IN-VIVO EVALUATION OF *PLEUROTUS SAJORCAJU* MYCELIUM EXTRACT FOR ANTI-INFLAMMATORY ACTIVITY

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Summary

The present study was performed to evaluate the anti-inflammatory activity of fruiting body of *Pleurotus sajorcaju* mycelium. Plant was collected from Anand district and extracted with water and methanol separately. For anti-inflammatory activity 500mg/kg and 1000mg/kg concentration of both extract were prepared and administered by oral route. Indomethacin (10 mg/kg) was used as a standard drug. Anti-inflammatory activity was determined by carrageenan induce paw edema and formaline induce paw edema method, inflammation was produced by subcutaneous injection of 0.05 ml 1% carrageenan suspension and 0.1 ml 2% formaldehyde on the left hind paw respectively. Paw thickness was measured using a plethysmometer. In carrageenan induce inflammation aqueous and methanolic extracts shows 25.4% and 22.04% inhibition ($p < 0.01$) at 1000 mg/kg respectively while with indomethacin was found 46.5%. In formalin induce inflammation aqueous and methanolic extracts shows 55.13% and 48.0% inhibition ($p < 0.01$) at 1000 mg/kg respectively while with indomethacin was found 73.07%. Thus aqueous extract had more potent anti-inflammatory activity than methanolic extract and this activity probably due to presence of polysaccharides in the plant extract.

Keywords: Anti-inflammatory activity, Carrageenan-induced paw edema, formalin-induced paws edema, *Pleurotus sajorcaju*

Introduction

The use of medicinal herb in the treatment and prevention of diseases is attracting attention by scientists worldwide. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed [1].

Many drugs are use for the treatment of inflammation but they produce some unwanted side effect which require drug that have minimum side effect. While herbal drug have minimum side effect so finding of such drug require for the treatment of analgesic and inflammation effect.

Mushrooms are used in folk medicine throughout the world since ancient times as nutritionally functional food. *Pleurotus sajorcaju* is an edible and highly priced mushroom. Hypotensive activity, hypocholestremic activity, phenoloxidase and lignolytic activities *of P. sajorcaju* has been proved [2,3,4]. In this study aqueous and methanolic extracts of *P. sajorcaju* was tested for its Anti-inflammatory activity.

Material and Methods

Collection of plant materials

Fresh fruiting body of *P. sajocaju* was collected from Anand district, Gujarat, India and the plant was identified by Prof. Shubhash J. Patel, Anand Agriculture University, Anand. Fruiting body was dried under shade and then stored in airtight container.

Preparation of fruiting body extract

The dry fruiting body was reduced to powder and subjected to extraction with distilled water and methanol by Soxhlet apparatus for 8 hour separately under reduce pressure at 90ºC and 50ºC respectively and then filtered with cotton wool. Nearly 85% solvents were recovered by distillation over a boiling water bath at atmospheric pressure and temperatures maintain at 95ºC and 65ºC for aqueous and methanolic extract respectively. The yield was 200 mg/kg and 80.5 mg/kg for aqueous and methanolic extract respectively. These extracts were performed for preliminary phytochemical test.

Animals used

Female albino rats of Wistar strain weighing around 200-250 gm were procured from Central Animal Facility, S. K. Patel College of Pharmaceutical Edu. and Research, Gujarat and were approved by Institutional Animal Ethic Committee (Approved no. IAEC/2007/04). The rats were maintained under standard animal housing conditions (23±1 $^{\circ}$C, 40–70% RH, 12 h light/dark cycle) and had access to food and water ad libitum.

Anti-inflammatory activity on carrageenan-induced paw edema

The animals were divided into six groups of six rats. The negative control group received distilled water (1% Carboxy methyl cellulose (CMC) in distilled water 2 ml/kg, orally), the positive control group received the NSAID Indomethacin (10 mg/kg, orally) and the test groups received the extracts at the doses of 500 and 1000 mg/kg, orally. The test was conducted using an electric plethysmometer 7140 (Ugo Basile, Italy). Carrageenan 1% (0.05 ml) was injected subcutaneously in the plantar surface of the rat's left hind paw 1 h after oral administration of drugs to induce a progressive swelling of the paw. The paw volume, up to the tibiotarsal articulation, was measured at 0 h (before carrageenan injection) and 1, 2, 3, 4, 5, 6, 7, 8, 9 h later. Increase in paw thickness and per cent inhibition was calculated as follows:
Increase in paw thickness in control/treatment $PC/PT = Pt - P0$.

Per cent inhibition = (PC – PT) * 100
PC

Where Pt is paw thickness at time t, P0 is initial paw thickness, PC is increase in paw thickness of the control group and PT is the increase in paw thickness of the treatment groups [5].

