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Antinociceptive activity of *Pleurotus cystidiosus*, an edible mushroom in rats

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ABSTRACT

The antinociceptive potential of *Pleurotus cystidiosus*, an edible mushroom (family: Tricholomataceae) was investigated in male rats (doses used: 125, 500, and 1000 mg/kg) and female rats in the di-oestrous stage using the standard hot plate and tail flick tests. In the hot plate test, the reaction time was significantly ($P < 0.05$) prolonged in male rats after 2 h of administration (18 % mid and 93% high dose). The di-oestrous female rats also showed significant ($P < 0.05$) prolongation in the reaction time on the hot plate test (22 %) upon administration of the high dose. This antinociceptive activity had the peak effect at 2 h in male rats and the activity was dose dependent ($r^2 = 0.81$, $p < 0.05$). In contrast, none of the rats showed an increase in reaction time in the tail-flick test. The acetone and methylene chloride extracts of *P. cystidiosus* was also orally administered to the rats and antinociceptive activity investigated. Only the acetone extract showed a marked and significant increase in reaction time on the hot plate test at 2 h (23% mid and 49% high dose). We conclude that the acetone extract retain the pain alleviating properties, however the whole mushroom has a better effect with 1000 mg/kg dose.

KEY WORDS : Antinociception, Hot plate, Morphine, Pleurotus cystidiosus, Tail flick method

INTRODUCTION

Pleurotus cystidiosus (family: Tricholomataceae) commonly known as Abalone is an edible mushroom; a popular delicacy among Sri Lankans. These mushrooms are large and fleshy, and grow on tree trunks or stumps in shelf-like layers. The pileus is shell-shape. The young cap is deep brownish. The surface is smooth and moist with the edge turned downwards. The stipe is dark brown. *P. cystidiosus* differs from the other members of Tricholomataceae (1) in that its stipe is not central but lateral, excentric or even absent.

Mushrooms belonging to the genus *Pleurotus* have been investigated in search of biologically active compounds. Blood cholesterol lowering properties of *P. ostreatus* is reported (2, 3, 4) in the literature. The antioxidant property of the same mushroom is also reported (5). An antifungal peptide has been isolated from *Pleurotus eringii* (6). Anti-inflammatory activity of *Pleurotus florida* has been reported (7). We have reported the anti-nociceptive activity of *P. ostreatus* in our earlier study (8).

A possibility thus exists that *P. cystidiosus* may also possesses antinociceptive activity. The aim of this study was to evaluate antinociceptive potential of *P. cystidiosus*. This was tested in rats first with a freeze dried mushroom and then with different solvent extracts of the mushroom.

MATERIALS AND METHODS

Animals

Healthy adult male Wistar rats (weight: 230-250g) were used in the study. The animals were kept in standardized animal house conditions (temperature: 28-31^{0}C, photoperiod: approximately 12 hours natural light per day). All rats had access to water and pelleted food (Vet House Ltd., Colombo, Sri Lanka).

Animal experiments were done in accordance with the internationally accepted principles for animal use and care and rules of the Faculty of Science, University of Colombo.

Preparation of an oral suspension from Mushroom

Fresh *P. cystidiosus* mushroom was collected from a farmer and the identification and authentication was performed by Mr. A.R. Marashinghe, Mushroom development and training centre, Export development board, Ratmalana. A voucher specimen (P. cys. 2006) is deposited at the research laboratory, Department of Chemistry, University of Colombo, Sri Lanka. Fresh *Pleurotus cystidiosus* (1 kg) was washed with water to remove any soil particles, freeze dried (LFD-600EC,

Laytant life science Co. Ltd, Tokyo, Japan), and ground with a commercial blender (Food Machine International, HGB-SS, Osaka, Japan) to obtain (82 g, yield:8.2%) a yellowish powder and was stored in an air-tight container at 4^0 C.

Suspension of freeze dried mushroom was freshly prepared with tap water (1 g in 10 ml of water for 1000 mg/kg dose) prior to feeding of the rats. The three doses used in this experiment are 1000 mg/kg, 500 mg/kg and 125 mg/kg. These doses were chosen based on one of our previous experiments conducted on *P. ostreatus* (8). In all concentrations the volume of oral administration was 2.5 ml.

