IN VIVO ANTI-INFLAMMATORY ACTIVITY OF *SYZYGIUM AROMATICUM* IN RODENTS

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ABSTRACT

Background: *Syzygium aromaticum* (clove) has a conventional therapeutic anti-inflammatory use. Cold ethanolic extraction of *Syzygium aromaticum* was aimed to investigate the anti-inflammatory activity in animal model. In vivo inflammation was observed by carrageenan. Anti-inflammatory activity of *Syzygium aromaticum* was tested & compared with standard (ibuprofen 10mg/kg).

Objective: The objective of this study is to evaluate the anti-inflammatory effect of *Syzygium aromaticum* extract on carrageenan induced paw edema in mice.

Method: The anti-inflammatory potential of drug was tested in mice. A combination of drug was set as high dose & low dose with standard. Animals were divided into 6 groups each containing 2mice. Group 1 was administered normal saline considering it as controlled. Remaining groups were administered standard (ibuprofen), low dose extract of *Syzygium aromaticum* , high dose extract of *Syzygium aromaticum* , combination of low dose extract with standard & combination of high dose extract with standard to groups II, III, IV, V, VI respectively.

Results: The effect of ethanol extract of *Syzygium aromaticum* as anti-inflammatory was evaluated. Extracts having two different strengths i.e., 250mg/kg (low dose) & 500 mg/kg (high dose) were used for study which produced significant effect against carrageenan induced paw edema. Distinctively, combination of high dose with standard & low dose syzygium aromatic gave best anti-inflammatory effect.

Conclusion: The effective results of *Syzygium aromaticum* substantiate its traditional use as anti-inflammatory agent.

Keywords: *Syzygium aromaticum*, clove extract, carrageenan, anti-inflammatory.

INTRODUCTION

Inflammation is a nonspecific, physical and defensive response of body when tissue injury (caused by bacteria, trauma, chemicals, heat, etc) occurs, many substances are released by the injured tissues and cause dramatic changes in the surrounding un-injured tissues. The goal of inflammation is to abolish the original cause of cellular damage, eliminate necrotic cells and tissues damaged by the original injury and inflammation, and initiate tissue repair. (1-2) The cardinal sign of inflammation comes from Latin language: Dolor (pain), calor (heat), rubor (redness), tumor (swelling) & function leasa (loss of function). The inflammation may be acute or chronic. (3) Acute inflammation is the preliminary defense system against injury/trauma which onsets within few seconds of injury. This type of inflammation begins when immune cell like, dendritic cells, macrophages, histocytes, kupffer cells and the mast cells containing pattern recognition receptors (PRRs recognize the pathogen- associated molecular patterns; PAMPs and DAMPs; damage associated molecular patterns), activated due to injurious stimulus and

release inflammatory mediators that cause vasodilation and increase blood flow resulting redness and increased heat, increased permeability of the blood vessels results in an exudation of plasma protein and fluid into the tissue which manifests itself as swelling, increase the sensitivity to pain and alter the blood vessels to permit the migration of leucocytes mainly macrophages and neutrophils, outside of the blood vessel into the tissue that is injured and diminished the cause of injury. (4) While chronic inflammation is prolong inflammation that leads to progressive shift in the type of cells present at the site of inflammation such as, mononuclear cells. (4)

Herbal drugs have been utilized for several years to treat and prevent many diseases. There are also a few herbal remedies that show a positive effect to counter inflammation. But further testing is needed to determine the side effects, effectiveness and toxicity. Conventionally an entire plant or its part used as a medicine. But among modern times, the chemical components of plants and their metabolites (primary / secondary) are derived, broken down, and refined for use. (5) Phyto-constituents show an anti-inflammatory response that includes the following: polyphenols, flavonoids, steroids, terpenoids, alkaloids, withaferin A, coumarin, xanthone, and aliphatic alcohol among others. If parts of a plant or an entire plant are used, its constituents & metabolites produce synergite or counteract the effects or may give a false impact. Countless phytochemicals have been studied until now with anti-inflammatory effect. The herbal medicines currently used have fewer side effects, are more expensive and more readily available than man-made drugs. For this reason, there is a need to test the herbal anti-inflammatory drug which is better as compared to the allopathic drug. (5)

