

Investigation of Anti-Inflammatory Activity of Some Traditionally Important Medicinal Plants of Chunar Region of Uttar Pradesh

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Abstract

Medicinal plants have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants. These plant metabolites, according to their composition, are grouped as alkaloids, glycosides, saponins, corticosteroids, essential oils etc. In this study anti-inflammatory activity of *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, *Leucas cephalotes* Roxb. (Gumma) Flowers and *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers were investigated and reported. The findings indicates that EE and AE at the test doses 500 mg/kg b.w. reduced the oedema induced by at 5 h is more, when compared with the PEE and CE at the test doses 500 mg/kg b.w. when compared to standard drug and control group. The maximum activity was shown by LCF followed by BLF and PBF against tested bacterial and fungal strain when compared to standard drug.

Key-words: Medicinal Plants, Anti-inflammatory, Extract, Paw Volume

Introduction

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow. Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation whereas prostaglandins are detectable in the late phase of inflammation. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs. Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethnobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. [1-7]

The present work carries the results of anti-inflammatory activity of some traditionally important medicinal plants of Chunar region of Uttar Pradesh. It indicates the utilization of selected plants for the treatment of various ailments among the inhabitants as mentioned in folk-lore and to validated scientifically.

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Material and Methods

Selection and Collection of Plant Material

Three plants i.e., *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, *Leucas cephalotes* Roxb. (Gumma) Flowers and *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers were selected from Chunar region of Uttar Pradesh, were selected for the present study.

Authentication of Plant/Plant Material

The plant parts viz., BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers and PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers were collected from local sites of Chunar region of Uttar Pradesh, India and identified morphologically, microscopically and compared with standard pharmacopoeial monograph. The sample of drug was also identified & authenticated by Dr. S. N. Dwivedi, Retd. Prof. and Head, Department of Botany, Janta PG College, A.P.S. University, Rewa, (M.P.)

Pharmacological Screening

Acute Toxicity studies [8-9]

Procurement of experimental animals

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization. IEAC approval no.....

Anti-inflammatory activity [10-12]

Carrageenan induced paw oedema

Animals

Adult albino rats of both sex (200-250 gm) were procured from Veterinary College, Mhow, Indore, (M.P.) maintained under ideal feeding and management practices in the laboratory. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate

cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee, after scrutinization.

Study Design

The animals were divided into different groups (Control, treated with different extract & Standard) each containing six animals. Group I served as untreated control and received 0.9 normal saline, group II served as positive control and received Indomethacin (10 mg/kg, i.p.) and others group were treated with different doses of Pet. Ether, Chloroform, ethanolic and aqueous extract of BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers and PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers .

Anti-inflammatory Screening

The Pet. Ether, Chloroform, ethanolic and aqueous extract of BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers and PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers and standard drug Indomethacin were administered in prescribed doses. Control received 0.1 ml of 1% carrageenan in normal saline. The administration of extract and drug was 30 min prior to injection of 0.1 ml of 1% carrageenan in the right hind paw sub platar of each rat. The paw volume was measured plethysmometrically (model 7140, Ugo Basil, Italy). Prior to injection of carrageenan, the average volume of the right hind paw of each rat was calculated. At 1, 3 and 5hr after injection paw volume was measured. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response.

Statistical analysis

All the values were statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. Comparison between control and drug treated groups were considered to be significant (*P<0.01). All values are expressed as mean \pm SEM.

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Results and Discussion

The PEE, CE, EE and AE of BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers and PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers were screened for acute toxicity study by OECD guideline no. 423 for determination of LD₅₀. The results showed that the PEE, CE, EE and AE of BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers and PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers were belonging to category-5(unclassified). Hence, LD₅₀ was 5000 mg/kg, therefore, ED₅₀ was 500 mg/kg. Therefore doses of 500 mg were selected for present investigation. The results were presented in table 1, 2 and 3.

