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Research Article

COMPARATIVE STUDIES OF ANTI-INFLAMMATORY PROPERTIES OF *Cymbopogon citratus* (DC.) Stapf AND *Piper longum* L. FOLLOWING MICE PAW OEDEMA TEST

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ABSTRACT

Inflammation is a protective mechanism of immune system characterised by secretion of pro-inflammatory substances like nitric oxide, Interleukin 1, Interleukin 6, TNF-α, Leukotrines, Prostaglandins and vasoactive amines. Though it is aimed towards protection against invading pathogens or neutralization of toxicity precipitated by chemicals; its manifestation is observed in harmful manner. Most of the times it acquires fulminating symptoms due to impaired pathway for production of PRDX5 proteins. On this back ground comparative study of aqueous extract of *Cymbopogon citratus* (DC.) Stapf and *Piper longum* L. was carried out with respect to potential anti-inflammatory properties. Carrageenan Induced Mice Paw Oedema Test revealed statistically significant anti-inflammatory properties of *Cymbopogon citratus* (DC.) Stapf.

INTRODUCTION

Scholars of life sciences or medical sciences who deals with animal study still are unable to discriminate a very thin line between inflammation and allergy. For example, results dealt with mice paw oedema test often are considered as symptoms of inflammation and not the allergy. Immunological base of allergy or hypersensitivity beautifully defines the term as any unwanted reaction exhibited by host following antigenic stimulus. During 'Carrageenan Induced Mice Paw Oedema Test', inflammation is observed as a symptom following stimulus with carrageenan. Keeping aside dubious line of demarcation, no doubt the inflammation is a protective mechanism characterised by secretion of pro-inflammatory substances like nitric oxide, Interleukin 1, Interleukin 6, TNF-α, Leukotrines, Prostaglandins and vasoactive amines. Though it is aimed towards protection of host, its manifestation is precipitated in harmful manner. The process of inflammation include the activation of complement pathways to produce chemo attractant complements like C3a, C5a and subsequent extravasation of neutrophils and other leukocytes[(23)]. Inflammation also involve proteins like MIP 1 -α, MIP 1 -β to attract macrophages at the site of inflammation. IL 1 produced during inflammation causes over expression of ICAM-1 and VCAM-1 on the surface of endothelial cells. Such adhesion molecules subsequently allow interaction with integrines of phagocytic cells and lymphocytes. Overall mechanism is aimed to eradicate invading pathogens or harmful chemicals. Drugs used for treatment of inflammation includes steroid drugs, non-steroid drugs and are the cause of serious human health hazards[(2,6)]. It was claim by W.H.O. for the use of plant derived medicines by major global population[(1)]. Ayurveda has sustained by the use of blended formulations of medicinal plants and few may carry anti-inflammatory properties. Different plants including *Terminalia chebula, Cynodon dactylon, Terminalia belerica, Cassia fistula, Emblica officinalis, Sphaeranthus indicus, Terminalia indica, Holarrhena antidysentrica, Syzigium cumini, Mesua ferrea,* have been evaluated for potential anti-inflammatory activities[(10)].

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Carrageenan is a well-known sulphated polysaccharide whose source is *Rhodophyceae* (sea weeds)[3]. It is widely recommended for evaluation of anti-inflammatory properties [14, 15, 16].

Petroleum ether extract of *Dendrophthoe falcata* (Linn) has been reported for significant anti-inflammatory properties [7]. Other compounds which are used for the assessment of anti-inflammatory properties are indomethacin, morphine, dextran, 'acetyl salicylic acid' [5]. Most preferred site for developing inflammation using carrageenan is subplanter region of right hind paw[4]. Different approaches have been claimed for accurate measurement of oedema and use of plethysmometer is one of them[10].

The present study is aimed to find safer substitute for allopathic anti-inflammatory drugs. *Cymbopogon citratus* (DC.) Stapf which commonly known as lemmon grass is a perennial grass plant variety commonly found in tropical and subtropical regions[24]. *Cymbopogon citratus* (DC.) Stapf (Family-Poaceae) has been reported to carry myrcene, nerol, neral, citronell, geranyl acetate, limonene and citral[25]. Another selected plant *Piper longum* L. whose vernacular name is Pippali, belongs to the Family Piperaceae and is in common use in Asia including India[26]. The plant is perennial climber with heart shaped leaves and woody roots. *Piper longum* L. carries 3 to 5% piperine as a major active constituents. Oil extracted from fruit of the plant shows presence of pentadecane, caryophyllene, thujine, bisaboline, zingiberine, terpinoline, dihydrocarveol, P-cymene, P-methoxyacetophenone[27].