Syzygium aromaticum ; a tree belongs to Myrtaceae family, and native in Indonesia. The flower / bud has an aromatic nature, called as Clove. Clove is traditionally being used as spice. It is commercially being cultivated in Indonesia, India, Pakistan, Comoro islands, Sri lanka, Seychelles, Madagascar and Tanzania. Traditionally clove was used for multiple remedies and because of its identified biological activities it can be considered as a potential drug for several diseases (6). The synonym for clove is *Eugenia Cariophylata* (7). It appears as a median sized tree (i.e., 8-12 m). The tree grows at altitude of 200m above sea level (8).

The word Carrageenan comes from the Irish language which means ―Irish Moss‖. This name refers to specie of red alga ―Chondrus crispus‖, whose habitat is rocky areas of Atlantic coast of the British Isles, Europe, and North America. Its name also refers to its muco-polysaccharide extract, which was discovered by a British Pharmacist Stanford in 1862. (5) Structurally, Carrageenan is a complex group

of polysaccharides which is composed of continuous or repeating galactose-related monomers, and they are of three types, which are: ¬ Lambda. ¬ Kappa. ¬ Iota. All these forms have gel like characteristics. The lambda form does not strongly form gel at room temperature and is injected to induce inflammation. The inflammation induced by Carrageenan is acute, non-immune, well researched and highly reproducible. On the administration of carrageenan, several mediators are released like, bradykinin, histamine, ROS (reactive oxygen species), nitrogen specie and several others as well and all these mediators contribute to the onset of inflammation. Erythema, hyperalgesia & edema are also observed that are the cardinal signs of inflammation. (9-10)

The objective of this study is to evaluate the anti-inflammatory effect of *Syzygium aromaticum* extract on carrageenan induced paw edema in mice.

MATERIAL AND METHODS

Animals Selection: Animal selection has been carried out after looking over of all ethical aspects. After getting approval from department of Pharmacology of university of Karachi, the activity was conducted out on white albino mice (breed locally) of either sex weighing in between 25-30 gm, divided into 6 groups with 6 animals in each group. They were procured from Karachi, Pakistan. Animals selected for study received proper handling and a standard diet with free access of purified and clean water and were placed in individual cages. But at day before experiment, they were kept in fasting condition overnight with access of fresh water and labitinum. Animals were kept in standard environmental conditions i.e., temperature 23˚C & humidity was about 50 – 60%, along with 12hrs light and dark cycles. All Animals selected for studies were handled according to specification providing in Helsinki Resolution 1964 and the whole study was approved by our BASR (Board of Advanced Studies and Research) Committee.

Drugs and chemicals:

1. Ibuprofen as the standard drug at a dose of 10mg/kg; (brufen, Abbott laboratories Pakistan limited) was purchased from local chain of Noor medicos Karachi, Pakistan.

2. Test drug at doses of 250 and 500mg/kg; Ethanolic extracted sample of *Syzygium aromaticum* (clove) was tested for anti-inflammatory activity. The ethanolic extract was prepared in distilled water which was prepared freshly in research lab.

3. 1% w/v carrageenan; Carrageenan is a white amorphous powder diluted in sterile water for injection obtained from Sigma Lambda, USA.

4. Normal saline; commercially available sterile normal saline was used throughout the experiments for control group.

Clove Extract Preparation: The extraction method was followed as described by CortesRojas et al. The crushed clove buds were macerated in 70% ethanol with a ratio of 1:5 (sample: solvent). The mixture was soaked for a day (i.e., 24 hours) and stirred on an interval of 12 hours. The process repeated two times with the same quantity of solvent. The mixture is than placed in rotary evaporator to obtain the extract after condensation. The collected extracted is than concentrated through evaporation at room temperature. The ethanol derived extract obtained in the form of paste having yellowish-brown color.

Experimental Design: Animals were isolated and marked randomly in to 6 different groups with 6 mice in each for treatment as follows:

¬ **Group I**: Control received normal saline, 0.1 ml.

¬ **Group II**: Standard (ibuprofen) with standard adult at recommended dose of 10 mg/kg, mice dose was calculated according to body weight.

