A Comparison of Antinociceptive Activity of Mycelial Extract from Three Species of Fungi of Basidiomycetes

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Abstract: The antinociceptive activity of mycelial extract from fermented mushroom of *Ganoderma lucidum, Coprinus comatus* and *Grifola frondosa* were studied in this paper. The writhing test, formalin test and hot-plate test were used to evaluate the antinociceptive activity of the acetone extracts of the three Species of Fungi of Basidiomycetes in the study respectively. The extract of *Ganoderma lucidum* inhibited in a dose-dependent manner the acetic acid-induced abdominal constrictions in mice ($p<0.05$). Also both phases of the response were significantly inhibited in the formalin test ($p<0.05$). In the hot-plate test, results showed that extract of *Ganoderma lucidum* significantly inhibited the reaction time to thermal stimuli at 30, 60, and 90 min ($p<0.05$). The preliminary toxicologic study demonstrated the safety of it. However the extract from fermented mushroom of *Coprinus comatus* and *Grifola frondosa* did not show significantly expectative antinociceptive effect. Our results show the acetone extract of Ganoderma lucidum is a potent analgesic drug.

Key Words: Antinociceptive activity, *Ganoderma lucidum, Coprinus comatus, Grifola frondosa*.

1. INTRODUCTION

Various edible mushrooms have a long history of use in traditional Chinese medicine [1]. Some further mushroom extracts and compounds have been found with special central effects that could be of pharmacological interest. Marc Stadler [2] reported that acetone extract from several edible mushrooms of basidiomycetes, showed strong activities against neurolysin, a protease involved in the regulation of dynorphin and neurotensin metabolism. There have been report that the anti-inflammatory and antinociceptive properties of the methanol extract of I. obliquus may be due to the inhibition of inducible nitric oxide (NO) synthase (iNOS) and cyclooxygenase-2 (COX-2) expression *via* the down-regulation of nuclear factor kappaB (NF-kappaB) binding activity [3]. The n-BuOH subfraction of Phellinus linteus also showed highest inhibitory activity on the chick embryo chorioallantoic membrane (CAM) angiogenesis in a dose-dependent manner. The results suggest that Phellinus linteus has anti-inflammatory and antinociceptive activities, in addition to its anti-angiogenic activity [4]. *Ganoderma lucidum, Coprinus comatus* and *Grifola frondosa* are three Fungi of Basidiomycetes that have a long history of use in traditional Chinese medicine. An earlier study [5] performed with the CH2Cl2 extract of Ganoderma lucidum demonstrated that these extracts possess antinociceptive activity. But there were no reports about the acetone extract of Ganoderma lucidum, *Coprinus comatus* and *Grifola frondosa* on antinociceptive activity. The objectives of the present study were to compare the antinociceptive activity of mycelial extract from the three Species of Fungi of Basidiomycetes.

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2. MATERIALS AND METHODS

2.1. General

If not indicated otherwise, all chemicals were provided by Sigma Aldrich (Deisenhofen, Germany), while media ingredients was purchased from Tianjin

Xing Yu Chemical Co.,Ltd, China. Yuanhuzhitong capsules (YHZT) were purchased from Jinan Limeng Pharmaceutic Factory, China. Yuanhuzhitong capsule is a kind of Chinese medicine used in the treatment of pain. It is composed of two traditional Chinese herbs, including Rhizoma-CorydalisYanhusuo and Radixngelicae Dahuricae.

2.2. Fermented Mushroom of *Ganoderma lucidum, Coprinus comatus* and *Grifola frondosa*

Fermented mushroom of *Ganoderma lucidum* , *Coprinus comatus* and *Grifola frondosa* were produced in the Pharmaceutic Laboratory of Shandong University of Traditional Chinese Medicine, China.

The seed of *Ganoderma lucidum*, *Coprinus comatus* and *Grifola frondosa* was purchased from the Agricultural Culture Collection of China.

First, the seed was grown at 28°C for 5 days on PDA slants (1,000mL 20% potato extract liquid +20.0g dextrose +20.0g agar) and then maintained at 4°C in a refrigerator. Five to six pieces of the mycelia of the mushrooms were transferred from a slant into 250mL Erlenmeyer flasks containing 100mL liquid medium (20% potato extract liquid +2.0% dextrose +0.1% KH_2PO_4+0.05% $MgSO_2$). The culture was incubated at 27℃ on a rotary shaker at 180rmp for 3 days.

A 72-h-old liquid culture was homogenized using a sterilized blender and then inoculated to 500 mL Erlenmeyer

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flasks containing 300 mL of fermented culture medium (20% potato extract liquid +2.0% dextrose +0.1% KH_2PO_4+0.05% $MgSO_2$). The volume of inoculum was 15 mL, which was then cultivated under the same condition. The 72-h-old fermented liquid culture was the fermented mushroom.

