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EVALUATION OF ANALGESIC, CYTOTOXIC AND ANTIOXIDANT ACTIVITIES OF *BLUMEA MEMBRANACEA* DC

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ABSTRACT

In the present study, the crude methanolic extract of whole plant part of *Blumea membranacea* (Asteraceae) and its fractions obtained by Kupchan method were screened for their possible analgesic, cytotoxic and antioxidant activities. The analgesic activity of the samples was evaluated using acetic acid induced writhing method in Swiss-albino mice. In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. As a positive control, diclofenac sodium was used to serve the purpose. The cytotoxic activity was evaluated by brine shrimp lethality bioassay while antioxidant effect was measured by 1,1-diphenyl-2-picrylhydrazyl-hydrate (DPPH) free radical scavenging assay. The crude extract and it's all Kupchan fractions were found to have significant ($p<0.001$) analgesic activity at the oral dose of 100 mg/kg body weight. The ethyl acetate soluble fraction of crude methanolic extract demonstrated significant analgesic activity with writhing inhibition of 59.17% compared to 66.66% exhibited by standard diclofenac sodium. In brine shrimp lethality bioassay, the petroleum-ether soluble fraction exhibited maximum toxicity towards the shrimp with LC_{50} value of 0.841 µg/ml. In case of antioxidant screening, all the partitionates revealed mild to moderate free radical scavenging activity.

KEYWORDS : ***Blumea membranacea,*** **asteraceae, writhing, brine shrimp lethality bioassay, free radical scavenging assay**

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INTRODUCTION

Blumea membranacea DC (Local name- Almish) grows in waste places, both in shady and sunny situations, sometimes in pure stands. The essential oil of the plant produces a marked and long-lasting fall in the blood pressure of anaesthetized dogs, exerts a direct depressant action on frog hearts, and a spasmolytic effect on rabbit ilea[1]. The essential oil also shows significant antifungal activity[2].

As a part of our ongoing investigations on local medicinal plants of Bangladesh[3,4] in this paper, we report the analgesic, cytotoxic and antioxidant activity of the whole plant part of *B. membranacea* DC.

MATERIALS AND METHODS

Drugs and chemicals

Both the acetic acid and dimethyl sulfoxide (DMSO) were obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Normal saline solution was purchased from Orion Infusion Ltd. Bangladesh. Diclofenac sodium (Voltalin) was obtained from Novartis Ltd., Bangladesh.

Plant materials

For this present investigation, *Blumea membranacea* DC was collected from Dhaka, July 2008 and was identified at Bangladesh National Herbarium, where a voucher specimen has been deposited for future reference (DACB-34731). The collected plant parts were dried for one week and ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of the extract

About 630 gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 1.5L of methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper number 1. The extract was concentrated with a rotary evaporator at low temperature (40-45 ºC) and reduced pressure. The concentrated methanolic extract was partitioned by the modified Kupchan method[5] and the resultant partitionates i.e., petroleum-ether, carbon tetrachloride, chloroform, ethyl acetate and aqueous soluble fractions were used for the experiment.

Animals

Young Swiss-albino mice of either sex aged 4-5 weeks, average weight 20-25 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were kept in standard environmental condition (at 24.0±0 °C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDR,B formulated rodent food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee[6].

Analgesic activity

Acetic acid induced writhing method

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice[7]. In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. As a positive control, any standard NSAID drug can be used. In the present study, diclofenac sodium was used to serve the purpose. About 100 mg/Kg body weight of the plant extract was administered orally to the Swiss Albino mice after an overnight fast. Test samples and vehicle were administered orally

30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g) but Diclofenac sodium was administered 15 minutes prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Diclofenac sodium (10 mg/kg) was used as a reference substance (positive control).

Cytotoxic activity

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds[8] (Meyer et al., 1982). Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. For cytotoxicity screening, DMSO (Dimethyl sulfoxide) solutions of the plant extractives were applied to *Artemia salina* in a one day *in vivo* assay. For the experiment, 4 mg of each of the extracts were dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.123, 1.563, 0.781 µg/ml) were obtained by serial dilution technique. The solutions were then added to the pre-marked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the test samples were obtained by plotting percentage of the shrimp killed against the logarithm of the sample concentration.

Antioxidant activity

Free radical scavenging activity

The free radical scavenging activity (antioxidant capacity) of the plant extractives on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method established by Brand-Williams et al[9] . Here, 2.0 ml of a methanol solution of the sample (extractive/standard) at different concentration (500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min of reaction at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by a UV-Visible spectrophotometer. Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$$(I\%) = (1 - A_{sample}/A_{blank}) \times 100$$

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test material) and A_{sample} is the absorbance of the sample. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted with inhibition percentage against extractive/standard concentration.