¬ **Group III**: Test group received 0.1 ml of low dose (250mg/kg) of ethanolic extract of *Syzygium aromaticum* (clove).

¬ **Group IV**: Test group received. 0.1 ml of high dose (500mg/kg) of ethanolic extract of *Syzygium aromaticum* (clove).

¬ **Group V**: Test group received a combination of low dose of *Syzygium aromaticum* extract (250mg/kg) along with standard ibuprofen (10mg/kg), about 0.1 ml.

¬ **Group VI**: Test group received a combination of high dose of *Syzygium aromaticum* extract (500mg/kg) along with standard ibuprofen (10mg/kg), about 0.1 ml.

Carrageenan induced paw edema: In the beginning of this study paw volume of all the animals were measured with the help of digital Vernier caliper. Carrageenan was administered to all groups of mice by plantar route in the plantar surface of paw at a dose of 0.1ml of 1% w/v solution. The inflammatory

response was measured soon after the administration of carrageenan. After that anti-inflammatory drugs were introduced to each group of animals except the control group and the anti-inflammatory effect was noted after every 01 hour for about 03 hours and percent inhibition of paw volume was calculated. The decrease in paw edema of treated groups was compared with the control group. Edema % inhibition is calculated via the given formula:

% inhibition = 100 (1-Vt/Vc)

Where, Vt = test group edema volume

Vc = control group edema volume

Statistical Analysis: The data was analyzed statistically through One-way analysis of variance (ANOVA) & stated as mean ± S.D followed by Dunnet's t-test using IBM SPSS Statistic 22. Significance level was selected as $p < 0.05$.

RESULT

CARRAGEENAN PROVOKED PAW EDEMA - STATISTICAL ANALYSIS: Anti-inflammatory activities of *Syzygium aromaticum* in four unlike doses are compared to both the groups i.e., standard and control group. Statistical One-way analysis was performed using IBM SPSS Statistic 22 tracked by a Dunnet's test at a significance level 0.05. If the value of p is 0.05 the null hypothesis would be accepted. N = 6 in each group. The null hypothesis states Ho: there is a significant difference between the test group and the control group. Although, alternative hypothesis states HA: there is no significant difference between the test group and the control group.

% inhibition = 100 (1-Vt/Vc)

Where, Vt = test group edema volume

Vc = control group edema volume

FIGURE 1- GRAPH OF MEAN TIME OF ANTI-INFLAMMATORY ACTIVITY OBSERVED THROUGH CARRAGEENAN PROVOKED PAW EDEMA IN DIFFERENT GROUPS