Hot Plate and Tail Flick tests

Twenty four male rats were randomly divided into four equal groups (n = 6/group). The rats in group 1 were orally treated with 1000 mg/kg of freeze dried *P. cystidiosus*. Groups 2 and 3 were orally administrated with 500 and 125 mg/kg of freeze dried *P. cystidiosus* respectively. The last group severed as the control and 2.5 ml of tap water was orally administrated per rat.

Twelve female rats in the di-oestrous stage of oestrous cycle were selected by a vaginal smearing. They were randomly divided into two equal groups (n = 6/group). Rats in the first group were orally treated with 1000 mg/kg of *P. cystidiosus* suspension. Other group was used as a control and was orally treated with 2.5 ml of tap water. The other two doses were not used in the study of antinociceptive potential as the highest dose did not show promising activity.

The reaction times of these rats were measured 1 h prior to the treatment and 1 to 5 h after the treatment at hourly intervals using hot plate and tail flick test methods (9). In the hot plate test, rats were placed in a hot plate analgesia meter (Model MK 35 A, Muromachi Kikai Co.Ltd., Tokyo, Japan) at 50^0 C and the time taken to lick either of the hind paws or to jump was recorded. The rats showing a pre treatment reaction time greater than 15 s in the hot plate test were not used in the experiment. A cut off time of 20 s was set to avoid tissue damages. In the tail flick test, the tail of the rat up to 5 cm from its tip was immersed in a water bath at 55^0 C and time taken to flick the tail was recorded using a stopwatch. Rats showing a pre treatment reaction time greater than 5 s in the tail flick test were not selected for the experiment. A cut off time of 5 s was set to avoid tissue damages.

Morphine was used as the reference drug and male rats (n = 6) were orally administered with 15 mg/kg of morphine.

Preparation of extracts of Pleurotus cystidiosus

Acetone extract

Fresh mushroom (2 kg) was homogenized with a homogenizer (Janke and Kunkel TR 50, Staufen, Germany) for two days in 3 l of distilled acetone and filtered. The filtrate (acetone extract) was concentrated using a rotary evaporator and freeze dried for two days to obtain (33.9 g, yield: 3.3%) a brown colour gum. This was reconstituted in tap water to study the antinociceptive activity.

Methylenechloride extract (CH_2Cl_2 extract)

The residue obtained from the above extraction was homogenized for three days in 3l of distilled methylenechloride and filtered. The filtrate (methylenechloride extract) was concentrated and evaporated all the methylene chloride to obtain (5.4 g, yield: 0.5%) a yellow solid after lyophilization. This solid was reconstituted in 5% Dimethylsufoxide (DMSO) prior to the administration of rats in studying the antinociceptive activity.

Treatment of rats with acetone and methylenechloride extracts

Two groups of male rats (n = 6) were treated with freeze dried acetone extract. The rats in group 1 were orally treated with 1000 mg/kg and group 2 was orally administrated with 500 mg/kg of freeze dried acetone extract, suspended in water (2.5 ml).

The rats administered with 1000 mg/kg dose (the highest dose) of the acetone extract was observed for any overt signs of clinical toxicity (in terms of salivation, diarrhoea, yellowing of fur, loss of fur, postural abnormalities, behavioral changes, impairment in food and water intake and body weight). A group of male rats (n = 6) was treated with freeze dried methylenechloride extract (500 mg/kg) dissolved in 2.5 ml of 5% DMSO. Another group of rats was given 2.5 ml of 5% DMSO solution and served as the control. The reaction times of these rats were measured 1 h prior to the treatment and at hourly intervals for 5 h after the treatment using hot plate and tail flick test methods.

Analysis of data

Data is given as mean ± standard error of mean. Data were analyzed with Mann-Whitney U-test. Statistical significance was set at $P \leq 0.05$.

