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Antioxidative and Antiinflammatory Activities of the Chloroform Extract of *Ganoderma lucidum* Found in South India

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Abstract

Antioxidative and anti-inflammatory activities of *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae) from tropical South India were investigated. The chloroform extract of the mushroom showed marked free radical scavenging activities. The anti-inflammatory activity of the extract at concentrations of 100 and 50 mg/kg was evaluated in carrageenan induced acute and formalin induced chronic inflammatory models in mice. The extract showed remarkable antiinflammatory activity in both models, comparable to the standard reference drug diclofenac. The results suggest that anti-inflammatory activity of the chloroform extract of *G. lucidum* is possibly attributed to its free radical scavenging properties. This study also reveals the potent therapeutic uses of *G. lucidum* from South India.

Keywords

Medicinal mushrooms • *Ganoderma lucidum* • Chloroform extract • Antioxidative activity • Antiinflammatory activity

Introduction

Mushrooms represent a major and as yet largely untapped source of potent pharmaceutical products. Nearly 10,000 mushroom species are known, of which 2000 are safe for humans and about 300 of them possess medicinal properties. *Ganoderma lucidum,* has been used in folk medicine in China and Japan for over 2000 years for a wide range of ailments. In Chinese folklore, fruiting bodies of *Ganoderma* have been regarded as a panacea for all types of diseases, perhaps due to its demonstrated efficacy as a popular remedy to treat a large number of diseases, namely chronic hepatitis, arthritis, hypertension, hyperlipidemia, insomnia, bronchitis, neoplasia, asthma, gastric ulcer, atherosclerosis, diabetes, debility due to prolonged illness etc [1]. Almost all medicinal properties have been attributed to *G. lucidum* and thus, it is known as 'mushroom of immortality' in China, Japan and Korea.

Ganoderma spp. occur in morphological types such as black, light black, red, purple, yellow and white. Each type of *Ganoderma* has its own characteristic biological properties. The commonly used medicinal *Ganoderma* include *G. lucidum*, *G. tsuge, G. capense* and *G. applanatum*. The fruiting bodies of *G. lucidum* contain a variety of chemical substances, major components are terpenoids and polysaccharides. Currently 130 triterpenoids, and more than 100 types of polysaccharides are reported from *G. lucidum* [2].

In biological systems potentially harmful reactive oxygen species (ROS) are produced during the normal aerobic metabolism. Antioxidants are deployed to prevent generation of ROS or to scavenge those formed. Deficiency of antioxidative defenses may lead to oxidative stress, which might be associated with a variety of disorders such as coronary heart diseases, neural disorders, diabetes, arthritis and cancers [3, 4]. For chronic diseases, such as osteoarthritis and rheumatoid arthritis, life long dependency on anti-inflammatory drugs is necessary. The most widely used non-steroidal antiinflammatory drugs (NSAID) suffer from several side effects. Hence, the search for effective antiinflammatory agents that could be safely used on a long-term basis is a priority [5].

G. lucidum has been found to occur widely in India particularly in the tropical areas. Previous studies at Amala Cancer Research Centre showed that the methanolic extract of *G. lucidum* occurring in tropical South India possessed significant antioxidative and antiinflammatory activities [6],[7]. Some physiological effects and distinctive properties of *Ganoderma* are strain dependent and evidence for strain specific terpenoids has been reported in this mushroom [8]. In this communication, we report the antioxidative and antiinflammatory activities of the chloroform extract of *G. lucidum* occurring in the tropical South India.

Experimental

Plant collection and identification

Fruiting bodies of *G. lucidum* growing on gulmohar trees (*Delonix regia* Raf.) were collected from the out skirts of Thrissur, Kerala, South India. The specimen was identified by Prof. K.M. Leelavathi (Dept. of Botany, Calicut University, Calicut, India). A voucher specimen is deposited in the herbarium of Centre for Advanced Studies in Botany, University of Madras, Chennai (HERB.MUBL-3172).

