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*Original article*

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# Anti-neuroinflammatory of Chloroform Extract of *Panax ginseng* Root Culture on Lipopolysaccharide-stimulated BV2 Microglia Cells

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## Abstract

***Background:*** It is believed that activation of microglia in the central nervous system upon detection of stimulus like lipopolysaccharides provokes neuroinflammation via the production of pro-inflammatory mediators and cytokines. The cytoprotective and anti-inflammatory properties of various folk medicine has been gaining attention as a strategy to combat various disease. This study aimed to assess the anti-neuroinflammatory properties of chloroform extract of *in vitro Panax ginseng* root culture based on nitric oxide and cytokines production.

***Methods:*** The study was initiated with the determination of maximum non-toxic dose (MNTD) of *P. ginseng* root culture chloroform extract using the MTT assay. The lipopolysaccharides-stimulated BV2 microglia cells were treated with MNTD and ½MNTD of the extract and its anti-neuroinflammatory properties were assessed by measuring the production of nitric oxide (NO) via Griess assay, as well as TNF-α, IL-6 and IL-10 using Quantikine ELISA.

***Results:*** It was found that the MNTD and ½MNTD of the extract did not play a significant role in the production of pro-inflammatory cytokines such as NO, TNF-α and IL-6. However, the MNTD and ½MNTD of chloroform extract significantly increased the anti-inflammatory IL-10 compared to the untreated cells.

**Conclusions:** With this, the chloroform extract of *P. ginseng* root culture potentially exerts anti-neuroinflammatory properties.

**Keywords:** Anti-inflammatory cytokines, Ginsenosides, Microglia cells, Neuroinflammation, Pro-inflammatory cytokines.

## Introduction

Neurodegeneration is a chronic and progressive event in the central nervous system (CNS), in which neuronal and glial material loss occurred, leading to impairment in cognitive function and mental stability (1). Uncontrolled and prolonged neurodegeneration appeared to be the commonest manifestation of various neurodegenerative diseases (ND), which is commonly affecting the aging population (1). The clinical presentation of ND is dependent on the foci of the CNS that is affected by neurodegeneration, for instance, neurodegeneration in the substantia nigra is the hallmark of Parkinson's disease in which the motor system of the patient is jeopardised (2).

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The aetiology of neurodegeneration is not entirely confirmed as it is a complicated pathological process involving genetic predisposition, traumatic injury and infection (3–5). The CNS is an immune-privileged organ system which the access of peripheral innate and acquired immune cells are tightly regulated (6). However, studies suggested that systemic lipopolysaccharides (LPS) is able to induce inflammatory responses in the CNS (7) and microglia cells are believed to be the myeloid cells that exerts important role in the event of neuroinflammation (7,8). Primarily, the inflammation process is neuroprotective as it serves to eliminate harmful stimulus by two mechanisms, either the direct cell-to-cell contact or through the release of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) as well as cytotoxic factor such as nitric oxide (NO) and reactive oxygen species (ROS) (9). However, chronic and prolonged neuroinflammation may induce neurodegeneration causing ND.

Despite the prevalence of ND and its morbidity, there are no effective and disease modifying treatment available for ND to date. Currently available treatments on ND are palliative and it only suppresses the symptoms without targeting the root cause of the disease. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the drugs that are used in suppressing the inflammation in the CNS. However, alleviation of symptoms using NSAIDs does not promote neurogenesis and reverse or modify neurodegeneration (10). Moreover, prolonged consumption of NSAIDs is found to be closely associated with side effects such as gastrointestinal discomfort, ulceration and bleeding due to the lack of PG required for normal physiology (10). This creates an interest and attention in the studies of anti-neuroinflammatory properties from various safer compounds and plants is among the greatest interest (10,11).

