

Original Article

**IN VITRO ANTIOXIDANT AND IN VIVO ANTI-INFLAMMATORY ACTIVITY OF THE AERIAL PART OF *BLUMEA ERIANTHA DC***

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**ABSTRACT**

**Objective:** Objective of the present study was to carry out *in vivo* anti-inflammatory and *in vitro* antioxidant activity of methanol extract of aerial part of the *Blumea eriantha DC* belonging to family *Asteraceae*.

**Methods:** The shade dried aerial part of *B. eriantha* (0.5 kg) was powdered and extracted with methanol (1.5 x 3L) at room temperature (24h x 3). After filtration combined all the three extracts and were concentrated on rotary evaporator under reduced pressure at 40 °C, thereby providing crude methanol extract which was subsequently employed for further studies. Anti-inflammatory effect was studied by carrageenan-induced paw edema model in rats at dose level 100, 200, and 400 mg/kg. Acute oral toxicity study and *in vitro* antioxidant potential of the extract was also studied. The *in vitro* antioxidant activity of methanol extract of aerial part of *Blumea eriantha DC* was evaluated against 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl (OH) radical scavenging and reducing power assays.

**Results:** The results indicate that methanol extract of *Blumea eriantha* (BEME, 400 mg/kg) exhibited significant inhibition ( $p < 0.001$ ) of increase in paw edema at 5<sup>th</sup> h. IC<sub>50</sub> value of BEME showed significant antioxidant activity. The extract exhibits promising free radical scavenging effect of DPPH, H<sub>2</sub>O<sub>2</sub>, OH and reducing power in a dose-dependent manner up to 100µg/ml concentration while the reference standard Ascorbic acid demonstrated more scavenging potential than the methanol extract of *Blumea eriantha*. The methanol extract was found to be safe at the dose of 2000 mg/kg.

**Conclusion:** The results of the experimental study confirmed that methanol extract of *Blumea eriantha DC* possesses significant anti-inflammatory and antioxidant activity.

**Keywords:** *Blumea eriantha DC*, Anti-inflammatory, Antioxidant

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**INTRODUCTION**

The assault of contagious microorganisms such as bacteria, viruses or fungi on host usually leads to Inflammation reside in particular tissues. Inflammation plays an important role not only in tissue injury, cell death, cancer, ischemia but also in degeneration and tissue enhancing treatment. Inflammation and pain allied with each other. Reactive oxygen species (ROS) play a crucial role in the instigation of free-radical reaction [1]. Antioxidants are compounds that can prevent or inhibit oxidation chain reaction process in living cells. The free radicals involved in the oxidative damage of DNA, proteins, lipids, and lipoproteins. Inflammation coupled with oxidative stress can influence much neurogenerative disorder like arthritis, cancer of lungs, cervix, hepatotoxicity, neurotoxicity, nephrotoxicity, Alzheimer's, etc. Application of traditional medicinal plants with analgesic, anti-inflammatory effects has recently gained popularity world wide over non-steroidal anti-inflammatory drug available in the market because of their natural origin and fewer side effects [2]. Therefore plants have served as a source of natural and safer new drugs for the treatment of inflammation and pain [3].

The genus *Blumea* includes 25-30 species. The other species of *Blumea* like *Blumea lacera* shows anthelmintic, anti-diarrhoeal, antidiabetic activity [4] analgesic, hypothermic, and tranquilizing activities have been reported from the essential oil of *Blumea* [5, 6] *Blumea balsamifera* biologically studied for their cytotoxicity against cancer cells. *Blumea eriantha DC* is commonly known as 'Nimurdi' (Marathi) and Kukronda in Hindi, a small perennial herb, up to 1m in height covered with white and silky hair, dichotomously branched, dispersed in Karnataka, Maharashtra, Uttar Pradesh, Madhya Pradesh, Bihar, and Orissa. Juice of the herb used as 'carminative.' The essential oil extracted from leaves and stem

showing potent antibacterial, antifungal and insecticidal activity. The plant has been applied to treat cholera and diarrhea traditionally and also used as a diuretic. It also used in Rheumatic pain, cough and the common cold. Antioxidant potential and cytotoxicity of leaf extract were reported [7]. The plant also exhibits significant cholesterol-lowering effect [5, 7].