Phytochemical procedures

Preparation of extract

Fruiting bodies of the mushroom were cut into small pieces, dried at 45–50°C for 48 h and powdered. The powdered material (200 g) was extracted with petroleum ether using a Soxhlet apparatus for 24 h. The defatted material was then extracted with chloroform by the same process. The chloroform extract was evaporated to dryness at 40°C using a rotary vacuum evaporator. The residue (3g) thus obtained was used for the experiments.

Phytochemical screening

Phytochemical screening for secondary metabolites in the chloroform extract was carried out by Mayer's and Dragendorff's tests (alkaloids), Shinoda's test (flavonoids), ethanolic KOH test (coumarins), Libermann-Burchard test (terpenoid/steroids) and froth formation test (saponins) [9–11]. The chloroform extract of *G. lucidum* dissolved in chloroform and applied onto the silica gel HPTLC plate (60 F 254, E. Merck, Germany, 10 × 10 cm) as 6 mm wide bands with an automatic Linomat V applicator with N_2 flow (Camag, Switzerland). The HPTLC plate was developed to a height of 80 mm in hexane-chloroform-methanol (1.5 : 7.5 : 1) with pre-saturation for 15 min. in a Camag twin trough glass tank. After development, the plate was derivatized in anisaldehyde-sulfuric acid, dried and spots were visualized in white light and scanned at 580 nm (visible, tungston lamp) using Camag TLC Scanner 3 equipped with Wincat software at slit width 5 × 0.45 mm.

Pharmacological evaluation

Determination of antioxidant activity

In vitro antioxidant activity of the extract was determined by DPPH (1,1 diphenyl 2-picryl hydrazyl) scavenging activity using AEAC (Ascorbic acid equivalent antioxidant capacity) as standard. AEAC is the concentration of ascorbic acid required to give the same antioxidant capacity as test substance [12]. Superoxide radical, lipid peroxidation and nitric oxide scavenging activities of the extract were determined by finding out IC_{50} values.

DPPH radical scavenging activity

In this method DPPH (2,2-diphenyl-1-picrylhydrazyl) dissolved in methanol was used [13].

Superoxide anion scavenging activity

Superoxide anion scavenging activity was determined according to the method of McCord and Fridovich (1969) [14]. Quercetin was used as standard.

Inhibition of lipid peroxidation

Lipid peroxidation induced in rat liver homogenate [15] and its inhibition by the extract was determined by the method of Ohkawa et al. [16]. Ascorbic acid was used as standard.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined by the method of Sreejayan and Rao (1997) [17]. Quercetin was used as standard.

Determination of antiinflammatory activity

Acute and chronic antiinflammatory activities of the extracts were evaluated by carrageenan-induced acute and formalin-induced chronic inflammatory models in mice. The extract was administered orally.

Carrageenan-induced paw edema

Male Swiss albino mice were divided into four groups of six animals in each group. In all groups the inflammation was induced by single sub-plantar injection of 20 µl of freshly prepared 1% carrageenan suspension in normal saline [18]. Group 1 treated with carrageenan alone served as control. Group 2 and 3 received *G. lucidum* extract at concentrations of 50 and 100 mg/kg body wt. orally 1 h before the carrageenan injection. The extract was presolubilized in 0.2% dimethyl sulfoxide (DMSO) and a fine suspension was prepared in phosphate buffered saline. Group 4 was administered with reference drug diclofenac (10 mg/kg body weight) also orally 1 h before carrageenan injection [18]. The paw thickness of animals in all groups was measured using vernier calipers before and 3 h after carrageenan injection.

Formalin – induced paw edema

Experimental procedure was the same as described above except that single dose of 0.02 µl of formalin (2%) was used to induce inflammation [18]. The extract was administered once daily for 6 consecutive days [19].

In the above two models, the degree of edema formation was determined as increase in paw thickness. In the case of acute anti-inflammatory activity, paw thickness was measured once daily for 6 days. The increase in paw thickness and percent inhibition were calculated as follows.

