Evaluation of Acute, Sub-chronic Oral Toxicity Studies and Anti-inflammatory Activity of *Blumea mollis* in Experimental Animals

ABSTRACT

Objective: The goal of this study was to test the ethanol extract of the aerial part of the *Blumea mollis* (Asteraceae) for acute and sub-chronic toxicity as well as anti-inflammatory efficacy.

Methods: The shade dried aerial part of *Blumea mollis* (0.5 kg) was powdered and extracted with ethanol. Ethanol extracts was used for these studies. Acute oral and sub-chronic toxicity studies were performed as per OECD guidelines. The anti-inflammatory effect was studied by carrageenan-induced paw edema model in rats at dose levels 100, 200, and 400 mg/kg, orally.

Results: The results indicate that ethanol extract of *Blumea mollis* was found to be safe at the dose of 2000mg/kg. The EBM 100, 200 and 400 mg/kg exhibited significant inhibition ($p<0.001$) of increase in paw edema in rats.

Conclusion: The results of the experimental study confirmed that ethanol extract of *Blumea mollis* is devoid of toxicity and possesses significant anti-inflammatory activity.

Keywords: Acute toxicity study; Sub-chronic toxicity study; *Blumea mollis*; Asteraceae; Anti-inflammatory activity

1 INTRODUCTION

As natural herbal treatments are increasingly widely used, experts are focusing their efforts on determining the efficacy and safety of medicinal plants. Because medicinal herbs are used in people for a long time, they should be minimal in toxicity. Various medicinal plants utilised in folkloric medicine, on the other hand, have been documented to have harmful consequences [1,2].

Inflammation can be divided into two types: acute and chronic. Acute inflammation is the body's first reaction to harmful stimuli, and it is marked by increased blood flow of plasma and leukocytes (especially granulocytes) into the injured tissues. The inflammatory response is propagated and matured by a sequence of biochemical events involving the local vascular system, the immune system, and diverse cells inside the wounded tissue. Chronic inflammation results in a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and is characterised by simultaneous tissue death and recovery from the inflammatory process. Type 1 and Type 2 inflammation are distinguished by the types of cytokines and helper T cells (Th1 and Th2) involved [2-4]. Acute inflammation happens right after an injury and lasts only a few days. The migration of neutrophils and macrophages to the site of inflammation is aided by cytokines and chemokines. Acute inflammation is commonly caused by pathogens, allergies, toxins, burns, and frostbite. Microbial infections are recognised by Toll-like receptors (TLRs). Acute inflammation can serve as a protective mechanism against harm. Subacute inflammation is defined as inflammation that lasts 2–6 weeks [5,6].

The plant *Blumea mollis* belongs to the Asteraceae family. It's a fragrant annual plant with upright stems and silky glandular hairs. The leaves are irregularly serrated, ovate-oblong, petiolate, and ovate-oblong [7]. *Bumea molilis* can be found in tropical southern India, Myanmar, China, the Sahara Desert, Malaysia, and South America. The plant have long been used to cure a variety of maladies, including skin infections, diarrhoea [8], asthma, dropsy, wounds [9], and parasites. Antioxidant, anticancer [10], antibacterial, larvicidal, hepatoprotective, and anti-inflammatory activities have also been documented for the plant's leaves [11]. Literature survey suggest that there are few studies on the extract of aerial part of *Blumea mollis* on the scientific basis. Keeping this, the present study was undertaken to carry out acute oral and sub-chronic toxicity studies and anti-inflammatory activity of ethanol extract of aerial part of the *Blumea mollis* in the experimental animals.

2 MATERIALS AND METHODS

2.1 Plant material

The medicinal plant *B. mollis*. traditionally used for the treatment of neuralgia, microbial infection, viral infection and anthelmintic infection as well as treatment of piles. The plant was collected from Nirkhi, Amauli, a town of district Fatehpur UP (26.0479° N, 80.2962° E) and authenticated by a Botanist Dr. Ashok Kumar IFTM University Moradabad (UP) India (Reference no. 2018/SOS/BOT/68).

2.2 Preparation of extract

Dried and powdered aerial part of plant was firstly defatted with petroleum ether followed by ethyl alcohol. extracts and were concentrated on a rotary evaporator under reduced pressure. The extract obtained were preserved in a desiccator for further study. (Percentage yield=7.5 % w/w)

2.3 Animals

Wister albino rats of both sexes weighing between 150-180 g were used for the study. Animals were housed under standard conditions of temperature (25±2°C), 12 h/12 h light/dark cycles and fed with standard pellet diet and RO water for drinking were available all the time [12].

2.4 Acute oral toxicity study

Healthy male and female rats were subjected to acute oral toxicity studies as per OECD guidelines-423 [13,14]. The three female rats were fasted overnight and ethanol extract of *Blumea mollis* was administered orally at one dose level of, 2000 mg/kg body weight. The rats were observed continuously for behavioral, respiratory or autonomic responses, restlessness, convulsions, tremors, salivation, diarrhoea, and mortality for 2 h and any sign of toxicity or mortality up to 48h [15].