Traditionally, plants and herbs have been used in treating various diseases with lesser side effects, proven that the plants is an invaluable subject in pharmacological studies (11). *Panax ginseng*, also known as Asian or Korean ginseng, is a common folk medicine used in traditional Chinese medicine (12). *P. ginseng* is commonly used as a general health tonic, in the association of memory boosts and cognitive function improvement (12). Studies revealed that *P. ginseng* showed anti-inflammatory properties by reducing pro-inflammatory cytokines in the inflammation of liver (13). Besides, it was reported that *P. ginseng* was able to modulate oxidative stress by inhibiting the expression of cyclooxygenase-2 (COX-2) and iNOS (14). There were also studies conducted on SH-SY5Y neuroblastoma cells where ginsenosides were found to upregulates the brain derived neurotrophic factor, thus induced neurogenesis and demonstrated neuroprotective properties 15). Another studies confirmed that *P. ginseng* showed anti-cancer anti-inflammatory effect through interaction with COX-2, which play important roles in suppressing tumorigenesis via inflammatory pathway, upon the treatment on MCF-7 cells (16).

Despite the popularity of *P. ginseng* as Chinese folk medicine, as well as its well characterised pharmacological properties and bioactivity, there is lack of information on the anti-neuroinflammatory mechanism of *P. ginseng*. Besides, this study has used *in vitro P. ginseng* root culture, which is not widely used in the previous studies, allowing standardization of bioactive compounds found in *P. ginseng*. Hence, this study was designed to investigate the anti-neuroinflammatory responses of chloroform extract of *in vitro P. ginseng* root culture on LPS-stimulated BV2 microglia cells based on nitric oxide, pro-inflammatory and anti-inflammatory cytokines production.

## Materials and Methods

### *Chloroform extraction of Panax ginseng root culture*

*Panax ginseng* root culture was derived from 20-L advance bioreactor system (Malaysian Nuclear Agency, Malaysia) and it was cultured in modified Murashige & Skoog medium, supplemented with ½ nitrate, 5 mg/L of indole-3-butric acid (IBA) and 5% sucrose. The 4-

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week old root culture was harvested from the bioreactor and was pulverized into fine powder, followed by the addition of 99.8% chloroform (Friendemann Schmidt, Australia) at ratio of 1 g: 5 mL. The extraction mixture was then incubated in the dark, under room temperature for 3 days. After that, the mixture was filtered and the filtrate was subjected to evaporation by rotary evaporator (Buchi, Switzerland) and oven at 60 °C.

***Preparation of stock and working solution of the extract***

The stock solution was prepared by addition of 210 µL of dimethyl sulfoxide (DMSO) (Sigma Aldrich, USA) into 52.42 mg of root culture chloroform extract. The stock solution was further subjected to dilution using complete Dulbecco's Modified Eagle medium (DMEM) (Gibco, USA) in order to prepare desirable concentration for cell treatments.

***Preparation of positive drug control***

The 10 mM stock solution of indomethacin (Sigma Aldrich, USA) was prepared by dissolving 3.58 mg of indomethacin (Sigma Aldrich, USA) with 1 mL of DMSO (Sigma Aldrich, USA). Then, 2.5 µL of 10 mM stock solution of indomethacin was mixed with 997.5 µL of complete DMEM to prepare 1mL of 25 µM indomethacin as working reagent.

***BV2 microglia cell culture and maintenance***

BV2 microglia cells were provided by Dr Sharmili Vidyadaran from Universiti Putra Malaysia (Selangor, Malaysia). The cells were cultured in DMEM (Gibco, USA) supplemented with 10% FBS (Gibco, USA), 1% penicillin and streptomycin (Gibco, USA), 0.1% fungizone (Gibco, USA) and 0.1% gentamicin (Gibco, USA) and subsequently incubated in the 5% carbon dioxide ($CO_2$) incubator at 37 °C.

***Determination of cytotoxicity using MTT assay***

The assessment of MNTD was done to determine the highest concentration of chloroform extract of *P. ginseng* root culture that does not exert cytotoxic effect to BV2 microglia cells. This was done by seeding the cells into flat bottom 96-well plate (Corning, USA) at the density of $5x10^4$ cells/well. The cells were incubated for 24 hours (>70% confluent) in 5% $CO_2$ incubator at 37 °C. Next, the cells were treated with 2-fold serially diluted extract ranging from 0 to 250 µg/mL and it was further incubated for 24 hours.

