WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 18, 579-594. Research Article ISSN 2277–7105

THE ANTI-INFLAMMATORY EFFECT OF AQUEOUS EXTRACT OF *OCIMMUM SANCTUM* LEAVES AND FLOWERS ON RATS

Sagir Ahmad*[1], Md. Quamuddin[2] and Somnath Kharwar[3]

Department of Pharmacology: HIMT College of Pharmacy, Knowledge Park-I, Greater Noida, Uttar Pradesh, India.

Article Received on
23 July 2024,

Revised on 13 August 2024,
Accepted on 03 Sept. 2024

DOI: 10.20959/wjpr202418-33836

***Corresponding Author**
Sagir Ahmad
Department of Pharmacology: HIMT College of Pharmacy, Knowledge Park-I, Greater Noida, Uttar Pradesh, India.

ABSTRACT

We investigate the anti-inflammatory effect of freshly aqueous extract of *Ocimum Sanctum* leaves and flowers in carrageenan induced acute inflammation and formalin induced chronic inflammation. Oral administration of freshly prepared aqueous extract of *Ocimum Sanctum* to each animal was given to carrageenan induced acute inflammation until four hours. After the carrageenan administration at dose of *Ocimum Sanctum* 200 mg/kg/hr. and 400 mg/kg/hr. Treating with these aqueous extract of *Ocimum Sanctum* significantly reduces the acute inflammation in rats. In chronic inflammation in rat the oral administration of the aqueous extract of *Ocimum Sanctum* to each animal was given to formalin induces chronic inflammation until 10 days. After formalin administration the linear cross section of ankle joint was measured by using vernier calliper and the percentage anti inflammation was calculated. The higher dose 400mg/kg/hr. and 400mg/kg/day of aqueous extract of *Ocimum Sanctum* therapy works better than that of lower dose (200mg/kg/hr. and 200mg/kg/day) against acute inflammation and chronic inflammation induced by carrageenan and formalin respectively. Therefor the use of the aqueous extract of this plant leaves and flower as anti-inflammatory is justified. Aspirin (300/kg/day) is a NSAID. It used as standard drug and maintain the inflammation level in rats.

KEYWORDS: *Ocimum Sanctum*, Carrageenan, Formalin, Acute inflammation and chronic inflammation.

INTRODUCTION

Inflammation

Inflammation is defined as the biological response of body which is part of complex response of body tissue to any harmful stimuli like irritants, pathogens, or destructed body cells is a defensive response involving blood vessels, molecular mediators and immune cells. The result of inflammation clear out necrotic cells and damage tissue healing from inject of original inflammation process, and initiate tissue healing.

Sign and symptoms

- Heat, pain, swelling, redness and loss of function are the classical signs of inflammation.
- Heat (Calor): Blood flows is increase at affected area due to which it feel warm to the touch.
- Pain (Dolor): The affected area is become to be painful, during and after touching specially. The chemicals are released which stimulate the nerve endings which makes the affected area more sensitive.
- Swelling (Tumor): Swelling takes place at the affected site due to formation of the fluid.
- Redness (Rubor): The blood become unusual flow within the capillaries which makes redness of the affected area.
- Immobility (Functio laesa): Due to the inflammation the affected part of the body become functionless.

Since inflammation is a specific response, so inflammation is considered as mechanism of innate immunity, as compared to adaptive immunity, and its unique for each pathogen. Deficient inflammation can lead to continuous tissue loss by the harmful stimulus like bacteria and compromise the survival of that living organism. In counterpoint the ailments can be hosted by the chronic inflammation, like rheumatoid arthritis, periodontitis, allergic rhinitis, arterial sclerosis and sometime it hosts even cancer for example gallbladder carcinoma. Therefore the body regulate the inflammation in normal and close way.

Causes

Physical

- Foreign bodies, including splinters, dirt, and debris
- Physical injury, blunt or penetrating
- Frostbite

- Burns
- Trauma
- Different ionizing radiation

Biological

- Immune reactions due to hypersensitivity
- Infection by pathogens
- Stress

Chemical

- Alcohol
- Chemical irritants
- Toxins

Psychological

- Excitement

Classification

Inflammation can be classified as either acute inflammation or chronic inflammation.

Acute inflammation

The primary response of the body to any harmful stimuli is called acute inflammation. This is occurred through increased flow of leukocytes and plasma (Mainly granulocytes) to the injured tissues from blood. The response of inflammation become mature and propagated by a series of biochemical phenomenon in which the immune system, local vascular system and different types of cells are involved within the affected tissues.

