



EVALUATION OF ANTI-INFLAMMATORY EFFECT OF FRESH TULSI LEAVES (*OCIMUM SANCTUM*) AGAINST DIFFERENT MEDIATORS OF INFLAMMATION IN ALBINO RATS

*Kothari Ankur, Sharma Sheetal

Swami Vivekanand College of Pharmacy, Indore, M.P., India.

*Corresponding author's E-mail: ankur.kothari3@gmail.com

Accepted on: 10-04-2012; Finalized on: 25-05-2012.

ABSTRACT

Ocimum Sanctum (Linn) had been widely used for its reported biological activities in indigenous system of medicine. The present investigation was carried out to find the effect of Fresh tulsi leaves extract of *Ocimum Sanctum* for its anti-inflammatory activity in rodents. The anti-inflammatory activity was evaluated using acute inflammatory models like; carrageenan induced paw oedema, and chronic models like; cotton-pellet induced granuloma, Turpentine oil-induced granuloma pouch and Formalin-induced oedema in rats. Oral administration of the extract at the doses 500 and 600 mg/kg b.w.p.o. exhibited dose dependent and significant anti-inflammatory activity in acute (carrageenan-induced hind paw oedema, $p < 0.05$) and chronic (cotton pellet granuloma, Turpentine oil-induced granuloma pouch and Formalin-induced oedema models, $p < 0.05$) of inflammation. Hence, present investigation established some pharmacological evidences to support the folklore claim that *Ocimum Sanctum* is used as anti-inflammatory agent.

Keywords: *Ocimum Sanctum*, Anti-inflammatory effect, Albino rats.

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 inflammation¹. 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 flow². 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 inflammation³. Whereas prostaglandins are detectable in the late phase of inflammation⁴. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics. 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 2 to 8 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 formulations⁵. 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 drugs⁶.

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. There are two types of plant chemicals, primary metabolites and secondary metabolites. These chemicals exert a significant physiological effect on the human system. A lot of references are available in the field of ethnomedicinal plants used as anti-inflammatory drugs⁷. These have reported that anti-inflammatory activity of two Ayurvedic formulations containing roots of *Glycyrrhiza glabra* (Linn) and fruits of *Coriandrum sativum* (Linn). The present study was carried out to evaluate the antiinflammatory activity of the leaves extract of *Ocimum Sanctum* against acute inflammatory models like; carrageenan induced paw oedema, and chronic models like; cotton-pellet induced granuloma, Turpentine oil-induced granuloma pouch and Formalin-induced oedema in rats.

MATERIALS AND METHODS

Plant material: The Fresh leaves of *Ocimum Sanctum* were collected from the local market areas of Indore, and they were sent for authentication and confirmed by Dr. S.P. Sharma, Department of Botany, Faculty of Science, Agriculture College, Indore. A voucher specimen is kept in the SVCP, Indore.

Preparation of extract: Preparation of extract- Fresh tulsi leaves were taken and triturated in a mortar with a pestle and made into a fine paste. This was then suspended in gum acacia and at doses of 500 & 600mg/kg (a pilot study was done to assess the dose) was administered orally to the animals with the help of oral feeding tube.



Animal care and handling: The experiment was carried out on Wistar albino rats of 4 months, of both sexes, weighing between 100 to 150 gm. They were provided from Sapience Bio-analytical Research Lab, Bhopal, (M.P.). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature $25\pm 2^{\circ}\text{C}$ relative humidity 44–56% and light and dark cycles of 12:12 hours, fed with standard pellet diet and water *ad libitum* during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1413/a/11/CPCSEA).

Anti-inflammatory activity: The animals were divided into four groups (n = 6). Group I served as Control, received the vehicle only (1% Carboxymethylcellulose, CMC, 10 ml/kg p.o.). Group II served as Standard, received Diclofenac Sodium at dose of 100 mg/kg b.w. Group III and IV served as test, received Fresh tulsi leaves extract at doses of 500 and 600 mg/kg b.w.p.o. respectively.

Carrageenan-induced rat paw edema: The test was used to determine the anti-inflammatory activity of the extract by the reported method¹⁰. The animals pretreated with the extract of *Ocimum Sanctum* seed (500 and 600 mg/kg) or diclofenac sodium (100 mg/kg) one hour before were injected with 0.1 ml of 1% carrageenan (in 1% CMC) solution into the sub-plantar region of right hind paw. Paw volume was measured by displacement of the water column in a plethysmometer using plethysmographic reported method¹¹. Immediately after carrageenan application at 0, 1, 2, 3, 4, 5 and 6 h after the stimulus. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response.