Increase in paw thickness in control/treatment $\frac{P_c}{P_T} = P_t - P_0$

$$Percent\ inhibition = \left(\frac{P_c - P_T}{P_c} \right) \cdot 100$$

Where P_t is paw thickness at time t, P_o 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 [16].

Animal Experiments

All animal experiments were carried out according to the guidelines of the Committee for the Purpose of Control of Experiments on Animals (Reg. No. 149/1999/CPCSEA) and approval of the Institutional Animal Ethics Committee was obtained.

Statistical analysis

Experimental data are expressed as mean ± SD. Student's t test was applied for expressing the significance and P value less than 0.05 was considered as significant.

Results

Phytochemical screening

The chloroform extract of *G. lucidum* was tested positive for terpenoids and alkaloids in preliminary phytochemical tests. The HPTLC fingerprint of the chloroform extract is shown in Fig. 1.

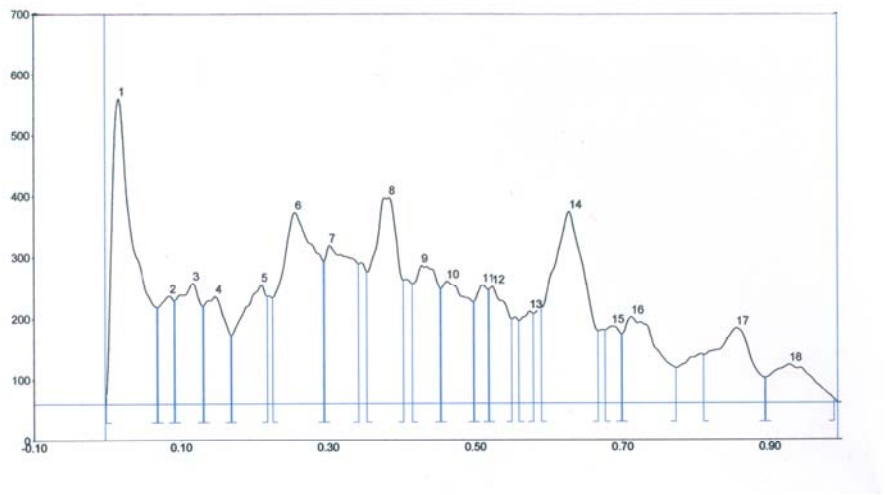

Fig. 1. HPTLC profile of the chloroform extract of *G. lucidum* in 1.5 : 7.5 : 1 hexane-chloroform-methanol, derivatized in anisaldehyde-sulfuric acid and scanned at 580 nm.

Antioxidant capacity of extract: DPPH radical scavenging activity

The results showed that the chloroform extract of *G. lucidum* showed potent radical scavenging activity. The activity of the extract at different concentrations is presented in Table 1. The result indicated free radical scavenging activity of the extract in a dose dependent manner.

Superoxide radical scavenging activity

The chloroform extract of *G. lucidum* was found to scavenge superoxide generated by photoreduction of riboflavin (Table 2). The extract showed significant superoxide scavenging activity (IC_{50}: 144.6 ± 1.5 μg/ml).

Inhibition of lipid peroxidation

Chloroform extract of *G. lucidum* was effective in inhibiting the lipid peroxidation induced by Fe^{2+}-ascorbate system in rat liver homogenate (Table 2). The generation of malondialdehyde (MDA) and related substances that react with thiobarbituric acid (TBARS) was found to be inhibited by the extract. This indicated lipid peroxidation inhibiting activity of the extract (IC_{50}: 593.3 ± 25.1 μg/ml).

Nitric oxide radical scavenging activity

The chloroform extract of *G. lucidum* effectively reduced the generation of nitric oxide radicals from sodium nitroprusside solution (Table 2). This showed marked nitric oxide scavenging activity of the extract (IC_{50}: 21.6 ± 1.5 µg /ml).