Chronic inflammation

As per the term chronic it is clear that the prolonged inflammation is termed as the chronic inflammation, and this inflammation process leads to a progressive shift in the form cells which are present at the affected site of inflammation, like mononuclear cells, and it is characterized by simultaneously breaking up and healing of the tissues from the process of inflammation.

Inflammation is not the similar to the infection. the term infection elaborate the connection between the reaction of the inflammatory reaction of body and the aggression action of the

microbes, when infection is discussing the two components are observed together, and the term is used to imply a microbial aggressive cause for the observed reaction of the inflammation. On the other way inflammation elaborate entirely the immune vascular response of the body, whatever the situation may be. But for the reason that of how frequently the correlated the two, sometimes the words which ends with the suffix- itis (this indicates to inflammation), are described informally as indicating to infection. For example urethritis is a word whichis specifically used for the "urethral inflammation". But urethritis is usually discussed as the urethral infection in the clinical health care and it is because the most common cause of this ailment is urethral microbial aggression**.**

MATERIALS AND METHODS

Instruments

- Digital Plethysmometer (Campden Instruments Ltd.)
- Vernier Caliper
- Electronic weight box
- Volumetric Flask
- Beaker
- Conical Flask
- Morter and Pestle
- Muslin Cloths
- Syringe
- Refrigerator (LG Company)

Animals used in experiment

8-10 weeks old albino male/ female rats (initially) weighing 150- 200 g were used. Animals were housed 6 per cage, under standard laboratory conditions (Temperature:25 ± 2ºC, humidity 30- 70 % and lighting condition 12 hr. dark and 12 hr. light (artificially), and were given food and water ad libitum. The animals acquired from Animal house of HIMT college of Pharmacy Gr. Noida. The experiment is to be performed in animal facility centre, HIMT College of pharmacy Gr. Noida. All animal experiment protocols were approved by the Institutional Animal Ethics Committee (IAEC). The rats of both sexes were used.

Plant materials

Ocimum Sanctum commonly known as TULSI belongs to the family: Lmiaceae are procured from wholesale spice market Khari Baoli of Delhi India. The plant and rhizomes were authenticated with the help of a scientist & Head Dr. Sandeep Chauhan, Ministry & Environment, Forest and Climate Change, Botanical Survey of India, BGIR, Sec.-38A Noida 201303.

Preparation of plant extract

The aqueous extract of tulsi leaves and flowers were carried out according to the method described by the Mitra et al. the collected leaves of tulsi plants were shade dried and grinded to make powder form. The powder of dried Tulsi leaves and flowers were soaked overnight in doubled distilled water (15 gm. per 100 ml), filtered through loin cloth (fine cotton cloth). The filtrate was centrifuged 5000rpm for 10 min (by REMI cold centrifuge). The supernatant which is obtained after centrifugal process was filtered again through loin cloth and the filtrate is collected in sterile polypropylene tubes and frozen at -20˚C. A definite amount of tulsi leaf extract was always freshly dissolved in double distilled water to give a particular concentration and aliquot of this solution (not more than 0.5 ml) was fed to the rats with the help of feeding needle. Any leftover of this solution was discarded**.**

Phytochemical Analysis

The test sample was subjected to phytochemical analysis in order to find out the presence of phytochemical constituents. The phytochemical tests employed for fixed oils and fatty acid, carbohydrates, alkaloids and proteins, Cardiac glycosides and flavonoids.

- Fixed oils and fatty acid (Spot test)

A small quantity of crude extract was pressed between two filter papers separately. An oily appearance on filter paper indicated the presence of fixed oil and fats.

- **Test for carbohydrates (Fehling's test)**

Few drops of extract are heated with Fehling's A and B solution. Appearance of orange red precipitate indicates presence of carbohydrates.

- **Test for alkaloids(Wagner's test)**

20mg of turmeric was dissolved in 2ml of methanol. Few drops of 1% HCl added to it. Then the mixture was heated, kept in steam and after cooling. Then the mixture was treated with few drops of Wagner's reagent. The sample was observed for turbidity or precipitation.

- **Test for proteins(Biuret's test)-**

Add 2ml of Biuret reagent to 2ml of extract. Shake well and warm it on water bath.
Appearance of red or violet colour indicates presence of proteins.

- **Test for cardiac glycosides**

20mg of turmeric was dissolved in 1ml of glacial acetic acid and 1-2 drops of ferric chloride solution was added. 0.5ml of concentrated sulphuric acid was slowly added along the sides of the test tube. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides.