Cotton pellet induced granuloma: The test was performed on the rats using the cotton pellet induced granuloma method. The rats were anesthetized under light ether and an incision was made on the lumbar region by blunted forceps, a subcutaneous tunnel was made and a sterilized cotton pellet (100 ± 1 mg) was inserted in the groin area using the method of D'Arcy¹². All the animals received either extract or Diclofenac sodium or vehicle (1% CMC) orally depending upon their respective grouping for seven consecutive days from the day of cotton pellet insertion¹³. On the 8th day, animals were anesthetized again and cotton pellets were removed and dried to constant mass.

Formalin-induced edema in the rat paw: Formalin 0.1 ml (2% in distilled water) was injected into the subplantar area of right hind paw of albino rats¹⁴. The extract at doses of 500 and 600 mg/kg or diclofenac sodium 100 mg/kg were given 30 min prior to formalin injection and subsequently for 7 consecutive days. The paw volume was determined by plethysmographic method initially and on seventh day to measure degree of inflammation

Turpentine oil-induced granuloma in the rat: Subcutaneous dorsal granuloma pouch was prepared in the rat by injecting 2ml air, followed by injection of 0.5 ml of turpentine oil in it. The extract at doses of 500 and 600mg/kg and diclofenac sodium 100 mg/kg were administered orally 30 min prior to turpentine injection and for 7 consecutive days¹⁵. The pouch was opened on seventh day under anaesthesia and exudate was taken out by syringe, and the volume of exudate was compared in various groups. The leukocyte count from exudate was carried out by Neubauer chamber using micropipette.

Statistical analysis: Values are expressed as mean \pm SEM of six observations. Data was analysed by Student *t* test using computerised software SPSS (version 10). $P \leq 0.05$ (a) and $P \leq 0.001$ (b) were considered to be statistically significant.

RESULTS

The fresh leaves extract of *Ocimum Sanctum* was evaluated for anti-inflammatory activity in acute and chronic experimental animal models and the results are summarized in Table 1, 2 and 3.

Carrageenin-induced rat paw oedema: Anti-inflammatory effect of SC-I against carrageenin-induced inflammation is shown in table 1 and figure 1. The Fresh tulsi leaves extract on carrageenan induced paw oedema in rats is shown in table 1. The result obtained indicates that the extract found to have significant ($P < 0.05$) anti-inflammatory activity in rats. The extract at the test doses 500 and 600 mg/kg b.w. reduced the oedema induced by carrageenan by 65.09 and 76.98% respectively, whereas the standard drug showed 78.14% of inhibition as compared to the control group. It significantly reduced the paw volume as compared to the control rats. Diclofenac showed similar type of reduction.

Cotton pellet induced granuloma: The fresh leaves extract was screened for cotton pellet induced granuloma in rats and the results are shown in table 2 and figure 2. The extract exhibited 47.02 and 55.49% inhibition of granuloma formation at the doses 500 and 600 mg/kg b.w respectively, whereas diclofenac sodium showed 73.69% when compared to control.

Turpentine oil-induced granuloma in the rat: The fresh leaves extract was screened for Turpentine oil-induced granuloma pouch in rats and the results are shown in table 3 and figure 3. MCP significantly reduced the exudate volume ($P < 0.05$) dose dependently (table 3), which was comparable with the effect of diclofenac ($P < 0.05$).

Formalin-induced edema in the rat paw: Table 4 and figure 4 shows that *Ocimum Sanctum* Fresh leaves extract was also effective in chronic inflammation. Formalin-induced pedal oedema was inhibited significantly by *Ocimum Sanctum* Fresh leaves extract ($P < 0.05$) as compared to the control rats in dose dependent. Diclofenac also exerted inhibitory action on oedema formation.