MATERIALS AND METHODS

The Collection of samples

1. *Cymbopogon citratus* (DC.) Stapf was collected from Shivane, Taluka-Haveli, District-Pune, Maharashtra, India. The plant was shed dried and its herbarium was prepared. *Cymbopogon citratus* (DC.) Stapf was authenticated from Agharkar Research Institute, Pune, Maharashtra vide Auth 17-204 with the reference of Maharashtra state (1996), IC MR (2012).
2. *Piper longum* L. was collected from authorised Ayurvedic supplier and the root parts were critically studied with macroscopic and organoleptic characters. The sample was authenticated by Agharkar Research Institute, Pune, Maharashtra vide Auth 17-205 with the reference of Sarin (1996), API (1999) IC MR (2003).

Preparation of plant extracts

Cymbopogon citratus (DC.) Stapf and root parts of *Piper longum* L. were carefully washed with tap water and it was followed by washing with sterile distilled water. The samples were shed dried. Leaves of *Cymbopogon citratus* (DC.) Stapf were separated and subjected to aqueous extraction process using Soxhlet apparatus. Similar process was followed for roots of *Piper longum* L. Both the extracts were homogenised using magnetic stirrer and the process was followed by concentration of extracts using rotary vacuum evaporator to dryness. The extracts were stored at 4ºC.

Carrageenan Induced Mice Paw Oedema Test

Potential anti-inflammatory properties of *Cymbopogon citratus* (DC.) Stapf and *Piper longum* L. were assessed by using Carrageenan Induced Mice Paw Oedema Test following Winter *et al*. 1962 method as modified by Johny Olukunle 2011. Albino mice under study were starved for 24 hours. The mice were divided into four different groups such as Group-I, Group-II, Group-III and Group-IV. Each group included 3 males and 3 females. Group-I was used as control group (no medication), Group-II as standard group (Trypsin 1 lakh units + Chymotrypsin 2 lakh units as a blended dose as a standard medicine). Group-III as a test (A) group (aqueous extract of *Cymbopogon citratus* (DC.) Stapf as obtained above), Group-IV as test group (B) (aqueous extract of *Piper longum* L. as obtained above.) Male and female mice were kept in different cages and the protocol was followed for each group. Using marking ink each animal in each group was marked on different body parts and the record was kept. It was counterchecked before the experiment. Body weight of each mouse was recorded for adjusting animal dose. For dried extracts *Cymbopogon citratus* (DC.) Stapf and *Piper longum* L., 150 mg dose was used as a standard human dose and it was converted to animal dose using the formula:

Human dose × 0.0026 = x
Animal dose is x ×50 = y

The value of y as obtained was mixed with 10 ml of sterile water. For every 10 gm of body weight of selected mouse, 0.1 ml of above homogenized mixture was used as single animal dose. Thus considering body weight of respective mouse, animal dose was calculated every time. For every group including control group, 0.1 ml of 1% freshly prepared carrageenan (w/v) in pyrogen free distilled water was injected into the sub-planter surface of the right hind paw of each mouse. Similar volume of saline was injected into the other hind paw. The paw volume was measured before the administration and the record was kept.

After administration of the carrageenan dose, mice were kept in their natural habitat for 60 min. After 60 min, Group-I animals were given oral dose of saline, (0.1 ml/10 gm); Group-II animals were given blended dose of Trypsin+ Chymotrypsin as calculated by standard formula. Similarly Group-III were administered the animal dose of *Cymbopogon citratus* (DC.) Stapf and Group-IV with animal dose of *Piper longum* L. For the administration of oral dose, sterile mouse oral gavage feeding needles were used. The paw volume was measured using 'Mitutoyo Digimatic Caliper', for the time intervals of 30 min, 1 h, 2 h, 4 h and 6 h. Results obtained were analysed using Univariate Analysis of Variance and Levene's Test of Equality of Error Variences.

OBSERVATIONS AND RESULT

Observation after 30 minuits

Following Univariate Analysis of Variance and Levene's Test of Equality of Error Variences after 30 m, there was no significant reduction in the swelling in control group. *Cymbopogon citratus* (DC.) Stapf had shown remarkable reduction (mean swelling reduction = 1.228 mm) after half an hour as compared to *Piper longum* L. Both males and females showed significant reduction in the swelling. For standard drug,

reduction in the swelling was (mean swelling reduction = 0.590 mm) more in females than in males.

Observation after 1 hr

Following Univariate Analysis of Variance and Levene's Test of Equality of Error Variences no significant reduction in the swelling was observed in the control group. Mean swelling reduction for standard drug was 1.22 mm and for *Cymbopogon citratus* (DC.) Stapf it was 1.072 mm. For *Piper longum* L. The value was 0.550 mm. Thus, standard drug and aqueous extract of *Cymbopogon citratus* (DC.) Stapf showed statistically significant reduction in swelling after 1 h.