However, there have been no studies on its *in vivo* anti-inflammatory and *in vitro* antioxidant activity of methanol extract of (aerial part) of *Blumea eriantha DC*. Hence the objective of the study was to investigate the anti-inflammatory activity of the extract of *Blumea eriantha* in animal model, and *in vitro* evaluation of antioxidant activity of the extract.

**MATERIALS AND METHODS**

**Procurement and authentication of plant**

*Blumea eriantha DC* was identified and authenticated by A. Benniamin, Scientist D, Botanical Survey of India, Pune and voucher specimen (No. BSI/WRC/cert/2015/43) was deposited at that institute.

**Drugs and chemicals**

Carrageenan, DPPH (1, 1-diphenyl-2-picrylhydrazyl), Methanol (Molychem, India), Diclofenac (gift sample from Emcure Pharmaceuticals Ltd., Pune) and all other chemicals and solvents used were of analytical grade.

**Preparation of extract**

The aerial part of *B. eriantha* was shade dried and powdered. The total 0.5 kg powder was extracted by maceration with using

methanol (1.5 lit x3) solvent at room temperature (24hx3). Then combined all the three collected extracts after filtration and concentrated on a rotary evaporator under reduced pressure at 40 °C to obtain 35.0 gm, 7.0% (BEME) greenish viscous methanol extract.

#### Experimental animals and approval

Female Wistar rats (10-12 w of age, 150-200 g) and Swiss albino mice (male and female, 4 w of age, 20-25 g) were acquired from National Institute of Biosciences, Pune. Animals were housed at 24±1 °C and relative humidity of 65±10% and standard environmental conditions (12 h light and 12 h dark cycle) in the animal house. The animals were fed with standard pellet rodent diet and water was provided *ad libitum*. All the experimental protocols used in this study were approved by Institutional Animal Ethical Committee (CPCSEA/PCH/02/2014-15).

#### Acute oral toxicity study

Healthy male and female Swiss albino mice were subjected to acute oral toxicity studies as per OECD guidelines-425 [8-10]. The animals were fasted overnight and divided into a group of 5 animals. Methanol extract of *Blumea eriantha* DC was administered orally at one dose level of, 175 mg/kg, 550 mg/kg, 1750 mg/kg, and 2000 mg/kg body weight. The mice were observed continuously for behavioral, respiratory or autonomic responses, restlessness, convulsions, tremors, salivation, diarrhea, and mortality for 2 h and any sign of toxicity or mortality up to 48 h.

#### Anti-inflammatory activity

##### Carrageenan induced rat paw edema

Wistar rats of either sex weighing 170-200 g were divided in following groups (n=6) viz;

Group I-Vehicle (2% Tween 80),

Group II-Standard (Diclofenac 10 mg/kg, p. o.),

Group III-BEME (100 mg/kg, p. o.).

Group IV-BEME (200 mg/kg, p. o.).

Group V-BEME (400 mg/kg, p. o.).

Inflammation was produced by injecting 0.1 ml of 1.0 % lambda carrageenan (Sigma Co; USA) in sterile normal saline in to the sub plantar region of the right hind paw of the rat. Rats were presented orally with BEME, and Diclofenac 1h before the carrageenan injection. The paw volume of the rat was measured from 0-6h, at an hourly interval using plethysmometer (Model: 2888, Almemo, Germany). The mean changes in injected paw volume with respect to initial paw volume were calculated. Percentage inhibition of paw volume between treated and control group was calculated by the following formula.

$$\% \text{ Inhibition} = (1 - VT/VC * 100)$$

Where, VT and VC are the mean increase in paw volume in treated and control groups, respectively.

##### In vitro antioxidant activity of *Blumea eriantha*

The ability of the methanol extract (BEME) of the aerial part of the plant and ascorbic acid to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl (OH) radical and reducing power was determined according to the methods described [1,11,12].

##### DPPH free radical scavenging assay

The antioxidant activity of the methanol extract (BEME) and ascorbic acid were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. The (10–100 µg/ml of each extract or standard was added to 2 ml of DPPH in methanol (0.33%) in a test tube. After incubation at 37 °C for 30 min, the absorbance of each solution was determined at 517 nm using a spectrophotometer. The corresponding blank reading was also taken and the remaining DPPH was calculated by using the following formula,

$$\text{DPPH radical scavenging activity (\%)} = [\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{standard})} / \text{Abs}_{(\text{control})}] \times 100.$$

Where, Abs<sub>(control)</sub>: Absorbance of DPPH radical+methanol

Abs<sub>(standard)</sub>: Absorbance of DPPH radical+extract/standard.

