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Evaluation of the analgesic activity of ethyl acetate, methanol and aqueous extracts of *Pleurotus eous* mushroom

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ABSTRACT

Objective: To evaluate the analgesic activity of the ethyl acetate, methanol and aqueous extracts of *Pleurotus eous (P. eous)* mushroom. **Methods:** The dried fruiting bodies were extracted with ethyl acetate, methanol and water. The analgesic effect of extracts of *P. eous* were investigated at doses 250 500 and 1 000 mg/kg body weight, using acetic-acid induced writhing, hot-plate, tail immersion and tail-clip tests. **Results:** *P. eous* extracts produced significant reduction in number of writhes induced by intraperitoneal injection of acetic-acid ($P<0.05$). Moreover, in hot-plate and tail immersion test, all the three extracts significantly raised the pain threshold at different time of observation (0-60 min) in comparison with control ($P<0.05$). In tail-clip test the extracts also caused a significant inhibition of pain at both the doses used ($P<0.05$). **Conclusions:** The results of present study suggest that extracts of *P. eous* possess potent analgesic property and could serve as a base for future drugs.

1. Introduction

Pain is an unpleasant sensation, but it is usually beneficial to man and animal. It is mainly a protective mechanism for the body, whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus. Typically, it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation but severe pain can arise independently of any obvious predisposing cause (*e.g.* trigeminal neuralgia), or persistent long after the precipitating injury has healed (*e.g.* phantom limb pain). It can also occur as a consequence of brain or nerve injury (*e.g.* following a stroke or herpes infection). With many pathological conditions, tissue injury is the immediate cause of the pain, and this results in the local release of a variety of chemical agents, which are assumed to act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation[1,2]. 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.

Mushrooms are the fleshy and edible fruiting bodies of several species of fungi, typically produced above ground on soil or on its food source[3]. Mushrooms have high nutritive and medicinal values, and contribute to healthy diet because of their rich source of vitamins, minerals and proteins[4]. They comprise a vast and yet largely untapped source of powerful new pharmaceutical products[5,6]. They are low calorie food with very little fat and are highly suitable for obese persons[7]. *Pleurotus* species are commonly known as oyster mushrooms and are edible. They are reported to possess several medicinal properties. On preliminary phytochemical screening the extracts of *Pleurotus eous (P. eous)* were found to contain flavonoid compounds (Mg+HCl reduction test) which are known to target prostaglandins involved in the late phase of acute inflammation and pain perception. *Pleurotus* mushrooms are the second most important mushrooms in production in the world, 25% of total world production of cultivated mushrooms. *P. eous* mushroom belongs to Class *Agaricomycetes* and Family *Pleurotaceae*. This species has been of interest to researchers because its phytochemical constituents are similar to those of *Pleurotus florida*, *Pleurotus ostreatus* and *Pleurotus sajor-caju*, *Pleurotus pulmonarious* which are commonly used in medicines[4-6,8,9]. A bibliographic survey showed that there are no reports on the analgesic activity of *P. eous* in spite of its medicinal applications. This prompted us to investigate the effects

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of pharmacological activities of *P. eous* mushroom in experimental models of analgesia.

2. Materials and methods

2.1. 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, Department of Plant Biology, College of Agriculture, Vellayani, Kerala Agricultural University, Trivandrum. Mushroom fruiting bodies were dried at 40–50 ℃ for 48 h and powdered. The powdered material (250 g) was 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 (70–80 ℃) for 8–10 hrs. The extract was collected after filtering through Whatmann No.1 filter paper. The solvents were completely evaporated at 40 ℃ using a rotary vacuum evaporator. The residues were designated as ethyl acetate (EA) extract and methanol extract (MeOH) and aqueous extract (Aqs), respectively.

2.2. Animals

Swiss Albino mices were purchased 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).

2.3. Acute toxicity studies

Swiss albino mice of either sex (18–25 g of 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 4 g/kg p.o., during the 24 h observation period. Based on the results obtained from this study, the dose for analgesic activity was fixed to be 250, 500 and 1 000 mg/kg body weight, for dose dependent study.

2.4. Analgesic activity

The animals were divided into 12 groups (n=6). Group I served as control received the vehicle only (1% carboxy methyl cellulose, 10 mL/kg p.o.). Group II and III served as standard, received indomethacin (10 mg/kg b.w.) or diclofenac sodium (10 mg/kg b.w.) Groups IV–XII served as test received EA, MeOH and Aqs extracts of *P. eous* at doses of 250, 500 and 1 000 mg/kg b.w. p.o., respectively.

2.4.1. Writhing test

The test was carried out according to Koster *et al*[10]. Animals were orally administered with indomethacin and diclofenac sodium (10 mg/kg b.w.) as the standard drug, *P. eous* (250, 500 and 1 000 mg/kg b.w.) and vehicle. Thirty minutes after treatment, the mice were given an intraperitoneal (i.p.) injection of 0.6% v/v acetic acid in a volume of 10 mL/kg to induce the characteristic writhing. The No. of writhing occurring between 5 and 15 min. after acetic acid injection was recorded. The response of the extract treated animals was compared with that of control[11].

