PRO-INFLAMMATION CYTOKINE SECRETION OF PERIPHERAL BLOOD MONONUCLEAR CELLS BY EDIBLE MUSHROOM

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Abstract:

Inflammation is a natural process of the innate immune system that associated with the increase in the level of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6). Prolonged inflammation, known as chronic inflammation, related to many chronic diseases such as autoimmune diseases, wound healing, rheumatoid arthritis and cardiovascular disease. The present study aims to investigate the effect of mushroom extracts on the cytokines secretion from lipopolysaccharide (LPS) stimulated peripheral blood mononuclear cells (PBMCs) involved in immune regulation. The secretion of TNF-α, IL-1β and IL-6 was measured by ELISA. The ethanol extract of all mushroom significantly reduced the production of TNF-α. Furthermore, *Lentinula edodes*, *Isaria tennipes*, *Pleurotus osttreatus* and *Lentinus squarrosulas* Mont. were decreased IL-1β level and *Pleurotus osttreatus*, *Isaria tennipes* and *Lentinus squarrosulas* Mont. were significantly suppressed IL-6 secretion in LPS-treated PBMCs. The application of these extracts showed important anti-inflammatory activity which involved in the treatment and prevention of inflammation and associated diseases.

Keywords: Cytokine; Anti-inflammatory activity; ELISA; Mushroom

Introduction

Inflammation is a complex biological process of the body that response to pathogens, damaged cells, irradiation, toxins, heat, or any other cause (Elsayed *et al.*, 2014). It is a protective attempt that is characterized by pain, redness, heat, swelling, disturbance of function and inflammation causes fever (Javed *et al.*, 2019). The releasing of pro-inflammatory cytokines is occurred after the inflammatory response. Cytokine is a polypeptide which is created and secreted by body cells. It plays an important role in both non-specific and specific immunity. For specific immunity, cytokines are released after T lymphocyte and most of the non-specific immunity cytokines are secreted from mononuclear phagocyte but it also stimulated by T lymphocyte. Hence, pro-inflammatory cytokines are secreted in conditions that stimulate the immune system, such as infectious and most of the cytokines that are released are interleukins (ILs), tumor necrosis factors (TNFs), and interferon (IFN)-γ (Luo & Zheng, 2016). Nowadays, several studies which are related to anti-inflammatory activity of various medicinal plant and fungi are reported.

Mushroom is one of the most common fungi grown on every continent except Antarctica and grows throughout the year (Bellettini *et al*. 2019). This plant is the conspicuous

umbrella-shaped fruiting body (sporophore) of certain fungi belonging to Basidiomycetes and Ascomycetes. Several bioactive compositions and nutritional values were found in mushrooms and therapeutic benefits of mushrooms specific bioactive nutraceuticals have been investigated. The antitumor, immunomodulatory and anti-inflammatory activities of mushrooms extracts were the most investigated health benefits by researchers (Ma *et al*. 2018). Several studies reported the bioactive properties of mushroom extracts such as anti-inflammatoryactivities (Choi *et al*. 2014; Han *et al*. 2013; Taofiq *et al*. 2015), antitumour/anticancer (Sliva *et al*. 2102; Zong *et al*. 2012; Carocho M & Ferreira. 2013), antioxidant (Ferreira *et al*. 2009; Heleno *et al*. 2015; Mu *et al*. 2012), immunomodulatory (Yamanaka *et al*. 2012; Han *et al*. 2011; Enshasy & Hatti-Kaul.2013), antibacterial and antiviral (Adotey *et al*. 2011; Alves *et al*. 2012; Alves *et al*. 2013).

The aim of the present study was to investigate the in vitro pro-Inflammation cytokine secretion of mushroom. In order to contribute the knowledge concerning benefit of mushroom extracts on medical purpose as well as for economical purpose in the future.

Materials and Methods

Mushroom extraction

The plant samples were washed with fresh water, sliced and crushed. Cleaned mushroom samples were transferred to oven (MEMMERT) for drying. The 100 g of dried samples were macerated into 95% ethanol at room temperature. Then the extracts were filtered using the filter paper, and the filtrate was furthered for evaporation to remove the solvent through a rotary evaporator. The residual crude distilled extracts were freeze dried and weighed. The samples were stored at -20°C for further study.

Isolation of murine PBMC

The peripheral blood mononuclear cells (PBMC) of mice were isolated from EDTA-treated venous blood by Ficoll density gradient centrifugation method. The sample was diluted 1:1 with sterile phosphate-buffered saline (PBS), layered over Ficoll-Hypaque (Sigma-Aldrich, U.S.A.), and centrifuged at 400xg for 15 min at room temperature. The supernatant was removed and the pellet was resuspended in 6 ml of PBS. The mixture was centrifuged at 400xg for 10 min at room temperature and the supernatant was removed by pipetting. Finally, the cell pellet was resuspended in RPMI 1640 (Gibco Laboratories, Grand Island, N.Y., U.S.A.), supplemented with 1% antibiotic-antimycotic (Invitrogen, U.S.A.), 2 mM glutamine (Invitrogen, U.S.A.), and 10% fetal bovine serum (Invitrogen, U.S.A.).

