

Antiinflammatory and Antioxidant Effects of Aqueous Extract from *Phellinus gilvus* in Rats

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This study evaluated the antiinflammatory activities of an aqueous extract of *Phellinus gilvus* (PG) against carrageenan- and cotton pellet-induced acute and chronic inflammation in rats. Four acute and chronic inflammation groups included the vehicle control group, positive control group (aminopyrine, 100 mg/kg), PG10 group (PG, 10 mg/kg), and PG20 group (PG, 20 mg/kg). Oral administration of PG extract produced dose-dependent antiinflammatory effects in both the acute and chronic groups. In the carrageenan-induced paw edema, significant inhibitions were observed at 0.5 and 1 hr in the PG10 group and at 0.5, 1, 2, 4, 5, and 6 hr in the PG20 group. In the cotton pellet-induced granuloma formation, PG extract at 10 and 20 mg/kg per day also showed significant inhibition in the wet and dry weights of granuloma. The free radical scavenging assay indicated a dose-dependent scavenging activity of PG against 2,2-diphenyl-1-picrylhydrazyl free radicals. PG extract may be beneficial as an antiinflammatory agent by virtue of antioxidant action.

Key words——*Phellinus gilvus*, antiinflammatory effect, antioxidant activity

INTRODUCTION

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.¹⁾ Although it is a protective response, continuous inflammation is deleterious and is a major contributor to the development of many chronic diseases. Most clinically important medications for treatment of inflammation-related diseases belong to either steroidal or non-steroidal antiinflammatory chemical therapeutics. Although non-steroidal antiinflammatory drugs (NSAIDs) have potent activity, long-term administration is required for the treatment of chronic diseases. However, the long-term use of these drugs can have deleterious effects on the gastrointestinal tracts including effects on the kidney, liver, immune system, and central nervous system.^{2,3)} NSAIDs with specific cyclooxygenase-2 inhibitory action have fewer gastrointestinal side effects than other NSAIDs⁴⁾ but they are associated with higher rates of hypertension and cardiovascular complications.⁵⁾ Therefore, natural products and traditional medicines with few adverse effects are needed as a substitute for chemical therapeutics.^{6,7)}

Mushrooms have become attractive as a functional food and as source of drugs and nutraceuticals.^{8,9)} *Phellinus* species of mushroom have traditionally used as a folk medicine for a variety of human diseases in several Asian countries. Extracts obtained from *Phellinus* mushrooms have received special attention due to their potent pharmacological activities, including immunostimulation, antitumor activity, antioxidant, and antihepatotoxicity.^{10,11)} The immunomodulatory activities and antioxidant effects of *Phellinus* extracts suggest that these natural products may be beneficial in the treatment of acute and chronic diseases induced by immunosuppression and oxidative stress. The aqueous extract of *Phellinus gilvus* (PG) inhibits the increase of inflammatory cells and interleukin (IL)-1 β concentration in bronchial lavage fluid in lipopolysaccharide-challenged rats, and might be useful in preventing acute pulmonary inflammation in human diseases.¹²⁾ Therefore, it is conceivable that aqueous extracts of PG may have the anti-inflammatory effects. PG has also significant adhesion- and abscess-reducing effects in a rat peritonitis model,^{13,14)} and has significant dermal wound healing effects clinically.¹⁵⁾

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The present study investigated the anti-inflammatory effects of an aqueous extract of PG against various inflammation models (carrageenan- and cotton pellet-induced acute and chronic inflammation) in rats, in view of the fact that PG have the inhibitory effects of increase in inflammatory cells and antioxidant activities. Among *Phellinus* spp., PG was selected because it has a very short growth period (3 months) compared to *Phellinus linteus* (2–3 years) and *Phellinus baumii* (1 year), making it cheaper to produce.

MATERIALS AND METHODS

Animal Husbandry and Maintenance—— Male Sprague-Dawley rats 6-weeks-of-age were obtained from a specific pathogen-free colony at Orient-Bio (Seoul, Korea) and used after 1 week of quarantine and acclimatization. The animals were housed in a room maintained at a temperature of $23 \pm 3^\circ\text{C}$ and a relative humidity of $50 \pm 10\%$, with artificial lighting from 08:00–20:00 and with 13–18 air changes per hour. Forty-eight healthy rats weighing 170–210 g were housed in pairs in stainless steel wire mesh cages and were given tap water and commercial rodent chow (Samyang Feed, Wonju, Korea) *ad libitum*. The protocols for the animal study were approved by the Institutional Animal Care and Use Committee, Chonnam National University, and the animals were cared for in accordance with the Guidelines for Animal Experiments, Chonnam National University.

