synthesis of prostaglandin. The EEBD gradually reduces dody temperature in our experimental animal (swiss albino mice). Most of the NSAIDs inhibited prostaglandin synthesis which showed the antipyretic activity. The EEBD exhibited antipyretic action as that of paracetamol which inhibited synthesis prostaglandin can be achieved blocking the cyclo-oxygenase enzyme activity.[19] There are many mediators are responsible for pyrexia and the inhibition of these mediators is the antipyretic effect. Thus EEBD might have interfered the release of prostaglandins.

The antidiarrheal activity test, we used castor oil which may be causes diarrhea mainly to the fact of active metabolite, ricinolic acid as a result of the action of lipases on castor oil. The ricinoleic acid produces irritating and inflammatory action to release prostaglandins.[20] Castor oil delayed diarrheal action if prostaglandins synthesis is inhibited.[21] Inhibit synthesis of prostaglandins to delay diarrhea after induce castor oil. The EEBD may be inhibited prostaglandin synthesis which leads to the antidiarrheal effect.

The anxiolytic like effects of the EEBD was studied in different animal models of anxiety such as open field, hole cross and swing test. The EEBD and diazepam produced a significant ($P<0.05$) reduction of the field cross and increase in the time spent at the centre of the field which provides a better indication of the animal emotional state. In our second hole cross test, it indicated that the hole cross behavior was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state may be reflected by decrease hole cross behavior which is significant ($P<0.05$). The EEBD activities were studied by our third animal experiment of the numbers of swing inside the box. The administration of the EEBD and diazepam produced a significant ($P<0.05$) decrease number swing inside the box. This behavior clearly indicated that the plant extract has anxiolytic activity. The anxiolytic effect of EEBD is similar to diazepam, a typical benzodiazepine drug. The significant decrease in the number of field cross, line cross and swing were treated by animal with EEBD suggests that it has depressant effect at the dose used. The opening of the GABA activated chloride channel is highly responded to GABA activator which facilitated by anxiolytic agents. Therefore we can be hypothesized that EEBD may be acting like a benzodiazepine like substance.

CONCLUSION

Through of our study on the basis of above experiment, we can be concluded that EEBD have shown a better potential of analgesic, antipyretic, antidiarrheal and anxiolytic activity which is compare with the standard drugs. Further research would know the exact mechanism action of such activities. In addition, to identify chemical constituents through isolation process for further study which will be confirm of this effect.

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