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Anti-inflammatory effect of mushrooms in dengue-infected human monocytes

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Abstract. Pathogenesis of dengue fever has been associated with the activation of the cytokine cascade that triggered inflammatory responses. The inflammatory reactions in dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) are the main cause of haemorrhagic manifestations, coagulation disorders, vascular permeability, hypotension and shock which could exacerbate the condition of the disease. In an earlier study, extracts belonging to *Lignosus rhinocerotis*, *Pleurotus giganteus*, *Hericium erinaceus*, *Schizophyllum commune* and *Ganoderma lucidium* mushrooms were screened for anti-dengue virus activities. We found that hot aqueous extract (HAE) and aqueous soluble separated from ethanol extract (ASE) exhibited their potential to reduce dengue viral load which were observed in plaque reduction assay and real-time RT-PCR. In continuation of our previous findings, this study was initiated to further investigate the other aspect; the anti-inflammatory activities of HAE and ASE of *L. rhinocerotis*, *P. giganteus*, *H. erinaceus*, *S. commune* and *G. lucidium* on human monocytes infected with dengue virus-2 (DENV-2) New guinea C strain. Human monocytes infected with DENV-2 were treated with mushroom extracts for 48 hours. The cytokine profile coincides with dengue infection, i.e. IFN-γ, TNF-α, IL-1β, IL-6, IL-8, and IL-10 were measured by BD OptEIA™ Elisa Kit. The expression of these cytokines was significantly elevated in untreated infected cells two days after infection. However, after treated with mushroom extracts prominent anti-inflammatory effect were detected towards IFN-γ, IL-10, TNF-α, IL-6, and IL-1β. The most significant anti-inflammatory effects were detected in HAE of *G. lucidium*, *S. commune*, *P. giganteus* and ASE of *L. rhinocerotis* and the effects were comparable with dexamethasone, the reference inhibitor. These results demonstrated that mushroom HAE or ASE could successfully have suppressed cytokine production in dengue-infected monocytes and has a great potential to develop an anti-inflammatory agent from mushroom extract for the treatment of dengue infection.

INTRODUCTION

Immunological mechanisms play a crucial role in the pathogenesis of dengue disease. The immunological response during dengue infection is induced by a cytokine cascade. Activation of CD4+ (T helper) Th cells during dengue replication stimulates the production of human cytotoxic factor (hCF) which triggers a cytokine cascade that may lead to Th1-type response causing a mild illness, i.e. the dengue fever; or to a Th2-type response resulting in various grades of severe illness, i.e. the dengue haemorrhagic fever (DHF) (Chaturvedi *et al.*, 2000). Other human viruses, such as human immunodeficiency virus, herpes simplex, and influenza viruses, also present a severe disease progression due to shifting of Th1-type response to Th2-type response (Raphael *et al.*, 2015). During the Th1-type response, the Th1 cell secretes tumor necrosis factor-β (TNF-β), interferon-

gamma (IFN-γ) and Interleukin-2 (IL-2) which induced a cell-mediated inflammatory reaction, tissue injury, and delayed-type hypersensitivity. Meanwhile, during Th2 -type response, interleukins such as IL-4, IL-5, IL-6, IL-10 and IL-13 which are associated with B cells antibody production are secreted from Th2 cells. A cross regulating network between Th1 and Th2 cell is mediated by IL-10 and IFN-γ, respectively. The upregulation of IL-1, TNF-α, IL-8, and hydrogen peroxide in macrophage is not linked to T cell response but it is directly induced by free radicals, nitrite, and peroxynitrite (Chaturvedi *et al.*, 2000).

Tumour necrosis factor-α (TNF-α), IFN-γ, IL-6 and IL-8 are the cytokines that are profoundly expressed in patients with dengue infection (John *et al.*, 2015; Mangione *et al.*, 2014). Over-regulation of these cytokines has been known to cause haemorrhagic manifestations, platelet decay, coagulation activation, fibrinolysis and may instigate vascular permeability in dengue patient and aggravate disease severity (Costa *et al.*, 2013; Green & Rothman, 2006). Search for an active compound that could downregulate the overexpressed cytokine in dengue patient is crucial to reduce the severity of dengue fever.