After 24 hours of incubation, 20 µL of supernatant was removed and replaced by 20 µL of 5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (Bio Basic, Canada) in dark condition, followed by incubation of 4 hours in 5% $CO_2$ incubator at 37 °C. All the supernatant was subsequently discarded and replaced with 100 µL of solvent grade DMSO (Friendemann Schmidt, Australia) for the dissolution of formazan crystals. The 96-well plate was then well mixed for 5 seconds, followed by the measurement of absorbance at the wavelength of 570 nm with 650 nm as the reference wavelength using a microplate reader (Spectramax Molecular Devices, USA). Determination of percentage of cytotoxicity of the extracts was followed by plotting the graph of cytotoxicity against the chloroform extract concentration. The graph was used in the identification of MNTD of the chloroform extract of *P. ginseng* root culture, whereby the concentration of the extract at the point where it shows≤ 0% of cytotoxicity is identified as the MNTD.

***Lipopolysaccharides stimulation and cell treatments with extract***

Firstly, the healthy and viable cells were seeded into a flat bottom 24-well plate with the density of $1x10^5$ cells/well and it was incubated for 1-2 days (>70% confluent) in 5% $CO_2$ incubator at 37 °C. Next, the cells were treated with seven treatment groups, followed by 3 hours of incubation in 5% $CO_2$ incubator at 37 °C. After the incubation, 0.1 µg/mL of LPS (Sigma Aldrich, USA) was added into the cells to evoke inflammation in the cells, followed by 24 hours incubation in 5% incubator at 37 °C. Lastly, the supernatants were collected and subjected to further studies on nitric oxide and cytokines level. The seven treatment groups consisted of untreated cells,

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cells treated with ½ MNTD, cells treated with MNTD as well as all above treatments that were stimulated with 0.1 µg/mL LPS.

***Determination of nitric oxide (NO) level using Griess assay***

In the determination of NO levels, freshly collected supernatant from treated cells was used as prolonged incubation may leads to degradation of NO. A total of 50 µL of Griess reagent (Sigma Aldrich, USA) were mixed with 50 µL of supernatant in the dark, followed by absorbance measurement at the wavelength of 540 nm at 5 minutes interval for total of 30 minutes, using a microplate reader (Spectramax Molecular Devices, USA). With the aid of a sodium nitrite ($NaNO_2$) standard curve constructed at the concentration of 0 to 250 µM, the concentration of nitrite in the treated cells was determined.

***Determination of cytokines level using ELISA***

The tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10) levels were determined by subjecting 50 µL of supernatant collected from each treatment groups to the Quantikine ELISA kit (R&D System, USA), according to the manufacturer's instructions.

***Statistical analysis***

The results shown were summarized from three independent experiments with triplicates. The data was represented as mean ± standard deviation. The results were analysed with Statistical Package for the Social Sciences (SPSS) 11.0 software using one-way ANOVA while the significant differences of $p< 0.05$ were examined using Tukey's HSD Multiple Comparison Test.

## Results

***Determination of maximum non-toxic dose (MNTD)***

The MTT assay is used in determining the MNTD of chloroform extract of *P. ginseng* root culture on BV2 microglia cells. As shown in Figure 1, the MNTD obtained from the root culture chloroform extract was 24.3±5.5 µg/mL. As such, the ½ MNTD was identified as 12.2±2.8 µg/mL. The chloroform extract of root culture with concentration below 24.3±5.5 µg/mL did not exhibit cytotoxicity effect to BV2 microglia cells but rather promoted the cell growth up to 37.56% at the concentration of 1.95 µg/mL. On the other hand, extract with concentration more than 24.3±5.5 µg/mL showed gradual increase in cytotoxicity as the concentration increased.