Culture of murine PBMC

PBMCs were cultured in RPMI 1640, supplemented with 1% antibiotic-antimycotic, 2 mM glutamine, and 10% fetal bovine serum at 37°C with 5% CO_2. Cell count and viability were measured by trypan blue exclusion assay.

Measurement of Pro-inflammatory cytokine

To determine the effect of the mushroom extracts *in vitro*, PBMCs were seeded into a 24-well plate with the density of 1×10^6 cells/well and cultured at 37°C with 5% CO_2 for 24 h. On the next day, when the cells reach confluence, the cells were treated with mushroom extracts at concentrations of 0.001, 0.01, 0.1, 1 mg/ml and dexamethasone (1 µM) with another two more

wells left untreated. After 2 h, all treated wells and an untreated well were supplemented with Lipopolysaccharide (LPS; 10 nM) to encourage inflammation. An untreated well without LPS remained as the control group. Cultures were incubated in a humidified atmosphere of 37°C with 5% CO_2 overnight. The supernatant was collected for quantitative analysis of proinflammatory cytokines (IL-1β, IL-6 and TNF- α) using an enzyme linked immunosorbent assay kit with a specific antibody to each cytokine according to the manufacturer's protocol (BioLegend, Canada). Briefly, supernatants from treated samples and control were added to each well of 96-well plate coated with the capture antibody against the respective cytokine provided in the kit and all incubation steps were performed at room temperature. The optical density at 450 nm was measured with microplate reader Greiner bio-one, Germany). Consequently, the graph of the level of cytokines against concentrations of treatment was plotted.

Results and Discussion

Extraction yield

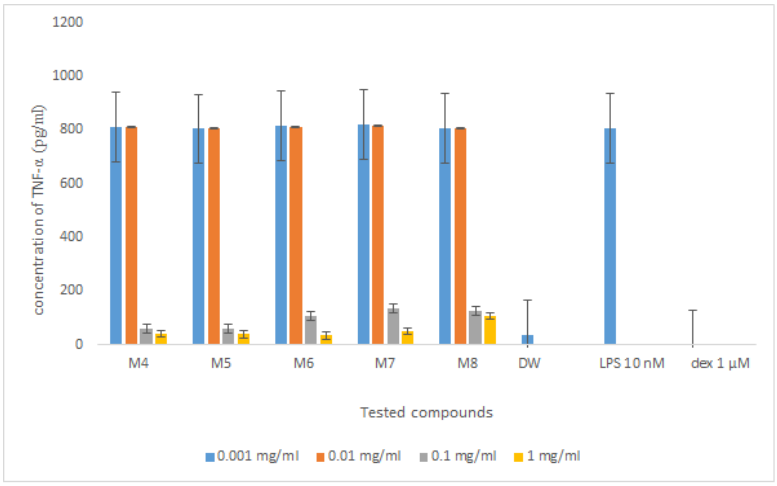
The percentage of mushroom yields in ethanolic extract were shown in Table 1.

Table 1: The percentage of ethanolic extract content of the mushroom extract.

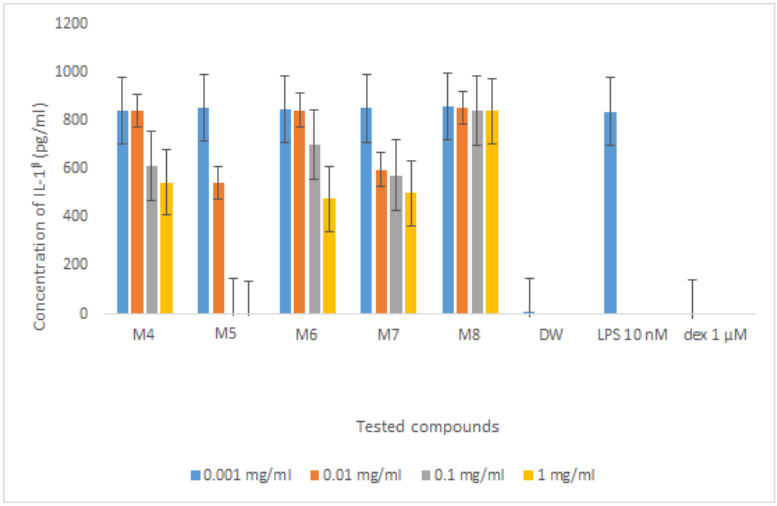
Plant name	Amount of extracts (g)	Amount of extracts obtained (g)	% yield
Lentinula edodes	100	23.67	23.67
Pleurotus osttreatus	100	7.44	7.44
Isaria tennipes	100	11.73	11.73
Lentinus squarrosulas Mont.	100	14.2	14.2
Agaricus Blazei Murrill	100	21.34	21.34