2.4.2. Hot-plate test

Mice were placed on an aluminum hot plate kept at (55 ±0.5) ℃ for a maximum time of 30 s[12]. Reaction time was recorded when the animals licked their fore and hind paws and jumped at before (0) and 15, 30, 45 and 60 min after i.p. administration of *P. eous* at doses of 250, 500 and 1 000 mg/kg b.w. to different groups. Indomethacin and diclofenac sodium (10 mg/kg b.w.) is the standard drug.

2.4.3. Tail clip method

A metal artery clip was applied to the root of the mouse's tail to induce pain[13]. A sensitivity test was carried out and animals that did not attempt to dislodge the clip within 10 s were discarded. The responsive mice were allotted to groups of six animals each. The tail clip was applied 60 min after oral administration of extract (500 and 1 000 mg/kg b.w.), indomethacin and diclofenac (10 mg/kg b.w.). Whereas, vehicle treated group was served as control.

2.4.5. Tail immersion method

Prior to this experiment the animals were screened for the sensitivity test by immersing the tail of mice gently in hot water maintained at 55–55.5 ℃. The animal dislodging the tail from hot water within 5 seconds was selected for the study[14]. After oral administration of extracts (500 and 1 000 mg/kg b.w.), indomethacin and diclofenac (10 mg/kg b.w) the reaction time was recorded at 0, 5, 10, 15, 30, 45 and 60 minutes[15].

2.5. Statistical analysis

Results are expressed as Mean±SEM. 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.

3. Results

The effects of extract on acetic-acid induced writhes in mice have been shown in Table 1. *P. eous* at dose (250, 500 and 1 000 mg/kg b.w.) produced a significant (P<0.05) decrease in number of writhes in comparison with the control group. Diclofenac sodium and indomethacin (10 mg/kg b.w.) also showed significant (P<0.05) decrease in number of writhes.

Table 1
Effect of EA, MeOH and Aqs extracts of *P. eous* on acetic-acid induced writhes in mice.

Treatment	Dose(mg/kg)	Number of writhes	Inhibition (%)
Control	–	36.83±0.60	
Diclofenac sodium	10	12.17±0.31*	66.96
Indomethacin	10	14.83±0.31*	59.73
Aqs extract	250	26.33±033*	28.51
Aqs extract	500	23.33±0.21*	36.65
Aqs extract	1 000	20.83±0.31*	43.44
MeOH extract	250	21.17±0.31*	42.52
MeOH extract	500	19.33±0.21*	47.52
MeOH extract	1 000	17.17±0.31*	53.38
EA extract	250	25.67±0.21*	30.30
EA extract	500	22.33±0.21*	39.37
EA extract	1 000	19.83±0.31*	46.16

Each value is the Mean±SEM for 6 mice; *:P<0.05 vs control.

Hot-plate test was also assayed to threshold at different time of observation (0-60 min) in comparison with control. The results presented in Table 2 showed that diclofenac sodium and indomethacin (10 mg/kg b.w.) were used as standard drug, also that the oral administration of the *P. eous* produced a significant ($P<0.05$) analgesic effect at doses 250, 500 and 1 000 mg/kg b.w. in a time-dependent way ($P<0.05$). The effect of *P. eous* on tail clip test is shown as in Table 3. The extract caused a significant inhibition of pain at both used doses (500 and 1 000 mg/kg b.w.), diclofenac sodium (10 mg/kg b.w.), a standard drug, was highly effective ($P<0.05$).

The effects of extract on tail immersion test in mice have been shown in Table 4. Oral administration of *P. eous* produced a significant analgesic effect at both doses of 500 and 1 000 mg/kg b.w. in a time-dependent way ($P<0.05$). Diclofenac sodium and indomethacin (10 mg/kg b.w.) were used as standard drug.

Table 2

Effect of EA, MeOH and Aqs extracts of *P. eous* on mice subjected to the hot-plate test.

Treatment	Dose(mg/kg)	Reaction time in seconds at time (minutes)				
		0 min	15 min	30 min	45 min	60 min
Control	–	1.50±0.22	2.00±0.26	1.83±0.31	2.17±0.17	2.00±0.00
Diclofenac sodium	10	2.17±0.31	5.83±0.31	12.00±0.63	17.33±0.33	21.83±0.47
Indomethacin	10	1.67±0.21	4.50±0.22	7.33±0.49	12.83±0.47	14.50±0.74
Aqs extract	250	1.83±0.31	3.83±0.31	5.67±0.21	8.00±0.36	10.67±0.33
Aqs extract	500	1.83±0.31	4.33±0.21	7.33±0.33	10.67±0.42	13.33±0.33
Aqs extract	1 000	1.50±0.43	4.00±0.26	6.83±0.47	11.17±0.47	14.67±0.21
MeOH extract	250	1.50±0.22	4.50±0.22	8.00±0.26	10.83±0.31	13.83±0.31
MeOH extract	500	1.83±0.31	4.83±0.31	8.50±0.43	11.50±0.43	14.67±0.42
MeOH extract	1 000	1.83±0.17	4.50±0.22	8.33±0.33	12.33±0.33	16.50±0.43
EA extract	250	1.33±0.33	3.67±0.21	6.17±0.17	8.83±0.31	11.50±0.43
EA extract	500	1.67±0.42	3.83±0.31	6.50±0.22	8.83±0.31	12.17±0.31
EA extract	1 000	2.00±0.26	4.50±0.22	6.83±0.31	9.67±0.21	12.67±0.21

Each value is the Mean±SEM for 6 mice.

