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Anti-inflammatory and Anti-nociceptive Activities of *Ganoderma lucidum* Occurring in South India

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Abstract

The seventy percent methanol and ethyl acetate extracts of *Ganoderma lucidum* Reishi, occurring in South India, were investigated for anti-nociceptive and anti-inflammatory activities in animal models. Both extracts produced significant dose-dependent inhibition of carrageenan-induced acute and formalin-induced chronic inflammation in mice. The extracts also showed marked analgesic activity comparable to the standard reference drug, diclofenac. The methanol extract possessed significantly higher activity than the ethyl acetate extract. These findings indicate the potential therapeutic use of extracts of South Indian *G. lucidum* as an anti-inflammatory and anti-nociceptive agent.

Keywords: Anti-inflammatory, anti-nociceptive, medicinal mushroom, *Ganoderma lucidum.*

Introduction

The fruiting bodies of *Ganoderma lucidum* (Fr.) P. Karst (Aphyllophoromycetideae), commonly known as Reishi, have long been prescribed in Chinese medicine as a tonic and sedative (Shiao et al., 1994). Reishi has been used in folk medicine in China and Japan for 4000 years for a wide range of ailments. In Chinese folklore it has been regarded as panacea for all types of diseases, perhaps due to its demonstrated efficacy as a popular remedy to treat a large number of diseases (Jong & Birmingham, 1992). However, the extensive range of traditional medical treatment with *Ganoderma* has not yet been fully substantiated by modern scientific standards. The fruiting bodies of *G. lucidum* contain a variety of chemical substances; the major components are terpenes and polysaccharides. The mushroom is a rich source of triterpenes, as it is currently reported to contain 119 different triterpenes (Kim & Kim, 1999). *Ganoderma* species were classified into several types and each type has its own characteristic and biological activities (Liu, 1999). It is reported that some of the physiological effects and distinctive properties of *Ganoderma* are strain dependent (Nishitoba et al., 1986). For chronic diseases, such as osteoarthritis and rheumatoid arthritis, life-long dependency on anti-inflammatory drugs is necessary. The most widely used nonsteroidal anti-inflammatory drugs (NSAID) suffer from several side effects. Hence, the search for effective anti-inflammatory agents that could be safely used on a long-term bases recently focused on this ancient medicinal mushroom (Chen, 2001). In this communication, we report the anti-inflammatory and anti-nociceptive activities of the methanol and ethyl acetate extracts of *Ganoderma lucidum* occurring in the tropical South India.

Materials and methods

Plant material

Fruiting bodies of *G. lucidum* were collected from different locations in the Thrissur District, Kerala, South India. The specimen was identified with the help of the literature and the identification was confirmed by comparison with type specimens. A voucher specimen is deposited in the herbarium of Centre for Advanced Studies in Botany, University of Madras, Chennai (HERB MUBL-3172).

Preparation of extracts

Fruiting bodies of the mushroom were cut into small pieces, dried at 45–50 °C for 48 h, and powdered. One hundred

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grams of the powdered material was extracted with petroleum ether using a Soxhlet apparatus. The defatted material was then extracted with ethyl acetate by the same process. The ethyl acetate extract was evaporated to dryness at 40 °C using a rotary vacuum evaporator (2 g). After ethyl acetate extraction, the sample was air-dried, suspended in 70% methanol and boiled for 8 h. The solvent was removed and the extraction repeated once again. The methanol extracts were combined, filtered through Whatman No. 1 filter paper, concentrated on a waterbath at 60–70 °C, and finally lyophilised (4 g).

For animal experiments, the ethyl acetate extract was pre-solubilised in 0.1% ethanol and a fine suspension was prepared in 2% olive oil. The methanol extract was pre-solubilised in distilled water.

Test animals

Male Balb/c mice (18–20 g) were used to study anti-inflammatory activity and male Swiss albino mice (25–30 g) were used for anti-nociceptive activity. The animals were purchased from Small Animal Breeding Centre of Kerala Agricultural University, Mannuthy, Thrissur. The animals were maintained in environmentally controlled conditions, fed standard diet and given water *ad libitum*.

Anti-inflammatory activity

Acute and chronic anti-inflammatory activities of the extracts were evaluated by carrageenan-induced acute and formalin-induced chronic inflammatory models in mice.