- **Test for flavonoids(Ferric chloride test)**

20mg of turmeric was dissolved in 1ml of distilled water. 0.5ml of dilute ammonia solution was added to it. Conc. Sulphuric acid was added later. A yellow colour indicated the presence of flavonoids. The yellow colour disappeared on allowing the solution to stand**.**

Induction of Acute Inflammation

0.05 ml of 1% Carrageenan was injected aseptically into the sub plantar surface of right hind paw of each rat. Paw edema was measured by mercury plethysmograph (IITC 520). The hind paw volume obtained at 1 h, 2 h, 3 h and 4 h after Carrageenan injection both in control and test animals. By comparing the edema produced in control rats and in those treated with drugs, percentage inhibition of edema was calculated as follows.

$$\text{Percentage inhibition} = Vc- Vt /Vc\times 100$$

Where Vc = Volume of paw edema in control animals. Vt = Volume of paw edema in treated animal.

Induction of Chronic Inflammation

0.1 ml of 2% formalin was injected aseptically into the sub plantar surface of right hind paw of each rat. Paw edema was measured by Vernier Calliper**.**

Experimental design for study of acute anti- inflammatory study (Groups are treated with carrageenan 1% to induce inflammation)

Group I: Control Group- receive vehicle (distilled water) only

Group II: Standard Group: - Receive 300 mg/kg Aspirin for 4 hrs. In equal interval of time.

Group III: Test Group-I: - Receive aqueous extract of O.sanctum 200 mg/kg.

Group IV: Test Group-II: - Receive aqueous extract of O.sanctum 400 mg/kg.

Acute anti-inflammatory activity (paw edema test)

Paw edema was induced by an intrademal injection of 0.1 ml of carrageenan (1% in normal saline) into the plantar surface of the right hind paw of rats (method of winter, risely and nuss). The acute phase of inflammatory reaction i.e. edema volume of right hind paw was determined using a plethysmometer. Prior to and 1 h, 2 h, 3 h and 4 h after Carrageenan injection both in control and test animals. All the drugs were administered one prior to carrageenan. % in inhibition of paw edema was calculated using the following formula

% inhibition in given time interval

$$\frac{\text{Paw volume in control group-Paw volume in test group}}{\text{Paw in control group}} \times 100$$

Experimental design for study of Chronic anti- inflammatory sudy(Groups are treated with formalin solution 2% to induce inflammation)

Group I: Control Group- receive vehicle (distilled water) only for 10 days

Group II: Standard Group: - Receive 300 mg/kg Aspirin for 10 days per day.

Group III: Test Group-I: - Receive aqueous extract of O.sanctum 200 mg/kg for 10 days per day.

Group IV: Test Group-II: - Receive aqueous extract of O.sanctum 400 mg/kg for 10 days per day.

Chronic anti-inflammatory activity test

In this method, a chronic phase of inflammatory was induced by subcutaneous injection of 0.1 ml of 2% of formalin under the plantar Apo neurosis of right hind paw of rats on first and third day of the experiment. Treatment was started on day one and continued daily for ten days. The linear cross section (LCS) immediately below the ankle joint was measured daily with the vernier caliper. The difference in LCS on day one and day ten was calculated for all groups. Percentage anti-inflammatory effect of particular drug was calculated as follows.

% Anti-inflammatory effect

$$\frac{\text{Mean difference in LCS in control group- Mean difference in LCS in test group}}{\text{Mean difference in LCS in control Group}} \times 100$$

RESULTS

The results obtained were compared with control and also with known anti -inflammatory agent, Aspirin.

The mean paw volumes at hourly interval in milliliters (ml) of each group are represented in Table 1 and Graph 1.

In control group there was a progressive increase in mean paw volume, where as in standard and test groups there was progressive decrease in mean paw volume from 1h to 4h.

Anti-inflammatory activity is expressed as percent inhibition. The results obtained are shown in (Table 2) and also depicted in bar diagram (Graph 2). The mean paw volume of the control group reached its peak at about 4h after the administration of Carrageenan. Groups treated with O.sanctum 200mg/kg, O.sanctum 400mg/kg and Aspirin 300mg/kg displayed values of 18.13%, 30.41% and 63.45% percentage inhibition of paw edema at 4h respectively. The anti-inflammatory effect of the above mentioned treatment groups were significant i.e. ($p<0.05$) at time intervals of [2h] and highly significant ($p< 0.001$) at time intervals of [3h and4h] but not at the 1h interval ($p>0.05$).

Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. 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 (especially granulocytes) from the blood into the injured tissues. A cascade 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 and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Anti-inflammatory drugs inhibit different stages of inflammation.

Carrageenan induced edema in the Rat Paw

Results of the present study in comparison with control clearly indicate that the O.sanctum showed significant anti-inflammatory activity when administered alone for reducing paw edema in acute model induced by Carrageenan. The edema results from the action of inflammatory mediators such as histamine, serotonin, kinins and prostaglandins at the site of a local inflammatory insult.[90] The early phase of edema, beginning from 1h after administration of the irritant, is due to the release of histamine and serotonin, while the later phase, occurring from 3 h to 4 h after administration of the irritants induced by bradykinin, protease, prostaglandin and lysosome.

Linolenic acid could significantly inhibit the edema induced by PGE2, LTB4 and arachidonic acid. Linolenic acid present in fixed oil of O. sanctum could account for the anti-inflammatory activity of the oil by dual inhibition of arachidonic acid metabolism. The fixed oil of O. sanctum possesses significant anti-inflammatory, analgesic, anti-arthritic, antipyretic, antiulcer, antimastitic and antimicrobial properties without any noticeable toxicity.

The lipids present in O. sanctum contain relatively large amount of gammalinolenic acid (GLA), an omega-6 (18:3, n-6) fatty acid (all cis-6, 9, 12 octadecatrienoic acid) which contains the first double bond at 6th carbon atom from the methyl end of the fatty acid chain. GLA is rapidly converted to dihomogammalinolenic acid (DGLA) (20:3, n-6) (a precursor of anti-inflammatory prostaglandin E1) which competes with arachidonate for oxidative enzymes thereby reducing production of cyclooxygenase enzyme.

Table 1: Effect of O.sanctum and indomethacin administered alone and in combination at various time intervals of Carrageenan-induced paw edema in rats.

Treatment group	Paw volume(edema) in ml (mean ± SD)			
	1hr	2hr	3hr	4hr
Control D/W	3.65±0.60	3.56±0.10	3.60±0.06	3.42±0.12
Carrgn.	3.68±0.20	3.62±0.12	3.74±0.15	3.82±0.10
Carrgn.+OS 200mg/kg	3.10±0.10	2.95±0.06	2.92±0.12	2.80±0.07
Carrgn.+OS 400mg/kg	2.70±0.06	2.58±0.18	2.55±0.15	2.38±0.10
Aspirin 300mg/kg	2.54±0.16	2.46±0.16	1.48±0.18	1.25±0.14

Each value represents the mean ± SD (N = 6). Statistical analysis by One-way ANOVA followed by turkey's multiple comparisons. P value * < 0.05 is significant; ** < 0.001 is highly significant. Abbreviations: carrgn, carrageenan; D/W, distilled water; OS, Ocimum sanctum.