Test Substances and Treatment—— PG was kindly provided by Gyeongbuk Agricultural Technology Administration (Daegu, Korea). A seed culture was grown in a 250 ml flask containing 50 ml of PMP medium (2.4% potato/dextrose broth plus 1% malt extract and 0.1% peptone) at 28°C on a rotary incubator at 150 rpm for 4 days. To obtain fruiting bodies of PG, a culture was grown in a sawdust block consisting of 90% oak and 10% rice bran for 90 days. The yield of fruiting bodies was 97 g dried weight per block. The preparation of an aqueous extract of PG used in this study followed our previously established methods.¹²⁾ Aminopyrine and carrageenan lambda Type IV were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). All chemicals were dissolved in distilled water and were prepared immediately before treatment. The rats were administered the test compounds orally because this is typically the main clinical route. The individual

dose volume (5 ml/kg body weight) was calculated based on the body weight on the treatment day.

Experimental Groups and Selection of Doses—— Twenty-four healthy rats were randomly assigned to four experimental acute or chronic inflammation groups ($n = 6$ per group): group 1 (vehicle control) received distilled water as a vehicle, group 2 (positive control) received 100 mg/kg aminopyrine, group 3 received 10 mg/kg PG (PG10 group), and group 4 received 20 mg/kg PG (PG20 group). The effective doses of PG were based on an earlier work.¹²⁾

Carrageenan-induced Paw Edema Test—— In this acute inflammation model, rats were fasted overnight prior to dosing and the test articles were administered once by oral gavage. Thirty min after administering the PG, 0.1 ml of 1% carrageenan solution in distilled water was injected subcutaneously into the plantar surface of the right hind paw. The level of inflammation was quantified by measuring the volume of the paw using a plethysmometer (Ugo Basile, Comerio, Italy). The paw volume was measured shortly before administering the test article and at 0.5, 1, 2, 3, 4, 5, and 6 hr after carrageenan injection. The acute antiinflammatory activities in the animals that received PG were compared with those in the vehicle and positive control groups, and the percentage inhibition of edema was normalized to the vehicle control animals.

Cotton Pellet Granuloma Test—— In this chronic inflammation model, the effects of PG on the proliferation phase of inflammation were examined using a cotton pellet granuloma test. The male rats ($n = 6$ per group) were anesthetized intraperitoneally with 10 mg/kg ketamine hydrochloride (Yuhan, Seoul, Korea). Under sterile conditions, cotton pellets weighing 30 mg each were implanted at the interscapular distance under the skin. After a 1 hr recovery period, PG was administered by an oral gavage for 7 consecutive days. One day after the final administration, all the rats were anesthetized by ether inhalation, and euthanized by blood withdrawal from the abdominal aorta. The pellets surrounded by granuloma tissues were dissected and weighed. The moist pellets were dried in an oven (50°C) for 3 days and then re-weighed. The chronic antiinflammatory effects of PG were compared with those of the vehicle and positive control groups, and the percentage inhibition of granuloma formation was normalized to the vehicle control animals.

Assay for 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity—— The free radical

Table 1. The Antiinflammatory Effects of PG Extract in Carrageenan-induced Paw Edema