Observation after 2 hr

Following Univariate Analysis of Variance and Levene's Test of Equality of Error Variences it was observed that there was no significant reduction in swelling of control group. Mean reduction value for standard drug was 1.252 mm and for *Cymbopogon citratus* (DC.) Stapf it was 1.228 mm. For *Piper longum* L., mean swelling reduction value was 0.807 mm. For *Cymbopogon citratus* (DC.) Stapf both males and females showed same response. But for *Piper longum* L. reduction in the swelling in females was better than males.

Observation after 4 hr

Following Univariate Analysis of Variance and Levene's Test of Equality of Error Variences it was observed that there was no significant reduction in the swelling of control group. For standard drug (1.353 mm) and *Cymbopogon citratus* (DC.) Stapf (1.322 mm) swelling remarkably reduced after 4 h as compared to *Piper longum* L. For *Cymbopogon citratus* (DC.) Stapf both males and females showed significant results. For *Piper longum* L. reduction in females was better (1.170 mm) than in males.

Observation after 6 hr

Following Univariate Analysis of Variance and Levene's Test of Equality of Error Variences, it was observed that there was no significant reduction in swelling of control group. Standard drug and *Cymbopogon citratus* (DC.) Stapf both showed remarkable reduction in swelling after 6 h. (mean swelling reduction value for standard drug was 1.487 mm, for *Cymbopogon citratus* (DC.) Stapfit was 1.486). For standard drug and also for aqueous extract of *Cymbopogon citratus* (DC.) Stapf both males and females showed statistically significant reduction in erythema. For *Piper longum* L. mean swelling reduction was 1.273 mm. Reduction in swelling was better in females than in males.

DISCUSSION

Essential oil of *Cymbopogon citratus* (DC.) Stapf exhibit anti-inflammatory activities but exact mechanism of its effect needs better evaluation. (Mohammad *et al*. 2014)[(11)]. Role of *Cymbopogon citratus* (DC.) Stapf in the reduction of TNF α associated neutrophil adhesion was studied by Maruyama *et al*. (2003)[(13)]. Lemmon grass essential oil inhibits Interleukin 1-β and Interleukin 6 as reported by Tiwari M. *et al* 2010[(12)]. Most of the scholars have confined their studies to individual activity of *Cymbopogon citratus* (DC.) Stapf. Present research gave comparative analysis of anti-inflammatory activity of *Cymbopogon citratus* (DC.) Stapf and *Piper longum* L.

Many scholars have reported the potential of *Piper longum* L. in curbing autoimmune diseases. Most of autoimmune diseases shows inflammation as one of the symptoms hence for the comparison purpose authors selected the *Piper longum* L. The *Piper longum* L. also affects unique G protein related efflux mechanism of antibiotics. Hence such a comparative account of *Cymbopogon citratus* (DC.) Stapf and *Piper longum* L. may provide different approaches for drug designing purposes. Though the study revealed better anti-inflammatory properties of *Cymbopogon citratus* (DC.) Stapf, in blended formulation with *Piper longum* L. it may provide better chemotherapeutic values for certain diseases including autoimmune diseases. Anti-inflammatory properties of *Piper longum* L. were studied by A. Kumar *et al* (2009) using buprofen as a standard drug. The authors targeted dried fruit oil of the plant and claimed significant activity[(19)]. Sarvesh Kumar *et al*. (2015) modified functional groups of *Piper longum* L. derived lead molecules and reported better anti-inflammatory activities[(20)]. Namrata Sengupta (2018)[(22)] reported dual role of nitric oxide, one as a pro-oxidant and another as antioxidant. Nitric oxide triggers two different proteins named KEAP 1 and NRF 2. Later both the proteins shows synergistic action to activate another protein called PRDX 5[(22)]. The protein suppresses harmful activity of nitric oxide and decreases level of inflammatory cytokines. In our opinion there is a wide scope to judge anti-inflammatory activities of selected plants, beyond the conventional 'Carrageenan Induced Mice Paw Oedema Test': Potential activities of such plants to trigger PRDX 5 protein may be futuristic research plan of the scholars of life sciences.

CONCLUSION

During comparative analysis of *Cymbopogon citratus* (DC.) Stapf and *Piper longum* L. following 'Carrageenan Induced Mice Paw Oedema Test', statistically significant anti-inflammatory activity was observed of *Cymbopogon citratus* (DC.) Stapf. The activity was as par with FDA approved blended formulation of Chymotrypsin and Trypsin. *Cymbopogon citratus* (DC.) Stapf showed better anti-inflammatory activities than that of *Piper longum* L.

Conflict of interest

Present research was not funded by any funding agency. Therefore, authors do not claim for conflict of interest.

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