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Original article

Anti-inflammatory effect, total polysaccharide, total phenolics content and antioxidant activity of the aqueous extract of three basidiomycetes

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Abstract

Inflammation is a part of the non-specific immune response which occurs in reaction to any type of injury. Medicinal mushrooms have had application in various disorders including cancer, liver injuries, inflammation and diabetes. In the present study, the anti-inflammatory effects of the aqueous extracts of medicinal mushrooms (*Fomes fomentarius*, *Ganoderma applanatum* and *Trametes hirsuta*) were evaluated using carrageenan method. In addition, total polysaccharide, total phenolics contents and the radical scavenging activity of the extracts have also been examined. Mushrooms were extracted with distilled water in 100 °C for 4 hours and then the extracts were freeze dried. Indomethacin was considered as the positive control in the anti-inflammatory evaluation. Polysaccharide contents of *F. fomentarius*, *G. applanatum*, and *T. hirsuta* extracts were assessed as 53.3±0.2, 31.7±0.03, and 19.1±0.6 glucose equivalent µg/100 µgEXT and total phenolic contents of them were successfully revealed as 9.9±0.2, 8.2±0.1, and 8.8±0.2 µgGAE/100 µgEXT, respectively. Furthermore, the IC_{50} values for *F. fomentarius*, *G. applanatum*, and *T. hirsuta* extracts in DPPH assay, were calculated as 90.9, 108.6, and 908.3 µg/mL, respectively. The results of the experiment showed that the extracts possessed potent anti-inflammatory effect which was comparable to indomethacin.

Keywords: anti-inflammatory, antioxidant, basidoimycetes, total phenol, total polysaccharide,

Introduction

Inflammation, a part of the non-specific immune response, involves changes in blood flow, increased vascular permeability and destruction of tissues via the activation and migration of leucocytes with synthesis of reactive oxygen derivatives [1]. Peripheral inflammation involves an increase in cyclooxygenase-2 (COX-2)-mediated prostaglandin (PG) synthesis in the central nervous system (CNS), which contributes to allodynia and hyperalgesia. Carrageenan-induced inflammation in the rat paw represents a classical model of edema formation and hyperalgesia, which has been extensively used in the development of non-steroidal anti-inflammatory drugs and selective COX-2

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inhibitors. Carrageenan-induced edema in the paw, elicits an early phase of COX-2 induction in the CNS leading to an increase synthesis in PGD_2, 6-keto-$PGF_{1\alpha}$, and TXB_2 in addition to the major PGE_2 response. The data also indicate that the up-regulation of mPGES-1 contributes to COX-2-mediated PGE_2 production in the CNS during peripheral inflammation [2].

For millennia, mushrooms have been valued by humankind as edible and medicinal resources [3]. They are a potential source of dietary fibers with beta-glucans as their most interesting functional components which are supposed to play an important role in some beneficial properties of mushrooms, such as enhancement of macrophage function and host resistance to many microbial infections, as well as activation of a non-specific immune stimulation [4]. Traditionally, many medicinal mushrooms have been used for a wide range of diseases. The main areas of medicinal studies include anticancer, cholesterol and blood pressure lowering, protection of liver, anti-fibrotic, anti-inflammatory, anti-diabetic and antimicrobial activity [5].

Fomes fomentarius (L.) Fr. commonly known as Tinder fungus, is a common polypore and economically important wood-rotting mushroom in deciduous forests [6]. In Japan, it has been used to make a popular drink which is believed to be tonic and to have anticancer effects, but there are a few studies about its pharmacological effects [7]. *Ganoderma applanatum* (Pers.) Pat., a perennial wood decaying mushroom and its related species, had been used for over 2000 years in China to prevent and treat various diseases [8]. New researches have reported that its major constituents (including polysaccharides and sterols) have beneficial effects for some diseases such as bronchitis, hepatitis, hypertension, diabetes, tumors and immunological disorders. Its terpenes significantly have inhibited inflammation by pressing the expression of IL-1β and COX-2 and inhibiting NF-κB translocation in the liver of BaP-treated [8]. *Trametes hirsuta* (Wulfen) Pilát, a very common polypore is a saprophyte on all kinds of dead broad-leaved wood trees [9], and has been used for the decolorization of a wide variety of dyes, including textile dyes. It had been used as a ligniperdous fungi for production of xylanase and for microbial metabolism of organo-sulfur compounds [10].