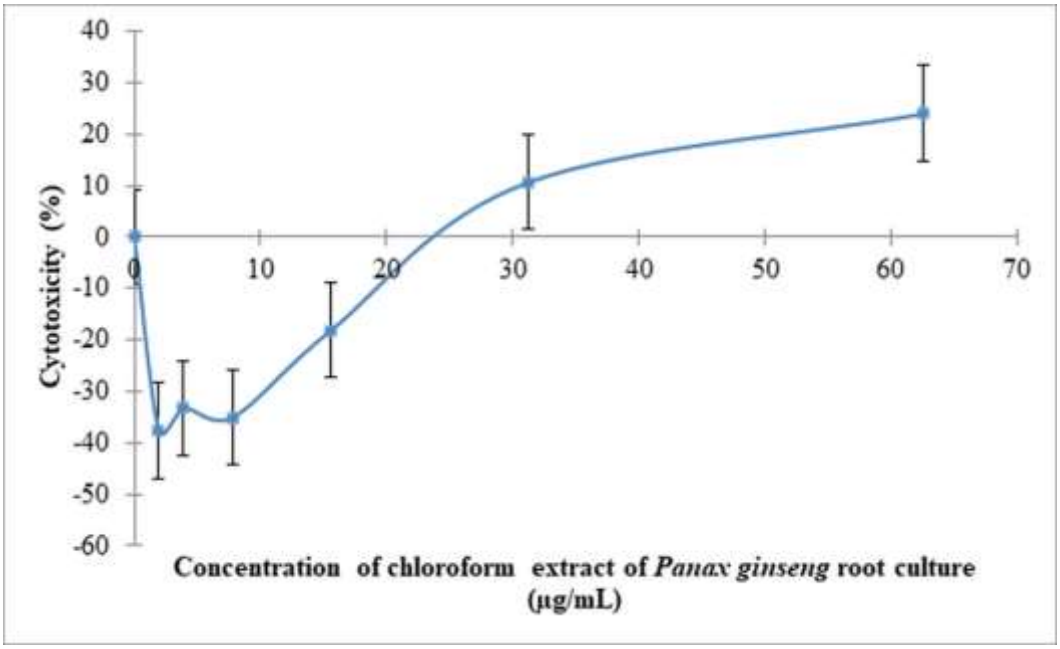


**Fig. 1.** The percentage of cytotoxicity of chloroform extract of *P. ginseng* root culture on BV2 microglia cells after 24 hours of incubation. Bar indicates the means ± standard deviation. Red arrow indicates the maximum non-toxic dose (MNTD) of chloroform extract of *P. ginseng* root culture.

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***Determination of intracellular nitrosative stress upon treatment***

Measurement of the NO in the form of $NaNO_2$ using Griess assay allows the assessment of nitrosative stress of BV2 microglia cells upon the treatment with various concentrations of chloroform extract of *P. ginseng* root culture. According to Figure 2, the untreated cells were shown to produce 10.53±4.55 µM of $NaNO_2$. When the cells were stimulated with 0.1 µg/mL of LPS, the concentration of $NaNO_2$ produced by the cells was 20.19±1.76 µM, which was the highest concentration in relative to other treatment groups. As the LPS-stimulated cells were treated with 25 µM indomethacin, the concentration of $NaNO_2$ produced by the cells was significantly reduced by 53 08% as opposed to LPS-stimulated cells, showing that indomethacin is a potent positive drug control in mediating the inflammatory production of NO. Besides, the LPS-stimulated cells treated with MNTD and ½ MNTD of chloroform extract of *P. ginseng* root culture produced 15.36±2.74 µM and 13.76±3.04 µM of $NaNO_2$, respectively. This shows that the $NaNO_2$ level in MNTD and ½ MNTD-treated LPS-stimulated cells was reduced by 23.90% and 31.83%, accordingly as compared to LPS-stimulated cells alone. Despite the reduction of $NaNO_2$ level observed in MNTD and ½ MNTD-treated LPS-stimulated cells, the value recorded were not significantly different as compared to untreated LPS-stimulated cells and the potency was lower as compared to the indomethacin-treated LPS-stimulated cells.