STATISTICAL ANALYSIS:

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle group. *p* values <0.05 were considered to be statistically significant compared with the control.

RESULTS

Table 1 shows the effect of the crude extract and their Kupchan fractions of *B. membranacea* on acetic acid-induced writhing in mice. The crude extract along with its all Kupchan fractions showed significant reduction of writhing induced by the acetic

acid after oral administration in a dose dependent manner. The carbon tetrachloride and ethyl acetate soluble fraction of crude methanolic extract demonstrated significant analgesic activity with writhing inhibition of 52.5%, 59.17%, respectively compared to 66.66% exhibited by standard diclofenac sodium. The reference drug diclofenac sodium was found more potent than both the plant extracts at all of the dose levels.

Table 1
Effect of the crude methanolic extract and different fractions of B. membranacea on acetic acid-induced writhing in mice.

Test samples	Group	Writhing count					Writhings* (Mean ± SEM)	% of writhing	% of inhibition
		M-1	M-2	M-3	M-4	M-5			
Control	A	23	24	24	25	24	24 ± 0.62	100	0
Standard	B	8	9	8	6	9	8 ± 0.84**	33.33	66.66
CM	C	13	15	14	16	13	14.2 ± 2.07	59.16	40.83
PESF	D	21	18	17	22	18	19.2 ± 1.13*	80	20.0
CTSF	E	10	10	14	11	12	11.4 ± 0.45	47.5	52.5
EASF	F	10	11	9	9	10	9.8 ± 0.72	40.83	59.17
AQSF	G	11	10	13	9	12	11 ± 0.42	52.5	47.5

Here, CM = Crude methanolic extract; PESF = Petroleum-ether soluble fraction; CTSF = Carbon tetrachloride soluble fraction; EASF= Ethyl acetate soluble fraction, AQSF = Aqueous soluble fraction of the methanolic extract of B. membranacea. M-1= Mice 1, M-2 = Mice 2, M-3 = Mice 3, M-4 = Mice 4, M-5 = Mice 5 and Number of animal each group = 5. Results are presented as mean ± SEM, (n=5), *: p<0.05, **: p<0.001 Dunnett's t test as compared to control.

Table 2
Cytotoxic and antioxidant activities of test samples of B. membranacea

Sample	LC_{50} (µg/ml)	IC_{50} (µg/ml)
VS	0.544 ± 0.32	Not determined
BHT	Not determined	71.02 ± 0.56
CM	1.26 ± 0.78	494.47 ± 0.56
PESF	0.841 ± 0.21	181.4 ± 1.15
CTSF	2.13 ± 1.2	315.07 ± 0.45
EASF	1.09 ± 0.28	151.78 ± 1.18
AQSF	1.52 ± 1.32	242.74 ± 0.71

The values of LC_{50} and IC_{50} are expressed as mean ± SD (n=3); VS = Vincristine sulphate (Std.); BHT = Butylated hydroxy toluene (Std.)

In the brine shrimp lethality bioassay, the crude extracts along with their Kupchan fractions showed significant toxicity against *Artemia salina* (Table 2). Among all the fractions, the petroleum-ether soluble fraction exhibited maximum toxicity towards the shrimp with LC_{50} value of 0.841 µg/ml. In case of antioxidant screening, all the partitionates revealed mild to moderate free radical scavenging activity (Table 2).

DISCUSSION

Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs[10]. The crude extract and its all Kupchan fractions showed significant analgesic action compared to the reference drug diclofenac sodium against acetic acid induced pain in mice at dose level of 100 mg/kg body wt. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid [11] via cyclooxygenase (COX), and prostaglandin biosynthesis[12]. In other words, the acetic acid induced writhing has been associated with increased level of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids as well as lipoxygenase products[13]. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability[14]. The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition[12,15]. The significant pain reduction of both the plant extracts might be due to the presence of analgesic principles acting with the prostaglandin pathways. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[16]. Most of the polar compounds such as phenolic and flavonoid substances are potent inhibitors of reactive oxygen species attack[17]. The biological properties, including cytotoxic and antioxidant properties, of flavonoids are considered in an evaluation of the medicinal and nutritional values of these compounds[18].

CONCLUSION

In conclusion, we can say that *Blumea membranacea* DC contains chemical constituents having analgesic, cytotoxic and antioxidant activity. This could provide a rationale for traditional uses of this plant and suggests for further investigation and isolation of biologically active constituents responsible for the activity.

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