Antioxidant and anti-inflammatory activities of methanol extract of *Phellinus rimosus* (Berk) Pilat

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The methanolic extract of a macrofungus, *P. rimosus* possessed significant *in vitro* superoxide anion, hydroxyl radical and nitric oxide scavenging and lipid peroxidation inhibiting activities. The anti-inflammatory activity of the extract was evaluated in carrageenan and dextran induced acute and formalin induced chronic inflammatory models in mice. The extract showed remarkable anti-inflammatory activity in both models, comparable to the standard reference drug diclofenac. The results suggest that the anti-inflammatory activity of the methanol extract of *P. rimosus* is possibly attributed to it’s free radical scavenging properties. The findings also reveal the potential therapeutic value of *P. rimosus* extract as an anti-inflammatory agent.

Mushrooms are nutritionally functional food as well as source of physiologically beneficial and nontoxic medicines. Since ancient times, they have been used in folk medicine throughout the world. Attempts have been made in many parts of the world to explore the use of mushrooms and their metabolites for treatment of a variety of human sufferings. *Phellinus rimosus* is a wood inhabiting polypore macrofungus. The basidiocarps of this fungus have been used by some local tribes in Kerala for the treatment of mumps.

Reactive oxygen species such as superoxide anion \((O_2^-)\), hydroxyl radical \((OH)\), and hydrogen peroxide \((H_2O_2)\) play an important role in the inflammatory process induced by ethanol, carbon tetrachloride or carrageenan. The most widely used nonsteroidal anti-inflammatory drugs (NSAID) suffer from inherent side effects, most important being the gastrointestinal irritation. For chronic diseases such as osteoarthritis and rheumatoid arthritis, life long dependency on drugs are necessary. Therefore, the search for an ideal anti-inflammatory drug which is safe and effective is still continuing. An anti-inflammatory drug, activity of which is based on free radical scavenging mechanism is considered the most suitable candidate.

In this communication, the antioxidant and anti-inflammatory activities of methanol extract of a tropical macrofungus, *Phellinus rimosus* are reported.

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Preparation of the extract—Sporocarps of *P. rimosus* growing on the tree trunks were collected from the outskirts of Thrissur. Identification of the fungus was authenticated by Prof. K.M. Leelavathi, Department of Botany, Calicut University, Calicut. Sporocarps were cut into small pieces and dried at 45-50°C for 48 hr. Powdered material (200g) was extracted with petroleum ether and the defatted material was then extracted with methanol using a Soxhlet apparatus for 8-10hr. The methanol extract was completely evaporated at 40°C under vacuum using a rotary evaporator. The residue (4g) designated as methanol extract was employed for the experiment.

The methanol extract was presolubilized in 10% dimethyl sulphoxide (DMSO) or in phosphate buffered saline for determining the *in vitro* antioxidant activities. For animal experiments, the extract was presolubilised in 0.2% DMSO and a fine suspension was prepared in 2% gum acacia.

*In vitro antioxidant activity—*Superoxide radical \((O_2^-)\) generated from the photoreduction of riboflavin, lipid peroxidation induced by \(Fe^{2+}\)-ascorbate system in the rat liver homogenate, hydroxyl radical \((OH)\) generated from \(Fe^{3+}\)-ascorbate-EDTA-\(H_2O_2\) system (Fenton's reaction) and nitric oxide \((NO)\) generated from sodium nitroprusside were estimated. The antioxidant activity of the extract was determined by comparing absorbance of the controls with that of treatments.

*In vivo anti-inflammatory activity—*Male Swiss albino mice were purchased from animal breeding
center, Kerala Agricultural University, Mannuthy and were kept for a week on a commercial diet under environmentally controlled conditions with free access to food and water. Mice weighing 18-20g were used for studying the anti-inflammatory activity. The animals were maintained according to the guidelines recommended by Animal Welfare Board.

**Carrageenan induced paw oedema**—Animals were divided into 4 groups of 6 animals each. In animals of all the groups, acute inflammation was produced by subplantar injection of 20μl of freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of mice. The paw thickness was measured using a vernier calipers before and 3hr after carrageenan challenge in each group. Animals were pre-medicated with vehicle (0.2% DMSO in 2% gum acacia, group I), *P.rimosus* methanol extract (10 and 20 mg/kg body weight, group 2 and 3) and the reference drug diclofenac (10mg/kg body weight, group 4) i.p one hour before carrageenan injection.

**Dextran induced paw oedema**—The animals were treated as in case of carrageenan induced paw oedema model, expect that in place of carrageenan, dextran was administered.