Herbal medicines have a great potential as a remedy for treating various infectious disease. Mushrooms have been essential components of traditional Chinese herbal medicines for thousands of years and recognized as effective to treat and prevent numerous human diseases which include diseases associated with inflammatory response (Gunawardena *et al.*, 2014; Phan *et al.*, 2017). The anti-inflammatory effect of mushroom extracts occurs due to inhibition of NF-kB activation, which suppresses the gene expression of IL-6, IL-1β, TNF-α, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) that play important roles in apoptosis, in the immune system, as well as in the inflammation (Elsayed *et al.*, 2014; Taofiq *et al.*, 2016). The anti-inflammatory effect of mushroom extract mostly due to the presence of polysaccharides, phenolic acids, steroids, terpenes, fatty acids and other metabolites with the first three being the most significant (Taofiq *et al.*, 2016).

In an earlier study, extracts prepared from selected culinary and medicinal mushrooms comprised of *Lignosus rhinocerotis (Cooke) Ryvarden*, *Pleurotus giganteus (Berk) Karunarathna & K.D.Hyde*, *Hericium erinaceus (Bull.) Persoon*, *Schizophyllum commune* (Fr.) and *Ganoderma lucidium* (Curtis) P. Karst were evaluated for *in-vitro* anti-dengue virus effect. The hot aqueous extract (HAE) and aqueous soluble extract (ASE) separated from ethanol extract had exhibited prominent anti-dengue virus activity in plaque reduction assay and real-time RT-PCR (Ellan *et al.*, 2019). These extracts were further selected to study the anti-inflammatory effect in human monocytes infected with dengue virus by measuring the level of cytokine coinciding with dengue infection, IFN-γ, TNF-α, IL-1β, IL-6, IL-8 and IL-10 using BD OptEIA Elisa Kit. To our knowledge, this is the first report on anti-inflammatory effect of mushroom extracts in dengue infected cells.

MATERIALS AND METHODS

Culinary and medicinal mushrooms

Five mushrooms which are known for their culinary and medicinal values were selected for this study. Four of the mushrooms were authenticated by mycologists from Mushroom Research Centre, University of Malaya and voucher specimens were deposited in the Herbarium of University of Malaya (KLU-M). The fruiting bodies of *Hericium erinaceus* (KLU-M 1232) were purchased from Highland Mushroom Farm (Genting Highlands, Pahang). The fruiting bodies of *Pleurotus giganteus* (KLU-M 1227) were obtained from Nas Agro Farm (Sepang, Selangor). Fruiting bodies of *Schizophyllum commune* (KLU-M 1389) were purchased from Glami Lemi Biotechnology research centre (Jelebu, Negeri Sembilan). *Ganoderma lucidium* (KLU-M 1233) was purchased in dried form from Vita Agrotech Sdn. Bhd. (Tanjung Sepat, Selangor). The fruiting bodies were sliced, freeze-dried (Christ, Germany)

for 1-2 days and powdered. The *Lignosus rhinocerotis* was purchased in freeze dried form from Ligno Biotek Sdn. Bhd. (Balakong Jaya, Selangor).

Cells and viruses

African green monkey kidney cells (Vero, ATCC® CCL-81™) and *Aedes albopictus* clone (C6/36, ATCC® CRL-1660™) were obtained from the National Public Health Laboratory, Sungai Buloh, Malaysia. Vero cells were grown in Eagle's minimum essential medium (Sigma Aldrich, St. Louis, MO) containing 2.5% sodium bicarbonate, 1% Hepes 1M, 1% penicillin & streptomycin and 10% heat-inactivated foetal bovine serum (FBS) (Gibco, USA). The C6/36 mosquito cell line was cultured in RPMI 1640 (Gibco, USA) supplemented with 1% sodium bicarbonate and 10% FBS. The cell growth was retained in maintenance medium supplemented with 2% of serum concentration. Dengue Virus serotype-2 strain New Guinea C (GeneBank Accession No. M29095) was obtained from Institute for Medical Research, Kuala Lumpur, Malaysia. The viruses were cultured and propagated in C6/36 cell line with RPMI 1640 medium containing 2% FBS at 28°C. The supernatants were collected by centrifugation after two rounds of freeze-thawing and were stored at -80°C for further experiments.

Preparation of mushroom extracts

The hot aqueous extraction method was done as previously described (Eik *et al.*, 2012). The mushroom powder was soaked overnight in distilled water (1:20 ratio, w/v). The suspension was double boiled in water bath for 30 minutes. The suspension was filtered and freeze-dried to collect the HAE. The ASE was prepared by solvent extraction method. Initially, ethanol extract was prepared by soaking 100 g of mushroom freeze-dried powder in 80% ethanol (1:10 ratio, w/v) for two days. The filtrate was collected by vacuum filtration. The procedure was repeated twice. The pooled filtrate was evaporated using a rotary evaporator to collect crude ethanol extract. The crude ethanol extract was mixed with hexane and subsequently with ethyl acetate. The substances insoluble in both solvents were collected as ASE.

