STUDIES ON ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITIES OF FRUITING BODIES OF PLEUROTUS EOUS IN EXPERIMENTAL ANIMALS

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Summary

Anti-inflammatory and antipyretic potential of ethyl acetate, methanol and aqueous extracts of fruiting bodies of *Pleurotus eous* were investigated. The dried fruiting bodies of *P.eous* were extracted with ethyl acetate, methanol and water. The anti-inflammatory effect of the extracts of was performed by carrageenan induced rat paw edema and formalin induced paw edema at doses 500 and 1000mg/kg b.w. The antipyretic effects of extracts of *P. eous* were investigated at doses 250,500 and 1000mg/kg b.w. using yeast induced pyrexia in rats. *P. eous* extracts produced significant ($P<0.05$) dose-dependent inhibition of both phases of the formalin induced pain response in comparison with control. In carrageenan test the extracts caused a significant ($P<0.05$) inhibition of pain at both the doses used. It also showed significant ($P<0.05$) reduction in hyperpyrexia in rats throughout the observation period of 5hrs.The results of present study suggest that extracts of *P. eous* possess potent anti-inflammatory and antipyretic effects and could serve as a base for future drugs.

Keywords: pyrexia, inflammation, carrageenan induced rat paw edema, formalin induced paw edema, yeast induced pyrexia test.

Introduction

Mushrooms have high nutritive and medicinal values, and contribute to a healthy diet because of their rich source of vitamins, minerals and proteins. They comprise vast and largely untapped source of powerful new pharmaceutical products [1, 2]. They are low calorie food with very little fat and are highly suitable for obese persons. Mushrooms belonging to the genera *Pleurotus* are also known as oyster mushrooms. *Pleurotus* mushrooms are the second most important mushrooms in production in the world. On preliminary phytochemical screening the extracts of *P.eous* were found to contain flavonoid compounds which are known to target prostaglandins involved in the late phase of acute inflammation and pain perception.

Chronic inflammatory diseases remain one of the world's major health problems. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair. Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. As a result of adverse effects such as gastric lesions caused by non-steroidal anti-inflammatory drugs (NSAID), tolerance and dependence were induced by opiates. The use of these drugs as anti-inflammatory agents has not been successful in all cases. Therefore, new anti-inflammatory drugs lacking these side effects are being researched as alternative to NSAID and opiates [3]. Attention is being focused on the investigation of the efficacy of mushroom-based drugs used in the traditional medicine because they are cheap and have little side effects.

This species has been of interest to researchers because its phytochemical constituents are similar to those of *P.florida, P.ostreatus and P.sajor-caju* which are commonly used in medicines such as analgesic, anti-cholesteremic, anti-inflammatory and antitumor agents etc [4,5]. The bibliographic survey showed that there are no reports on the anti-inflammatory and antipyretic activities of *P.eous* in spite of its bioremediation strategies. This prompted us to investigate the effects of pharmacological activities of *P.eous* mushroom in experimental models of pyrexia and inflammation.

Materials and Methods

Preparation of the Extract: The fruiting bodies of the mushroom *P. eous* were obtained from Kerala Agricultural University, Trivandrum and authenticated by Dr.Lu Lu Das, Professor, Dept of Plant Biology, College of Agriculture, Vellayani, Kerala Agricultural University, Trivandrum(Reg:No.T.5365/06 :61;27/08/2009). Mushroom fruiting bodies were dried at 40-50ºC for 48h and powdered. 500 gms of the powdered material were extracted with petroleum ether. The defatted material was extracted with ethyl acetate and then with 70% methanol for 8-10 h using Soxhlet apparatus. For the preparation of aqueous extract defatted material was extracted with hot water (70-80ºC) for 8-10hrs.The extract was collected after filtering through Whatmann No.1 filter paper. The solvents were completely evaporated at 40ºC using a rotary vacuum evaporator. The residues were designated as ethyl acetate (EA) extract and methanolic extract (MeOH) and aqueous extract (Aqs) respectively.

Animals: Swiss Albino mices (18-25g) and Wistar rats (180-200g) were procured from Animal Breeding Centre, Kerala Agricultural University, Trivandrum. They were randomly distributed into groups and housed in cages (6 per cage) and maintained under standard conditions. All animals were fed the standard diet and water *ad libitum.* This project was cleared by Institutional Animal Ethical Committee (Approval No: IEAC NO.03/001/10).

