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Research Article

Evaluation Of Antioxidant and Anti-Inflammatory Activity of Blumea Moliis

G. KAMALYADAV*
Vaagdevi Pharmacy College, Bollikunta Warangal-506015.
Email:gaddak70@gmail.com
*Corresponding Author
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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 ethanol extract of aerial part of the Blumea moliis belonging to family Asteraceae.
Methods: The shade dried aerial part of B. moliis (0.5 kg) was powdered successively extracted with ethanol (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 ethanol 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 ethanol extract of aerial part of Blumea moliis was evaluated against 1,1- diphenyl-2-picryl hydrazyl (DPPH), hydrogen peroxide (H2O2) and hydroxyl (OH) radical scavenging and reducing power assays.
Results: The results indicate that ethanol extract of Blumea moliis (BMEE, 400 mg/kg) exhibited significant inhibition ($p<0.001$) of increase in paw edema at 5th h. IC50 value of BMEE showed significant antioxidant activity. The extract exhibits promising free radical scavenging effect of DPPH, H2O2, 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 ethanol extract of Blumea Moliis The ethanol extract was found to be safe at the dose of 2000 mg/kg.
Conclusion: The results of the experimental study confirmed that ethanol extract of Blumea Moliis possesses significant anti-inflammatory and presence of antioxidant activity.

Key Words: Activity, Evaluation, Antixidant

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 intissue 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, antidiarrhoeal, 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. 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 ethanol extract of (aerial part) of Blumea Moliis . Hence the objective of the study was to investigate the anti-inflammatory activity of the extract of Blumea Moliis in animal model, and in vitro evaluation of antioxidant activity of the extract.

MATERIALS AND METHODS

Procurement and authentication of plant

Blumea Moliis was identified and authenticated by A. Benniamin, Scientist D, Botanical Survey of India, Pune and was deposited at that institute.

Drugs and chemicals

Carrageenan, DPPH (1, 1-diphenyl-2-picrylhydrazyl), Ethanol (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. Moliis was shade dried and powdered. The total 0.5 kg powder was extracted by maceration with using
ethanol (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% (BMEE) greenish viscous ethanol extract.

Experimental animals and approval

Female Wistar rats (10-12 w of age, 150-200 g) and Swiss albino rats (male and female, 4 w of age, 20-25 g) were acquiredfrom 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 ().

Acute oral toxicity study

Healthy male and female Swiss albino rats 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. Ethanol extract of Blumea Moliis was administered orally at one dose level of, 175 mg/kg, 550 mg/kg, 1750 mg/kg, and 2000 mg/kg body weight. The rats 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-BMEE (100 mg/kg, p. o.).
Group IV-BMEE (200 mg/kg, p. o.). Group V-BMEE (400 mg/kg, p. o.).
Inflammation was produced by injecting0.1 ml of 1.0 % lambada 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 BMEE, and Diclofenac 1h before the carrageenan injection. The paw volume of the rat was measured from 0-6h, at an hourly interval using plethsmometer (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.
% Inhibition = (1-VT/VC* 100)
Where, VT and VC are the mean increase in paw volume in treated and control groups, respectively.

RESULTS AND DISCUSSION RESULTS

Acute oral toxicity study

Administration ethanol extract (BMEE, 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 BMEE (100,200 and 400 mg/kg b. w) were selected for the anti-inflammatory study.
Anti-inflammatory activity of BMEE extract in carageenan-induced paw edema
Effect of BMEE on inhibition of right hind paws edema on carrageenan-induced inflammation in rats

Table 1: Effect of ethanolic extract of Blumea mollis on carrageenan inflammation in rats

Treatment	30 Min	1 h	2 h	3 h	4 h
Control	0.241±0.004	0.254±0.003	0.433±0.01	0.638±0.01	0.782±0.004
Ibuprofen	0.218±0.006	0.205±0.011*	0.221±0.009***	0.223±0.01***	0.67±0.005*
BMEE 100	(9.54) 0.223±0.006	(19.29) 0.214±0.007*	(48.96) 0.332±0.007***	(65.05) 0.285±0.008***	(14.32) 0.75±0.014*
	(7.47)	(15.75)	(23.33)	(55.33)	(4.09)
BMEE 200	0.22±0.009 (8.71)	0.211±0.01* (16.93)	0.228±0.008*** (47.34)	0.233±0.007** (63.48)	0.69±0.012* (11.76)
BMEE 400	0.219±0.006 (9.13)	0.209±0.008* (17.72)	0.224±0.006** (48.27)	0.231±0.006** (63.79)	0.68±0.008* (13.04)