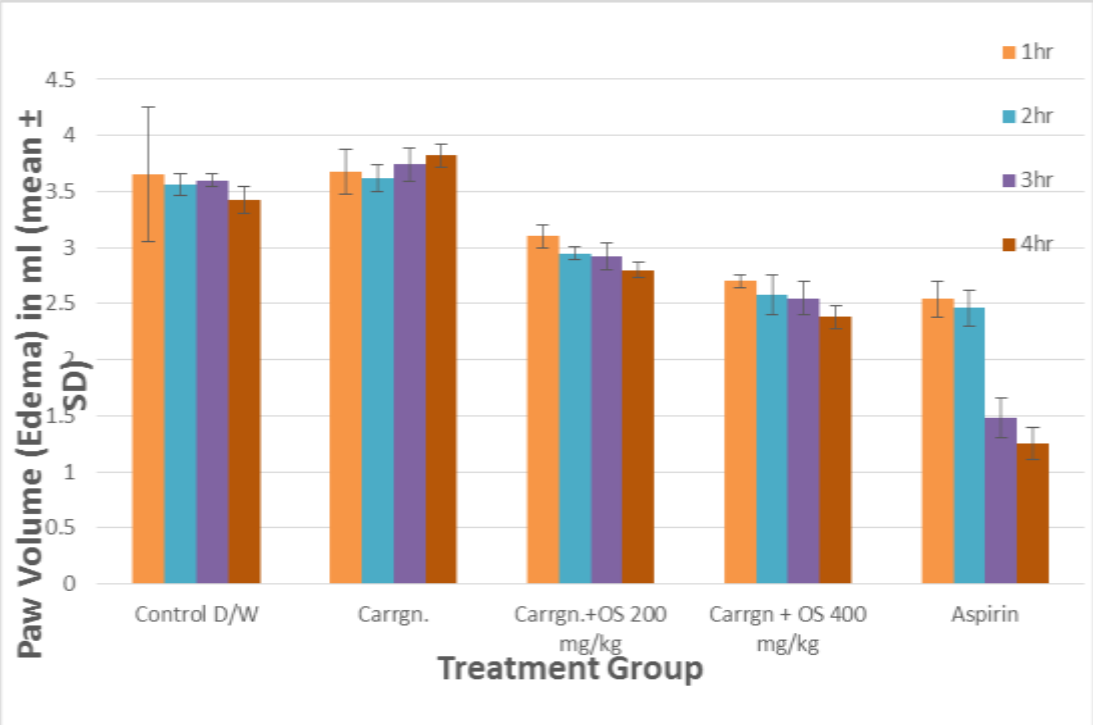

Fig 1: Effect of O. Sanctum and Aspirin administered alone at various time intervals of carrageenan induced paw edema in rats.

Table 2: Percentage inhibition of edema produced by O.sanctum and Aspirin alone at various time intervals of Carrageenan-induced rat paw edema Abbreviations: Carrgn, Carrageenan; OS, Ocimum sanctum.

Treatment group	Percentage of anti-inflammatory effect			
	1 hr	2 hr	3 hr	4hr
Carrgn.+OS 200mg/kg	15.07%	17.14%	18.89%	18.13%
Carrgn.+OS 400mg/kg	26.03%	27.53%	29.17%	30.41%
Aspirin 300mg/kg	30.41%	30.90%	58.89%	63.45%

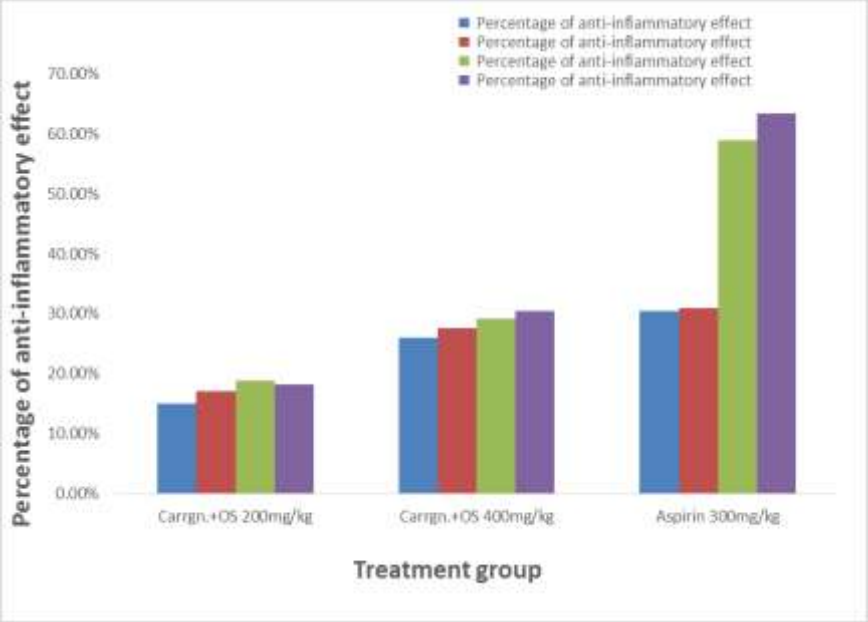

Fig. 2: Percentage inhibition of edema produced by O.sanctum and Aspirin alone at various time intervals of Carrageenan-induced rat paw edema.

Formalin induced edema in the Rat Paw

Table 3 and figure 3 shows that Ocimum Sanctum Fresh leaves extract was also effective in c hronic inflammation. Formalin induced pedal edema was inhibited significantly by Ocimum Sanctum Fresh leaves extract (P <0.05) as compared to the control rats in dose dependent.

Effect of aqueous extract of Ocimum Sanctum on Linear Cross Section below the ankle joint in formalin induced arthritis in rats.

Table 3: Effect of aqueous extract of O.Sanctum on Linear Cross section below the ankle joint in formalin induced arthritis in rats Aand Percentage inhibition of edema produced by O.sanctum and Aspirin alone during interval of 0 to 10 days of formalin-induced rat paw edema.