Polysaccharides are the best known and most potent mushroom derived substances with antitumor and immune modulating properties [3]. They have been used as medicine in the Far East, where using medicinal mushrooms primarily originated [11,12]. In forty years and after the first three polysaccharides were developed from medicinal mushrooms (krestin from *T. versicolor*, lentinan from *L. edodes* and schizophyllan from *S. commune*), many new pharmacologically active polysaccharides have been identified and put into practical use [3].

According to the literature surveys, there are few studies about the pharmacologic effects of these three mushrooms and there is no study about the anti-inflammatory effect of their aqueous extract.

Experimental

Materials

Fomes fomentarius, *Ganoderma applanatum*, and *Trametes hirsuta* were collected from Neka forests (Mazandaran province, Iran) in May 2011 and were deposited in the Herbarium of Passand Forest and Rangeland Research Station (Agriculture and Natural Research Center of Mazandaran). The voucher specimen numbers are: Iran, MZ. 266F; Iran, MZ. 272F, and Iran, MZ. 285F, respectively.

Extraction

250 g of air-dried and ground mushrooms were separately extracted with 2.5 L hot distilled water at 95-100 °C [13]. The extracts were concentrated using a rotary evaporator, freeze dried and weighed. The yields were 3, 9, and 6.2 %w/w for *F. fomentarius*, *G. applanatum* and *T. hirsuta*, respecively. The extracts were kept in opaque containers under cold and dry condition in -20 °C until analyzed.

Carrageenan induced paw edema

Male wistar rats (180-200 g) were obtained from the Animal House of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. The rats were maintained in a temperature-controlled (22±1

°C) room, on a 12 h light/dark cycle, were acclimated to their environment for 1 week and given free access to food and water. Each rat was used only once during the study. Inflammation in the rat paw was produced by the method described by Winter *et al.* [14]. Paw edema was induced by a sub-plantar injection of 50 µL of sterile saline containing 1% λ-carrageenan (Sigma, USA) into the right hind paw. Paw volume was measured by a caliper at given times (1, 2, 3, 4 and 5 h) after injection. The increase in paw volume was evaluated as the difference between the paw volume measured at the each time point and the basal volume measured immediately before carrageenan injection [15]. Seven rats were evaluated in each group including: extract group with three doses (50, 100 and 500 mg/kg of rat body weight), control group (distilled water) and positive control group (15 mg/kg indomethacin). Indomethacin (Liometacin®, 50 mg/2 mL) was purchased from Cheisi (Italy). The rats received the extract doses (dissolved in distilled water) intraperitoneal (*i.p.)* 60 min before subcutaneous injection of carrageenan. The percentage of the inhibitory activity was calculated using the following equation:

% Inhibition= [(C-T)/C] ×100

C and T represent paw edema in the negative control and samples, respectively [16].

Total polysaccharide assay

Total sugar content (including polysaccharides) was determined according to previous studies [17]. All the glycoside linkages in the presence of sulfuric acid were broken and a colored aromatic complex was achieved between phenol and the carbohydrate, afterwards the absorbance of the complex was measured at 490 nm. 100 µg of the aqueous extract of the mushrooms was thoroughly mixed with 500 µL of 4% phenol followed by 2.5 mL 96% sulfuric acid. 5 minutes later, the UV absorptions were measured. The samples absorptions were compared to the calibration curve (figure 2) of different concentration of glucose (5-50 µg/mL).