2.5 Sub-chronic toxicity study

Sub-acute toxicity of ethanolic extract of *B. mollis* was carried out per the OECD guideline; Test Guidelines 407 [16]. Either sex rats (thirty) were selected and separated into 3 groups. The groups designed for the study is as follows

Table 1 Experimental protocol for evaluation of Sub-acute toxicity

Group	Treatments

Group- I	Rats were treated with 5ml/kg saline, 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 rats were sedated and blood samples for haematological and biochemical examination were taken by heart puncture method into tubes with and without Ethylenediamine tetra acetate (EDTA) on the 28th day of sub-chronic oral poisoning, after an overnight fast. Animals in the study were also subjected to a full, detailed gross necropsy. Organ weights were also recorded [16].

2.6 Anti-inflammatory Activity

The animals with a bodyweight ranging from 150-180 g were orally administered for 7 days with different concentrations of test drug extract (*B. mollis* extract). A suspension of 0.1 ml carrageenan (type IV; 1% w/v in saline solution) was injected in the sub plantar region of the left hind paw of the rats; 0.1 ml saline solution as a control. The vehicle carboxyl methylcellulose 1% w/v (0.1 ml) was used for the control group of rats. The reference drug Ibuprofen (10 mg/kg) was administered orally after 20 minutes of the carrageenan injection as an anti-inflammatory agent. The hind paw volume was measured according to the method of [17,18]. The volume of edema in each rat was calculated from the initial and final volume of the hindfoot by employing plethysmograph (Tech, Ambala). The percentage inhibition of the increase in the volume of the injected foot edema was calculated for each animal group by the following formula: Paw edema inhibition = (Vc – Vt) / Vc×100; Where Vc = mean increase of paw volume control animals; Vt = mean increase of paw volume of treated animals.

3 RESULT

3.1 Acute oral Toxicity study

The dose (2000 g/kg) of orally administered EBM did not produce any signs of acute toxicity or mortality in rats and different parameters were recorded up to 14 days and presented in the table 2.

Table 2. Behavioral patterns of rats during acute toxicity studies.

Parameters	**Observations at time from dosing**					
	30minutes	**4 h**	**24 h**	**48 h**	**7 days**	**14 days**

Parameters	Observations at time from dosing					
	30minutes	4 h	24 h	48 h	7 days	14 days
Fur & skin	N	N	N	N	N	N
Eyes	N	N	N	N	N	N
Salivation	N	N	N	N	N	N
Respiration	↑	N	N	N	N	N
Urination(color)	N	N	N	N	N	N
Faeces consistency	N	N	N	N	N	N
Somatomotor activity & behavior pattern	↑	↑	N	N	N	N
Sleep	N	↑	N	N	N	N
Mucous membrane	N	N	N	N	N	N
Convulsions & tremors	N.F	N.F	N.F	N.F	N.F	N.F
Itching	N.F	N.F	N.F	N.F	N.F	N.F
Coma	N.F	N.F	N.F	N.F	N.F	N.F
Mortality	N.F	N.F	N.F	N.F	N.F	N.F

Key: N = Normal, P = Present, ↑ = Increased, N.F = Not found

3.2 Sub-chronic toxicity study

3.2.1 Effect on body weight

There is no significant difference in the bodyweight of rats in comparison to control rats and presented in the table 3.

Table 3 Effect of EBM on body weight during sub-chronic toxicity study

Groups	Dose	Bodyweight (g)			
		Initial	7^{th} day	14^{th} day	28^{th} 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

Data are expressed as mean± SEM; n=10 (5 male and 5 female rats) in each group. When compared to the control group (One-way ANOVA followed by Dunnett's test)

3.2.2 Effect on relative organ weight

The intact weight of organs was converted to a relative weight of 100 g body weight as shown in the table 4. 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 weights compared to the control group.

Table 4 Result of relative organ weight of EBM treated rats during sub-chronic 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

Data are expressed as mean± SEM; n=10 (5 male and 5 female rats) in each group. When compared to the control group (One-way ANOVA followed by Dunnett's test)

3.2.3 Effect on hematological parameters

The effect of EBM on hematological indices was examined at the end of treatment (Table 5). Treatment for 28 days has a non-significant effect on Hb, RBC, platelet count, WBC and eosinophil.

Table 5 Effect of EBM on the hematological profile of rats during sub-chronic toxicity study

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

Data are expressed as mean± SEM; n=10 (5 male and 5 female rats) in each group. When compared to the control group (One-way ANOVA followed by Dunnett's test)

3.2.4 Effect on serum biochemical parameters

The effect of EBM during sub-chronic toxicity study doesn't show significant changes in glucose, urea, creatinine, albumin, total protein, Aspartate Aminotransferase, Alanine transaminase and Alkaline phosphatase at doses 500 and 1000 mg/kg per day compare to control and presented in the table 6 and table 7.