Groups	Mean initial LCS	Mean day 10 LCS	Mean difference in LCS	% Anti-inflammatory effect
Control D/w 2ml/kg	3.958±0.192	7.207±0.182	3.249±0.162	--------
Formalin	3.875±0.234	8.275±0.110	4.400±0.120	---------
Formalin+ aqueous extract of O.S 200mg/kg	3.795±0.148	5.690±0.112	1.895±0.121	41.69%
Formalin+ aqueous extract of O.S 400mg/kg	3.920±0.190	4.980±0.130	1.06±0.145	67.37%
Aspirin 300mg/kg	3.878±0.051	4.232±0.038	0.354±0.056	89.10%

Each value represents the mean ± SD (N = 6). Statistical analysis by One-way ANOVA followed by turky's multiple comparisons. P value * < 0.05 is significant; ** < 0.001 is highly significant. Abbreviations: LCS, Linear Cross Section; D/W, distilled water; OS, Ocimum sanctum.

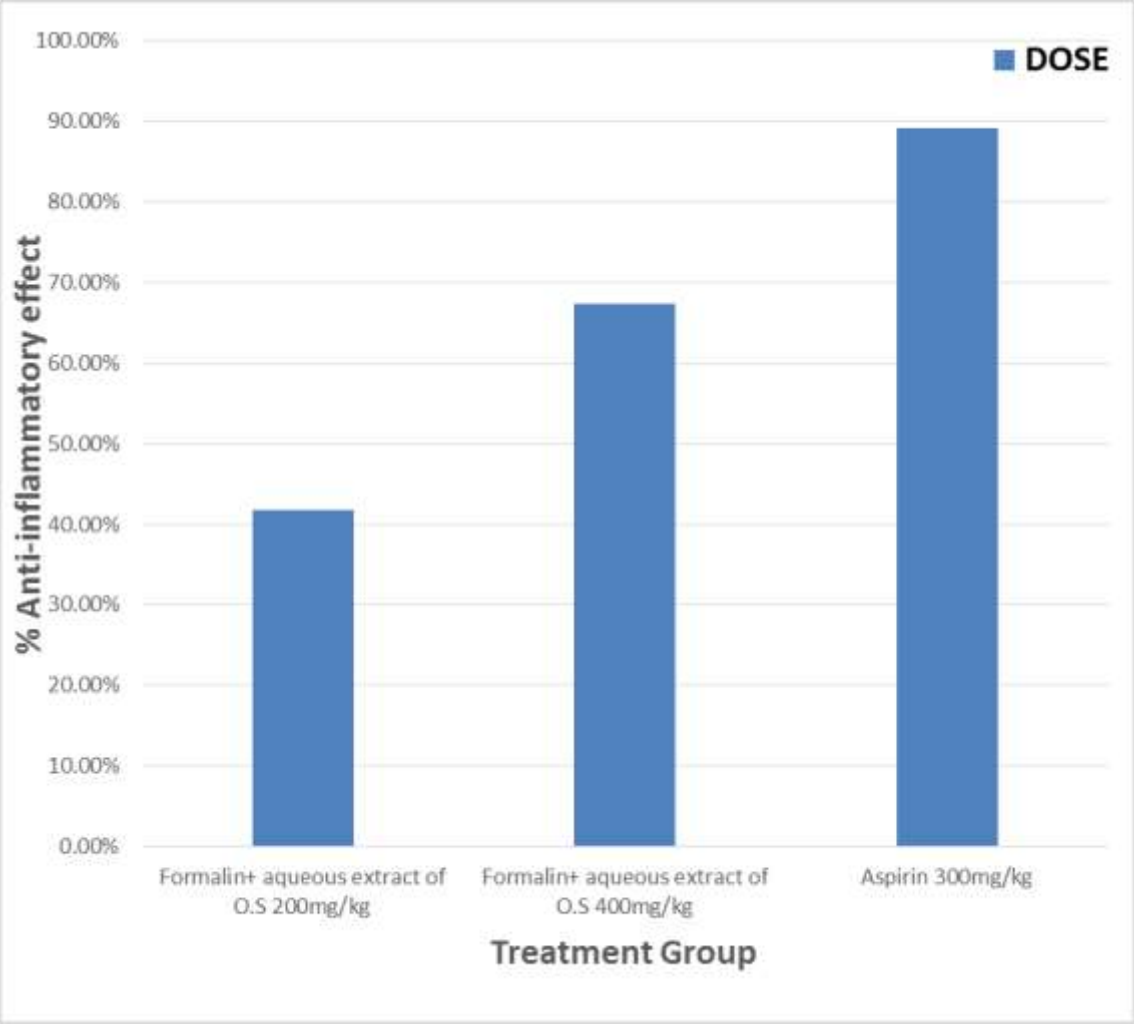

Fig. 3: Effect of aqueous extract of O.Sanctum on Linear Cross section below the ankle joint in formalin induced arthritis in rats.