Total phenolics content assay

Total phenolics contents of the aqueous extracts of the mushrooms were examined as gallic acid equivalent (GAE), expressed as µg GAE/100 µgEXT [18]. The aqueous extracts of the mushrooms (400 µg/mL) were transferred to glass tubes, to which 5 mL Folin-Ciocalteu reagent (diluted 1:10) was subsequently added and incubated at room temperature for 10 minutes. 4 mL of sodium bicarbonate (75 mg/mL) was added to the mixture and it was diluted to 10 mL with distilled water. Each solution was incubated for 30 min at room temperature, and its absorbance was measured at 765 nm thereafter. The sample absorbance was compared to gallic acid absorption.

Free radical scavenging activity

Free radical scavenging activity was evaluated by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method [18]. DPPH, a free stable radical, has been widely used to evaluate the free radical scavenging activity of natural antioxidants. 1 mL of various concentrations of the extracts (200-1000 µg/mL) was added to 2 mL of DPPH (4×10^{-2} mg/mL in methanol). The absorptions were measured at 517 nm after 30 min. Free radical 50% inhibition (IC_{50}) provided by the extracts was determined from the plot of inhibition percentage against extracts concentration. Vitamin E and BHA were used as positive controls.

Statistical Analysis

Results are expressed as the mean±S.E of n=7 rats. The anti-inflammation activity was analysed using Graph-Pad Prism (GraphPad Software Inc.). Comparisons between polysaccharide, phenol contents, and anti-oxidation activity of the extracts have been done in triplicate sets. Significant differences between groups were determined by one-way ANOVA followed by tukey's post hoc test for multiple comparisons. The level of statistical significance was $p<0.05$.

Results and Discussion

Pretreatment with all aqueous extracts resulted in a significant but dose-independent reduction in carrageenan-induced paw edema in rats after 5 hours of injections (table 1 and figure 1). Carrageenan injection caused skin

inflammation in the rat paw, it enlarged progressive paw diameter to its maximum 1.13 mm at 5 h. However, the aqueous extracts administrations of these three mushrooms, except for *G. applanatum* (50 mg/kg), significantly decreased the inflammation in the rat paw, time dependably.

At the second hour, all of the extracts exhibited reduction in the paw edema significantly.

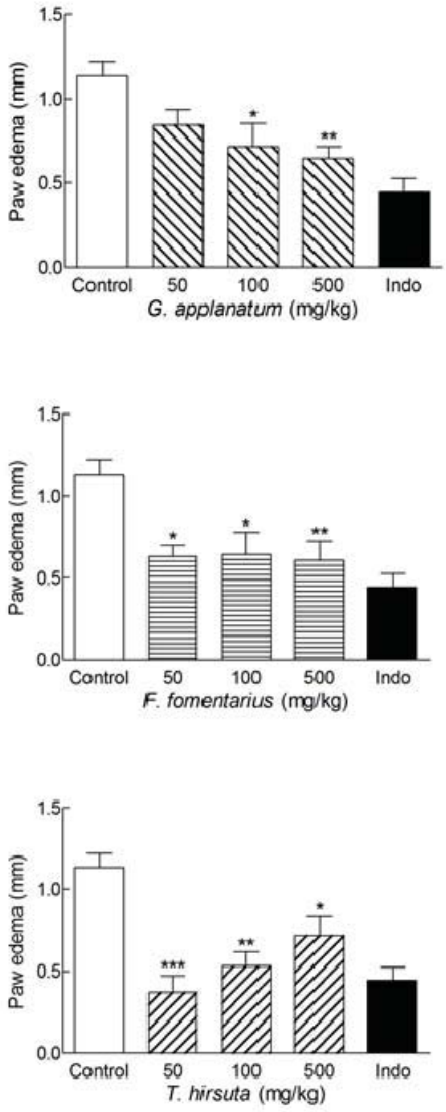

Figure 1. The anti-inflammatory effects of different doses of *F. fomentarius*, *G. applanatum*, and *T. hirsuta* aqueous extracts in a carrageenan-induced paw edema model after 5 h of injection. Normal saline was considered as the negative and indomethacin as the positive control. Significance levels: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to control group.