Table 1: Determination of LD₅₀ and ED₅₀ of Extract of BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers

S/No.	No. of Animals	Extract Dose (mg/kg)	No. of death of animals			
			PEE	CE	EE	AE
1.	3	5	0	0	0	0
2.	3	50	0	0	0	0
3.	3	300	0	0	0	0
4.	3	2000	0	0	0	0
5.	3	5000	0	0	0	0

Table 2: Determination of LD₅₀ and ED₅₀ of Extract of LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers

S/No.	No. of Animals	Extract Dose (mg/kg)	No. of death of animals			
			PEE	CE	EE	AE
1.	3	5	0	0	0	0
2.	3	50	0	0	0	0
3.	3	300	0	0	0	0
4.	3	2000	0	0	0	0
5.	3	5000	0	0	0	0

Table 3: Determination of LD₅₀ and ED₅₀ of Extract of PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers

S/No.	No. of Animals	Extract Dose (mg/kg)	No. of death of animals			
			PEE	CE	EE	AE
1.	3	5	0	0	0	0
2.	3	50	0	0	0	0
3.	3	300	0	0	0	0
4.	3	2000	0	0	0	0
5.	3	5000	0	0	0	0

The PEE, CE, EE and AE of BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers and PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers evaluated for anti-inflammatory activity in animal models and the results are summarized in Table 4, 5 and 6. The result obtained indicates that the extract found to have significant ($P < 0.01$) anti inflammatory activity in rats. The EE and AE at the test doses 500 mg/kg b.w. reduced the oedema induced by at 5 h is more, when compared with the PEE and CE at the test doses 500 mg/kg b.w. when compared to standard drug and control group .

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents. The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymerphuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthesis) to prostaglandins, which are major components that induce pain and inflammation. It is well known that carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in

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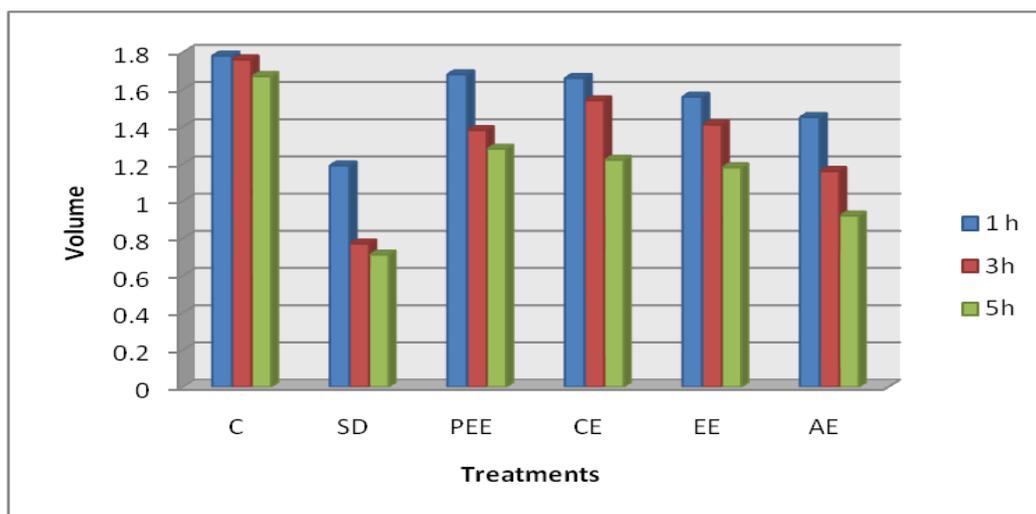
second phase (3 – 4 h after carrageenan injection). Kinin and prostaglandins are involved. From the above studies it is quite apparent that the aqueous extract possesses significant anti-inflammatory activity. The study justifies its use in inflammation as suggested in the folklore medicines.

Table 4: Effect of BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers extracts on carrageenan induced oedema

Treatment	Dose (mg/kg)	Right hind paw volume (mL)		
		1 h	3h	5h
C	-	1.78±0.11	1.76±0.22	1.67±0.14
SD	10	1.19±0.31	0.77±0.12	0.71±0.21
PEE	500	1.68±0.32	1.38±0.21	1.28±0.32
CE	500	1.66±0.21	1.54±0.31	1.22±0.29
EE	500	1.56±0.25	1.41±0.21	1.18±0.22
AE	500	1.45±0.03	1.16±0.04	0.92±0.08

Values are expressed as X (Mean) ±SEM, n=6. (One way ANOVA followed by Dunnett Multiple Comparison Test). Statistically significance *P<0.01 in comparison to control.