DISCUSSION AND CONCLUSION

Discussion

The results of this study showed that aqueous extract occimmum sanactum leaf and flower significantly suppressed the rat hind paw edema induced by intraplantar injection of carrageenan, 'indicating marked anti-acute inflammatory efficacy. In the current study, the anti-inflammatory indicating marked anti-acute inflammatory efficacy. In the current study, the anti-inflammatory effect of aqueous extract of occimmum sanactum leaf and flower was

further evaluated to elucidate the underlying mechanisms involved in this animal model. We demonstrated that the effect may be due to inhibition of the pro-inflammatory cytokines aqueous extract of occimmum sanactum leaves and flowers, inflammatory enzymes (iNOS and COX-2) expression and also their products (NO and PGE2).

CONCLUSION

In conclusion, the results of the current study suggested that aqueous extract of occimmum sanactum leaf and flower possessed anti-inflammatory effects in carrageenan-induced rat paw edema, which is comparable to indomethacin. The anti-inflammatory mechanism of aqueous extract of occimmum sanactum leaf and flower may be related to the reduction of TNF-α and IL-1 β that could result in inhibition of iNOS and COX-2 expression and its product (NO and PGE2). Furthermore, the protective effect of aqueous extract of occimmum sanactum leaf and flower on liver damage may result from anti-oxidative effects. Our dindings provide new perspectives on the therapeutic use aqueous extract of occimmum sanactum leaf and flower in the management of inflammatory.

ACKNOWLEDGEMENT

The authors are thankful Department of Pharmacology, HIMT College of Pharmacy, Knowledge Park-I, Greater Noida, Uttar Pradesh, India for providing research facilities and encouragement and to our friends those who helped us to complete this research.

REFERENCES

1. Linlin Chen, Huidan Deng, Hengmin Cui, Jing Fang, Zhicai Zuo et.al Inflammatory responses and inflammation-associated diseases in organsOncotarget, 2018; 9(6): 7204–7218.
2. Padalia, Rajendra C.; Verma, Ram S. "Comparative volatile oil composition of four Ocimum species from northern India". Natural Product Research, 2011; 25(6): 569–575.
3. Abbas A.B.; Lichtman A.H. (2009). "Ch.2 Innate Immunity". In Saunders (Elsevier) (ed.). Basic Immunology. Functions and disorders of the immune system (3rd ed.). ISBN 978-1-4160-4688-2.
4. Mitra E, Ghosh AK, Ghosh D, Mukherjee D, Chattopadhyay A, Dutta S, Pattari SK, Bandyopadhyay D. Protective effect of aqueous Curry leaf (Murraya koenigii) extract against cadmium-induced oxidative stress in rat heart. Food Chem Toxicol, 2012; 50: 1340-53.
5. Punyaratabandhu, Leela. Bangkok (1 ed.). New York: Ten Speed Press, 2017; 338.