Acute toxicity studies: Swiss albino mice of either sex (18-25g weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD and no adverse effects or mortality were detected in the mice up to 4g/kg, p.o., during the 24h observation period. Based on the results obtained from this study, the dose for anti-inflammatory activity was fixed to be 500mg/kg and 1000mg/kg for dose dependent study.

Anti-inflammatory activities

Carrageenan-induced rat paw edema: Anti-inflammatory activity of extracts of *P.eous* was assessed by carrageenan induced paw edema method. Rats were divided into 8 groups (6 animals in each group).Animals of all the groups were injected with 0.1 ml of 1% carrageenan in 0.9% normal saline, under the plantar aponeurosis of right hind paw. Group I (control) received 0.5% of Tween – 80 and Group II, the standard, an aqueous solution of Phenyl butazone (PB 200 mg/kg) and Groups III to VIII received 500 and 1000mg/kg of EA, MeOH and Aqs extracts of *P.eous* respectively, p.o, 30 min prior to carrageenan injection. The paw volume was measured plethysmographically at 1hr and 3hr after carrageenan injection. The percentage inhibition of edema was calculated for each group with respect to its vehicle treated control group. [6, 7]

Formalin induced paw edema in mice: Anti-inflammatory activity was studied by formalin induced hind paw edema in mice, measured by plethysmograph (mercury displacement method). [8]Swiss albino mices of either sex weighing between 18-25 g were divided into 8 groups of six animals each. The first group served as the control and received the vehicle i.e. Tween-80, second group of animals were administered with standard drug phenyl butazone, 200 mg/kg b.w. Third to eighth groups of animals were treated with EA, MeOH and Aqs extracts of P.eous at doses of 500 and 1000mg/kg b.w. p.o. The volume of paw edema was measured in control, standard and treated groups accordingly 1, 2, 3 and 4 h after formalin injection. The percent inhibition of edema was calculated as per Saundane et al., 2000. [9]

Antipyretic activity: Antipyretic activity was measured by slightly modifying the method described by Adams. [10] Rats were fasted overnight with water *ad libitum* before the experiments. Pyrexia was induced by subcutaneously injecting 20% w/v brewer's yeast suspension (10ml/kg) into the animal's dorsum region. 17h after the injection, the rectal temperature of each rat was measured using a digital thermometer (SK-1250 MC, Sato keiryoki Mfg.). Only rats that showed an increase in temperature of at least 0.7 ℃ were used for experiments. *P.eous* (250, 500 and 1000mg/kg b.w.), Paracetamol (200mg/kg b.w.) or vehicle was administered orally and the temperature was measured at 0, 1, 2, 3, 4, and 5h after treatment.

Statistical analysis: Results are expressed as Mean ± S.E.M. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. The results were considered statistically significant when $P<0.05$. [11, 12]