Carrageenan-induced paw edema in mice

Animals were divided into six groups with six animals in each group. In all groups, acute inflammation was induced by subplantar injection of 20 µl of a freshly prepared 1% suspension of carrageenan in normal saline in the right hind paw of mice. The paw thickness was measured using vernier calipers before and 3 h after carrageenan challenge in each group. Animals were premedicated with vehicle (0.1% ethanol in 2% olive oil, group 1), ethyl acetate extract of the mushroom (500 and 1000 mg/kg bodyweight, groups 2 and 3) or methanol extract (500 and 1000 mg/kg body weight, groups 4 and 5) orally, 1 h before carrageenan injection. The reference drug, diclofenac (10 mg/kg body weight, group 6), was administered i.p. 30 min before carrageenan injection (Ajith & Janardhanan, 2001).

Formalin-induced paw edema

The animals were treated in the same way as in the above models, except that a single dose of formalin (20 µl of freshly prepared 2% formalin) was used as the edematogenic agent. The drug treatment continued for 6 consecutive days. Diclofenac (10 mg/kg body weight, i.p.) was used as the reference drug.

In all the above models, the degree of edema formation was determined as an increase in paw thickness. The increase in paw thickness and percent inhibition were calculated as follows.

Increase in paw thickness in control / treatment

$$P_C / P_T = P_t - P_0$$

$$\text{Percent inhibition} = \frac{P_C - P_T}{P_C} \times 100$$

Where P_t is paw thickness at time t, P_0 is initial paw thickness, P_C is the increase in paw thickness of the control group, and P_T is the increase in paw thickness of the treatment groups (Ajith & Janardhanan, 2001).

Anti-nociceptive activity

Writhing test

Animals were divided into six groups of six animals in each group. The writhing resulting from intraperitoneal injection of 0.2 ml acetic acid (0.6%), consisting of a contraction of the abdominal muscle together with a stretching of hind limbs, was carried out according to procedures described by de Souza et al. (2000). Animals were treated with the methanol and ethyl acetate extracts (500 and 1000 mg/kg) orally, 60 min prior to acetic acid injection. After acetic acid challenge, pairs of mice were placed in separate boxes. The number of writhing movements were counted 8 min after acetic acid injection in each mouse for 20 min. Diclofenac (10 mg/kg i.p.) was used as the reference drug. The percentage of the protection was calculated as follows.

$$\% \text{ Protection} = \frac{\text{Control mean} - \text{Treated mean}}{\text{Control mean}} \times 100$$

Statistical analysis

The data were statistically analyzed using Student's *t*-test and *P* values less than 0.05 were considered as significant. All data were represented as mean ± SD.

Results and discussion

The results of the investigations showed that ethyl acetate and 70% methanol extracts of *G. lucidum* possessed a significant effect on carrageenan-induced acute and formalin-induced chronic inflammation in mice (Tables 1 and 2). Anti-inflammatory activity of the extracts was remarkably high at a concentration of 1000 mg/kg body weight. The ethyl acetate and methanol extracts at this concentration inhibited 41.6 and 58.3% inflammation, respectively. The antiinflammatory effect of the extract in both models was comparable to that of the standard reference drug, diclofenac. The methanol extract showed higher activity than the reference drug.

The *in vivo* antiinflammatory activity of *G. lucidum* extracts was mediated in a dose-dependent manner. Car-

Table 1. Effect of *G. lucidum* extracts on carrageenan-induced paw edema in mice.

Treatment	Dose (mg/kg)	Initial paw thickness (cm)	Paw thickness after 3 hr (cm)	Increase in paw thickness (cm)	Inhibition (%)
Control	Vehicle	0.160 ± 0.011	0.28 ± 0.013	0.120 ± 0.022	–
Ethyl acetate	500	0.161 ± 0.012	0.257 ± 0.015	0.096 ± 0.077***	20.0
	1000	0.160 ± 0.011	0.230 ± 0.017	0.070 ± 0.011*	41.6
Methanol	500	0.161 ± 0.013	0.250 ± 0.013	0.089 ± 0.006**	25.8
	1000	0.170 ± 0.011	0.220 ± 0.011	0.050 ± 0.004*	58.3
Standard Diclofenac	10	0.177 ± 0.012	0.237 ± 0.015	0.060 ± 0.013*	50.0

Values are mean ± S.D. (n = 6) * P < 0.001, ** P < 0.005, *** P < 0.02, with respect to control.

Table 2. Effect of *G. lucidum* extracts on formalin-induced paw edema in mice.