Anti-inflammatory activity on formalin-induced paw edema

Wistar rats were randomly assigned to 6 groups of six animals each and received orally the vehicle, one of the extracts or indomethacin as we just described for the previous test for 10 days orally. Freshly prepared 2% formaldehyde (0.1 ml) was injected subcutaneously in the plantar surface of the rat's left hind paw on the first and third days 1 h after oral administration of drugs to induce a progressive swelling of the paw. The daily changes in paw size were measured by plethysmometer. The paw volume, up to the tibiotarsal articulation, was measured at 0 h (before formaldehyde injection) and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 day later. Increase in paw thickness and per cent inhibition was calculated as above method [6].

Statistical Analysis

The result was express as mean + Standard Error of Mean (S.E.M.) statistical difference between two means was determine by one-way ANOVA followed by Dunnett multiple comparisons test by using InStat 3 statistical computer software. Only those mean values showing statistical difference $p<0.01$ or $p<0.05$ was considered as statistically significant.

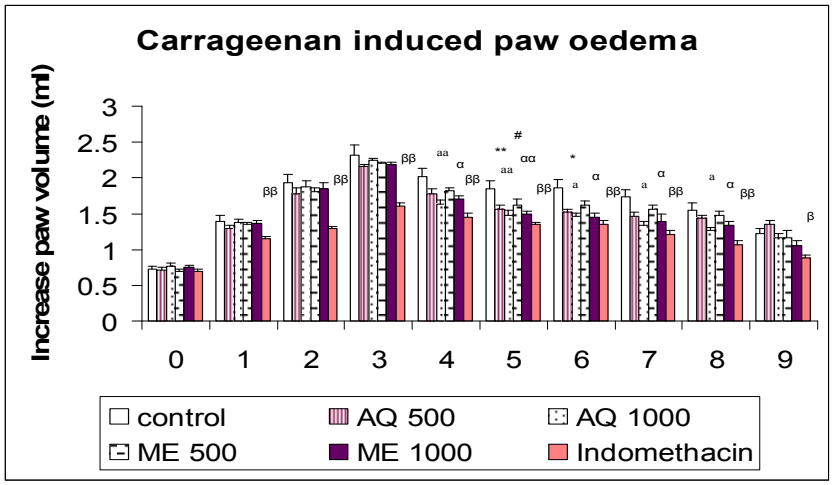
Results

Phytochemical Evaluation

Phytochemical screening of the aqueous extract indicated the presence of polysscharides, gum, lipid, protein, aminoacid, fats and oils, alkloid, flavones and saponins and methanolic extract containing polysscharides, gum, lipid, protein, aminoacid, fats and oils, alkloid, flavones and sterol.

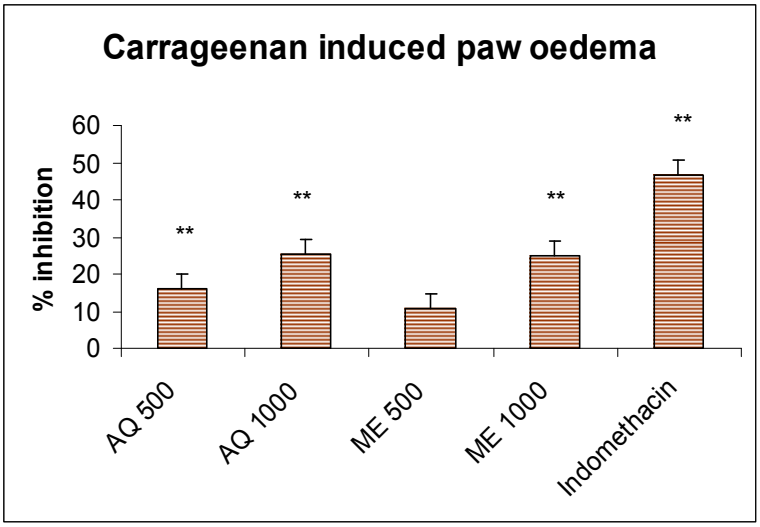
Anti-inflammatory activity on carrageenan-induced paw edema

Fig. 1 Effect of aqueous and methanolic extract of *P. sajorcaju* mycelium and Indomethacin on paw volume of carrageenan-induced acute inflammation



*, #, a, β denotes significance at the level of $p\leq0.05$. **, ##, aa, αα, ββ denotes the significance at the level of $p\leq0.01$.

Fig. 2. Effect of aqueous and methanolic extract of *P. sajorcaju* mycelium and Indomethacin on % inhibition of carrageenan-induced acute inflammation