The IC<sub>50</sub> value calculated denotes the concentration of the sample required to scavenge 50% of DPPH radical.

##### Hydrogen peroxide radical scavenging assay

A solution of hydrogen peroxide (2 m mol/l) was prepared in phosphate buffer (pH 7.4). Extracts (10–100 µg/ml) were added to hydrogen peroxide solution (0.6 ml). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage scavenging activity of hydrogen peroxide by compound 1 and ascorbic acid was calculated using the following formula,

$$\% \text{ scavenging activity [H}_2\text{O}_2] = [\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{standard})} / \text{Abs}_{(\text{control})}] \times 100$$

Where, Abs<sub>(control)</sub>: Absorbance of the control and

Abs<sub>(standard)</sub>: Absorbance of the extract/standard.

The IC<sub>50</sub> value calculated denotes the concentration of the sample required to scavenge 50% of hydrogen peroxide radical.

##### Hydroxyl radical scavenging assay

The assay was performed by adding 0.1 ml of 1 mmol EDTA, 0.01 ml of 10 mmol FeCl<sub>3</sub>, 0.1 ml of 10 mmol H<sub>2</sub>O<sub>2</sub>, 0.36 ml of 10 mmol deoxyribose, 1.0 ml of different dilutions of the extract and ascorbic acid (10-100 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mmol, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1 h. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% butylatedhydroxyanisole to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation and is calculated as,

$$\text{OH-scavenged (\%)} = [\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{standard})} / \text{Abs}_{(\text{control})}] \times 100.$$

Where, Abs<sub>(control)</sub>: Absorbance of the control reaction and

Abs<sub>(standard)</sub>: Absorbance of the extract/standard.

The IC<sub>50</sub> value calculated denotes the concentration of the sample required to scavenge 50% of hydroxyl radical.

#### Statistical analysis

Data are expressed as mean±SEM, and statistical analysis was carried out by two-way ANOVA followed by Bonferroni's post hoc test. All statistical analyses were performed using Graph Pad Prism software (Graph Pad Software, San Diego, California, USA). Differences with a value of *p*<0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

### RESULTS

#### Acute oral toxicity study

Administration methanol extract (BEME, 2000 mg/kg, p. o.) did not produce any behavioral abnormalities and mortality. Hence, the extract was found to be safe at the dose of 2000 mg/kg. Therefore, three doses of BEME (100,200 and 400 mg/kg b. w) were selected for the anti-inflammatory study.

#### Anti-inflammatory activity of BEME extract in carrageenan-induced paw edema

##### Effect of BEME on inhibition of right hind paws edema on carrageenan-induced inflammation in rats

The rats were pretreated with all the doses of extract and diclofenac for 1 h before the injection of carrageenan caused inhibition of the

increase in paw edema from 1 h to 5 h. Diclofenac (10 mg/kg) caused significant ( $p < 0.01$  and  $p < 0.001$ ) inhibition of the increase in paw edema at 3<sup>rd</sup> and 5<sup>th</sup> h respectively. The inhibitory effect of the diclofenac (10 mg/kg) was recorded (10.38%) at 3<sup>rd</sup>h and (30.94%) at 5<sup>th</sup> h. 400 mg/kg caused significant ( $p < 0.001$ ) inhibition of the increase in paw edema at

5<sup>th</sup> h. The inhibitory effect of BEME (400 mg/kg) was recorded at 3<sup>rd</sup> h (8.21%) and 5<sup>th</sup> h (21.69%) respectively. The inhibitory effect of BEME (100 and 200 mg/kg) were recorded at 3<sup>rd</sup> h (2.95 and 5.77%) and 5<sup>th</sup> h (10.16 and 14.16%) respectively. The inhibition elicited by the 400 mg/kg was comparable to that of diclofenac (Table 1).