RESULTS

Freeze dried P. cystidiosus

The reaction times after the oral administration of different doses of freeze dried mushroom *Pleurotus cystidiosus* are given in Table 1. The percentage increase in reaction time upon feeding 1000 mg/kg freeze dried *P.cystidiosus* in male rats is 21%, 93% and 20% (results compared to the pretreatment) at 1st, 2nd, and 3rd hours respectively (Table 1). This increase in the reaction time is marked and statistically significant ($p = 0.0025$) after the second hour of treatment. Upon feeding the male rats with mid dose (500 mg/kg dose) of *P.cystidiosus* suspension, significant ($p< 0.05$) prolongation of reaction time was observed at after 2 h (18%). In contrast lower dose (125 mg/kg) had no significant ($p< 0.05$) effect on the reaction time at any time point. The reference drug morphine profoundly and significantly ($p < 0.05$) increased the reaction time from the 1st to 4th h post treatment except at 3rd h.

The increase in the reaction time (Table 1) one hour after the treatment with freeze dried *P. cystidiosus* (1000 mg/kg) in female rats in the di-oestrous stage was marked (22%) and significant ($p < 0.05$).

On the other hand there was no significant increase in reaction time in the tail flick test upon feeding of 1000 mg/kg, 500 mg/kg and 125 mg/kg freeze dried *P.cystidiosus* (data not shown).

Acetone extract of *Pleurotus cystidiosus*

Upon feeding of 1000 mg/kg and 500 mg/kg of acetone extract (Table 2), percentage increase the reaction time on hot plate test at 2 h after the treatment was 49% ($p< 0.05$) and 23% ($p< 0.05$) respectively. Further, there was a statistical difference ($p< 0.05$) between the enhancements of reaction time 2 h after feeding of the two different doses. There was no significant increase in reaction time in the tail flick test upon feeding of 1000 mg/kg and 500 mg/kg of acetone extract. Further, the 1000 mg/kg dose of the acetone extract did not produce any overt signs of clinical toxicity.

Methylenechloride extract (CH_2Cl_2 extract) of *Pleurotus cystidiosus*

Methylenechloride extract at 500 mg/kg dose did not show a significant increase ($p>0.05$) in reaction time on the hot plate test (Table 2) as well as in the tail flick test.

Table 1. *Effect of oral administration of freeze dried P. cystidiosus on the hot plate reaction time of rats.*

Treatment (mg/kg)	Hot plate reaction time (s) (mean ± S.E.M.)					
	Pre-treatment	Post-treatment				
		1 h	2 h	3 h	4 h	5 h
Male						
Control	6.5 ± 0.6	5.5 ± 0.5	6.0 ± 0.5	5.0 ± 0.3	5.1 ± 0.7	5.2 ± 0.5
125	6.7 ± 0.4	7.1 ± 0.9	7.5 ± 0.6	6.5 ± 0.4	5.4 ± 0.5	5.1 ± 0.6
500	6.7 ± 0.4	6.3 ± 0.5	7.9 ± 0.2*	6.4 ± 0.5	6.7 ± 1.1	6.0 ± 0.7
1000	7.1 ± 0.6	8.6 ± 1.0	13.7 ± 1.2*	8.5± 0.6	7.9 ± 0.6	7.2 ± 0.6
Morphine	7.1 ± 0.4	14.4 ± 0.7*	15.8 ± 1.3*	9.2 ± 1.2	11.5 ± 1.0*	7.7 ± 0.6
Female						
Control	6.0 ± 0.5	7.8 ± 0.3	7.5 ± 0.7	6.7 ± 0.4	7.0 ± 0.5	7.1 ± 0.3
1000	6.3 ± 1.0	7.7 ± 0.7*	10.1 ± 2.2	8.2 ± 1.3	4.9 ± 0.8	6.2 ± 1.3

*n = 6 in all experiments ; * Significantly different ($p<0.05$) from the pre treatment values.*

Table 2. *Effect of oral administration of Acetone and CH_2Cl_2 extracts of P. cystidiosus on the hot plate reaction time of male rats.*