Table 1: Carrageenin-induced rat paw oedema

	MEAN &S.E. of PAW volume in ml of substance against CARRAGEENAN – INDUCED RAT PAW OEDEMA							% inhibition of oedema
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	
Control	0.835±0.008	0.87±0.004 ^a	0.893±0.006 ^a	0.927±0.003 ^a	0.937±0.006 ^a	0.962±0.006 ^a	0.941±0.006 ^a	0
Standard	0.94±0.006	0.823±0.004 ^b	0.717±0.007 ^b	0.543±0.008 ^b	0.452±0.009 ^b	0.382±0.005 ^b	0.315±0.007 ^b	78.14
Extract-I	0.947±0.005	0.865±0.008 ^c	0.773±0.007 ^c	0.712±0.01 ^c	0.608±0.008 ^c	0.515±0.004 ^c	0.438±0.005 ^c	65.09
Extract-II	0.963±0.005	0.837±0.004 ^b	0.72±0.004 ^a	0.573±0.006 ^b	0.463±0.006 ^b	0.405±0.008 ^b	0.325±0.004 ^b	76.98

α= 0.05, Values with different superscripts in same column differ significantly

Table 2: Cotton pellet induced granuloma

MEAN of PAW volume in ml of substance against COTTON PELLET INDUCED GRANULOMA				
	Control	Standard	Extract- I	Extract – II
Mean wt of granuloma	53.497±0.0364a	15.66±0.034b	29.34±.133c	25.42±.008
anti inflammatory %	0	73.069	47.02	55.493

α=0.05, Values with different superscripts in same column differ significantly

Table 3: Turpentine oil-induced granuloma in the rat

	MEAN &S.E. of PAW volume in ml of substance against TURPENTINE OIL – INDUCED RAT PAW OEDEMA							% inhibition of oedema
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	
Control	1.12±0.006	1.155±0.007 ^a	1.18±0.015 ^a	1.192±0.026 ^a	1.245±0.004 ^a	1.313±0.005 ^a	1.303±0.004 ^a	0
Standard	1.12±0.005	1.117±0.003 ^b	1.005±0.004 ^b	0.892±0.005 ^b	0.718±0.006 ^b	0.582±0.005 ^b	0.487±0.006 ^b	71.3
Extract-I	1.12±0.006	1.002±0.001 ^c	1.033±0.005 ^c	0.925±0.006 ^b	0.825±0.008 ^b	0.703±0.008 ^c	0.577±0.004 ^c	64.39
Extract-II	1.11±0.008	1.072±0.008 ^d	0.94±0.006 ^d	0.835±0.01 ^c	0.703±0.008 ^c	0.573±0.003 ^b	0.503±0.007 ^d	70.07

α= 0.05, Values with different superscripts in same column differ significantly

Table 4: Formalin-induced edema in the rat paw

	MEAN &S.E. of PAW volume in ml of substance against FORMALIN – INDUCED RAT PAW OEDEMA							% inhibition of oedema
	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr	
Control	0.83±0.006	0.87±0.02a	0.88±0.004a	0.902±0.003a	0.923±0.004a	0.933±0.005a	0.92±0.006a	0
Standard	0.845±0.004	0.72±0.006b	0.578±0.005b	0.468±0.007b	0.348±0.006b	0.26±0.006b	0.163±0.006b	82.28
Extract-I	0.805±0.008	0.717±0.007b	0.623±0.009c	0.52±0.006c	0.407±0.005c	0.362±0.01c	0.342±0.009c	68.83
Extract-II	0.822±0.005	0.708±0.003b	0.598±0.006d	0.498±0.008d	0.355±0.007b	0.283±0.005d	0.2±0.007d	80.11