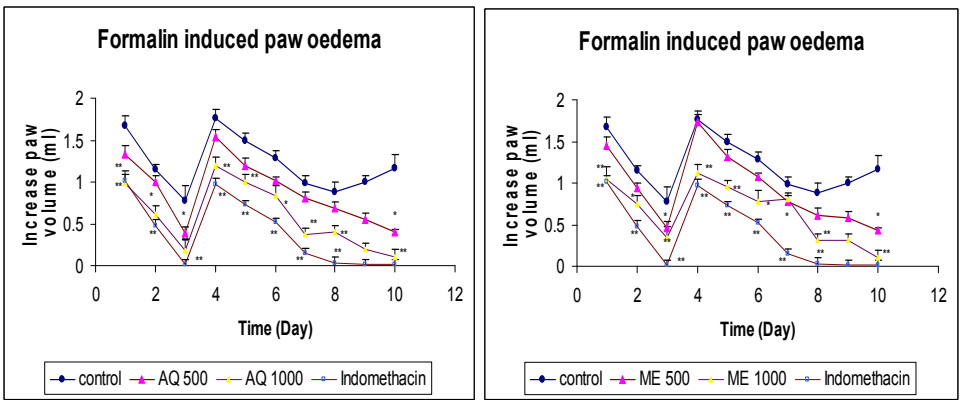


Values represented as mean+S.E.M. of six animals. Comparisons were made between: control and treated animals. ** denotes the significance at the level of $p \leq 0.01$.

In the carrageenan edema primary peak of swelling occurred within the third hour after that decrease in inflammatory swelling of rat paws. Percentage inhibition of edema by *P. sajorcaju* mycelium aqueous and methanolic extracts was found to be 25.4% and 22.04% ($p < 0.01$; Fig. 2) at 1000 mg/kg respectively. Indomethacin (10mg/kg) inhibited the edema volume by 46.5%. Thus aqueous and methanolic extract of *P. sajorcaju* mycelium significantly inhibited acute inflammation induced by carrageenan.

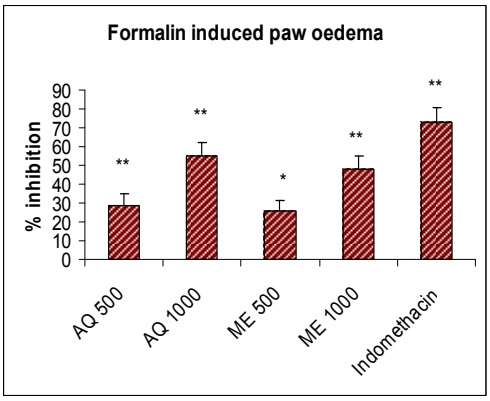
Anti-inflammatory activity on formalin-induced paw oedema

Fig. 3: Effect of aqueous and methnolic extract of *P. sajorcaju* and Indomethacin on formaldehyde induced arthritis in hind paw of rats



* denotes significance at the level of $p \leq 0.05$. ** denotes the significance at the level of $p \leq 0.01$.

Fig. 4: Effect of aqueous and methanolic extracts of *P. sajorcaju* mycelium and Indomethacin on formalin-induced chronic inflammation



* denotes the significance at the level of $p \leq 0.05$, ** denotes the significance at the level of $p \leq 0.01$.

For formalin induced paw edema, *P. sajorcaju* mycelium aqueous and methanolic extracts was found to be 55.13% and 48.0% ($p < 0.01$; Fig. 4) at 1000 mg/kg respectively. Indomethacin (10mg/kg) inhibited the edema volume by 73.07%. Thus aqueous and methanolic extract of *P. sajorcaju* mycelium significantly attenuated chronic inflammation induced by formalin in a dose-dependent manner. The extracts at a concentration of 1000 mg/kg body weight showed slightly lower activity than the reference drug, Indomethacin, in formalin induced inflammations.

Discussion and Conclusion

Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. Development of carrageenan-induced oedema involve three distinct phases; the first phase (0-2 hr) is attributed to the release of histamie, 5-HT, while second phase (3 hr) is related to the release of kinins and prostaglandin in third phase (>4 hr) [7,8,9]. It has been reported that the third phase edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents [10]. The suppression of the inflammatory edema by *P. sajorcaju* after the first 3 hr indicates a blockade of the action and/or inhibition of release of prostaglandin as an inflammatory mediator. First 3 hour the increase in edema at the carrageenan treated site may be due to activated complement, possibly not suppressed by the action of *P. sajorcaju*.

The extracts were further tested on chronic inflammation induced by formalin [11]. The nociceptive effect of formalin is also biphasic; an early neurogenic component followed by a later tissue-mediated response [12]. Arthritis induced by formalin is model used for the evaluation of an agent with probable antiproliferative activity [13]. Acute inflammation induced by formalin result from cell damage, which provokes the production of endogenous mediators, such as, histamine, serotonin, prostaglandins, and bradykinin [14].

The results of both extract shows profound anti-inflammatory activity against formalin induced edema. As the *P. sajorcaju* significantly inhibited this model of inflammation, it can be though to possess antiproliferative and antiarthritic activity.

The results shows methanolic extract evaluated were less potent than the aqueous extract on the edema induced by carrageenan and formalin in rat hind paw showed a significant anti-inflammatory activity ($p < 0.01$).

Various studies have demonstrated that various polysaccharides produced significant anti-inflammatory activities present in morel mushroom mycelium extract [15,16] and preliminary phytochemical results indicated the presence of polysaccharides in both the extracts which is responsible for anti-inflammatory effects.

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