Cytotoxicity assay

The HAE and ASE stock solutions (10,000 µg/ml) were freshly prepared in 2% DMEM and filter sterilized. The solutions were serially diluted to prepare concentrations ranging from 313 µg/ml till 10000 µg/ml. The cytotoxicity assay of HAE and ASE was carried out using the colourimetric MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) method using Cell Titre 96 Non-Radioactive Cell Proliferation assay kit (Promega, USA), according to the manufacturer's instructions.

Measurement of anti-inflammatory effects *in vitro* (Reis *et al.*, 2008)

Human peripheral blood mononuclear cells (PBMC) was prepared from blood collected from six healthy donors. The PBMC isolated from each donor were pooled into two groups. The purification of PBMC was carried out by diluting blood with phosphate buffered saline (1:1). A layer of Ficoll-Hypaque solution (Sigma Aldrich, St. Louis, MO) was transferred carefully underneath blood suspension and centrifuged for 30 min at 2000 rpm. The lowest density of platelets and plasma were collected on the top, PBMC were collected at the interphase of the Ficoll-Hypaque layer, in contrast, red blood cells, and granulocytes that have a higher density than Ficoll-Hypaque were collected at the bottom. The upper layer that contained the plasma and most of the platelets were removed. PBMC located at the interphase were carefully collected and transferred to a new centrifuge tube. Cells were washed with Hank balanced salt solution (HBSS) by centrifugation for 10 min at 1300 rpm. The supernatant was removed, cells were resuspended in HBSS, and the wash was repeated once more to remove most of the platelets. The mononuclear cells were suspended in RPMI-1640 containing 10% FBS, 1% penicillin and 1% streptomycin and transferred to tissue culture flask and incubated for 1 hour at 37°C, 5% CO_2 humidified incubator.

The majority of the PBMC population were comprised of lymphocytes and monocytes. The adherence characteristics of monocytes facilitate the depletion of monocytes from the isolated mononuclear cell suspension whereas lymphocytes do not adhere to plastic. After the incubation, the non-adherent lymphocytes were decanted into a centrifuge tube. The monocytes that adhered to the surface of the culture flask were resuspended in RPMI-1640 medium and incubated for overnight in 37ºC, 5% CO_2 humidified incubator. The following day, the monocytes cells were then seeded into 96 well plates (TPP, Switzerland) at the concentration of $2x10^6$ cells/mL. After overnight incubation, the cells were infected with DENV-2 New Guinea C. After 2 hours of adsorption at 37°C, the cell culture supernatant was replaced with non-cytotoxic concentration of mushroom extract diluted in RPMI medium containing 25 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma Aldrich, St. Louis, MO) and 1 µg/ml ionomycin (Sigma Aldrich, St. Louis, MO). The non-cytotoxic concentration of majority of mushroom extracts was 2000 µg/ml, except for HAE of *H. erinaceus* and *G. lucidium* was 1500 µg/ml and *G. lucidium* ASE was 500 µg/ml. Dexamethasone (0.05 mM) (Sigma Aldrich, St. Louis, MO), an anti-inflammatory and immunosuppressant, was used as a positive control. After incubation for 48 hours at 37°C in 5% CO_2 atmosphere, the culture supernatant was tested for the cytokine production of IFN-γ, TNF-α, IL-6, IL-10, IL-1β and IL-8 by BD Opt EIA ELISA Kit (BD Bioscience, USA) according to the manufacturer's protocol. The dose-dependent assay was carried out on the mushroom extracts that exhibited significant inhibitory activity (>50% inhibitory activity; $p < 0.05$) at maximum non-cytotoxic concentration. Three different concentrations were selected for each extract. For most of the extract, the selected concentrations were 2000 µg/ml, 1500 µg/ml and 1000 µg/ml. Meanwhile, for HAE *H. erinaceus* and *G. lucidium* the concentrations were 1500 µg/ml, 1000 µg/ml and 500 µg/ml and *G. lucidium* ASE were 500µ g/ml, 250 µg/ml and 125 µg/ml.

Ethical consideration

This study received approval and permission from the National Institute of Health, Ministry of Health (Ref. No.: (28) dlm. KKM/NIHSEC/07/0701 JLD9) and the Medical Research & Ethic Committee, Ministry of Health (Ref. No.: (6) dlm. KKM/NIHSEC/08/0804/P12-55). The healthy donors were medical lab technologist from National Public Health Laboratory, Sungai Buloh. Their participation in the study was entirely voluntary. Written informed consent was obtained from them and they were informed that their personal information will be kept confidential.