6. Abbas, Kumar, Vinay; Fausto, Nelso; Abul Robbins & Cotran Pathologic Basis of Disease (7th ed.). Saunders, 2004; 1230.
7. Cotran; Kumar, Collins Robbins Pathologic Basis of Disease. Philadelphia: W.B Saunders Company, 1998. ISBN 978-0-7216-7335-6.
8. Herrington, Simon. Muir's Textbook of Pathology (15th ed.). CRC Press, 2014; 59. ISBN 978-1444184990.
9. Becker W, Pfaltz CR, Naumann HH. Ear, nose, and throat diseases: with head and neck surgery (3rd ed.). Stuttgart: Thieme, 2009; 150.
10. Libby, P. "Inflammation in atherosclerosis". Nature, 19–26 December 2002; 420(6917): 868–74.
11. Ansorena, Montiel-Duarte, C.; E.; López-Zabalza, M. J.; Cenarruzabeitia, E.; Iraburu, M. J. "Role of reactive oxygen species, glutathione and NF-κB in apoptosis induced by 3,4-methylenedioxymethamphetamine ("Ecstasy") on hepatic stellate cells". Biochem Pharmacol, 2004; 67(6).
12. WERNER, Martin – GROSSMANN, John. Color Atlas of Pathology. 1. Edition., 2004; 457.
13. L.A., Rey-Cuillé, M. A.; Berthier, J. L.; Bomsel-Demontoy, M. C.; Chaduc, Y.; Montagnier, L.; Hovanessian, A. G.; Chakrabarti, 1 May 1998.
14. Teder, P. "Resolution of lung inflammation by CD44". Science, 2002; 296(5565): 155.
15. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end., 2005; 6(12): 1191-7.
16. Biswas, N. P.; Biswas, A. K. "Evaluation of some leaf dusts as grain protectant against rice weevil Sitophilus oryzae (Linn.)". Environment and Ecology, 2005; 23(3): 485–488.
17. Christopher P, Kiecolt-Glaser, Janice K.; Derry, Heather M.; Fagundes, "Inflammation: Depression Fans the Flames and Feasts on the Heat". American Journal of Psychiatry, November 2015; 172(11): 1075–91.
18. Parimisetty A, Dorsemans AC, Awada R, Ravanan P, Diotel N, Lefebvre d'Hellencourt C. "Secret talk between adipose tissue and central nervous system via secreted factors-an emerging frontier in the neurodegenerative research". J Neuroinflammation (Review), 24 March 2016; 13(1): 67.
19. Hall, John. Guyton and Hall textbook of medical physiology (12th ed.). Philadelphia, Pa.: Saunders/Elsevier, 2011; 428.
20. Wilmore, Jack. Physiology of Sport and Exercise. Champaign, IL: Human Kinetics, 2008; 26–36, 98–120, 186–250, 213–218.

21. Hendrik Ungefroren; Susanne Sebens; Daniel Seidl; Hendrik Lehnert; Ralf Haas. "Interaction of tumor cells with the microenvironment". Cell Communication and Signaling, 2011; 9(18).
22. Seunggu Han, Medically reviewed Understanding and Managing Chronic Inflammation, 2018.
23. William H. Blahd, E. et.al review of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs); (Healthwise staff), 2018.
24. William H, Blahd, Jr,; David Messenger,; Healthwise Staff.; Topic review on Nonsteroidal steroidal anti-inflammatory drugs(NSAID), 2011.
25. Nitin Shivappa, Edgar Denova-Gutiérrez, Paloma Muñoz-Aguirre, Nitin Shivappa, James R. Hébert, Lizbeth Tolentino-Mayo, Carolina Batis, and Simón BarqueraDietary Inflammatory Index and Type 2 Diabetes Mellitus in Adults.
26. Nordqvist Christian(2017).” Everything you need to know about inflammation” Medical News Today.
27. Wallace, J.M., Nutritional and botanical modulation of the inflammatory cascade:eicosanoids, cyclooxygenase and lipoxygenase- as an adjunct in cancer therapy. Inegr. Cancer. Ther., 2002; 1(1): 07-37.
28. Singh, S., Taneja M, and Majumdar, D.K. Biological activities of Ocimum sanctum L. fixedoil An overview. Indian J. Exp. Biol., 2007; 45: 403-412.
29. Singh, S, Majumdar, D.K, and Yadav, M.R. Chemical and pharmacological studies on fixed oil of Ocimum sanctum, Indian. J. Exp. Biol., 1996; 34: 12.
30. M. K. Gautam, A. Singh, C. V. Rao, and R. K. Goel, “Toxicological evaluation of Murraya paniculata (L.) leaves extract on rodents,” The American Journal of Pharmacology and Toxicology, 2012; 7(2): 62–67.
31. Winter CA, Riselay EA and Nuss GW: Carrageenan induced edema in the hind paw of the rats as an assay for anti–inflammatory drugs. Proc Soc Exp Biol Med., 1962; 111: 544-547.
32. Owoyele VB, Oloriegbe YY, Balogun EA, Soladoye AO. Analgesic and anti-inflammatory properties of Nelsonia canescens leaf extract. J. Ethnopharmacol, 2005; 99: 153-6.
33. Mondal S, Mirdha BR, Mahapatra SC the science behind sacredness of Tulsi (Ocimum sanctum Linn.). Indian J Physiol Pharmacol, 2009; 53: 291-306.

34. Godhwani S, Godhwani JL, Vyas DS. Ocimum sanctum: An experimental study evaluating its anti-inflammatory, analgesic and antipyretic activity in animals. J Ethnopharmacol, 1987; 21: 153-63.
35. Karin M, Liu Z, Zandi E. AP-1 function and regulation. Curr Opin Cell Biol., 1997; 9: 240–6.
36. Karin M, Cao Y, Greten FR, Li ZW Nat Rev Cancer, Apr. 2002; 2(4): 301-10.