Original Research Article

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

ABSTRACT

Objective: The present study was to carry out acute oral and sub-chronic toxicity studies and anti-inflammatory activity of ethanol extract of aerial part of the *Blumea mollis* belonging to family Asteraceae.

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

Since natural herbal remedies are being used on large scale, it is now the major focus of the researchers to conduct studies on efficacy and safety of medicinal plants. The plants having medicinal activity should have low toxicity because of their long-term use in humans. However, various medicinal plants used in folklore medicines have been reported to exhibit toxic effects [1,2].

Inflammation can be classified as either *acute* or *chronic*. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (in particular granulocytes) from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged

inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Inflammation has also been classified as Type 1 and Type 2 based on the type of cytokines and helper T cells (Th1 and Th2) involved [3].

Inflammation is not a synonym for infection. Infection describes the interaction between the action of microbial invasion and the reaction of the body's inflammatory response—the two components are considered together when discussing an infection, and the word is used to imply a microbial invasive cause for the observed inflammatory reaction. Inflammation, on the other hand, describes purely the body's immunovascular response—whatever the cause may be. But because of how often the two are correlated, words ending in the suffix *-itis* (which refers to inflammation) are sometimes informally described as referring to infection. For example, the word *urethritis* strictly means only "urethral inflammation", but clinical health care providers usually discuss urethritis as a urethral infection because urethral microbial invasion is the most common cause of urethritis. However, the inflammation-infection distinction becomes crucial for situations in pathology and medical diagnosis where inflammation is not driven by microbial invasions, such as the cases of atherosclerosis, trauma, ischemia, and autoimmune diseases (including type III hypersensitivity) [2,4].

Acute inflammation occurs immediately upon injury, lasting only a few days. Cytokines and chemokines promote the migration of neutrophils and macrophages to the site of inflammation. Pathogens, allergens, toxins, burns, and frostbite are some of the typical causes of acute inflammation. Toll-like receptors (TLRs) recognize microbial pathogens. Acute inflammation can be a defensive mechanism to protect tissues against injury. Inflammation lasting 2–6 weeks is designated sub-acute inflammation [5,6].

Chronic inflammation is inflammation that lasts for months or years. Macrophages, lymphocytes, and plasma cells predominate in chronic inflammation, in contrast to the neutrophils that predominate in acute inflammation. Diabetes, cardiovascular disease, allergies, and chronic obstructive pulmonary disease (COPD) are examples of diseases mediated by chronic inflammation. Obesity, smoking, stress, insufficient diet and poor diet are some of the factors that promote chronic inflammation. A 2014 study reported that 60% of Americans had at least one chronic inflammatory condition, whereas 42% had more than one [6]. On the basis of literature survey, 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.

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 [7].

2.4 Acute oral toxicity study

Healthy male and female rats were subjected to acute oral toxicity studies as per OECD guidelines-423 [8,9]. The animals 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 [10].

2.5 Sub-acute Toxicity Study

Sub-acute toxicity of ethanolic extract of *B.mollis* was carried out per the OECD guideline; Test Guidelines 407 [11]. Either sex rats were selected and separated into 3 groups. The groups designed for the study is as follows

Table No.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.

After 28th day, of the sub-chronic oral toxicity, after an overnight fast, the rats were anesthetized and blood sample for hematological and biochemical analysis were collected by cardiac puncture method into tubes with and without Ethylenediamine tetra acetate (EDTA), respectively. Animals in the study were also subjected to a full, detailed gross necropsy. Organ weights were also recorded [11].

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 [12,13]. The volume of edema in each rat was calculated from the initial and final volume of the hindfoot. 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	P	P	P	N.F	N.F	N.F
Itching	P	P	P	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	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

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

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

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

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± 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

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

3.3 Anti-inflammatory activity

The results of anti-inflammatory activity are presented in the table 9 and found to be dose dependent.

Table No.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 markers of the physiological changes in response to any environmental pollutant or toxic stress in animals [14]. In this study, there was no significant changes in hematological and biochemical parameters. These results are supported by the study of different researchers [15,16].

Oral administration of EBM at the concentrations of 100, 200 and 400 mg/kg showed the anti-inflammatory activity by inhibiting the acute phase of inflammation. Based on the inhibitory effect of the EBM seen at the 3rd Hr and 4th Hr, it suggests that the main mechanism of action may be due to inhibition of PGs synthesis. 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 [14].

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 study justified and supported the ethnopharmacological use of the plant scientifically as an anti-inflammatory agent to treat inflammation. A further attempt was made to isolate and characterize the active components which are responsible for the anti-inflammatory activity of the ethanol extract of *Blumea mollis*.

NOTE:

The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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