Statistical analysis

The equation for regression for each cytokine standard was obtained using MS Excel (Microsoft Office 2007 Professional). The equation was used to calculate the cytokine concentration. Data are expressed as mean ± standard errors, and the results are taken from two independent experiments performed in duplicate. ANOVA procedure was performed to compare the mean values of cytokines. A Dunnett test was used to compare all treatment groups vs the control group. P values less than 0.05 were considered to be statistically significant.

RESULTS

Cytokine profile of DENV infected human monocytes

Figure 1 shows the cytokine production profile in DENV infected monocytes. The expression of IFN-γ (P: 0.006), IL-10 (P: 0.019), TNF-α (P:0.05), and IL-6 (P: 0.05) in dengue-infected monocytes were significantly elevated than non-infected monocytes. The increase in cytokine production was between 1.18 to 1.4-fold, except for IL-1β (P: 0.371) and IL-8 (P: 0.176) where no significant effect was noted. IL-6 was the highly secreted cytokines with a value of 2218.1±55 pg/ml and, it was 1.31-fold higher than non-infected monocytes. A significant fold increase in infected cells was also noted IL-10 (1.28-fold increase) (P; 0.019) and IFN-γ (1.4-fold increase) (P: 0.006). Meanwhile, a moderate

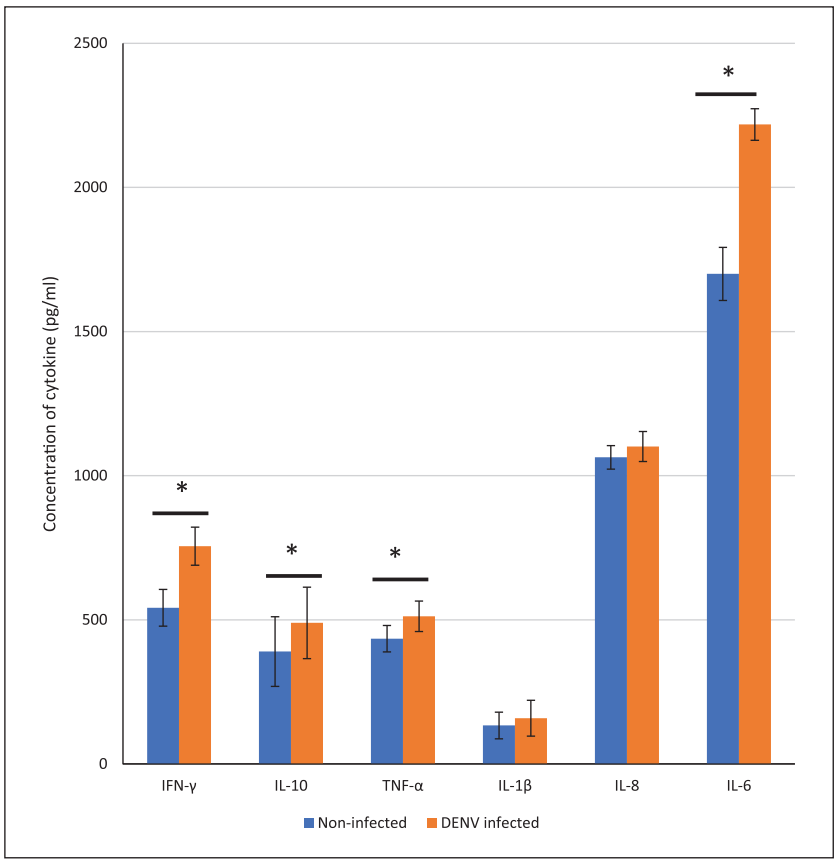

Figure 1. The expression of cytokines in dengue-infected monocyte.
The expression of cytokine in DENV infected monocytes after 48 hours of incubation at 37°C was determined by measuring the absorbance at 450 nm. The cytokine value was calculated from the equation for the regression obtained from standard curve. Data are expressed as mean ± SEM and the results are taken from two independent experiments performed in duplicate. P values less than 0.05 were considered to be statistically significant.

fold increase was noted in TNF-α production (1.18-fold increase) (P:0.05). The minimally expressed cytokine was IL-1β, the yield was only 158.7±62.1 and the fold increase was insignificant (P:0.371). Although IL-8 was highly expressed in monocytes, no significant difference detected between non-infected and infected monocytes in this study (P: 0.176).