**Table 1: Effect of BEME on inhibition of right hind paws edema on carrageenan-induced inflammation in rats**

Treatment Groups	Change in paw edema volume (ml)			% Inhibition at		
	1 h	3 h	5 h	1 h	3 h	5 h
Carrageenan control	0.79±0.010	1.30±0.029	1.46±0.028	-	-	-
Diclofenac 10 mg/kg	0.78±0.015	1.17±0.024**	1.01±0.034***	0.84	10.38	30.94
BEME (100 mg/kg)	0.79±0.015	1.26±0.015 <sup>ns</sup>	1.31±0.017***	0.00	2.95	10.16
BEME (200 mg/kg)	0.79±0.012	1.23±0.015 <sup>ns</sup>	1.25±0.015***	0.21	5.77	14.16
BEME (400 mg/kg)	0.79±0.024	1.19±0.031 <sup>ns</sup>	1.14±0.035***	0.63	8.21	21.69

Data are expressed as mean±SEM; n=6 rats per group. Two way ANOVA followed by Bonferroni's post hoc test when compared with carrageenan control \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

### **In vitro anti-oxidant activity of extract (BEME)**

#### **Effect of extract and ascorbic acid on DPPH free radical scavenging assay**

The extract exhibits promising free radical scavenging effect of DPPH in a dose-dependent manner up to a concentration of 100 µg/ml in this assay. The more radical scavenging potential of ascorbic acid was also demonstrated more which used as reference standard. The IC<sub>50</sub> values of extract and ascorbic acid were 91.5 and 56 µg/ml respectively (Table 2)

#### **Effect of extract and ascorbic acid on hydrogen peroxide free radical scavenging assay**

In this assay, the radical scavenging of hydrogen peroxide leads to exhibit promising free radical scavenging effect of extract in a dose-dependent manner up to a concentration of 100 µg/ml. The

reference standard ascorbic acid also exhibit more radical scavenging potential. The IC<sub>50</sub> values of extract and ascorbic acid were 82 and 74 µg/ml respectively (Table 2)

#### **Effect of extract and ascorbic acid on hydroxyl free radical scavenging assay**

The percentage scavenging of hydroxyl radicals by extract was increased in a dose-dependent manner. (10-100 µg/ml). The ascorbic acid (standard) also showed scavenging effect. The IC<sub>50</sub> values of extract and ascorbic acid were 61 and 44 µg/ml respectively (Table 2).

#### **Effect of extract and ascorbic acid on reducing power assay**

The reducing power of extract as a function of time is presented in Table 2. The reducing power of extract and ascorbic acid increased with increase in concentration.

**Table 2: Effect of extract (BEME) and ascorbic acid on DPPH free radical scavenging assay, H<sub>2</sub>O<sub>2</sub> scavenging assay and OH scavenging assay and reducing power assays**

Conc µg/ml	DPPH free radical scavenging assay. IC <sub>50</sub> µg/ml		Hydrogen peroxide radical scavenging assay. IC <sub>50</sub> µg/ml		Hydroxyl radical scavenging assay. IC <sub>50</sub> µg/ml		Reducing power assay (absorbance)	
	Extract	AA	Extract	AA	Extract	AA	Extract	AA
10	19.93	22.39	5.61	14.76	27.59	31.35	3.28	3.233
20	21.81	27.46	16.84	28.07	32.45	40.73	3.411	3.268
40	25.95	45.33	26.4	39.09	40.62	47.9	3.516	3.325
60	29.34	50.63	39.09	46.57	49.67	59.16	3.53	3.429
80	42.61	61.97	48.86	51.35	59.93	69.32	3.551	3.441
100	54.97	65.04	59.25	62.58	66.45	73.29	3.62	3.471
IC <sub>50</sub>	91.5	56	82	74	61	44	-	-

AA: Ascorbic acid; DPPH: 2,2 Diphenyl-1-picrylhydrazyl; H<sub>2</sub>O<sub>2</sub>: Hydrogen peroxide; OH: Hydroxyl. The reducing power of BEME as a function of time.

### **DISCUSSION**

Plants are a major key source of drug or treatment approach in different traditional medicinal systems. A large number of peoples believe in herbal based medicines for basic healthcare requirements. Several natural products are applied to release symptoms of pain and inflammation in various traditional medicinal systems. Pain and inflammation are associated with physiopathology of various diseases like arthritis, cancer and vascular diseases. *Blumea eriantha DC* is reported to antibacterial, antifungal and insecticidal, anticancer properties. This plant has been traditionally used as a diuretic and also for the treatment of cholera and diarrhea [7]. However, till now, there has been no investigation supporting the anti-inflammatory properties of this plant. Hence, in the present investigation, we have evaluated its *in vivo* anti-inflammatory potential against carrageenan-induced paw edema model in rats and *in vitro* antioxidant activity. Acute oral toxicity study performed at the dose of 2000 mg/kg revealed the non-toxic nature of methanol extract of this plant. There were no