Abbr.: C=Control, SD=Standard drug (Indomethacine)



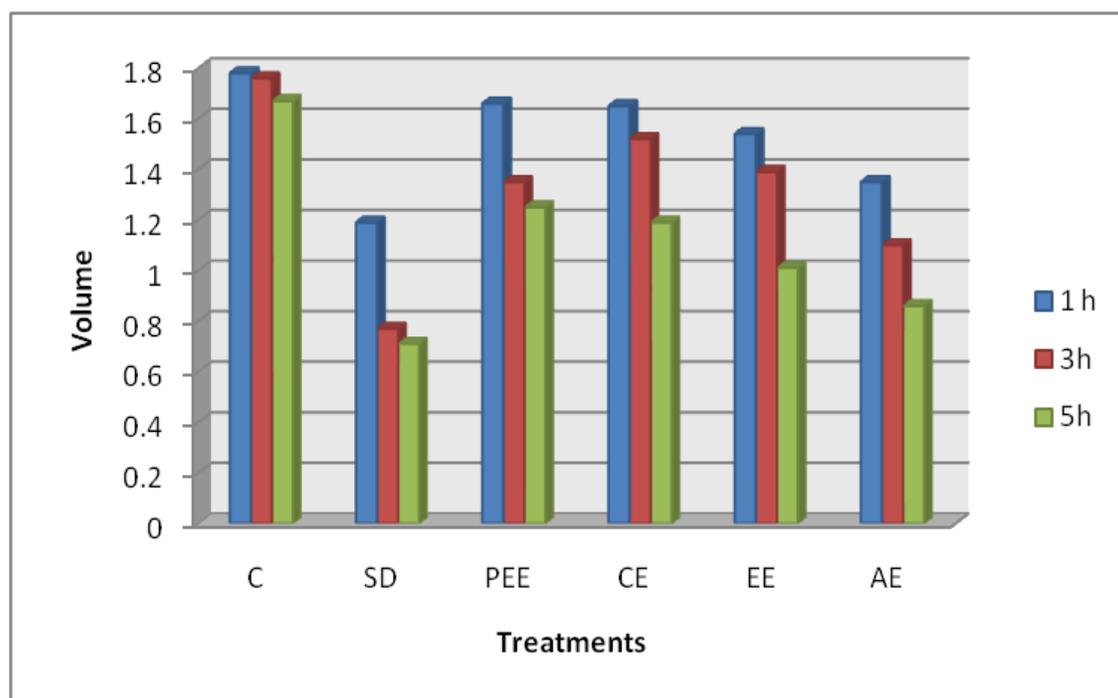
Graph 1: Paw Volume of BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers extracts

Table 5: Effect of LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers extracts on carrageenan induced oedema

Treatment	Dose (mg/kg)	Right hind paw volume (mL)		
		1 h	3h	5h
C	-	1.78±0.11	1.76±0.22	1.67±0.14
SD	10	1.19±0.31	0.77±0.12	0.71±0.21
PEE	500	1.66±0.30	1.35±0.23	1.25±0.02
CE	500	1.65±0.32	1.52±0.30	1.19±0.43
EE	500	1.54±0.25	1.39±0.04	1.01±0.20
AE	500	1.35±0.09	1.10±0.24	0.86±0.14

Values are expressed as X (Mean) ±SEM, n=6. (One way ANOVA followed by Dunnett Multiple Comparison Test). Statistically significance *P<0.01 in comparison to control.