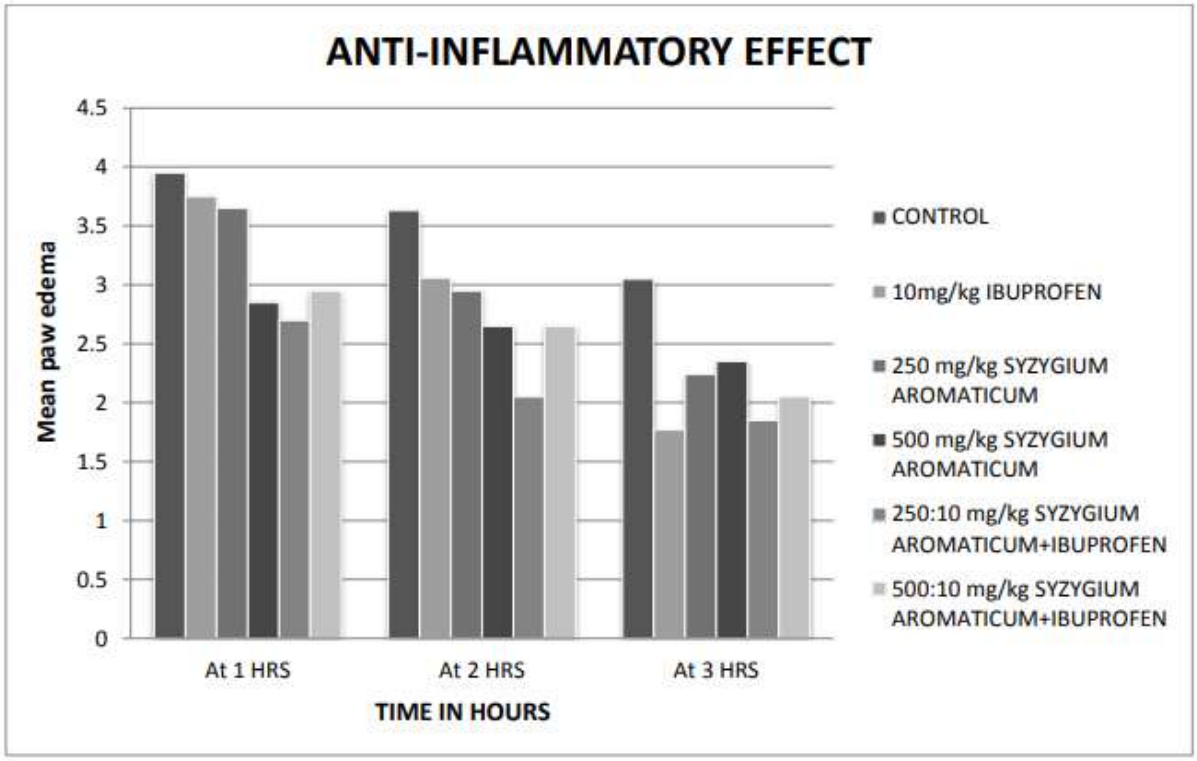

Figure 1: Bar diagram represents mean time of anti-inflammatory activity observed through carrageenan provoked paw edema in different mice groups. Control: normal saline, Standard: Ibuprofen=10mg/kg, low dose Syzygium aromaticum = 250 mg/kg, high dose Syzygium aromaticum = 500mg/kg, low dose of Syzygium aromaticum + ibuprofen= 250:10 mg/kg, high dose of Syzygium aromaticum + ibuprofen= 500:10 mg/kg. N: 6 mice for every group. Significant if $p<0.05$

FIGURE 2- GRAPH SHOWING PERCENTAGE SUPPRESSION IN CARRAGEENAN PROVOKED PAW EDEMA IN MICE BY DIFFERENT GROUPS:

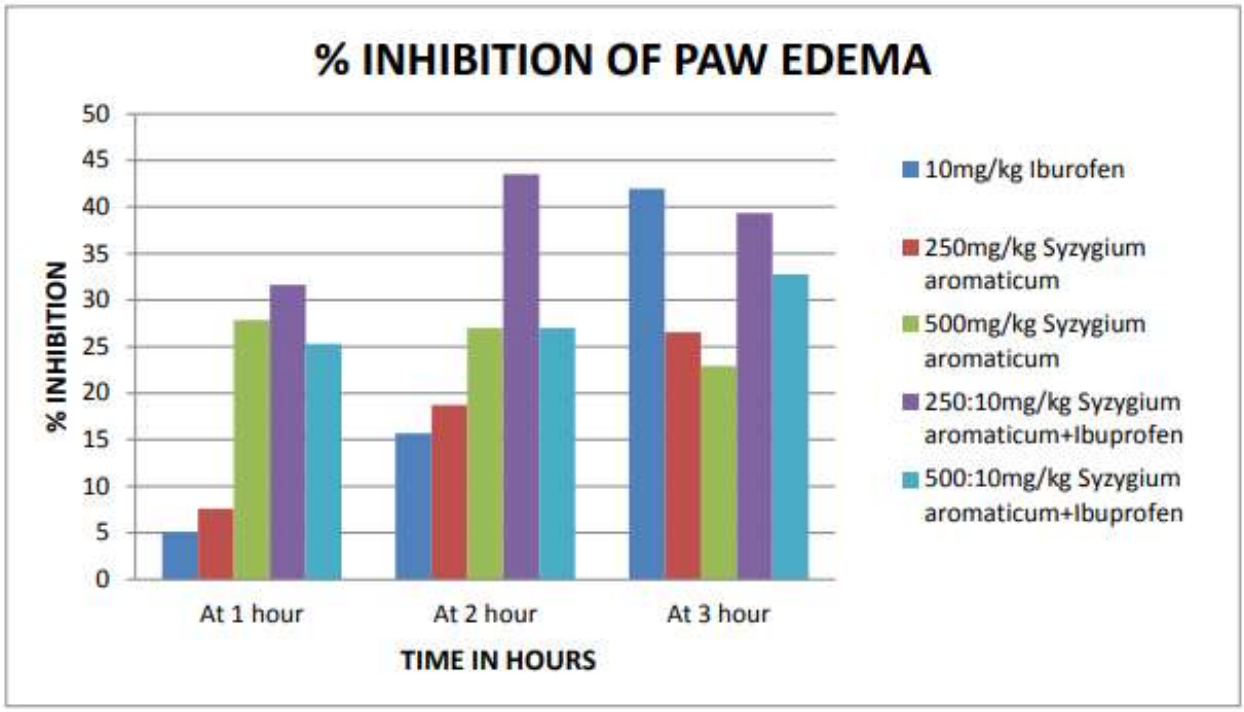

Figure 2: Bar diagram showing % inhibition in different groups. Standard: Ibuprofen=10mg/kg, low dose Syzygium aromaticum = 250 mg/kg, high dose Syzygium aromaticum = 500mg/kg, low dose of Syzygium aromaticum + ibuprofen= 250:10 mg/kg, high dose of Syzygium aromaticum + ibuprofen= 500:10 mg/kg. N: 6. Significant if $p<0.05$

DISCUSSION

CARRAGEENAN PROVOKED PAW EDEMA: Mean paw edema volume in mm through mice groups which receives group I: Normal saline 0.1 ml are 4.11, 3.95, 3.63, 3.05 mm at 0, 1, 2 and 3 hours correspondingly, group II: Ibuprofen at dose 10mg/kg are 3.95, 3.75, 3.06 and 1.77 mm at 0, 1, 2 & 3 hours correspondingly, group III: *Syzygium aromaticum* extract at dose 250mg/kg are 4.15, 3.65, 2.95 and 2.24 mm at 0, 1, 2 and 3 hours correspondingly, group IV: *Syzygium aromaticum* extract at dose 500mg/kg are 3.05, 2.85, 2.65 and 2.35 mm at 0, 1, 2 and 3 hours correspondingly, group V: Combination *Syzygium aromaticum* extract with Ibuprofen at a dose of 250:10 mg/kg are 2.90, 2.70, 2.05 and 1.85 mm at 0, 1, 2 and 3 hours respectively, group 6: Combination of *Syzygium aromaticum* extract with Ibuprofen at a dose of 500:10 mg/kg are 3.30, 2.95, 2.65 and 2.05 mm at 0, 1, 2 and 3 hours

correspondingly as revealed in figure 3. % inhibition of paw edema in mice in group II: Ibuprofen 10mg/kg are 5.06, 15.70 and 41.96 at 1, 2 and 3 hours correspondingly, group III: *Syzygium aromaticum* extract at dose 250mg/kg are 7.59, 18.73 and 26.55 at 1, 2 and 3 hours respectively, group 4: *Syzygium aromaticum* extract at a dose of 500mg/kg are 27.84, 26.99 and 22.95 at 1, 2 and 3 hours respectively, group 5: Combination of *Syzygium aromaticum* extract with Ibuprofen at a dose of 250:10 mg/kg are 31.64, 43.52 and 39.34 at 1, 2 and 3 hours respectively, group 6: Combination of *Syzygium aromaticum* extract with Ibuprofen at a dose of 500:10 mg/kg are 25.31, 26.99 and 32.78 at 1, 2 and 3 hours correspondingly as given in figure 2.

COMPARISION OF MEAN TIME OF STUDY GROUPS IN CARRAGEENAN PROVOKED PAW EDEMA IN MICE AFTER AN HOUR THROUGH ONE WAY ANOVA: Anti-inflammatory activity in carrageenan provoked edema in mice through standard: Ibuprofen is compared to other study groups afterwards 60mins. ANOVA test is applied & tracked by Dunnets t-test, in which $p<0.05$ is statistically significant. The p value is lesser than 0.05 as observed in the low dose of *Syzygium aromaticum* i.e. 250mg/kg is (sig: 0.000), high dose of *Syzygium aromaticum* i.e. 500mg/kg is (sig: 0.000), combination of low dose of *Syzygium aromaticum* with Ibuprofen i.e. 250:10 mg/kg is (sig: 0.000) and high dose of *Syzygium aromaticum* i.e. 500:10 mg/kg is (sig: 0.000), whereas control group is (0.23). Significance value is $p<0.05$ when compared with Ibuprofen which means *Syzygium aromaticum* shows anti-inflammatory effect at all doses.