Compared to the negative control (D.W). *T. hirsuta* extract showed significant decrease in the rat paw inflammations during the follow up period. Besides, *T. hirsuta* extract in concentration of 50 mg/kg after 5 hours revealed the highest anti-inflammatory activity in comparison to indomethacin ($p<0.001$), which exhibited edema inhibition of 67.2%, while indomethacin edema inhibition value was calculated as 61%.

Polysaccharide content of *F. fomentarius*, *G. applanatum*, and *T. hirsuta* aqueous extracts was evaluated as 53.3±0.23, 31.7±0.03, and 19.1±0.63 glucose equivalent µg/100µgEXT, respectively. Moreover, polysaccharide content of *F. fomentarius* was significantly higher than the other aqueous extracts. As could be seen in table 2, both *G. applanatum* and *T. hirsuta* phenol contents were examined as 8.2±0.14 and 8.8±0.2 µg GAE/100µgEXT, respectively. However, phenol contents of *F. fomentarius* (9.9±0.2 µg GAE/100µgEXT) was significantly more than the two other extracts. Antioxidant activities of the extracts were examined against free stable radical DPPH. Aqueous extract of *F. fomentarius* exhibited higher radical scavenging activity toward DPPH with the IC_{50} value of 90.9 µg/mL. Furthermore, the IC_{50} values of *G. applanatum*, and *T. hirsuta* were calculated as 108.6 and 908.3 µg/mL, respectively. Values of the IC_{50} for radical scavenging activity of vitamin E (14.2 µg/mL) and BHA (7.8 µg/mL) as positive controls were also measured.

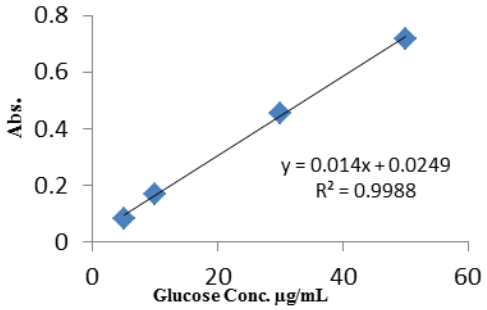

Figure 2. Glucose standard curve obtained for total polysaccharide assay.

In the present study, the anti-inflammatory activity of polysaccharide rich extracts of three basidiomycetes (*F. fomentarius*, *G. applanatum*, and *T. hirsuta*) was evaluated by carrageenan method and our results showed

that the aqueous extracts presented potent anti-inflammatory effects. There was no significant difference between the anti-inflammatory effect of the different doses (50, 100 and 500 mg/kg) of the three basidiomycetes and the positive control (indomethacin; 15 mg/kg) after five hours of carrageenan injection ($p<0.05$, except for 50 mg/kg of *G. applanatum*). Regarding the published literature, a few polysaccharides have been explored for the anti-inflammatory effect [19,20] and it is specified that this effect is through decreasing IL-4, IL-5 and eosinophils percentage as the main mechanisms of action [19,21]. Beta-glucans which are one of the most important polysaccharides in medicinal mushrooms [4,22], can significantly reduce the inflammatory infiltrate produced by thioglycolate-induced peritonitis and nitric oxide levels in carrageenan method [23]. It has been reported that the aqueous extracts of *Ganoderma tsugae* have showed anti-inflammatory/allergy effects [19] which may be due to the antihistaminic action. Other studies have also demonstrated that the ethanol extract and a proteoglycan from *Phellinus linteus* have demonstrated anti-inflammatory effects in collagen-induced arthritis and in the croton oil-induced ear edema test in mice [24]. The methanol extract of *Pleurotus pulmonarius* has reduced carrageenan-induced paw edema in mice which was comparable to 10 mg/kg diclofenac. The effect was ascribed to the antioxidant activity of the extract [24]. Ethanol 70% extract of *Trametes versicolor* has displayed anti-inflammatory properties in animal model of colitis induced by dextran sulfate sodium which has been attributed to the inhibition of STAT1 and STAT6 expression and resulting in inhibition of IgE production [25].