Anti-inflammatory effect of mushroom extract on the cytokine level produced in DENV infected monocytes

To evaluate the anti-inflammatory effect of mushroom extracts, the level of cytokine expression was measured in the supernatants of dengue-infected monocytes treated with a single dose of selected HAE or ASE. Figures 2a-e show the percentage of inhibitory effect of cytokine in DENV infected monocytes after treated with maximum non-cytotoxic concentration of mushroom extract. The IC_{50} values, the concentration of mushroom extracts that showed 50% of inhibitory effect in comparison with virus control group were estimated for active extracts (Table 1). We found that HAE showed a higher anti-inflammatory effect than ASE which were noted by a significant reduction in the production of cytokine ($P < 0.05$). The most potent anti-inflammatory effect was detected in HAE of *Ganoderma lucidium*, *Schizophyllum commune*, *Pleurotus giganteus* and ASE of *Lignosus rhinocerotis*. Their anti-inflammation effects towards some cytokines were comparable with dexamethasone, the reference inhibitor.

Table 1. Cytotoxic and anti-inflammatory effects of mushroom extracts

Mushroom extract	CC_{50} (µg/ml)	IC_{50} (µg/ml)				
		IFN-γ	IL-10	TNF-α	IL-1β	IL-6
P. giganteus HAE	4211.8±185.6	169.8±13.6	–	347.7±4.4	1055.1±109.9	–
P. giganteus ASE	10 000	734.8±445.3	–	–	–	–
L. rhinocerotis ASE	3051±244.6	119.4±87.7	596.6±290.7	803.1±477.4	406.2±25.9	–
H. erinaceus HAE	5174.4±1309.7	840.5±238.8	–	–	–	–
H. erinaceus ASE	10 000	742.5±228.6	–	–	–	–
S. commune HAE	4262.8±1345.7	541.4±348.5	131.8±70.2	–	–	946.4±469.2
S. commune ASE	10 000	392.3±146.4	–	–	1330.1±465.7	–
G. lucidium HAE	1748.1±189.8	178.7±67.5	378.9±62.2	604.4±191.1	965.1±371.7	1586.6±202.2
G. lucidium ASE	779.1±125	170.7±89.2	–	–	–	–

IC_{50}, the concentrations of extracts required to inhibit 50% of cytokine production compared with the virus control group and CC_{50}, 50% cytotoxic concentrations capable of reducing the absorbance by 50% in comparison to the negative control cell without extracts were calculated from dose response curve by probit analysis. ANOVA analysis was performed to compare the mean values of cytokines of different concentration of mushroom extract; a Dunnett test was then used to compare the cytokine value of treated groups vs the control group ($p < 0.05$).
"–" IC_{50} value was not defined due to insignificant inhibitory effect.

Majority of mushroom extracts exhibited most significant inhibitory effect against IFN-γ production (Figure 2a). The highest inhibitory effects were noted in HAE of *P. giganteus*, ASE of *L. rhinocerotis*, HAE and ASE of *G. lucidium*, the IC_{50} values were 169.8±13.6 µg/ml, 119.4±87.7 µg/ml, 178.7±67.5 µg/ml and 170.7±89.2 µg/ml, respectively. Moderate inhibitory effect was detected against TNF-α, IL-10 and IL-1β. Production of TNF-α was significantly inhibited by *P. giganteus* HAE, *G. lucidium* HAE and *L. rhinocerotis* ASE (Figure 2b). The HAE extract of *P. giganteus* gave the lowest IC_{50} value which was 347.7±4.4 µg/ml. For IL-10 production, significant inhibitory effect was noted in *L. rhinocerotis* ASE, HAE of *S. commune* and *G. lucidium* (Figure 2c). The lowest IC_{50} was found in *S. commune* HAE (131.8±70.7 µg/ml). The inhibitory effect on IL-1β production was noted in *P. giganteus* HAE, *G. lucidium* HAE, *L. rhinocerotis* ASE and *S. commune* ASE (Figure 2d). The most active extract was *L. rhinoceritis* ASE, the IC_{50} value was 406.2±25.9 µg/ml. Mushroom extracts showed weak inhibitory effect against IL-6 production (Figure 2e). Three mushroom extracts significantly suppressed the IL-6 production and *S. commune* HAE was the most active among them, with an IC_{50} value of 946.4±469.2 µg/ml. *Ganoderma lucidium* HAE and *L. rhinocerotis* ASE showed a broad spectrum of inhibitory effect by suppressing the production of most of the inflammatory cytokines. The SEM for few mushrooms in Figure 2b, c and e were huge due the level of cytokine expression which varies between two groups of PBMC donors. The inhibitory effect was insignificant in PBMC donor group with high expression of cytokines.

