

Anti-inflammatory response by ethanol extract of *Pleurotus ostreatus*.

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Abstract: Recent studies or development on mushroom shows the broad-spectrum values of mushroom which include not only its nutritive but therapeutically value too. In this paper we have seen how *P. ostreatus* work as anti-inflammatory agent. In vitro anti-inflammatory activity of the ethanol extract of *P.ostreatus* tested for HRBC membrane stabilization was found to be concentration dependent. The % protection is found 12.01, 24.06 & 51.07 at concentration of 0.25mg/ml, 0.5mg/ml and 1 mg/ml respectively. Its anti-inflammatory activity is compared with standard NSAID that is diclofenac sodium (Aspirin).

Keywords: *Pleurotus ostreatus*, Anti-inflammatory, concentration dependent.

Introduction

Oyster mushroom found to be of great importance because of its high nutritive values. But recent development in research shows its therapeutical importance along with its nutritive values. Mushrooms have been reported to exhibit an assortment of biological activities including but not limited to anticancer, antimicrobial, hypo-cholesterolemic, antioxidant, antihypertensive, antidiabetic, anti-obesity, hepato-protective, anti-aging, anti-allergic, and anti-coagulant activities.

Pleuran, isolated from fruiting bodies of Oyster Mushroom possesses anti-inflammatory activity.(Nosál'ová et al 2001) Extracts of many of them *P.florida*, *P.pulmonarius* etc give a lowering response in both acute as well as in chronic inflammation and when oral or percutaneous administration of extract of *P.eryngii* was done, it suppress the inflammation in delayed type allergy response in mice. Nozaki et al 2008 reported the mechanism and reported that glycosphingolipid isolated from *P.eryngii*, induced secretion of IFN-g and IL-4 from t cells whereas linked S-glucan isolated from *P.ostreatus* inhibited leucocyte migration to acetic acid-injured tissues. Recently a non-lectin glycoprotein (PCP 3-A) isolated by Chen et al 2011 from fresh fruiting body of *P.citrinopileatus* down regulated the pro inflammatory mediators, like iNOS and NF-kB in RAW 264.7 cells (mouse leukaemia monocyte macrophage cell line). Jedinak et al in their experiments observed that anti-inflammatory activity of OM that was mediated through the inhibition of NF-kB and AP-1 signalling.

Another potent anti-inflammatory agent, a polysaccharide has been extracted from the *P.pulmonarius* that acted against carrageenan and formalin induced paw edema in rats(Adebayo et al 2012).

Anti-inflammatory

Ethanol extract of the mushroom was assessed for its anti-inflammatory by In vitro methods. Anti-inflammatory activity of mushroom was investigated by Human Red Blood Cell Membrane (HRBC) stabilization method. [Gandhidasan R, Thamarachelvan A, and Baburaj S. 1991]

Sample Collection and preparation for Phytochemical analysis:

Fruit bodies of *Pleurotus ostreatus*, were collected from the local mushroom farms. Freshly harvested mushrooms were solar dried and finely powdered. Mushroom powder was stored in an air tight container in a refrigerator for phytochemical analysis. Twenty-five grams of the dried powder were extracted successively with ethanol overnight using soxhlet's apparatus. Extract was filtered through vacuum filter and the filtrate was concentrated in vacuum evaporator. The ethanol extract was used for further pharmaceutical studies.

In Vitro Anti-inflammatory Assay:

Human red blood cell membrane stabilization method was used for this study. The blood (10 ml) was collected from the healthy human volunteer who was not taken any NSAID's for 2 weeks prior to the experiment. Aseptically blood was transferred to the heparinized centrifuged tube. The tubes were centrifuged at 3000rpm for 10min and were washed three times with equal volume of isosaline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline. Various concentrations of extracts were prepared (0.25mg/ml, 0.5mg/ml, 1.0mg/ml) using distilled water and to each concentration 1ml of phosphate buffer, 2ml isosaline and 0.5 ml of packed cell suspension were added and incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was read at 560nm. The percentage haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization was calculated using the following formula.

$$\% \text{ Membrane Stabilization} = \frac{100 - \text{OD of Extract treated sample}}{\text{OD of control}} \times 100$$

Result:

In vitro anti-inflammatory activity of the ethanol extract of *P.ostreatus* tested for HRBC membrane stabilization was found to be concentration dependent (Table-1). Red blood cell membranes are similar to the lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity. The maximum activity at 1.0mg/ml for the ethanol fraction was 51.07%. This is comparable to that of the standard diclofenac sodium.

The phytochemicals or their secondary metabolites of mushroom present in the human diet possess a number of beneficial effects on human health such as anti-oxidant, anti-allergic, anti-viral, anti-diabetic, anti-inflammatory and anti-carcinogenic which was showed in the work of L.H.Yao et al in 2004. Similar results have also been observed especially in the *Pleurotus* spp collected. Anti-oxidant and anti-inflammatory activity might be responsible for *Gandoderma lucidium* as observed by R .Russel et al 2006. The relationship between the oxidative stress and inflammation has been investigated and reported in many edible mushrooms by Y. Ishitsku et al in 2007. With this background knowledge as supporting evidence in vitro pharmaceutical study was carried out.

Table No.1

Table showing Anti-inflammatory activity of ethanol extract *P.ostreatus*

S.No	Conc. mg/ml	% protection	
		<i>P.ostreatus</i> extract	Standard
1	0.25	12.01	-
2	0.50	24.06	81.3
3	1.00	51.07	-

Thus from the HRBC assay, stabilization of the RBC's membrane was evaluated for the anti-inflammatory activity of *P. ostreatus*. The extract was effective in inhibiting the heat induced haemolysis at different concentrations. These provide evidence for membrane stabilization as a mechanism of their anti-inflammatory effect.

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