toxic reactions or mortality found with extract. The rats were pretreated with all the doses of extract and diclofenac for 1 h before the injection of carrageenan caused inhibition of the increase in paw edema from 1 h to 5 h. Diclofenac (10 mg/kg) caused significant ( $p < 0.01$  and  $p < 0.001$ ) inhibition of the increase in paw edema at 3<sup>rd</sup> and 5<sup>th</sup> h respectively. The inhibitory effect of the diclofenac 10 mg/kg was recorded (10.38%) at 3<sup>rd</sup>h and (30.94%) at 5<sup>th</sup> h. 400 mg/kg caused significant ( $p < 0.001$ ) inhibition of the increase in paw edema at 5<sup>th</sup> h. The inhibitory effect of the 400 mg/kg was recorded at 3<sup>rd</sup> h (8.21%) and 5<sup>th</sup> h (21.69%) respectively.

The inhibitory effect of the 100 and 200 mg/kg were recorded at 3<sup>rd</sup> h (2.95 and 5.77%) and 5<sup>th</sup> h (10.16 and 14.16%) respectively. The inhibition triggered by the 400 mg/kg was comparable to that of diclofenac. According to literature, plant exhibit potential anti-inflammatory activities due to the presence of phenolic compounds, flavonoids and sesquiterpene lactones [13].

Escalating confirmation showed that free radicals lead to oxidative stress that plays a significant role in inflammatory processes whereas free radical and antioxidants scavengers played a crucial role in the reduction of inflammation [1, 14-19]. The close involvement of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radical (OH) resulted in many dreaded diseases like cancer, Alzheimer's disease, aging, inflammation, rheumatoid arthritis, and atherosclerosis [20, 21]. Now a day various studies have been reported on the crucial role of antioxidant and radical-scavenging mechanism in various anti-inflammatory, anti-arthritis, anti-diabetic, anti-convulsant, anti-ulcer, cardioprotective, hepatoprotective, neuroprotective, and wound healing potential of drugs [22-24]. The 2-diphenyl-1-picrylhydrazyl (DPPH), H<sub>2</sub>O<sub>2</sub> and hydroxyl radical scavenging assay are few of them. Several antioxidants that quickly reacts with peroxy radicals may be slowly reacted or even inert to DPPH [25]. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. In the present study, the methanol extract of *Blumea eriantha* showed scavenging activity in a concentration-dependent manner, and IC<sub>50</sub> of methanol extract for DPPH is 91.5µg/ml, Hydrogen peroxide appears naturally in various elements including air, water, the human body, plants, microorganisms, food, and beverages. [26-27]. However, the formation of hydroxyl radicals from its rapid decomposition in to oxygen and water can cause DNA damage via lipid peroxidation [28].

In the present investigations, IC<sub>50</sub> of methanol extract for H<sub>2</sub>O<sub>2</sub> is 82.0µg/ml and IC<sub>50</sub> of methanol extract for OH is 61.0µg/ml which suggest that *Blumea eriantha* has good antioxidant potential. It has been reported that the coupling of antioxidant activity and reducing power may result in significant reflection of the antioxidant activity [29-31]. The results of the present investigation are in accordance with the findings of the previous investigators. The methanol extract exhibits good reducing power ability which might potentiate its antioxidant property.

## CONCLUSION

The experimental study demonstrated the anti-inflammatory as well as the anti-oxidant activity of *Blumea eriantha* in a dose-dependent manner. Mainly BEME 400 mg/kg was found to be highly effective. The current study justified and supported the ethnopharmacological use of the plant scientifically as an anti-inflammatory agent to treat inflammation. Further attempts will be made to isolate and characterize the active component/s which are responsible for the anti-inflammatory activity of the methanol extract of *Blumea eriantha* DC.

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## AUTHORS CONTRIBUTIONS

Urmila Tambewagh conducted the experiments and also prepared the manuscript. Supada Rojatkar was involved in the planning of the experimental work and in assisting the manuscript preparation. Both the authors have read and approved the content of the manuscript.

## ABBREVIATION

*Blumea eriantha* methanol extract-BEME, MeOH-Methanol, Percentage-%, Temperature- °c, kg-kilogram, ml-milli litre, µg/ml-microgram/milli litre.

## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interest

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