The HAE and ASE obtained from *H. erinaceus* did not show a significant inhibitory effect against all the selected cytokines. Mushroom extracts did not alter the production of the IL-8 chemokine. The

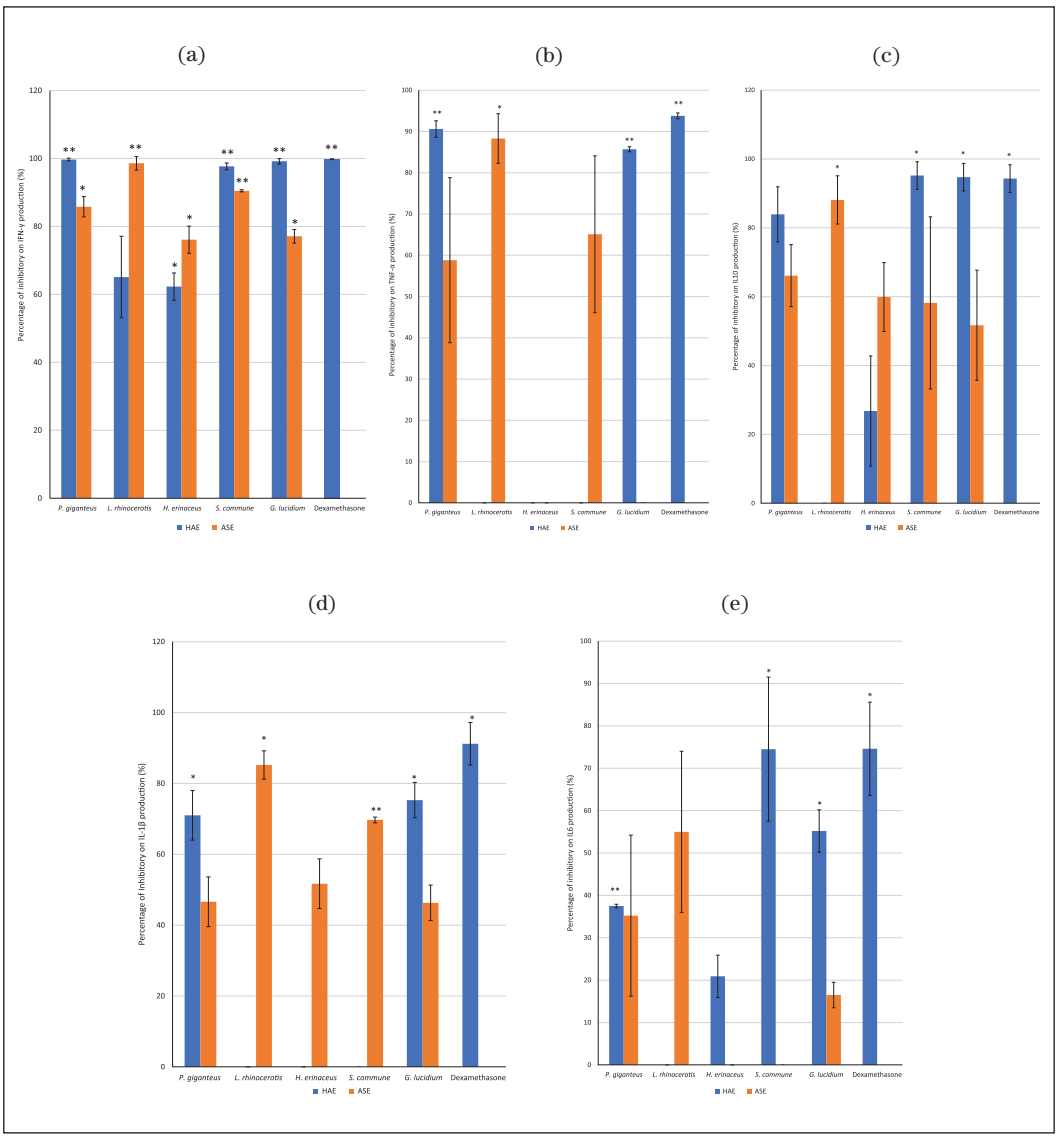

Figure 2. Percentage of inhibitory effect of a) IFN-γ, b) TNF-α, c) IL-10, d) IL-1β and e) IL-6 cytokines in DENV infected monocytes after treated with mushroom extract.