2.3. Extracts from Fermented Mushroom of *Ganoderma lucidum* (EGL), *Coprinus comatus* (ECC)and *Grifola frondosa*(EGF)

Mycelia were separated from the culture fluid by filtration and extracted twice with acetone for 30 minutes in an ultrasonic bath. The extract was filtered, and the acetone was removed *in vacuo* (*ca*. 40°C, 250 mbar) to yield an aqueous residue. Then it was dried to powers.

2.4. Animal

Kunming strain mice weighing 20-22g, were purchased from the Experimental Animal Center, Shandong University. The mice were maintained at room temperature under alternating natural light/dark photoperiod, and had access to standard laboratory food and fresh water *ad libitum*.

2.5. Evaluation of Antinociceptive Activity

2.5.1. Writhing Test

This test is used for the evaluation of analgesic activity. Mice were treated with EGL, ECC and EGF respectively (1, 5, and 10 mg/kg, orally), 60 min before receiving a 0.6% acetic acid injection (10 mL/kg, *ip*). The number of contractions or writhings, determined by abdominal muscle contractions and hind paw extension was recorded for 20 min, starting 10 min after the administration of acetic acid [6]. YHZT (5 mg/kg, *po*) was used as standard.

2.5.2. Formalin Test

This test, which causes a local tissue injury to the paw, has been used as a model for tonic pain and localized inflammatory pain. For this, 20 μL of a 1%-formalin solution was injected into the right hind paw of mice, and the licking time was recorded after the first 5 min (1st phase, corresponding to a direct chemical stimulation of nociceptors) and after 20 min (2nd phase, involving inflammation), for 5 min each time. Animals were pretreated respectively with EGL, ECC and EGF 60 min (*po*) before intraplantar formalin injection [7,8]. YHZT was used as standard.

2.5.3. Hot-Plate Test

In this test, mice were pre-selected according to their reactions to a thermal stimulus (jumping or licking of hind limbs when placed on a hot plate at 55ºC). Latency times were recorded immediately before and 30, 60 and 90min after drug administration, up to a maximum time of 40 s to avoid paw lesions [9].

2.5.4. Acute Toxicity

The acute toxicity of EGL was investigated using a single oral administration of EGL in mice. In this assay, increasing doses of the test substance were orally administered to animals (up to 150 mg/kg). The animals were observed for14 days and at the end of this period the number of survivors was counted and body and spleen weight was recorded.

2.6. Statistical Analysis

All data were analyzed by a one-way analysis of variance, and the differences between means were established by Duncan's multiple-range test [10]. The data represents means and standard deviations. The significant level of 5% ($p<0.05$) was used as the minimum acceptable probability for the difference between the means.

3. RESULTS

In the writhing test, EGL (1, 5, and 10 mg/kg) inhibited in a dose-dependent manner the acetic acid-induced abdominal constrictions in mice after *po* (41, 61, and 83%) administration (Table **1**). As well as EGL, YHZT also inhibited the acetic acid-induced abdominal constrictions in mice. However, ECC and EGF, given orally 60 min before receiving a 0.6% acetic acid injection did not significantly inhibited the acetic acid-induced abdominal constrictions in mice.

In the formalin test (Table **2**), although both phases of the response were significantly inhibited, the EGL effect was predominant in phase 2, causing 61 and 48% inhibition of licking time at the doses of 5 and 10 mg/kg, *po*, similarly to YHZT. In this test, neither in phase 1 nor in phase 2, did ECC and EGF inhibit significantly the response caused by formalin.

The hot-plate test was performed for the assessment of the central antinociceptive effect of extracts (Table **3**). Results showed that EGL significantly inhibited the reaction time to thermal stimuli at 30, 60, and 90 min after *po* administration of 1, 5, and 10 mg/kg compared to controls. So is the YHZT. However, ECC and EGF did not show significantly antinociceptive effect.

In preliminary toxicologic study, deaths were not observed even at the level of 150 mg/kg. In addition, EGL did not cause significant changes in body or spleen weight of the animals, demonstrating the safety of EGL (Table **4**).

4. DISCUSSION

The acetic acid-induced writhing reaction in mice, described as a typical model for inflammatory pain, has long been used as a screening tool for the assessment of analgesic or anti-inflammatory properties of new agents [11]. The constrictions induced by acetic acid in mice result from an acute inflammatory reaction related to the increase in the peritoneal fluid levels of PGE_2 and $PGF_2\alpha$ [12]. The fact that EGL was able to inhibit constrictions showed that this fraction has a peripheral antinociceptive effect.