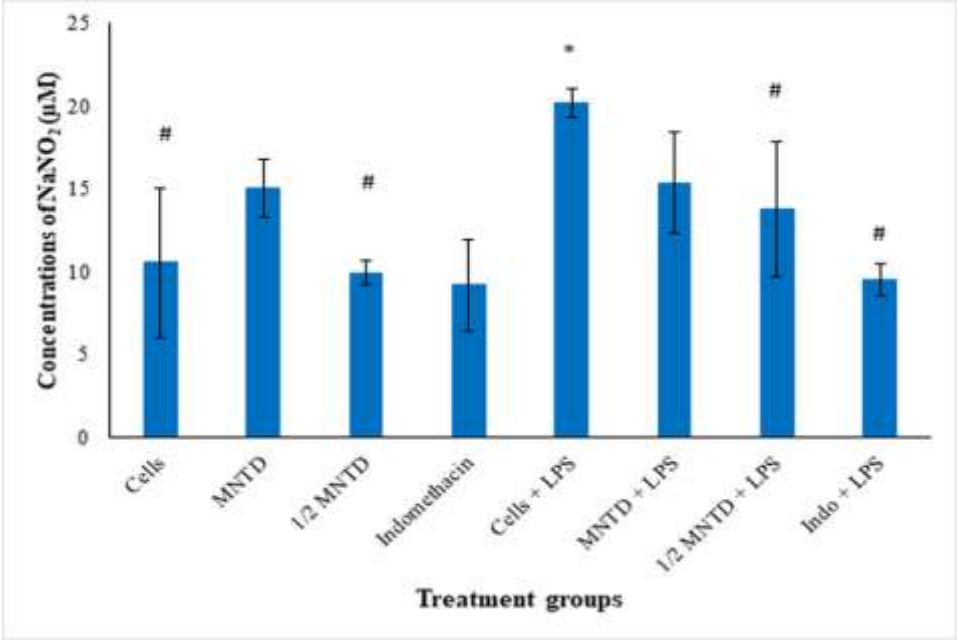


**Fig. 2.** The nitric oxide (NO) level in LPS-stimulated BV2 cells upon treatment with chloroform extract of *Panax ginseng*. Bars indicate the means±standard deviation. '*' indicates that the treatment was significantly different from the untreated cells using one-way ANOVA followed by Tukey's HSD Multiple Comparison Test at $p< 0.05$. '#' denotes the treatment was significantly different from the LPS-stimulated cells using one-way ANOVA followed by Tukey's HSD Multiple Comparison Test at $p< 0.05$.

***Determination of tumor necrosis factor-α (TNF-α) level upon treatment***

TNF-α is a pro-inflammatory cytokine that is expressed upon an inflammatory reaction. Measurement of TNF-α level using the Quantikine ELISA assay enables the determination of inflammatory condition of BV2 microglia cells upon the treatment with chloroform extract of *P. ginseng* root culture. As shown in Figure 3, the untreated cells produced 19.48±1.48 pg/mL of TNF-α. However, stimulation of these BV2 microglia cells using 0.1 µg/mL LPS increased the production of TNF-α up to 89.99±2.59 pg/mL. When the LPS-stimulated cells were treated with 25 µM of indomethacin, a significant reduction

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of 28.26% of TNF-α as compared to untreated LPS-stimulated cells was observed. Figure 3 also exhibited that LPS-stimulated cells treated with MNTD of chloroform extract of *P. ginseng* root culture produced 89.27±0.65 pg/mL of TNF-α, which was a slight and insignificant reduction of 0.8% as compared to untreated LPS-stimulated cells. In contrast, LPS-stimulated cells treated with ½ MNTD of chloroform extract of *P. ginseng* root culture produced 93.66±3.11 pg/mL which was 4.08% higher as compared to untreated LPS-stimulated cells.