The edema progression after carrageenan injection in the rat paw is a biphasic procedure initiated by histamine and serotonin release in the first phase (first hour). Over 1 h inflammation occurs due to the release of prostaglandins and cyclooxygenases [26]. The examined mushrooms in the present study displayed anti-inflammatory effects in 2 h and 5 h after carrageenan injection. Therefore, it could be concluded that these aqueous extracts prevented both phase of inflammation maybe

Table 1. Effects of different doses of the aqueous extracts of *F. fomentarius, G. applanatum,* and *T. hirsuta* on carrageenan induced paw edema in rats after 1, 2, 3, 4 and 5 hours of carrageenan injection. Normal saline was considered as the negative and Indomethacin as the positive control. Results are expressed as edema (mm) ±S.D.

Sample Dose (mg/kg)	Paw diameter (mm), edema inhibition (%)				
	1h	2h	3h	4h	5h
F. fomentarius (50)	0.73 ±0.2	0.99 ±0.41 (49.2%)	1.17 ±0.3	0.75 ±0.21	0.63 ±0.16 (44.2%)
F. fomentarius (100)	0.57 ±0.09	1.16 ±0.29 (40.5%)	1.07 ±0.34	0.89 ±0.35	0.64 ±0.36 (43.3%)
F. fomentarius (500)	0.63 ±0.43	1.19 ±0.71 (39%)	1.03 ±0.57	0.93 ±0.59	0.61 ±0.31 (46%)
G. applanatum (50)	0.74 ±0.21	1.11 ±0.21 (43.1%)	1.25 ±0.36	0.93 ±0.28	0.84 ±0.24 (25.6%)
G. applanatum (100)	0.61 ±0.14	1.13 ±0.41 (42%)	1.1 ±0.27	0.91 ±0.31	0.72 ±0.33 (36.3%)
G. applanatum (500)	0.61 ±0.27	1.06 ±0.19 (45.6%)	0.9 ±0.2	0.73 ±0.19	0.65 ±0.19 (42.4%)
T. hirsuta (50)	0.55 ±0.23	0.73 ±0.56 (62.5%)	0.72 ±0.47	0.46 ±0.23	0.37 ±0.27 (67.2%)
T. hirsuta (100)	0.68 ±0.24	0.8 ±0.22 (59%)	0.82 ±0.13	0.68 ±0.21	0.53 ±0.23 (53.1%)
T. hirsuta (500)	0.67 ±0.16	0.79 ±0.46 (59.5%)	0.87 ±0.41	0.77 ±0.37	0.72 ±0.32 (36.2%)
Indomethacin (15)	0.53 ±0.2	0.69 ±0.29 (64.6%)	0.67 ±0.22	0.55 ±0.24	0.44 ±0.21 (61%)
Control (D.W.*)	0.82 ±0.33	1.95 ±0.28	1.68 ±0.3	1.26 ±0.21	1.13 ±0.22

* distilled water

through inhibition of histamine and serotonin release and also prostaglandins and cyclooxygenases in the second phase.

The results of the present study showed that the considerable anti-inflammatory effect of the three basidiomycetes, may be due to polysaccharide components of the extracts. However, details of the anti-inflammatory effect should be examined through purification and isolation of the active components and evaluation of the inflammatory mediators.

Table 2. Total polysaccharide, total phenolic contents and radical scavenging activities of aqueous extracts of *F. fomentarius, G. applanatum* and *T. hirsuta*. Results are expressed as means ± SEM.

Assay	Aqueous extracts		
	F. fomentarius	*G. applanatum*	*T. hirsuta*
Total polysaccharide[1]	53.3±0.2	31.7±0.03	19.1±0.6
Total phenol content[2]	9.9±0.2	8.2±0.1	8.8±0.2
Radical scavenging activity (IC_{50})[3]	90.9	108.6	908.3

1: glucose equivalent µg/100 µg extract, 2: µg GAE /100 µg extract, 3: µg/mL.

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