Tab. 1. Free radical scavenging activity of chloroform extract of *G. lucidum* by DPPH reduction:

Concentration (µg/ml)	Radical scavenging (%)
100	24.46 ± 1.14
300	58.81 ± 3.79
500	72.86 ± 1.30
700	83.77 ± 1.82
1000	91.12 ± 1.10
Ascorbic acid (100µM)	95.55 ± 0.98

Values are mean ± SD., n =5

Tab. 2. *In vitro* antioxidant activity of chloroform extract of *G.lucidum* (IC_{50} µg/ml)

Activities	Chloroform extract	Ascorbic acid (Standard)	Quercetin (Standard)
Superoxide scavenging	144.66 ± 1.52		3.7 ±0.16
Lipid peroxidation inhibiting	593.33 ± 25.1	900 ± 15.0	
Nitric oxide scavenging	21.66 ± 1.52		500 ± 21

Values are mean ± SD., n= 5

Anti-inflammatory activity

The chloroform extract of *G. lucidum* showed significant inhibitory effect against induced inflammation in both the experimental models. The carrageenan induced acute and formalin induced chronic inflammation were significantly inhibited by the extract. The effect was evident from the inhibition of the paw edema (Fig. 2).

The effect was significantly high in 100 mg/kg body wt. extract treated group compared with standard reference drug, diclofenac. The carrageenan induced paw edema was reduced by 73.4% and 63.2% with treatment of chloroform extract of *G. lucidum* at concentrations of 100 and 50 mg/kg body weight respectively compared to that of the control group. Where as the inhibitory effect was 63.4% and 53.4% for formalin induced paw edema with the treatment of the extract at 100 and 50 mg/kg body weight respectively. Standard reference drug diclofenac showed an inhibition of 53.0% and 40.2% of carrageenan and formalin induced inflammation respectively (Fig. 2).

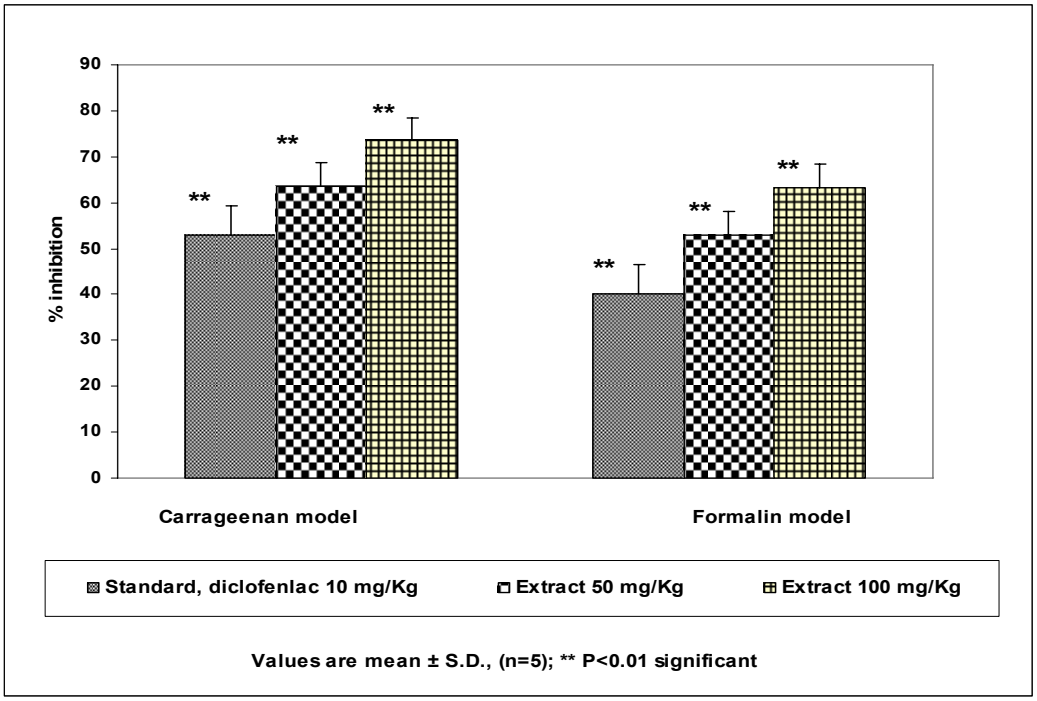

Fig. 2. Inhibition of acute and chronic inflammation by chloroform extract of *G.lucidum* (50 and100 mg/kg) administered orally.