Treatment (mg/kg)	Hot plate reaction time (s) (mean ± S.E.M.)					
	Pre- treatment	Post-treatment				
		1 h	2 h	3 h	4 h	5 h
Acetone						
Control (n = 6)	6.5 ± 0.4	5.5 ± 0.4	6.0 ± 0.4	5.0 ± 0.2	5.1 ± 0.5	5.2 ± 0.4
500 (n = 10)	7.9 ±0.6	8.7 ± 0.7	9.7 ± 1.2*	9.2 ± 1.5	7.7 ± 0.8	7.1 ± 1.7
1000 (n = 10)	7.5 ± 0.6	8.8 ± 0.3	11.2 ± 0.4*	9.3 ± 0.9	8.7 ± 0.5	7.6 ± 0.4
CH_2Cl_2						
5% DMSO(n = 6)	9.7 ± 0.8	11.1 ± 0.7	8.8 ± 0.9	8.5 ± 0.8	7.5 ± 0.7	6.5 ± 0.4
500 (n=6)	8.6 ± 0.5	8.5 ± 0.4	8.3 ± 0.6	7.4 ± 0.4	6.8 ± 0.6	7.3 ± 0.7

* *Significantly different ($p<0.05$) from the pre treatment values.*

DISCUSSION

This study examined the antinociceptive potential of freeze dried, acetone extract and methylenechloride extract of *P.cystidiosus* following oral administration to rats. Two analgesiometric tests, tail flick and hotplate, were used in this study. These two tests are reliable, sensitive and widely used in testing potential antinociceptive agents (10). The overall results of this study show that *P.cystidiosus* increases the reaction time on the hotplate test while significant increase in reaction time was not observed in the tail flick test. This suggests that the antinociceptive effect may be mediated via supraspinal mechanisms (11).

The percentage increase in reaction time upon feeding 1000 mg/kg freeze dried mushroom to male rats suggests that the peak effect of the antinociceptive action is at 2 h. The same dose (1000 mg/kg) on females in the di-oestrous stage did not show a significant increase in the reaction time after 2 h of administration. Only the di-oestrous females rats were chosen for this study, as the rats in this stage of the oestrous cycle showed the highest activity in our previous study (8) on *Pleurotus ostreatus*. In the present study the results deviate slightly from the results reported for the mushroom, *P. ostreatus* where the highest activity was observed, 1 h after administration in both males and females. This may be possibly due to the quick absorption of *Pleurotus ostreatus*. Gender differences in analgesic response exist with certain antinociceptives (12), and the antinociceptive activity of *P. cystidiosus* is gender dependent at 2 h after administration. The antinociceptive activity of *P.cystidiosus* in male rats, appears to be dose-dependent possibly indicating receptor mediation. However, antinociceptive potential of *P. cystidiosus* is inferior to one of the most potent analgesic, morphine. This is the first report on the antinociceptive activity of *P.cystidiosus*, further studies are however required to ascertain the precise mode of antinociceptive action of *P.cystidiosus*.

The two extracts of *P. cystidiosus* namely the acetone and methylenechloride extracts were used to get a preliminary idea about the polarity of the compounds responsible for this antinociceptive activity. The results from the methylenechloride extract was not positive on both hot plate and tail flick assays, upon administration of 500 mg/kg dose indicating that the compounds responsible for the activity are not present in this extract. However, the same dose of acetone extract showed a significant increase in the reaction time in hot plate test, 2 h after the oral administration to the rats showing that the active compound/s is/are of more polar in nature. This antinociceptive activity was stronger in the 1000 mg/kg dose of the acetone extract reinforcing our conclusion on polarity of active compounds. Polysaccharopeptides have been isolated from the mushroom *Coriolus versicolor* and it is shown to possess analgesic activity on hot-plate test upon intraperitoneal administration to mice (13). These polysaccharopeptides are very polar in nature and similar polymeric compounds may be present in the *P.cystidiosus* acetone extracts. Moreover, simple organic compounds with phenolic groups have been isolated from mushrooms, possessing analgesic properties. Two such cases reported in the literature are Scutigeral from *Scutiger ovinus* (14) and Albaconol from *Albatrellus confluens* (15). Further studies are underway in the purification of the acetone extract to identify the active ingredient responsible for the said activity.

If the results are applicable to humans, we could conclude that consumption of *P.cystidiosus* may be beneficial as a remedy for the body aches and pains arising due to daily activities. Further, it may also be possible to isolate and characterize antinociceptive agents from *P.cystidiosus* mushroom.