| Time after treatment | Experimental groups | | | |
|----------------------|---------------------|--------------------------|-------------------------|--------------------------|
| | Group 1 | Group 2 | Group 3 | Group 4 |
| 0.5 hr | 35.0 ± 15.61 | 8.0 ± 7.88** (77.1) | 9.8 ± 7.35** (72.0) | 6.6 ± 4.39** (81.1) |
| 1 hr | 56.2 ± 17.03 | 18.4 ± 9.88** (67.3) | 26.4 ± 8.40** (53.0) | 23.5 ± 10.82** (58.2) |
| 2 hr | 79.5 ± 23.20 | 22.9 ± 12.57** (71.2) | 56.6 ± 13.04 (28.8) | 46.4 ± 18.58* (41.6) |
| 3 hr | 92.9 ± 28.20 | 41.4 ± 13.55** (55.4) | 69.8 ± 19.65 (24.9) | 61.4 ± 22.10 (33.9) |
| 4 hr | 100.2 ± 24.97 | 44.6 ± 12.45** (55.5) | 76.8 ± 12.72 (23.4) | 67.7 ± 24.51* (32.4) |
| 5 hr | 93.9 ± 21.43 | 47.1 ± 9.53** (49.8) | 71.0 ± 13.53 (24.4) | 64.0 ± 24.86* (31.8) |
| 6 hr | 90.1 ± 25.16 | 43.7 ± 8.21* (51.5) | 62.9 ± 21.59 (30.2) | 55.2 ± 24.77* (38.7) |

Note: Group 1, distilled water; Group 2, 100 mg/kg aminopyrine; Group 3, 10 mg/kg PG; and Group 4, 20 mg/kg PG. Values (%) indicate the increment rates of paw volume compared to the volume before carrageenan injection (mean ± S.D., *n* = 6). Values (%) in parenthesis show the inhibition rates of paw edema. **p* < 0.05, ***p* < 0.01 vs. Group 1.

scavenging activity was assayed as described previously with slightly modification.¹⁶⁾ In brief, the aliquot of PG (30, 100, and 300 µg/ml) were mixed with 1 ml of 0.4 mM DPPH-ethanol solution and 0.9 ml of 10 mM acetate buffer (pH 5.6). After standing for 30 min at a room temperature, the absorbance of the mixture was measured at 517 nm and then converted into the percentage antioxidant activity, which was expressed as the percent decrease on the absorbance of mixture compared with the control. The experiments were performed in triplicate.

Statistical Analyses—All the variables were subjected to one-way analysis of variance. If a test showed a significant difference, the data was analyzed using the multiple comparison procedure of the Tukey-Kramer multiple comparison test. The *p* values < 0.05 (*) or *p* < 0.01 (**) are represented by asterisks. Statistical analyses were performed by comparing the treatment groups with the vehicle control group using GraphPad InStat v. 3.0 (GraphPad Software, LaJolla, CA, U.S.A.).

RESULTS AND DISCUSSION

The acute antiinflammatory effects of PG extract on carrageenan-induced paw edema are summarized in Table 1. The paw volume of the rats after the carrageenan injection increased by approximately 78% compared with the pre-test, demonstrating that carrageenan injection induced edema.

In addition, this test model was confirmed to be effective for evaluating acute inflammation and the antiinflammatory effect because the volume of paw edema in the positive control group administered with the antiinflammatory agent aminopyrine was significantly reduced (about 59%) compared with the vehicle control group. The oral administration of PG extract showed significant antiinflammatory effects at 0.5 and 1 hr in group 3, and at 0.5, 1, 2, 4, 5 and 6 hr in group 4 after carrageenan injection compared with the vehicle control group.

The chronic antiinflammatory effects of PG extract on cotton pellet-induced granuloma formation are presented in Table 2. The cotton pellet implanted into the subcutaneous tissues of the rats formed a granuloma with distinctive borders from the surrounding tissues, and both wet and dry weights increased considerably. In addition, the formation of the granuloma in the positive control group administered aminopyrine was significantly suppressed (about 22%) compared with the vehicle control group. Therefore, this test model was confirmed to be effective for examining chronic inflammation and evaluating the antiinflammatory effect. Seven day repeated oral administration of PG extract resulted in a significant decrease in the wet and dry weights of the granuloma formed by the cotton pellet implantation compared with the vehicle control group (about 21% in group 3 and 24% in group 4).