Table 3

Effect of EA, MeOH and Aqs extracts of *P. eous* on tail-clip test in mice.

Treatment	Dose (mg/kg)	Reaction time in seconds at time (minutes)						
		0 min	5 min	10 min	15 min	30 min	45 min	60 min
Control	–	1.67±0.42	2.17±0.17	2.00±0.26	2.17±0.17	2.33±0.21	2.17±0.31	2.00±0.26
Aqs extract*	500	2.17±0.17	3.33±0.21	6.00±0.26	7.50±0.22	10.00±0.26	11.67±0.21	13.33±0.33
Aqs extract*	1 000	2.00±0.26	3.17±0.17	6.50±0.22	9.33±0.21	11.50±0.22	13.50±0.22	14.67±0.21
MeOH extract*	500	2.00±0.26	3.67±0.42	7.50±0.22	9.67±0.21	12.67±0.21	15.00±0.26	16.67±0.42
MeOH extract*	1 000	2.17±0.31	4.83±0.31	8.00±0.36	11.00±0.36	13.50±0.22	15.17±0.31	17.50±0.34
EA extract*	500	2.00±0.42	4.00±0.26	6.33±0.21	9.00±0.36	11.00±0.36	12.67±0.21	14.50±0.22
EA extract*	1 000	1.83±0.42	4.00±0.00	7.17±0.17	9.50±0.22	11.67±0.21	14.00±0.36	16.50±0.22
Diclofenac sodium*	10	2.00±0.26	6.00±0.63	10.17±0.31	12.67±0.33	17.33±0.33	19.67±0.42	24.17±0.31

Each value is the Mean±S.E.M. for 6 mice; *:$P<0.05$ vs control.

Table 4

Effect of EA, MeOH and Aqs extracts of *P. eous* on tail-immersion test in mice.

Treatment	Dose(mg/kg)	Reaction time in seconds at time (minutes)						
		0 min	5 min	10 min	15 min	30 min	45 min	60 min
Control	–	2.00±0.26	3.17±0.17	2.33±0.21	3.00±0.00	2.83±0.17	2.00±0.26	2.00±0.00
Aqs extract	500	2.67±0.21	4.00±0.26	5.00±0.26	7.00±0.26	9.33±0.33	11.00±0.26	12.33±0.21
Aqs extract	1 000	2.17±0.31	4.00±0.26	6.00±0.26	7.67±0.21	10.17±0.31	12.17±0.31	13.67±0.21
MeOH extract	500	2.00±0.25	3.83±0.17	5.50±0.22	7.67±0.21	10.67±0.21	13.00±0.26	15.33±0.33
MeOH extract	1 000	2.00±0.00	5.00±0.00	6.33±0.21	8.67±0.21	11.83±0.31	14.50±0.22	17.00±0.26
EA extract	500	2.17±0.31	4.17±0.31	6.00±0.36	7.83±0.40	9.33±0.21	11.00±0.26	12.50±0.22
EA extract	1 000	2.50±0.22	4.17±0.17	6.33±0.21	8.17±0.17	10.33±0.21	12.00±0.26	14.00±0.26
Diclofenac sodium	10	2.00±0.36	5.17±0.40	9.17±0.40	15.17±0.47	22.17±0.79	25.67±0.42	27.67±0.42
Indomethacin	10	2.67±0.26	5.17±0.31	9.17±0.31	12.33±0.21	16.00±0.36	20.50±0.43	24.17±0.31

Each value is the Mean±SEM for 6 mice.

4. Discussion

The data presented here suggests that the *P. eous* possess anti-nociceptive activity. The extract at the doses tested was shown to possess anti-nociceptive activity in all the nociceptive models, signifying it possess both central and peripherally mediated activities. The abdominal constriction response induced by acetic acid is a sensitive procedure to

evaluate peripherally acting analgesics[16]. In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response[17]. The method has also been associated with prostanoids in general, that is, increased levels of PGE2 and PGF2 α in peritoneal fluids[18], as well as lipoxygenase products[19,20]. The significant reduction in acetic acid-induced writhes by *P. eous* suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances. The hot-plate, tail-immersion and tail-clip tests are useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level[21,22]. The significant increase in pain threshold produced by *P. eous* in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems[23-25].The analgesic effect produced by the extract may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes, and other endogenous substances that are key players in inflammation and pain[26].

In conclusion, the present study demonstrates that extracts of *P. eous* has potent analgesic property, which are mediated via peripheral and central inhibitory mechanisms. This could provide a rationale for the use of this mushroom in pain and inflammatory disorders in folk medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

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