COMPARISION OF MEAN TIME OF STUDY GROUPS IN CARRAGEENAN PROVOKED PAW EDEMA IN MICE AFTER 2 HOURS BY ONE WAY ANOVA: Anti-inflammatory activity in carrageenan provoked paw edema in mice models by standard: Ibuprofen is compared with the study groups after 2 hours. ANOVA test is applied which is tracked by Dunnets t-test, in which $p<0.05$ is statistically significant. The p value is lesser than 0.05 as observed in the low dose *Syzygium aromaticum* i.e. 250mg/kg is (sig: 0.000), high dose of *Syzygium aromaticum* i.e. 500mg/kg is (sig: 0.000), combination of low dose of *Syzygium aromaticum* with Ibuprofen i.e. 250:10 mg/kg is (sig: 0.000) and high dose of *Syzygium aromaticum* i.e. 500:10 mg/kg is (sig: 0.000), whereas control group (0.23). p value = 0.05 as compared with Ibuprofen which means there is no significant variation amid standard and test groups i.e. *Syzygium aromaticum* shows anti-inflammatory effect at all doses.

COMPARISION OF MEAN TIME OF STUDY GROUPS SPEND IN CARRAGEENAN PROVOKED PAW EDEMA IN MICE AFTER 3 HOURS BY ONE WAY ANOVA: Anti-

inflammatory activity in carrageenan provoked edema in mice by standard: Ibuprofen is compared with study groups after 03 hours. ANOVA test is applied which is further followed with Dunnets t-test, where $p<0.05$ is statistically significant. The p value is lesser than 0.05 as observed in the low dose *Syzygium aromaticum* i.e. 250mg/kg is (sig: 0.000), high dose of *Syzygium aromaticum* i.e. 500mg/kg is (sig: 0.000), combination of low dose of *Syzygium aromaticum* with Ibuprofen i.e. 250:10 mg/kg is (sig: 0.000) and high dose of *Syzygium aromaticum* i.e. 500:10 mg/kg is (sig: 0.000), whereas control group is (0.23). p value = *0.05* as compared to Ibuprofen which means there is no significant variation amid standard & test groups i.e. *Syzygium aromaticum* shows anti-inflammatory effect at all doses.

CONCLUSION

The effective results of *Syzygium aromaticum* substantiate its traditional use as anti-inflammatory agent.

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REFERENCES

1. Guyton and Hall textbook of Medical Physiology, 12th edition, chapter 33, page no. 428.

2. Piira OP,Mie&nen JA, Hulkuri HV, Tulpoo MP (2013) physiological responses to emotional Excitement in healthy subjects and patients with coronary artey disease. Auton Neurosci 2013 Oct, 177 (2) 280-5.

3. A massage therapist guide to pathology Ruth Werner (2009) , Philad MD: Wolters Kluwer.

4. Robbins pathological basis of Disease 1998.

5. Physiological and emotional responses to emotional excitement in healthy subjects and Patients with coronary artery disease, Auton Nerosci 2013 oct, 177.

6. A.T. Mbaveng, V. Kuete, in Medicinal Spices and Vegetables from Africa, 2017

7. Kamatou GP, Vermaak I, Viljoen AM. Eugenol--from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule. Molecules. 2012;17(6):6953–6981.

8. Filho GA, Cesar JO, Ramos JV. Itabuna: CEPLAC; 2013. Accessed on 21st April, 2013. Portuguese.

9. winter, C.A, Risley E.A, and Nuss G.W. (1962) Carrageenan induced edema in hind paw of the rat as an assay for anti-infammatory drugs. Proc .Soc . Exp. Biol. 111, 544, 547. 50

10. Rioja ,I, Ubeda A, Terrecino (2000) An in>ammatory ditriazine inhibiting nitric oxide synthase and cyclo oxygenase -2