α= 0.05, Values with different superscripts in same column differ significantly

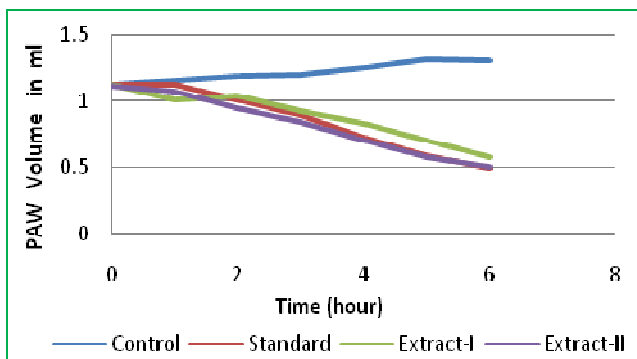


Figure 1: Anti inflammatory effect on carrageenin – induced rat paw oedema

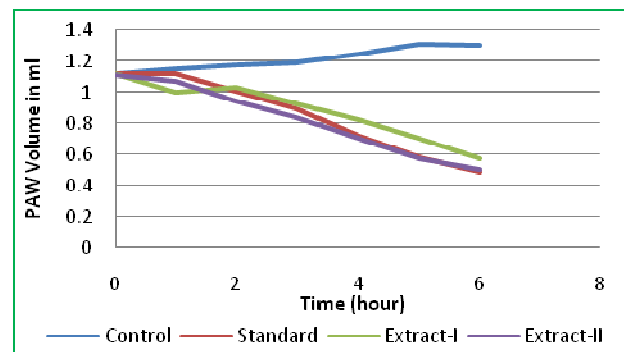


Figure 3: Anti inflammatory effect on turpentine oil – induced rat paw oedema

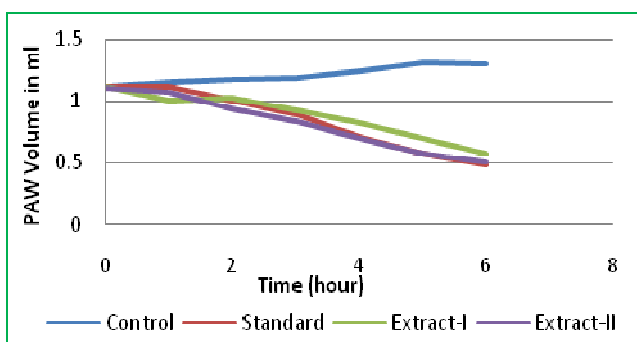


Figure 2: Anti inflammatory effect on cotton pellet induced granuloma

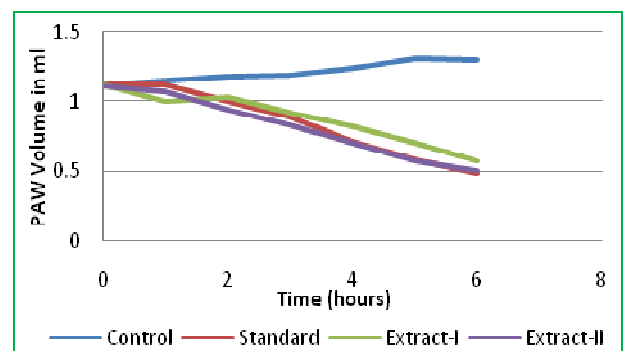


Figure 4: Anti inflammatory effect on formalin – induced rat paw oedema

DISCUSSION AND CONCLUSION

Inhibition of carrageenin-induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents. The development of carrageenin-induced oedema is bi-phasic, the first phase is attributed to the release of histamine, 5-HT and kinins, while, the second phase is related to the release of prostaglandins. Fresh tulsi fraction of the leaves extract of *Ocimum Sanctum* is highly effective in inhibiting carrageenin-induced oedema formation in rats). The leaves found to possess alkaloids, tannins, carbohydrates, phenols, flavanoids and volatile oils. These phytoconstituents could be responsible for anti-inflammatory activity. Granuloma pouch technique was modified⁶ using croton oil as irritant. In the chronic model of cotton pellet implantation the activity was dose dependant and significant reduction in granular tissue formation was recorded. The results were significant when analysed statistically. Thus, extract shows anti-inflammatory activity at various acute phases of inflammation and on formation of granular tissue.

An aseptic inflammation resulting in large volume of haemorrhage exudate is elicited which resembles the subacute type of inflammation. Instead of croton oil, turpentine oil or carrageenin can be used as an irritant. Therefore, turpentine oil-induced granuloma pouch offer a model for exudative type of inflammation. Though, the chemical mediators of this type of response are unknown, protein synthesis is necessary for the formation of granuloma¹⁵. *Ocimum Sanctum* Fresh leaves extract has shown potential inhibitory action on exudate formation. Kinin is said to be the main mediator of granuloma, as it both vasodilate and increase vascular permeability in the early stages of inflammation. It may also be responsible for the vascular flushing that occurs in the carcinoid syndrome. Besides, kinin formation is also implicated in endotoxin shock, hereditary angioneurotic oedema, anaphylaxis, arthritis and acute pancreatitis¹⁶. Gene sequence identity between a portion of this kininogen molecule, a major acute phase protein and a proteinase inhibitor suggests that kininogen inhibit acid proteases in an area of inflammation and may prolong the actions of the kinins¹⁸. Keeping all these in view it may therefore be said that *Ocimum Sanctum* Fresh leaves extract may possess anti-kinin like activity.