Discussion

The results of the present investigation reveal that chloroform extract of *G. lucidum* possesses significant capacity to inhibit free radical formation and scavenging activity. The extract acts at two different levels as primary antioxidant. Antioxidants show activities at different level of protection [20]. Although organisms are bestowed with antioxidant and repair systems that have evolved to protect them against oxidative damage, these systems are insufficient to prevent the damage totally [21]. Hence antioxidants in diet are of great importance as possible protective agents to help human body to reduce oxidative damage. Recently a large number of natural antioxidants have been isolated from different plants. [22–24]. Mushrooms are functional food and are traditionally used in folk medicine in several countries. Human diet containing medicinal mushrooms possessing antioxidative properties would be potentially useful to help human body to reduce oxidative damage. The broad-spectrum medicinal property of *G. lucidum* might be due its significant antioxidantive activity. Our earlier reports also confirm this conclusion [6, 25].

In the DPPH assay, the ability of antioxidant to scavenge stable purple–colored primary radical DPPH is tested by its depolarization spectrophotometrically at 515 nm [13]. This shows the ability of the extract to scavenge stable free radicals.

The chloroform extract of *G. lucidum* shows significant superoxide anion, nitric oxide scavenging and lipid peroxidation inhibiting activity in a dose dependent manner. Simultaneous generation of NO an O_2^- favours the production of a toxic reaction product, peroxynitrite ($ONOO^-$) [26]. The scavenging of the superoxide anion generated from the photoreduction of the riboflavin and nitric oxide from sodium nitroprusside indicate the possibility of preventing the peroxynitrite formation in the cell. Reducing the nitric oxide

generation in the digestive tract was found to be effective in preventing the reactions of nitrate with amines and amides to form carcinogenic nitrosamines and nitrosamides [27]. Hence the NO scavenging activity of *G. lucidum* extract could play a preventive role against nitrosamine mediated carcinogenesis.

The anti-inflammatory activity of chloroform extract of *G. lucidum* is dose dependent. Carrageenan induced acute inflammation in animals is one of the most suitable test procedures to screen anti-inflammatory agents. The carrageenan induced edema is mediated by activation of platelet activating factor (PAF), prostaglandins and other inflammatory mediators [28]. The first phase is attributed to the release of histamine 5-HT and kinins. The second phase is related to the release of prostaglandins [29–31]. Carrageenan also induces a protein rich exudate containing large number of neutrophills [32]. Formalin induced paw edema is also one of the most suitable test procedure to screen chronic anti-inflammatory agents as it closely resembled human arthritis [33]. The nociceptive effect of formalin is also biphasic, an early neurogenic component followed by tissue mediated response [34].

The preliminary phytochemical analysis reveals that the major chemical constituents of the chloroform extract of *G. lucidum* are terpenoids. The major chemical components of *G. lucidum* are known to be polysaccharides and triterpenoids [35]. Thus triterpenoids of chloroform extract might be responsible for the antioxidant and inflammatory activities [36]. Kim et al (1999) [37] identified ganoderic acid A, B, G, and H, the triterpene component of commercial strain of *G. lucidum*, for its antiinflammatory activity.

In conclusion, the chloroform extract of South Indian *G. lucidum* exhibited significant anti-inflammatory activity in mice. The chloroform extract also possessed significant antioxidative activity. However the findings suggest the therapeutic potentials of the chloroform extract of this mushroom for the prevention and the control of inflammation and diseases mediated through oxidative stress.

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Authors' Statements

Competing Interests

The authors declare no conflict of interest.

Animal Rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed. See the experimental part for details.

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