Table 6 Result of biochemical parameter of EBM on rats during sub-chronic toxicity study

Groups	Dose	Glucose (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Albumin (g/dL)	Total Protein (g/dL)
Control	5ml/kg	105.7±2.43	15.10±1.53	0.78 ±0.25	6.55 ±0.15	5.95 ±0.79
EBM	500 mg/Kg	102.5±1.33	16.44±1.75	0.60 ±0.64	6.68 ±0.49	5.76 ±0.64

EBM	1000 mg/Kg	101.4±1.24	14.28±1.30	0.75±0.28	6.50±0.50	5.64 ±0.45

Data are expressed as mean± SEM; n=10 (5 male and 5 female rats) in each group. When compared to the control group (One-way ANOVA followed by Dunnett's test)

Table 7: Result of biochemical parameter of EBM on rats during sub-chronic toxicity study

Groups	**Dose**	**AST (U/L)**	**ALT(U/L)**	**ALP(U/L)**
Control	5ml/kg	125.0± 2.2	42.8 ± 1.5	196 ±2.50
EBM	500 mg/Kg	125.6 ±1.5	50.0 ± 1.9	170 ± 2.5
EBM	1000 mg/Kg	121.8 ±1.5	52.5 ± 1.2	185± 1.8

Data are expressed as mean± SEM; n=10 (5 male and 5 female rats) in each group. When compared to the control group (One-way ANOVA followed by Dunnett's test)

3.2.5 Effect on lipid profile

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

Table 8: Results of EBM on lipid profile on rats during sub-chronic 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
EBM	500 mg/Kg	95.76±2.50	110.89±3.77	61.60±1.40	7.80±1.19
EBM	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; Data are expressed as mean± SEM; n=10 (5 male and 5 female rats) in each group. When compared to the control group (One-way ANOVA followed by Dunnett's test)

3.3 Anti-inflammatory activity

The results of anti-inflammatory activity are presented in the table 1 and found to be dose dependent.

Table 9 : 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*
	(9.54)	(19.29)	(48.96)	(65.05)	(14.32)
EBM 100	0.223±0.006	0.214±0.007*	0.332±0.007***	0.285±0.008***	0.75±0.014*
	(7.47)	(15.75)	(23.33)	(55.33)	(4.09)
EBM 200	0.22±0.009	0.211±0.01*	0.228±0.008***	0.233±0.007**	0.69±0.012*
	(8.71)	(16.93)	(47.34)	(63.48)	(11.76)
EBM 400	0.219±0.006	0.209±0.008*	0.224±0.006**	0.231±0.006**	0.68±0.008*
	(9.13)	(17.72)	(48.27)	(63.79)	(13.04)

Data are expressed as mean± SEM; n=6 in each group. Values in parenthesis are percentage inhibition in comparison to the control group. When compared to the control group (One-way ANOVA followed by Dunnett's test); *****: P<0.05, ******: P<0.01 and *******: P<0.001

4 DISCUSSION

Hematological parameters are sensitive indicators of physiological changes in animals in response to any pollutant or toxic stress [19]. In this study, there was no significant changes in hematological and biochemical parameters. These results are supported by the study of different researchers [20,21].

Oral treatment of EBM at doses of 100, 200, and 400 mg/kg inhibited the acute phase of inflammation, indicating anti-inflammatory efficacy. Based on the EBM's inhibitory effect at the 3rd and 4th hours, it's possible that the major mechanism of action is related to PGs synthesis inhibition. Moreover, the inhibitory effect of the EBM may partly involve other acute inflammatory mediators such as histamine, serotonin, bradykinin, and proinflammatory cytokines which are released during the 1st Hr after carrageenan injection [19]. Also, the anti-inflammatory activity may be due to presence of t 4-hydroxy-7-ethoxycarvotanacetone and 4-hydroxy-7-tigloyloxycarvotanacetone from the ethanolic extract of the aerial part of plant [22,23].

5 CONCLUSION

The experimental results demonstrates that ethanol extract of *Blumea mollis* is devoid of toxicity in rats. The ethanol extract of *Blumea mollis* possess the anti-inflammatory activity of in a dose-dependent manner. The current investigation validated and supported the plant's ethnopharmacological use as an anti-inflammatory agent in the treatment of inflammation. Another attempt was undertaken to isolate and describe the active components responsible for *Blumea mollis* ethanol extract's anti-inflammatory effect.

Ethical approval

The study was approved by the IAEC of IFTM University, Moradabad, affiliated to CPCSEA, New Delhi (837/PO/ReBiBt/S/04CPCSEA).

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Conflict of Interest

Declared None

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