It is well known that inhibition of formalin-induced pedal oedema in rats is one of the most suitable test procedures to screen anti-arthritis and anti-inflammatory agents as it closely resembles human arthritis¹⁸. Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response¹⁹. Thus formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation. Results with

Ocimum Sanctum Fresh leaves extract are quite compatible with those of the standard drug diclofenac in dose dependent manner. Therefore, the drug appears to be effective against formalin-induced arthritis. This finding justifies the usefulness of *Ocimum Sanctum* Fresh leaves extract in the treatment of inflammation associated diseases like arthritis. It is concluded that Fresh tulsi I fraction of leaves extract of *Ocimum Sanctum* possesses significant anti-inflammatory activity, which is comparable to diclofenac.

REFERENCES

- Mitchell RN, Cotran RS. In: Robinsons Basic Pathology, ed 7. Harcourt Pvt. Ltd., New Delhi, India. 2000; 33-42.
- Ialenti A, Ianaro A, Moncada S, Di Rosa M. Modulation of acute inflammation by endogenous nitric oxide. Eur. J. Pharmacol. 211:1995; 177-184.
- Di Rosa M, Giroud JP, Willoughby DA. Studies on the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol. 104(1):1971; 15-29.
- Salvemini D, Wang ZQ, Bourdon DM, Stern MK, Currie MG, Manning PT. Evidence of peroxynitrite involvement in the carrageenan induced rat paw edema Eur. J. Pharmacol. 303: 1996; 217-224.
- Cowan MM. Plants products antimicrobial agents. Clin. Microbial. Rev. 14:1999; 564-584.
- Ahmad F, Khan RA, Rasheed S. Study of analgesic and anti inflammatory activity from plant extracts of *Lactuca scariola* and *Artemisia absinthium*. J. Isl. Acad. Sci. 5:1992; 111-114.
- Bagul MS, Shrinivas H, Kanaki MS, Rajani M. Anti-inflammatory activity of two ayurvedic formulation containing guggul. Ind. J. Pharmacol. 37:2005; 299-400.
- Bhattacharya P, Lakshmi SM, Ashok kumar CK, Mandal SC. Conference proceeding on promotion and development of Botanicals with international coordination school of National Product, Jadavpur, Kolkota. 2005;383-385.
- Ammar NM, Alokbi SY, Mohamed DA. Study of the Antiinflammatory activity of some medicine edible plants growing in Egypt. J. Isl. Acad. Sci. 10:1997; 1-9.
- Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paws of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 111:1962; 544-547.
- Harris JM, Spencer PSJ. A modified plythesmographic apparatus for recording volume changes in rat paw. J Pharm Pharmacol, 14:1962; 464-6.
- D'Arcy PF, Howard EM, Muggleton PW, Townsend SB. The antiinflammatory action of griseofulvin in experimental animals. J Pharm Pharmacol, 12:1960; 659-65.
- Chau, T.T. Analgesic testing in animal models. In: Pharmacological Methods in the Control of Inflammation. Alan R Liss Inc., New York. 1989;195.



14. Selye, H. On the mechanism through which hydrocortisone affects the resistance of tissue to injury. An experimental study with granuloma pouch technique. *Journal of American Medical Association*.1953; 152:1207.
15. Robert, A., Nezamis, J.E. The granuloma pouch as a routine assay for anti-phlogistic compounds. *Acta Endocrinology*. 25:1957; 105.
16. Warren KS. In: inflammation. Lepaw IH, Waed PA. New York. Academic Press. 1972; 203.
17. Clark WG, Brates DC, Johnson AR. Kininins and other peptides. In: *Goth's Pharmacology* ed. 13th. Mosby Year Book 1991; 209-19.
18. Margolius HS. Tissue kallikrienes and kinins regulation and roles in hypertensive and diabetic diseases. *Annu Rev Pharmacol Toxicol*, 29:1989; 343-8.
19. Greenwald, R.A. Animal model for evaluation of arthritic drugs. *Methods and Findings in Experimental and Clinical Pharmacology*. 13: 1991; 75–83.