Treatment	Dose (mg/kg)	Initial paw thickness (cm)	Paw thickness after 3 hr (cm)	Increase in paw thickness (cm)	Inhibition (%)
Control	Vehicle	0.162 ± 0.017	0.410 ± 0.028	0.248 ± 0.028	–
Ethyl acetate	500	0.170 ± 0.016	0.330 ± 0.021	0.160 ± 0.021*	35.4
	1000	0.160 ± 0.012	0.280 ± 0.023	0.120 ± 0.024*	51.6
Methanol	500	0.170 ± 0.012	0.325 ± 0.019	0.155 ± 0.016*	37.5
	1000	0.160 ± 0.014	0.260 ± 0.013	0.100 ± 0.011*	59.6
Standard Diclofenac	10	0.170 ± 0.017	0.315 ± 0.015	0.145 ± 0.014*	41.5

Values are mean ± S.D. (n = 6). * P < 0.001 with respect to control.

rageenan-induced acute inflammation in animals is one of the most suitable test procedures to screen anti-inflammatory agents. The carrageenan-induced edema is caused by activation of platelet activating factor (PAF), prostaglandins and other inflammatory mediators (Hwang et al., 1986). The first phase is attributed to the release of histamine, 5-HT and kinins, while the second phase is related to the release of prostaglandins (Larsen & Henson, 1983; Brooks & Day, 1991; Vane & Booting, 1987). Carrageenan also induces a protein rich exudate containing a large number of neutrophills (Lo et al., 1982). Formalin-induced paw edema is also one of the most suitable test procedures to screen chronic anti-inflammatory agents, as it closely resembles human arthritis (Greenwald, 1991). The nociceptive effect of formalin is also biphasic, an early neurogenic component followed by a later tissue mediated response (Wheeler-Aceto & Cowan, 1991). The interference of vehicle in the inhibition of inflammation was also evaluated. The results indicate that vehicle does not show inhibition at the given concentration and therefore could be employed to pre-solubilise the extracts.

Experimental results showed that ethyl acetate and methanol extracts of *G. lucidum* inhibited the acetic acid-induced abdominal constriction response in mice. The methanol extracts, at a concentration of 1000 mg/kg, inhibited 40.6% abdominal constrictions (Table 3). This indicated that the methanol extract of the South Indian strain of *G. lucidum* possessed significant anti-nociceptive activity and the effect was dose-dependent. However, the ethyl acetate extract possessed only weak anti-nociceptive activity. Koyama et al. (1997) reported that methanol and aqueous extracts of the fruiting bodies of the commercial strain of *G. lucidum* possessed 33.9% and 37.9% protective effect against acetic acid-induced writhes after the administration of the nociceptive agent by s.c. injection. This indicates that the South Indian strain of *G. lucidum* has equally potent or higher anti-nociceptive activity than the commercial strain. The acetic acid-induced writhing method is used as a first screening test of analgesic activity and anti-inflammatory drugs, muscle relaxant and anti-histaminics, also show activity (Koyama et al., 1997).

The preliminary phytochemical analysis showed the presence of terpenoids in ethyl acetate and methanol extracts. The methanol extract was also found to contain polysaccharide as one of the major components. These phytochemicals might be responsible for the anti-inflammatory and anti-nociceptive activity of the extracts of *G. lucidum*. Koyama et al. (1997) identified ganoderic acids A, B, G and H, as triterpene

Table 3. Antinociceptive effects of extracts of *G. lucidum* against acetic acid-induced nociceptive response (writhing test).

Treatment	Dose (mg/kg)	Writhing movement	Inhibition (%)
Control	Vehicle	59 ± 6.58	–
Ethyl acetate	500	53 ± 3.01***	10.1
	1000	45 ± 4.50**	23.7
Methanol	500	38 ± 2.32*	36.0
	1000	35 ± 0.67*	40.6
Standard			
Diclofenac	10	21 ± 0.66*	64.4

Values are mean ± S.D. (n = 6), * P < 0.001, ** P < 0.005, *** P < 0.02, with respect to control.

components of a commercial strain of *G. lucidum* with anti-nociceptive activity. However, the methanol extract of South Indian *G. lucidum* showed higher anti-inflammatory and anti-nociceptive activities than the ethyl acetate extract, indicating the probable involvement of both terpenes and polysaccharides with these effects. Inflammation, a fundamental protective response, may be harmful in conditions such as life threatening hypersensitive reactions to insect bites, drugs, toxins, and in certain chronic diseases such as rheumatoid arthritis, atherosclerosis and lung fibrosis (Collins, 1999).

In conclusion, the ethyl acetate and methanol extracts of South Indian *G. lucidum* exhibited significant anti-inflammatory activity in acute and chronic inflammation models in mice. The methanol extracts possessed significant anti-nociceptive activity, while the ethyl acetate extract had only weak analgesic activity. These findings suggest the therapeutic potential of this mushroom and its metabolites for the prevention and control of diseases mediated through inflammation and nociception.

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