The formalin test is different from most models of pain. It is possible to assess the way an animal responds to moderate, continuous pain generated by injured tissue. This model is constituted by two distinct phases. The first phase represents the irritating effects of formalin at the sensorial fibers-C [13]. The second is an inflammatory pain response. Thus, it's possible to appraise the animal's answer to a moderate and continuous pain caused by the tissue lesion as well as the role of pain regulatory endogenous systems. The formalin test indicated that both peripheral analgesic properties and central analgesic effects are the antinociception mechanism of EGL. To corroborate that EGL also had central analgesic actions, hot plate test were conducted.

Table 1. Inhibitory Effect of Extracts in Mice Submitted to the Writhing test (N = 10)

Dose/Route of Administration	Number of Contraction (20 min)	Inhibition (%)
Control	23.5 ± 1.6	-
Distilled water 1 mL/kg, *po*		
EGL 1 mg/kg, *po*	13.9 ± 1.8	41%*
EGL 5 mg/kg, *po*	9.0 ± 1.9	62%*
EGL 10 mg/kg, *po*	4.1 ± 1.7	83%*
ECC 1 mg/kg, *po*	19.0±1.6	19%
ECC 5 mg/kg, *po*	18.6±1.4	21%
ECC 10 mg/kg, *po*	17.0±2.1	28%
EGF 1 mg/kg, *po*	18.8±1.3	20%
EGF 5 mg/kg, *po*	17.9±1.6	24%
EGF 10 mg/kg, *po*	17.1±1.5	27%
YHZT 5 mg/kg, *po*	3.9± 1.7	83%*

The asterisks in the last column indicate a statistical difference ($p<0.05$).

Table 2. Antinociceptive Effect of Extracts in Mice Submitted to the Formalin Test (N = 10)

Dose/Route of Administration	Licking Time (s)		Inhibition (%)	
	1st Phase	2nd Phase	1st Phase	2nd Phase
Control	61.2 ± 5.5	31.4 ± 3.0	-	-
Distilled water 1 mL/kg, *po*				
EGL 1 mg/kg, *po*	64.7 ± 3.6	46.5 ± 3.9	-	-
EGL 5 mg/kg, *po*	71.9± 4.7	12.4± 3.7	-	61%*
EGL 10 mg/kg, *po*	53.2 ± 2.9	16.7 ± 3.3	13%	47%*
ECC 1 mg/kg, *po*	74.7 ± 3.0	56.5 ± 3.8	-	-
ECC 5mg/kg, *po*	70.9± 4.7	43.4± 3.7	-	-
ECC 10 mg/kg, *po*	59.6 ± 3.7	29.5 ± 3.9	3%	6%
EGF 1 mg/kg, *po*	81.8± 4.7	43.2± 3.5	-	-
EGF 5 mg/kg, *po*	70.7± 3.6	33.4± 2.7	-	-
EGF 10 mg/kg, *po*	51.7± 4.2	28.4± 2.9	2%	10%
YHZT 5 mg/kg, *po*	50.2 ± 3.0	12.9± 3.6	11%	60%*

The asterisks in the last column indicate a statistical difference ($p<0.05$).

The hot-plate test is commonly used to assess narcotic analgesics or other centrally acting drugs [14]. The hot-plate test was performed for the assessment of the central antinociceptive effect of EGL. Results showed that EGL also had central analgesic actions.

Although Marc Stadler [2] reported that acetone extract from several edible mushrooms of basidiomycetes, showed antinociceptive effect, the acetone extract from *Coprinus comatus* and *Grifola frondosa* did not show the expectative antinociceptive effect.

Table 3. Effect of Extracts in Mice Submitted to the Hot-Plate Test (N = 10)

Dose/Route of Administration	Reaction Time to the Thermal Stimulus (s)			
	0 min	**30 min**	**60 min**	**90 min**
Control	11.8 ± 0.8	10.6 ± 0.9	8.3 ± 0.80	9.5 ± 0.6
Distilled water 1 mL/kg, *po*				
EGL 1 mg/kg, *po*	13.9 ± 1.1	8.2 ± 0.9	10.4 ± 1.7	7.5 ± 0.9
EGL 5 mg/kg, *po*	7.9 ± 0.9	10.5 ± 1.1	16.4 ± 0.8 *	15.8 ± 1.2*
EGL 10 mg/kg, *po*	10.9 ± 1.2	14.3 ± 0.8 *	15.8 ± 1.1 *	16.4 ± 1.1*
ECC 1 mg/kg, *po*	9.9 ± 1.0	9.2 ± 1.9	9.6 ± 1.8	8.5 ± 0.9
ECC 5mg/kg, *po*	10.9 ± 1.9	10.2 ± 0.9	10.5 ± 1.8	9.5 ± 1.9
ECC 10 mg/kg, *po*	12.9 ± 0.9	11.6 ± 1.7	11.4 ± 0.7	9.9 ± 1.4
EGF 1 mg/kg, *po*	8.9 ± 1.1	7.2 ± 1.9	9.4 ± 0.9	9.5 ± 1.7
EGF 5 mg/kg, *po*	9.9 ± 2.0	8.7 ± 0.7	10.8 ± 1.5	10.7 ± 0.9
EGF 10 mg/kg, *po*	10.8 ± 0.8	10.2 ± 1.5	11.4 ± 2.7	9.9 ± 012
YHZT 5 mg/kg, *po*	9.9 ± 1.0	13.5 ± 1.1*	16.4 ± 0.8*	16.4 ± 1.1*