DENV infected monocytes cells were treated with the non-cytotoxic concentration of mushroom extracts. The non-cytotoxic concentrations of majority of mushroom extracts were 2000 µg/ml, except for HAE of *H. erinaceus* and *G. lucidium* were 1500 µg/ml and *G. lucidium* ASE was 500 µg/ml. The expression of cytokine was measured after 48 hours of incubation at 37°C by reading the absorbance at 450 nm. The level of expression was compared with treated versus untreated DENV infected monocytes. Data are expressed as mean ± SEM and the results are taken from two independent experiments performed in duplicates. *denotes significant value at P <0.05 and ** P <0.01.

inhibitory effect demonstrated by mushroom extract comparable with the inhibitory effect shown by dexamethasone which exhibited a very prominent anti-inflammatory effect against all the selected cytokines except for IL-8.

DISCUSSION

Anti-inflammatory effects of mushroom were evaluated by measuring the expression of six different cytokines, IFN-γ, TNF-α, IL-6, IL-10, IL-8 and IL-1β in the culture supernatant

collected after two days of post-infection. All the selected cytokines could be detected after two days of post-infection as reported in the previous study (Reis *et al.*, 2008). We found that the cytokines were expressed at various detection levels. IL-6 showed up as fast kinetics, IFN-γ, TNF-α and IL-10 appeared as inter-mediate kinetics, and IL-1β as slow kinetics. Chaturvedi *et al.* (1999) also observed early appearance of IL-6 in the second day of dengue infection in peripheral blood leukocyte cultures. Meanwhile, IFN-γ and TNF-α showed up as inter-mediate kinetics, the highest concentration was detected in day three of infection. In contrast with our finding, the IL-10 only showed peaked value in day seventh of infection. We found a low expression of IL-1β. According to Moreno-Altamirano *et al.* (2004) the expression of IL-1β occurs at the very early phase of infection and gradually decreased over time but still over base levels at 72 hours post-infection. Kou *et al.* (2011) also reported a similar trend in IL-1β expression in dengue virus-infected PBMC by Cytometric Bead Array assay. In the monocytes isolated from Brazilian donors, IL-1β was not significantly detected, meanwhile TNF-α, IL-6 and IL-10 were upregulated as observed in our study (Reis *et al.*, 2007).

The cytokine pattern observed in dengue-infected monocytes correlated well with the inflammatory response in dengue patient. The elevated levels of TNF-α, IL-1β, IL-6, IL-8, IL10, and IFN-γ, have been detected in the serum of DF and DHF/DSS patients. Higher expression of TNF-α was more often detected in the plasma of DHF patients than DF patients (Soundravally *et al.*, 2014). Inyoo *et al.* (2017) also had observed an *in-vitro* synergistic effect between TNF-α and DENV-2 infection and they suggested that TNF-α may play a role in increasing endothelial permeability during DENV infection. Production of IFN-γ during dengue infection provide an early host defence that could inhibit the viral replication and regulate inflammatory response (Fagundes *et al.*, 2011; Pal *et al.*, 2014). IL-1β is also an important proinflammatory cytokine that plays an important role in the pathogenesis of DHF-DSS. Hottz *et al.* (2013) suggested that an inflammasome-dependent release of IL-1β from DENV-infected platelets contributes to increased endothelial cell permeability *in vitro*. IL10 is a late cytokine associated with dengue severity, which causes thrombocytopenia (Ma *et al.*, 2014). IL-10 may regulate the expression of suppressor-of-cytokine-signaling (SOCS) factors and suppressed the production of IFNs and nitric oxide, both known for their antiviral activities (Tsai *et al.*, 2013). Levy *et al.* (2010) reported that IL-6 is associated with severe dengue, as the levels of IL-6 in severe dengue was higher, especially in secondary infections. Rachman and Rinaldi (2006) also suggested that IL-6 could trigger higher production of antiplatelet or anti-endothelial cell auto-antibodies, upregulation of tissue plasminogen activator, and coagulation deficiency, leading to plasma leakage and bleeding.

Search for an active compound that possibly displays anti-inflammatory response by inhibiting the production of the cytokine associated with severity of dengue fever will be beneficial to prevent further complications. Here we had selected extracts from mushrooms to evaluate its capability to diminish the production of inflammatory cytokine response in dengue-infected monocytes. We had confirmed that the selected extracts showed the significant anti-inflammatory effect against IFN-γ, IL-10, TNF-α, IL-1β, and IL-6 in dengue monocytes treated with mushroom extracts. The prominent effect was observed in *Pleurotus giganteus* HAE, *Schizophyllum commune* HAE, *Ganoderma lucidium* HAE and *Lignosus rhinocerotis* ASE. The anti-inflammatory response of mushroom extract in dengue-infected monocytes correlates with other anti-inflammatory study associated with dengue infection (Schul *et al.*, 2007). They reported that 7-deaza-2'-C-methyl-adenosine (7-DMA) and N-nonyl-deoxynojirimycin (*N*N-DNJ) an anti-viral drug that significantly lowered the level of viremia, could bring down proinflammatory cytokine levels in AG129 mice. We also observed that not all extracts that exhibited anti-viral effect could reduce inflammatory response as observed in *Hericium erinaceus*

extracts. In contrast, *G. lucidium* HAE which had a low anti-viral effect in plaque reduction assay (Ellan *et al.*, 2019) showed a higher reduction in cytokine response.