Abbr.: C=Control, SD=Standard drug (Indomethacine)



Graph 2: Paw Volume of LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers extracts

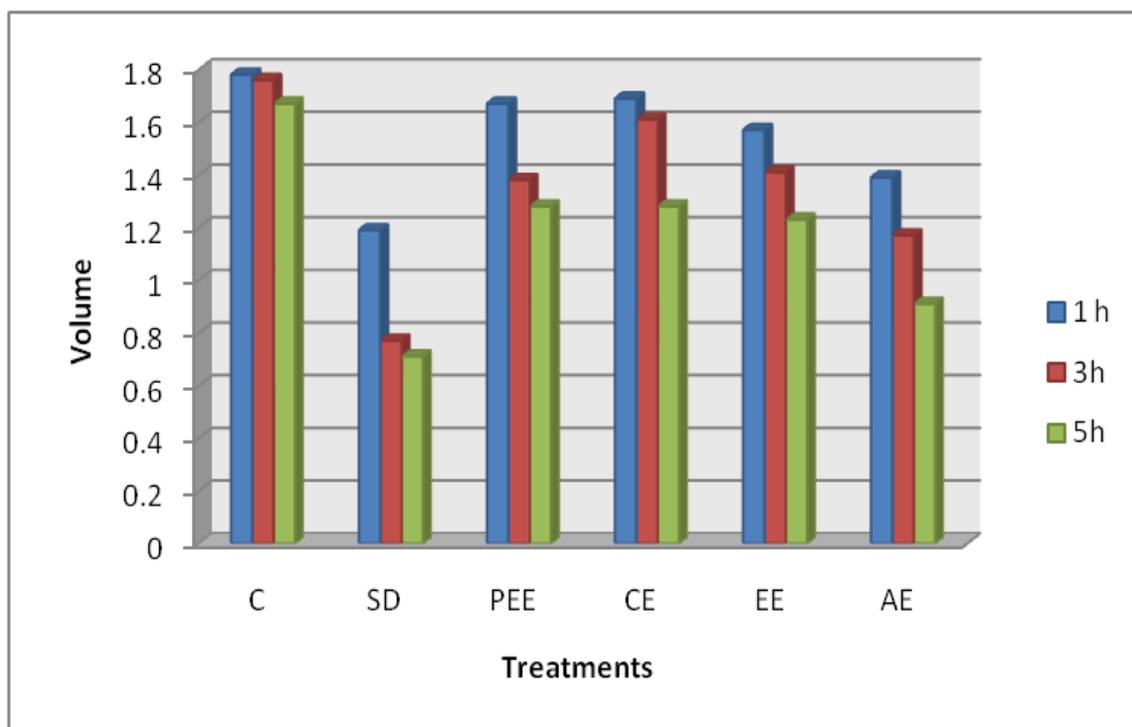
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Table 6: Effect of PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers on carrageenan induced oedema

Treatment	Dose (mg/kg)	Right hind paw volume (mL)		
		1 h	3h	5h
C	-	1.78±0.11	1.76±0.22	1.67±0.14
SD	10	1.19±0.31	0.77±0.12	0.71±0.21
PEE	500	1.67±0.45	1.38±0.09	1.28±0.31
CE	500	1.69±0.33	1.61±0.23	1.28±0.61
EE	500	1.57±0.21	1.41±0.23	1.23±0.47
AE	500	1.39±0.08	1.17±0.44	0.91±0.11

Values are expressed as X (Mean) ±SEM, n=6. (One way ANOVA followed by Dunnett Multiple Comparison Test). Statistically significance *P<0.01 in comparison to control.

Abbr.: C=Control, SD=Standard drug (Indomethacine)



Graph 3: Paw Volume of PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers extracts

Conclusion

The PEE, CE, EE and AE of BLF= *Blumea lacera* (Burm.f.) DC. (Nirmuli) Flowers, LCF= *Leucas cephalotes* Roxb. (Gumma) Flowers and PBF= *Peristrophe bicalyculata* (L) Merr. (Chotiharjori) Flowers evaluated for anti-inflammatory activity in animal models. The EE and AE at the test doses 500 mg/kg b.w. reduced the oedema induced by at 5 h is more, when compared with the PEE and CE at the test doses 500 mg/kg b.w. when compared to standard drug and control group. The maximum activity was shown by LCF followed by BLF and PBF against tested bacterial and fungal strain when compared to standard drug.

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