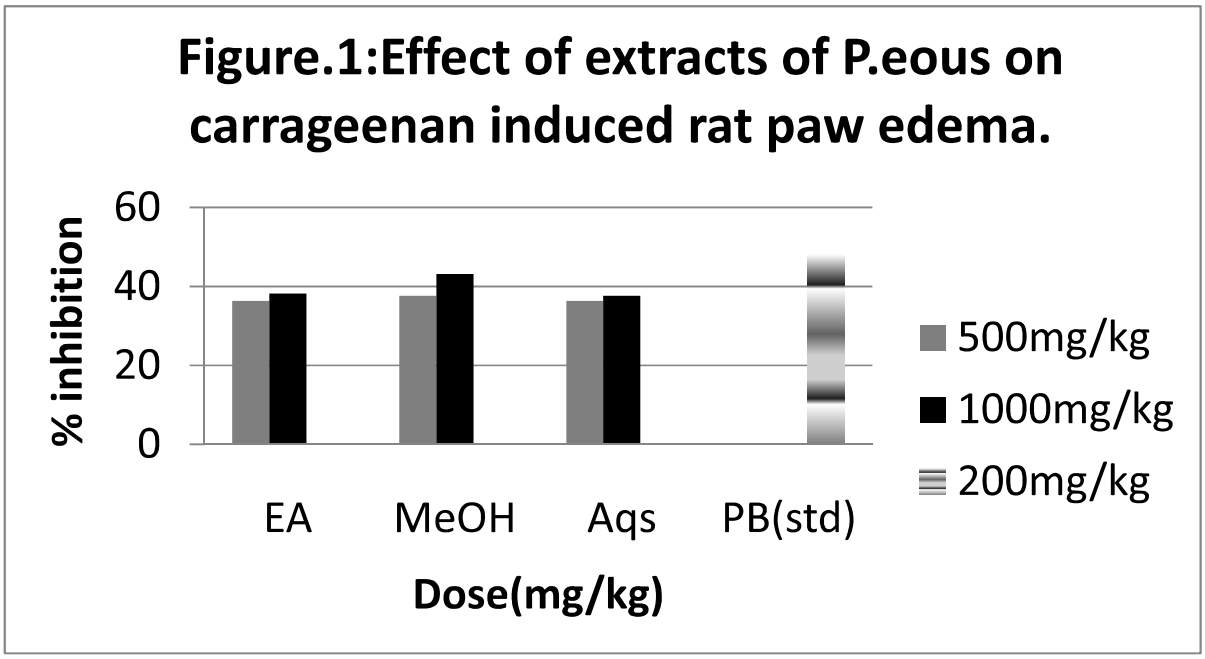
Results

Anti-inflammatory effect of P.eous: The amount of edema produced was quantitated at 1hr and 3hr of carrageenan injection. The group treated with PB showed maximum inhibition of edema formation followed by EA, MeOH and Aqs extracts of P.eous at both doses studied significantly inhibited the carrageenan induced paw edema in rats (Table 1 and Fig 1).

Table 1: Effect of MeOH, EA and Aqs extracts of *P. eous* on paw edema induced by carrageenan in rats

Treatment	Dose(mg/kg)	Paw volume at different time intervals (ml)		% inhibition of edema.
		1hr	3 hr	
Control	-	5.84±0.04	7.02±0.04	7.02±0.04
Aqueous extract	500	5.09±0.03	4.47±0.07	4.47±0.07
Aqueous extract	1000	5.07±0.14	4.38±0.14	4.38±0.14
Methanolic extract	500	4.89±0.04	4.11±0.04	4.11±0.04
Methanolic extract	1000	4.97±0.07	3.99±0.03	3.99±0.03
Ethyl acetate extract	500	5.07±0.05	4.47±0.04	4.47±0.04
Ethyl acetate extract	1000	5.02±0.05	4.34±0.04	4.34±0.04
Phenyl butazone	200	4.80±0.05	3.65±0.05	3.65±0.05

Each value is the Mean ± S.E.M. for 6 rats.



In formalin induced paw edema at 1st h, PB exhibited good anti-inflammatory activity compared to the extracts of *P.eous*. During 2nd h, PB and MeOH extracts showed good activity compared to other groups. Similarly in the 3rd h and 4th h PB and MeOH as well as EA and Aqs extracts exhibited good anti-inflammatory activity. It means, PB showed highest activity followed by methanolic extract, ethyl acetate and aqueous extract (Table 2).

Table 2: Effect of MeOH, EA and Aqs extracts of *P. eous* on formalin induced paw edema in mice.

Treatment	Dose(mg/kg)	Paw volume at different time intervals			
		1hr	2hr	3hr	4hr
Control	-	.2400±.0026	.2467±.0021	.2417±.0031	.2417±.0017
Aqs	500	.2233±.0021 (6.95%)	.2050±.0034 (16.90%)	.1667±.0033 (31.03%)	.1333±.0042 (44.85%)
Aqs	1000	.2217±.0031 (7.63%)	.1933±.0033 (21.65%)	.1567±.0033 (35.17%)	.1267±.0033 (47.58%)
MeOH	500	.2217±.0031 (7.63%)	.1933±.0033 (21.65%)	.1550±.0043 (35.87%)	.1233±.0033 (48.98%)
MeOH	1000	.2200±.0000 (8.33%)	.1867±.0021 (24.32%)	.1467±.0021 (39.30%)	.1083±.0031 (55.42%)
EA	500	.2233±.0021 (6.95%)	.2000±.000 (18.92%)	.1717±.0017 (28.96%)	.1417±.0017 (41.37%)
EA	1000	.2217±.0031 (7.63%)	.1933±.0033 (21.65%)	.1567±.0033 (35.17%)	.1250±.0034 (48.28%)
PB	200	.2133±.0021 (11.13%)	.1750±.0022 (29.06%)	.1333±0.0021 (44.85%)	.0850±.0034 (64.83%)

Each value is the Mean ± S.E.M. for 6 mices.