Among the selected mushroom, the anti-inflammatory effect of *Ganoderma lucidium* has been most extensively studied (Cai *et al.*, 2016). Anti-inflammatory study of selected mushroom extracts was mostly towards the inhibition of the NF-kB pathway, which suppresses the expression of cytokines, NO, iNOS, and COX-2. A triterpene and steroid purified from *G. lucidium* showed anti-inflammatory in LPS induced RAW264.7 cells by reducing NO production, suppressed iNOS and COX-2 expression and inhibited TNF-α and IL-6 cytokine production (Akihisa *et al.*, 2007; Choi *et al.*, 2014; Dudhgaonkar *et al.*, 2009; Liu *et al.*, 2015a). Ganoderic acid C1, a triterpenoid found in *Ganoderma lucidium*, suppressed the production of inflammatory cytokines (TNF-α, IFN-γ and IL-17A) found in Crohn Disease, inflammatory bowel disease and warrants further clinical investigation for the treatment of this disease (Liu *et al.*, 2015b). Anti-inflammatory effect of *G. lucidium* was also observed in a low molecular weight water-insoluble β-(1→3)-d-glucan (GLPs) which exhibited significant inhibition of inflammation in Raw 264.7 cells by decreasing the NO production, via blocking NF-κB and the inhibition of the phosphorylation of JNK MAPK signal pathways (Wang *et al.*, 2014).

Only a few studies on anti-inflammatory effects were reported for the other selected mushrooms. The protective effect of ethanol extract of *P. giganteus* (EPG) on RAW 264.7 was mediated via the suppression of the STAT 3, and COX-2 pathways (Baskaran *et al.*, 2017). The high content of glycine in *P. gigateus* might act on macrophages to suppress activation of transcription factors and the formation of free radicals and inflammatory cytokines (Phan *et al.*, 2014). An exopolysaccharide obtained from a mycelial culture of *S. commune* significantly ($p < 0.05$) inhibited lipopolysaccharides-induced iNOS expression levels in the RAW 264.7 cells in a dose-dependent manner (Du *et al.*, 2017). On the other hand, Li *et al.* (2014) isolated a novel ergosterol conjunction-type alkaloid, hericine from the dried fruiting bodies of *H. erinaceum*, it significantly inhibited protein expression of iNOS and COX-2 and reduced NO, PGE2, TNF-α, IL-6 and IL-1β production in RAW264.7 cells exposed to LPS.

Employing two different technique of extraction produced a distinct modulation effect, where a better effect was observed in HAE. Previous findings suggested that the anti-viral composition in mushroom extracts might be glucan and protein which present abundantly in HAE and ASE (Ellan *et al.*, 2019). Involvement of heat for preparation of HAE might release low molecular weight polysaccharide and protein. Meanwhile as ASE was prepared at room temperature it might contain higher molecular weight polysaccharide and protein. It had been reported that degraded form of polysaccharide had improved the suppressive effect on NO production than native polysaccharide due to the mild effect of intramolecular hydrogen bond that has more free hydroxyl and amino groups (Du *et al.*, 2016). In contrast with this finding, high molecular weight protein obtained from *Lignosus rhinocerotis* cultivar TM02 cold water extract showed a better anti-inflammatory effect against TNF-α production (Lee *et al.*, 2014). Besides the molecular size, the monosaccharide composition and linkage types also may affect polysaccharide's anti-inflammatory activities (Zhao *et al.*, 2014).

CONCLUSION

In conclusion, the HAE of *Pleurotus giganteus*, *Schizophyllum commune*, *Ganoderma lucidium* and ASE of *Lignosus rhinocerotis* had demonstrated anti-inflammatory activities by inhibiting proinflammatory cytokines in a DENV-infected monocyte, this leads to further evaluate the use of mushroom extract as an anti-inflammatory agent that could alleviate the severe inflammatory response during dengue haemorrhagic fever. However, further studies need to be carried out to investigate

the relationship between the structure of the active compound in the mushroom aqueous extracts and anti-inflammatory effect, elucidation of anti-inflammatory mechanism regarding signalling pathways on macrophages and testing this effect in the animal model.

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