Data are expressed as mean± SEM; n=6 in each group. Values in parenthesis are percentage inhibition in comparison to control group. When compared to control group (One-way ANOVA followed by

Dunnett's test);
*: P<0.05, **: P<0.01 and ***: P<0.001

Anti-Pyreticactivity

Antipyretic activity was performed by Yeast induced hyperthermia method. In this,15 rats were selected and made hyperthermic by subcutaneous injection of 12% yeast suspension at a dose of 1 ml per animal. Then the rats were divided into three equal groups. After 10 hours of yeast administration, saline was administered at a dose of 1ml per animal orally to one group. The second group required paracetamol 20 mg per animal orally. The third group was given the test drug (B. mollisextract), 100mg,200 mg, 400mg per animal orally. The mean rectal temperature of the rats was recorded at 0, 1½, 3, 4½ hours after drug administration8.

Table 2: Effect of ethanolic extract of Blumea mollis onYeast induced hyperthermia in rats

Group	Dose	Temperature in °C			
		Initial	1½ Hrs	3 Hrs	4½ Hrs
Control	Water 1ml	38.5±0.09	38.1±0.11	37.8±0.12	37.8±0.19
Standard	Paracetamol 10 mg	38.6±0.10	36.5±0.08	35.8±0.13	35.3±0.23
BMEE	100mg	38.2±0.12	38.0±0.15	37.8±0.14	37.2±0.24
BMEE	200mg	38.1±0.13	37.6±0.12	36.3±0.15	36.1±0.28
BMEE	400mg	38.4±0.12	37.1±0.11	36.4±0.10	35.4±0.32

Sub-Acute Toxicity Study

Sub-acute toxicity was carried out in accordance to the OECD guideline; Test Guide lines
407. Both sex wistar albino rats (weight range 150-180g) are selected and separated into 3 groups with 3 male and 3 female rats in each. The groups designed for the study is as follows

Table 3: Experimental protocol for evaluation of Sub-acute toxicity

Group	Treatments
Group- I	Rats were treated with 5ml/kg saline, per orally, Control group
Group- II	Rats were treated with extract of Blumea mollis(500mg/kg/body weight) in 0.5% w/v CMC, p.o.
Group- III	Rats were treated with extract of Blumea mollis(1000mg/kg/body weight) in 0.5% w/v CMC, p.o.

The subacute toxicity of ethanolic extract of B.mollis was carried out as per OECD- 407 guidelines for safe dose administration to animals and the study was carried out. From the subacute toxicity studies (as per OECD- 407 guidelines)

Effect on body weight

There is no significant difference in the body weight of rats in comparison to control rats.

Table 4 Changes in body weight of rats following treatment with different doses of ethanolic extract of Blumea mollis for 28 days repeated dose oral toxicity study

Groups	Dose	Body weight (g)			
		Initial	7th day	14th day	28th day
Control	5ml/kg	175 ± 1.3	189±1.2	194±1.4	199±1.5
EBM	500mg/Kg	174 ± 1.8	180±1.4	188±1.4	192±1.9
EBM	1000mg/Kg	172±1.5	184±1.5	190±1.5	195±1.8

Effect on relative organ weight

The intact weight of organs was converted to relative weight of 100 g body weight as shown in table: 3. The result showed that ethanolic extract of blumea mollis in different doses (500 and 1000 mg/kg/day) administered for 28 days has no significant effect on various organ weight compared to control group.

Table 3: Result of relative organ weight of Ethanolic extract of Blumea mollis treated rates in 28 days in repeated dose oral toxicity study.