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REFERENCES

1. H.C. Dube. An Introduction to fungi. (Vikas Publishing House Pvt. Ltd. ,Delhi, India. 1996) pp. 372
2. P. Bobek, L. Ozdin. Oyster mushroom (*Pleurotus ostreatus*) reduces the productivity and secretion of very low density lipoproteins in hypocholesterolemic rats. *Z Ernahrungswiss* **35(3)**: 249-252 (1996).
3. P. Bobek, L. Ozdin, I. Kajaba. Dose dependent hypocholesterolemic effect of oyster mushroom (*Pleurotus ostreatus*) in rats. *Physiol. Res.* **46 (4)**: 327-329 (1997).
4. S. Hossain, M. Hashimoto, E.K. Choudhury, N. Alam, S. Hussain, M. Hasan, S.K. Choudhury, I. Mahmud. Dietary mushroom (*Pleurotus ostreatus*) ameliorates atherogenic lipid in hypocholesterolemic rats. *Clin. Exp. Pharmacol. Physiol.* **30(7)**: 470-475 (2003).
5. P. Bobek, S. Galbavy. Effect of pleuran (beta-glucan from *Pleurotus ostreatus*) on the anti-oxidant status of the organism and on dimethylhydrazine induced precancerous lesion in rat colon. *Br. J. Biomed. Sci.* **58(3)**: 164-168 (2001).
6. H. Wang, T.B. Ng. A novel anti-fungal peptide from fruiting bodies of the edible mushroom *Pleurotus eringii*. *Peptides* **25(1)**: 1-5 (2004).

7. N. Jose, T. A. Ajith, K. K. Janardhanan. Methanol extract of the oyster mushroom, *Pleurotus florida*, inhibits inflammation and platelet aggregation. *Phytother. Res*. **18**: 43-46 (2004).
8. N.S. Vasudewa, D.T.U. Abeytunga, W.D. Ratnasooriya. Antinociceptive activity of *Pleurotus ostreatus,* an edible mushroom, in rats. *Pharm. Biol.* **45 (7)** 530-540 (2007).
9. L. Langerman, M.I. Zakouski, B. Piskoun, G.J. Grant. Hot plate versus tail flick evaluation of acute tolerance to continuous morphine infusion in the rat model. *J. Pharmacol. Toxicol. Methods* **34**: 23- 28 (1995).
10. W.D. Ratnasooriya, M.G. Dharmasiri. Water extract of leaves and stem of *Psychotria sarmentosa* has analgesic and antihyperalgesic activity in rats. *Med. Sci. Res.* **27**: 715-718 (1999).
11. C.H. Wong, P. Day, J. Yarmush, W. Wu, U.K. Zbuzek. Nifedipine-induced analgesic after epidural injections in rats. *Anesth. Analg*. **79**: 303-306 (1994).
12. R.W. Gear, N.C. Gordon, P.H. Heller, S.M. Paul, C. Miaskowski, J.D. Levine. Gender difference in analgesic response to the k-opioid pentazocine. *Neurosci. Lett.* **205**: 207-209 (1996).
13. T.B. Ng, W.Y. Chan. Polysaccharopeptide from the mushroom *Coriolus versicolor* possess analgesic activity but does not produce adverse effects on female reproductive or embryonic development in mice. *Gen. Pharmacol.: The vascular system* **29(2)**: 269-273 (1997).
14. A. Szallasi, T. Biro, T. Szabo, S. Modarres, M. Petersen, A. Klusch. A non-pungent triprenyl phenol of fungal origin, scutigeral, stimulates rat dorsal root ganglion neurons via interaction at vanilloid receptors. *Br. J. Pharmacol*.**126**: 1351–1358 (1999).
15. V. Hellwiq, R. Nopper, F. Mauler, J. Freitaq, L. Ji-Kai, D. Zhi-Hui, M. Stadler. Activities of prenylphenol derivatives from fruit bodies of *Albatrellus* spp. on the human and rat vanilloid receptor 1 (VR1) and characterization of the novel natural product, confluentin. *Arch. Pharm. (Weinheim).* **336 (2)**: 119-126 (2003).