**Formalin induced paw oedema**—The animals were treated in the same way as in above models, expect that formalin (20μl of freshly prepared 2% formalin) was used as the oedematogenic agent. The drug treatment continued for 6 consecutive days. Diclofenac (10mg/kg body weight) was used as the reference drug.

In all the above models the degree of oedema formation was assayed as increase in paw thickness. The increase in paw thickness and percent inhibition were calculated as follows.

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\text{Percent inhibition} = \frac{P_c - P_T \times 100}{P_c}
\]

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 treatments groups.

**Statistical analysis**—The data were statistically analysed using Student’s t test and P values less than 0.05 were considered significant. All data were represented as mean ± SD.

**In vitro antioxidant activity**—Methanol extract of *P.rimosus* showed significant superoxide radical, hydroxyl radical, and nitric oxide radical scavenging and lipid peroxidation inhibiting activities. Concentrations required for 50% inhibition (IC50) of superoxide, hydroxyl radical and nitric oxide

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<th>Table 1—Effect of methanol extract of <em>P.rimosus</em> on carrageenan, dextran and formalin induced paw oedema in mice</th>
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*P values <0.001 compared to control
*Paw thickness was measured after 6 days.
scavenging activities and lipid peroxidation inhibiting activity were 25.3±1.2, 93.0±10.3, 126.7±12.6 and 282.0±12.8 μg/ml respectively.

In vivo anti-inflammatory activity—Anti-inflammatory activity of *P. rimosus* in the three models is given in Table 1. The methanolic extract of *P. rimosus* significantly reduced the carrageenan and dextran induced paw oedema (P<0.001). The extract of *P. rimosus* was also effective in ameliorating formalin-induced chronic inflammation. Formalin induced paw oedema was inhibited significantly (P<0.001) by methanol extract of *P. rimosus*. The concentration required to inhibit paw oedema in both type of inflammations was comparable to the standard reference drug, diclofenac.

The reactive oxygen species (ROS) such as superoxide anion radical (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (·OH) have been implicated in pathophysiology of various clinical disorders, including ischemia, reperfusion injury, atherosclerosis, acute hypertension, haemorrhagic shock, diabetes mellitus and cancer. Recent studies suggest that the inflammatory tissue damages is due to the liberation of reactive oxygen species from phagocyes invading the inflammation sites. In addition to this, nitric oxide is also implicated in inflammation, cancer and other pathological conditions. Interaction between superoxide and nitric oxide regulates the vascular tone or inflammation.

The significant *in vitro* antioxidant activity of *P. rimosus* is in a concentration dependent manner. The possible interference of DMSO used as a pre-solubilizer of the extract for antioxidant assays was also evaluated. The results indicate that DMSO does not show antioxidant activity at the given concentration, and therefore could be employed to pre-solubilize the extracts.

Carrageenan induced acute inflammation in animals is one of the most suitable test procedures to screen anti-inflammatory agents. The development of carrageenan-induced oedema is biphasic, the first phase is attributed to the release of histamine, 5-HT and kinins, while second phase is related to the release of prostaglandins. Dextran induced paw oedema is known to be mediated both by histamine and serotonin. Carrageenan and dextran induce paw oedema by different mechanisms. Dextran induces fluid accumulation because of mast cell degranulation with little protein and few neutrophils, carrageenan induces a protein rich exudates containing large number of neutrophils.

Formalin induced paw oedema is one of the most suitable test procedures to screen chronic anti-inflammatory agents as it closely resembled human arthritis. The nociceptive effect of formalin is also biphasic, an early neurogenic component followed by a later tissue mediated response. The results suggest the usefulness of *P. rimosus* in the treatment of inflammation associated diseases like arthritis.

The methanolic extract of *P. rimosus* has significant anti-inflammatory effect against carrageenan, dextran or formalin induced paw oedema in mice in a dose dependent manner. The effect of the extract at a concentration of 20 mg/kg body wt. is comparable to that of standard reference drug diclofenac (10mg/kg body wt).

The antioxidant activity of *P. rimosus* explains at least in part the mechanism of its anti-inflammatory activity. Polyphenols have been shown to possess anti-inflammatory properties. The preliminary chemical examination of methanol extract shows presence of flavonoids and polyphenols. These compounds may be responsible for antioxidant and anti-inflammatory activities.

In conclusion, the methanol extract of *P. rimosus* exhibited significant anti-inflammatory activity in acute and chronic inflammations in mice. This effect is probably mediated through its significant antioxidant activity.

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References
NOTES

9 Ohkawa H, Ohishi W & Yagi K, Assay for peroxides in animal tissues by thiorbarbituric acid reaction, Anal Biochem, 95 (1979) 351.