Measurement of Pro-inflammatory cytokine

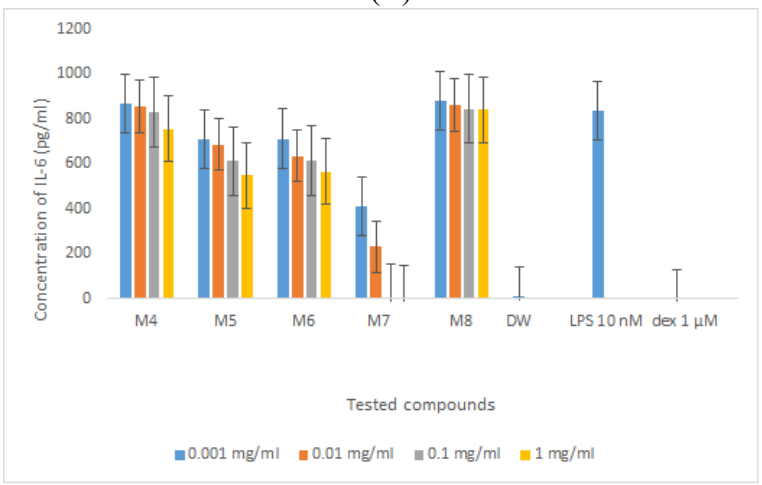
To assess the pro-inflammatory effects of mushroom extracts. Cytokine expression of LPS-stimulated PBMC was measured by ELISA method. LPS treatment alone significantly released IL-1β, IL-6 and TNF- α in PBMCs. The effect of mushroom extracts on the production of IL-1β, IL-6 and TNF- α were studied at different concentration (0.001, 0.01, 0.1 and 1 mg/ml). The results showed that all mushroom extracts were significant reduction in TNF- α at concentration 0.1 and 1 mg/ml (Figure 1A). *Lentinula edodes* and *Isaria tennipes* were slightly suppressed production of IL-1β at concentration 0.1 and 1 mg/ml, while *Pleurotus osttreatus* and *Lentinus squarrosulas* Mont. were significantly decreased the secretion of IL-1β at concentration 0.001, 0.1 and 1 mg/ml (Figure 1B). Furthermore, IL-6 secretion reduced after treat with *Pleurotus osttreatus*, *Isaria tennipes* and *Lentinus squarrosulas* Mont. at all concentration compared to control culture (Figure 1C).



(A)



(B)



(C)

Figure 1: Production of TNF- α (A), IL-1β (B) and IL-6 (C) were measured in the supernatant of LPS-stimulated PBMC by ELISA with specific antibodies to each cytokine. M4 = *Lentinula edodes*, M5= *Pleurotus osttreatus*, M6 = *Isaria tennipes*, M7 = *Lentinus squarrosulas* Mont., M8 = *Agaricus Blazei Murrill*, LPS = Lipopolysaccharide, dex = dexamethasone.

This study evaluated the production of pro-inflammatory cytokines including IL-1β, IL-6 and TNF-α by murine PBMC after *in vitro* treat with mushroom extracts. It is well known that LPS activates the signaling pathway such as NF-kB via the stimulation of Toll-like receptor 4 (TLR4) (Muniandy *et al*. 2018) which can induce inflammatory response that is characterized by increased pro-inflammatory cytokines (Lu *et al*. 2018). Cytokines and mediators are essential for the maintenance of optimum inner environment of our system from injurious agents and the pro-inflammatory play a key role in inflammation by its chemotactic and vasoactivator properties. IL-1β, IL-6 and TNF-α are pro-inflammatory mainly released by macrophages, and involved in inflammatory processes in many diseases such as rheumatoid arthritis (Lee *et al*. 2012; Assaf *et al*. 2016). In this study, we found that all mushroom extracts significantly decreased TNF-*α* level, *Lentinula edodes*, *Isaria tennipes*, *Pleurotus osttreatus* and *Lentinus squarrosulas* Mont. were reduced production of IL-1β and *Pleurotus osttreatus*, *Isaria tennipes* and *Lentinus squarrosulas* Mont. were significantly suppressed IL-6 secretion. From this result showed that mushroom extracts had anti-inflammatory effects on LPS-stimulated PBMC.

Conclusion

These results showed that mushroom extracts have significant anti-inflammatory effects *in vitro* through reduction of LPS-induced pro-inflammatory mediators. All the conducted experiments in the present study have demonstrated that ethanolic extract of mushroom reduce the level of pro-inflammatory cytokines that participated in the prolonging of chronic inflammation. Moreover, further investigation about different model of *in vitro* and *in vivo* are important to investigate the potential application of these extracts and need to be assessed prior to clinical treatment of inflammation in variety of diseases caused by a serve immune response.

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