Antipyretic effect of P.eous: The experimental rats showed a mean increase of about 0.7ºC in rectal temperature, 17hr after Brewer's yeast injection. P.eous extracts at 250,500 and 1000 mg/kg and paracetamol, 200mg/kg. (Table 3)

Table 3: Effect of MeOH, EA and Aqs extracts of *P. eous* on yeast induced pyrexia in rats.

Treat Ment	Dose (mg/ kg)	Mean ± S.E.M. Rectal temperature (ºC)						
		Before 18hr	0hr	1hr	2hr	3hr	4hr	5hr
Aqs	250	35.97±0.23	36.97±0.14	36.72±0.15	36.40±0.15	36.17±0.15	36.05±0.16	36.03±0.14
Aqs	500	36.42±0.14	37.35±0.15	37.08±0.13	36.77±0.14	36.47±0.14	36.37±0.13	36.37±0.14
Aqs	1000	36.15±0.12	37.07±0.11	36.68±0.11	36.32±0.13	36.15±0.09	36.10±0.11	36.02±0.12
MeOH	250	36.28±0.12	37.22±0.10	37.00±0.11	36.78±0.12	36.53±0.11	36.32±0.11	36.23±0.10
MeOH	500	36.18±0.12	37.05±0.10	36.78±0.10	36.47±0.10	36.22±0.09	36.13±0.10	36.15±0.13
MeOH	1000	36.45±0.08	37.33±0.06	37.02±0.05	36.67±0.07	36.38±0.08	36.38±0.06	36.40±0.07

EA	250	36.13±0.04	37.00±0.13	36.80±0.13	36.55±0.14	36.27±0.14	36.15±0.13	36.07±0.14
EA	500	36.42±0.14	37.35±0.15	36.75±0.20	36.60±0.16	36.47±0.14	36.37±0.13	36.37±0.14
EA	1000	36.18±0.12	37.05±0.10	36.80±0.10	36.52±0.10	36.30±0.09	36.18±0.09	36.12±0.11
Paracetamol	200	36.13±0.10	37.03±0.08	36.70±0.11	36.33±0.11	36.08±0.10	35.97±0.12	35.97±0.13
Control	-	36.45±0.16	37.37±0.16	37.53±0.15	37.47±0.15	37.43±0.15	37.33±0.17	37.32±0.18

Each value is the Mean ± S.E.M. for 6 rats.
Dose Mean ± S.E.M. Rectal temperature (ºC)

Discussion

Carrageenan assay is well suited for comparative bioassay of anti-inflammatory agents, since the relative potency estimates obtained from most drugs tend to reflect clinical experience. The time course of edema development in carrageenan induced paw edema model in rats is generally represented by a biphasic curve. The first phase occurs within an hour of injection and is partly due to the trauma of injection and also due to the serotonin component. Prostaglandins play a major role in the development of the second phase of reaction which is measured around 3 h times. The presence of prostaglandin in the inflammatory exudates from the injected foot can be demonstrated. The carrageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit cyclooxygenase involved in prostaglandin synthesis. Based on these reports, it is inferred that the inhibitory effect of extracts of *P.eous* on carrageenan induced inflammation in rats may be due to inhibition of the enzyme cyclooxygenase, leading to inhibition of prostaglandin synthesis [13].

It is well known that most of the anti-inflammatory analgesic drugs possess antipyretic activity. Yeast-induced fever is called pathogenic fever [14, 15].Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis [16]. Extracts of *P.eous* revealed weak antipyretic effect at low doses (250 and 500mg/kg) but at higher dose (1000mg/kg), it produced marked antipyretic activity in Brewer's yeast induced febrile rats. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through inhibition of prostaglandin synthetase within the hypothalamus. The present results show that *P. eous* possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, and its effect is comparable to that of paracetamol .So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol.

In conclusion, the present study demonstrates that extracts of *P.eous* has potent antipyretic and anti-inflammatory activities. This could provide a rationale for the use of this mushroom in pain, fever and inflammatory disorders in folk medicine.

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