It has been well-documented that PG has an antiinflammatory effect in various experimental

Table 2. The Antiinflammatory Effects of PG Extract in the Granuloma Formation Induced by a Cotton Pellet

| Parameters | Experimental group | | | |
|----------------------|--------------------|----------------|----------------|----------------|
| | Group 1 | Group 2 | Group 3 | Group 4 |
| Wet weights (mg) | 575.0 ± 118.45 | 455.0 ± 28.81* | 464.2 ± 25.38* | 448.3 ± 56.36* |
| Inhibition rates (%) | — | 20.9 | 19.3 | 22.0 |
| Dry weights (mg) | 123.3 ± 25.82 | 93.3 ± 12.11* | 95.0 ± 7.07* | 91.7 ± 10.33* |
| Inhibition rates (%) | — | 24.3 | 23.0 | 25.6 |

Note: Group 1, distilled water; Group 2, 100 mg/kg aminopyrine; Group 3, 10 mg/kg PG; and Group 4, 20 mg/kg PG. Values are presented as means ± S.D. for six male rats per group. * $p < 0.05$ vs. Group 1.

systems. Our previous studies showed that PG inhibits the increase of inflammatory cells and IL-1 β concentration in bronchial lavage fluid in lipopolysaccharide-challenged rats and might be useful in preventing acute pulmonary inflammation in human diseases.¹²⁾ It was also reported that PG has significant adhesion- and abscess-reducing effects in a rat peritonitis model,^{13, 14)} and has significant dermal wound healing effects clinically.¹⁵⁾ The results of the previous and present studies provide clear evidence for the antiinflammatory effect of PG in acute and chronic inflammatory diseases.

Experimental and clinical results have implicated oxygen-derived free radicals (especially superoxide and hydroxyl radical) and high energy oxidants (such as peroxynitrite) as mediators of acute and chronic inflammation.¹⁷⁾ Important proinflammatory roles for free radicals include endothelial cell damage and increased microvascular permeability,¹⁸⁾ recruitment of neutrophils at sites of inflammation,¹⁹⁾ and poly-adenosine 5'-diphosphate (ADP)-ribose-polymerase activation.²⁰⁾ Salvemini *et al.*²¹⁾ reported that the generation of nitric oxide, superoxide anion, and peroxynitrite contributes to edema in acute inflammation induced by carrageenan administration. Recently, we demonstrated that PG inhibits the increase of inflammatory cells, IL-1 β release, and nitric oxide production.^{12, 22)} Presently, all the samples at different concentrations of PG showed dose-dependent scavenging activity against DPPH free radicals (Fig. 1). Although the exact cause of the antiinflammatory effect is unknown at present, the present results strongly suggest that the antiinflammatory effect of PG may be related to the ability to scavenge free radicals and reactive oxygen species formed during acute and chronic inflammation. Therefore, it is considered that PG can be used effectively to prevent or at least ameliorate various types of inflammatory diseases caused by the excessive production of reactive oxygen species. Previous studies also demonstrated that a number of free radical scav-

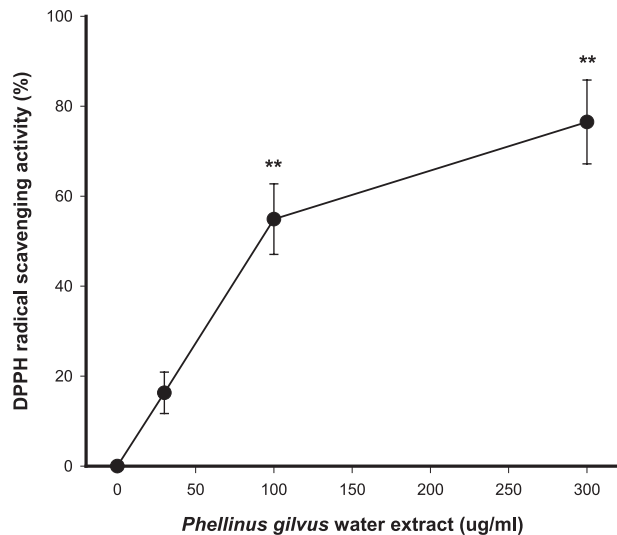


Fig. 1. DPPH Free Radical Scavenging Activity of PG extract. Values are presented as the mean of the percentage inhibition ± S.D. for three experiments, performed in triplicate.

engers are effective in carrageenan-induced acute edema and cotton pellet granuloma test.^{23–25)}

In conclusion, the present investigation demonstrates that the aqueous extract of PG produces significant antiinflammatory activity against acute and chronic inflammation. PG has potential as an anti-inflammatory agent.

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