Groups	Dose	Weight (g/100g of body weight)				
		Liver	Heart	Lungs	Kidney	Spleen
Control	5ml/kg	4.58±0.15	0.56±0.019	0.90±0.17	0.45±0.08	0.032±0.015
EBM	500 mg/Kg	4.55±0.60	0.45±0.046	0.88±0.035	0.53±0.73	.028±0.057
EBM	1000 mg/Kg	4.52±0.58	0.49±0.051	0.87±0.29	0.52±0.91	0.029±0.015

Effect on hematological parameters

The effect of various extracts of Blumea mollison hematological indices was examined at the end of treatment (Table 4). Treatment for 28 days has non-significant effect on Hb, RBC, platelet count, WBC and eosinophil.

Table 4: Result of hematological profile of ethanolic extract of Blumea mollis treated rates in 28 days repeated dose oral toxicity study

Groups	Dose	Hb (g/l)	RBC($10^6/\mu l$)	Platelets($10^3/\mu L$)	WBC($10^3/\mu l$)	Eosinophils (%)
Control	5ml/kg	13.24± 1.72	9.5 ± 0.35	1105 ± 15.8	12.38 ±1.44	1.7 ±0.44
BMEE	500 mg/Kg	12.48± 1.55	9.50 ± 0.29	1101 ± 12.8	12.46 ± 1.52	2.5±0.55
BMEE	1000 mg/Kg	11.45± 1.12	8.4 ± 0.25	1109 ± 12.7	12.72 ± 1.12	2.5 ±0.15

Table 5: Result of biochemical parameter of ethanolic extract of Blumea mollis treated rates in 28days repeated dose oral toxicity study

Groups	Dose	Glucose (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Albumin (g/dL)	Total Protein (g/dL)
Control	5ml/kg 500 mg/Kg 1000 mg/Kg	105.7±2.43	15.10±1.53	0.78 ±0.25	6.55 ±0.15	5.95 ±0.79
BMEE		102.5±1.33	16.44±1.75	0.60 ±0.64	6.68 ±0.49	5.76 ±0.64
BMEE		101.4±1.24	14.28±1.30	0.75±0.28	6.50±0.50	5.64 ±0.45

Effect on serum biochemical parameters

The effect of various extracts of Blumea mollis for 28days doesn't showed significant changes in glucose, urea, creatinine, albumin, totalprotein, Aspartate Amino transferase, Alanine transaminase and Alkaline phosphatase at doses 500 and1000mg/kg per day compare tocontrol.

Table 6: Results of effect of Ethanolic extract of Blumea mollis on biochemical parameters in rats

Groups	Dose	AST (U/L)	ALT(U/L)	ALP(U/L)
Control	5ml/kg	125± 2.2	42.8 ± 1.5	196 ±2.50
BMEE	500 mg/Kg	125.6 ±1.5	50.0 ± 1.9	170 ± 2.5
BMEE	1000 mg/Kg	121.8 ±1.5	52.5 ± 1.2	185± 1.8

Effect on lipid profile

The effect of various extracts of B.mollis for 28days doesn't showed significant changes in total cholesterol, phospholipids, triglycerides and free fatty acid at doses500 and 1000 mg/kg per day compare tocontrol.

Table 7: Results of lipid profile of various extracts of *Blumea mollis* treated rats in 28 days repeated dose oral toxicity study

Groups	Dose	TC (mg/dL)	PL (mg/dL)	TG (mg/dL)	FFA(mg/dL)
Control	5ml/kg	95.67±1.58	110.45±4.80	72.64±2.97	9.73±2.65
BMEE	500 mg/Kg	95.76±2.50	110.89±3.77	61.60±1.40	7.80±1.19
BMEE	1000 mg/Kg	98.50±4.60	112.76±5.50	59.85±1.66	9.05±1.56

TC: Total cholesterol; PL: Phospholipids; TG: Triglycerides and FFA: Free fatty acid

CONCLUSION

The experimental study demonstrated the anti-inflammatory as well as the anti-oxidant activity of Blumea Moliis in a dose-dependent manner. Mainly BMEE 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 ethanol extract of Blumea Moliis .

Abbreviation

Blumea Moliis ethanol extract-BMEE, MeOH-Ethanol, 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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