The asterisks in the last column indicate a statistical difference ($p<0.05$).

5. CONCLUSION

In the present study, we report a comparison of antinociceptive activity of mycelial extract from three Species of Fungi of Basidiomycetes, *Ganoderma lucidum*, *Coprinus comatus* and *Grifola frondosa*. Our results show that EGL is a potent analgesic drug. However the ECC and EGF did not show significantly expectative antinociceptive effect. Although Marc Stadler [2] reported that acetone extract from several edible mushrooms of basidiomycetes, showed antinociceptive effect, not all the acetone extract from edible mushrooms of basidiomycete show analgesic actions.

Table 4. The Acute Toxicity Trial of EFMG on Mice (N = 12)

	0d	**14d**
Deaths	-	0
Body weight(g)	21.2 ± 1.0	23.0 ± 1.3
Spleen weight(mg/g)	4.2 ± 1.6	4.4±1.0

No significant changes in body or spleen weight were observed at the dose level of 150mg/kg.

As an edible mushroom of basidiomycetes, acetone extract of Ganoderma lucidum, showed potent antinociceptive activity. The preliminary toxicologic study demonstrated the safety of EGL. It supports the folk medicinal use of this mushroom. Further studies currently in progress will enable us to understand the mechanisms of action underlying the effects observed in this investigation. And there is a need for further studies on the identification and isolation of biologically active natural products from Ganoderma lucidum.

REFERENCES

[1] Demirbas A. Heavy metal bioaccumulation by mushrooms from artificially fortified soils. Food Chem 2001; 74: 293-1.

[2] Stadler M, Hellwig V. Novel analgesic triglycerides from cultures of *Agaricus macrosporus* and other basidiomycetes as selective inhibitors of neurolysin. J Antibiot 2005; 58: 775-6.

[3] Kim HG, Yoon DH, Kim CH. Ethanol extract of inonotus obliquus inhibits lipopolysaccharide-induced inflammation in RAW 264.7 macrophage cells. J Med Food 2007; 10: 80-9.

[4] Kim SH, Song YS, Kim SK. Anti-inflammatory and related pharmacological activities of the n-BuOH subfraction of mushroom *Phellinus linteus*. J Ethnopharmacol 2004; 93: 141-6.

[5] Koyama K, Imaizumi T, Akiba M. Antinociceptive components of Ganoderma lucidum. Planta Med 1997; 63: 224-7.

[6] Koster R, Anderson M, DeBeer EJ. Acetic acid for analgesic screening. Fed Proc 1959; 18: 418-20.

[7] Fasmer OB, Berge OG, Hole K. Changes in nociception after lesions of descending serotonergic pathways induced with 5,6-dihydroxytryptamine. Different effects in the formalin and tail-flick tests. Neuropharmacology 1985; 24: 729-34.

[8] Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1987; 30: 103-14.

[9] Woolfe G, MacDonald AD. The evaluation of the analgesic action of pethidine hydrochloride (demerol). J Pharmacol Exp Ther 1944; 80: 300-7.

[10] Duncan DB. Multiple range tests for correlated and heteroscedastic means. Biometrics 1957; 13: 164-76.

[11] Collier HOJ, Dinneen JC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother 1968; 32: 295-10.

[12] Deraedt R, Jouquey S, Delevalée F *et al.* Release of prostaglandins-E and prostaglandins-F in an algogenic reaction and its inhibition. Eur J Pharmacol 1980; 61: 17-24.

[13] Hosseinzadeh H, Ramezani M, Salmani GA. Antinociceptive, anti-inflammatory and acute toxicity effect of Zataria multiflora Boiss extracts in mice and rats. J Ethnopharmacol 2000; 73: 379-85.

[14] Beirith A, Santos ARS, Rodrigues ALS, *et al*. Spinal and supraspinal antinociceptive action of dipyrone in formalin, capsaicin and glutamate tests. Study of the mechanism of action. Eur J Pharmacol 1998; 345: 233-45.

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