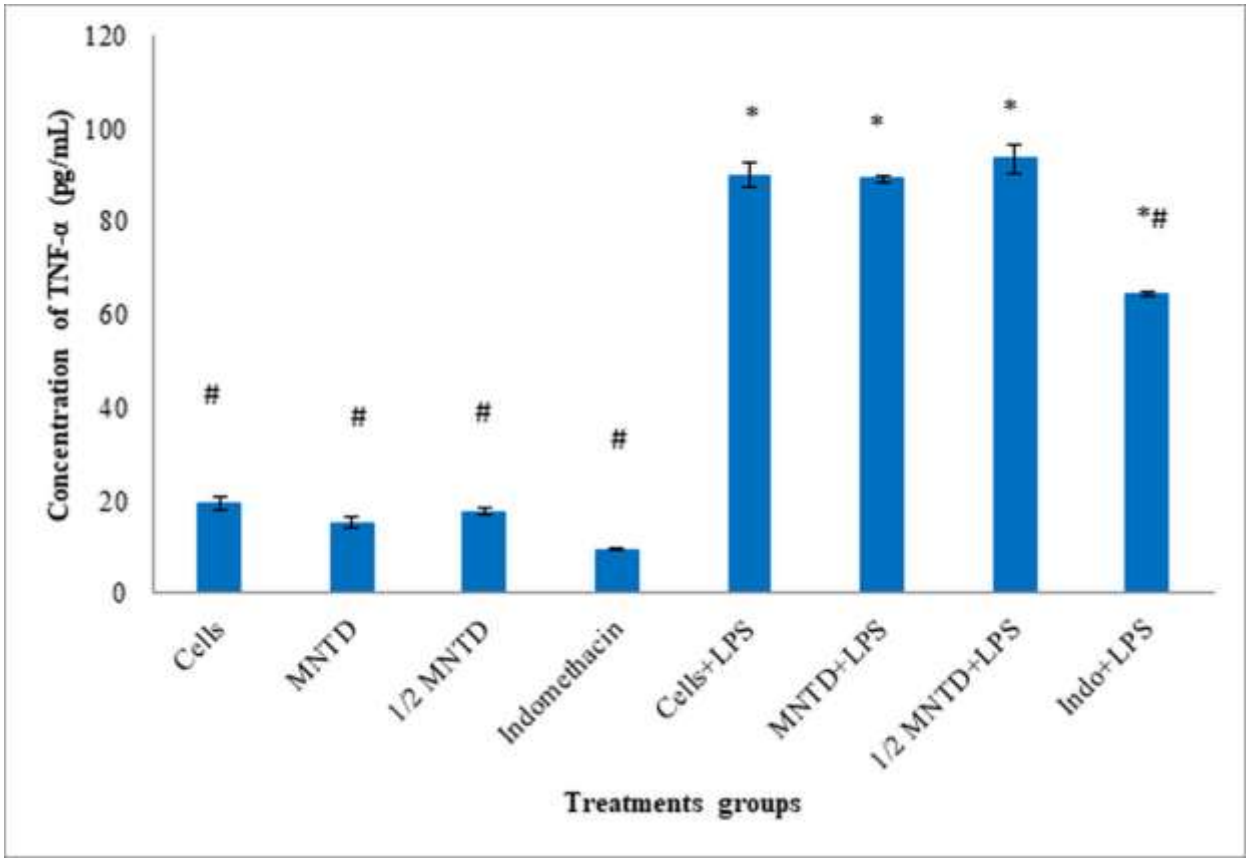


**Fig. 3.** The tumour necrosis factor-α (TNF-α) level in LPS-stimulated BV2 cells upon treatment with chloroform extract of *Panax ginseng*. Bars indicate the means±standard deviation. '*' indicates that the treatment was significantly different from the untreated cells using one-way ANOVA followed by Tukey's HSD Multiple Comparison Test at $p< 0.05$. '#' denotes the treatment was significantly different from the LPS-stimulated cells using one-way ANOVA followed by Tukey's HSD Multiple Comparison Test at $p< 0.05$.

### *Determination of interleukin-6 (IL-6) level upon treatment*

Interleukin-6 (IL-6) is another pro-inflammatory cytokine that is highly expressed during acute and chronic inflammation. Using Quantikine ELISA, the level of IL-6 was measured, and this correlated to the inflammatory condition of the BV2 microglia cells upon treatment with various concentrations of chloroform extract of *P. ginseng* root culture. As demonstrated in Figure 4, the untreated cells produced 417.54±26.37 pg/mL of IL-6. Upon the stimulation with 0.1 µg/mL of LPS, the concentration of IL-6 was found to spike up to 1063.23±3.59 pg/mL which was a 2.5 fold increase. It was also observed that when the LPS-stimulated cells were treated with 25 µM of indomethacin, the concentration of IL-6 has increased to 1077.93±5.52 pg/mL, by 1.38% as compared to LPS-stimulated cells. Contrarily, insignificant reduction of IL-6 concentration was observed when the LPS-stimulated cells were treated with MNTD and ½ MNTD of chloroform extract of *P. ginseng* root culture, from 1063.23±3.59 pg/mL to 1061.40±3.66 pg/mL and 995.94±116.25 pg/mL respectively.

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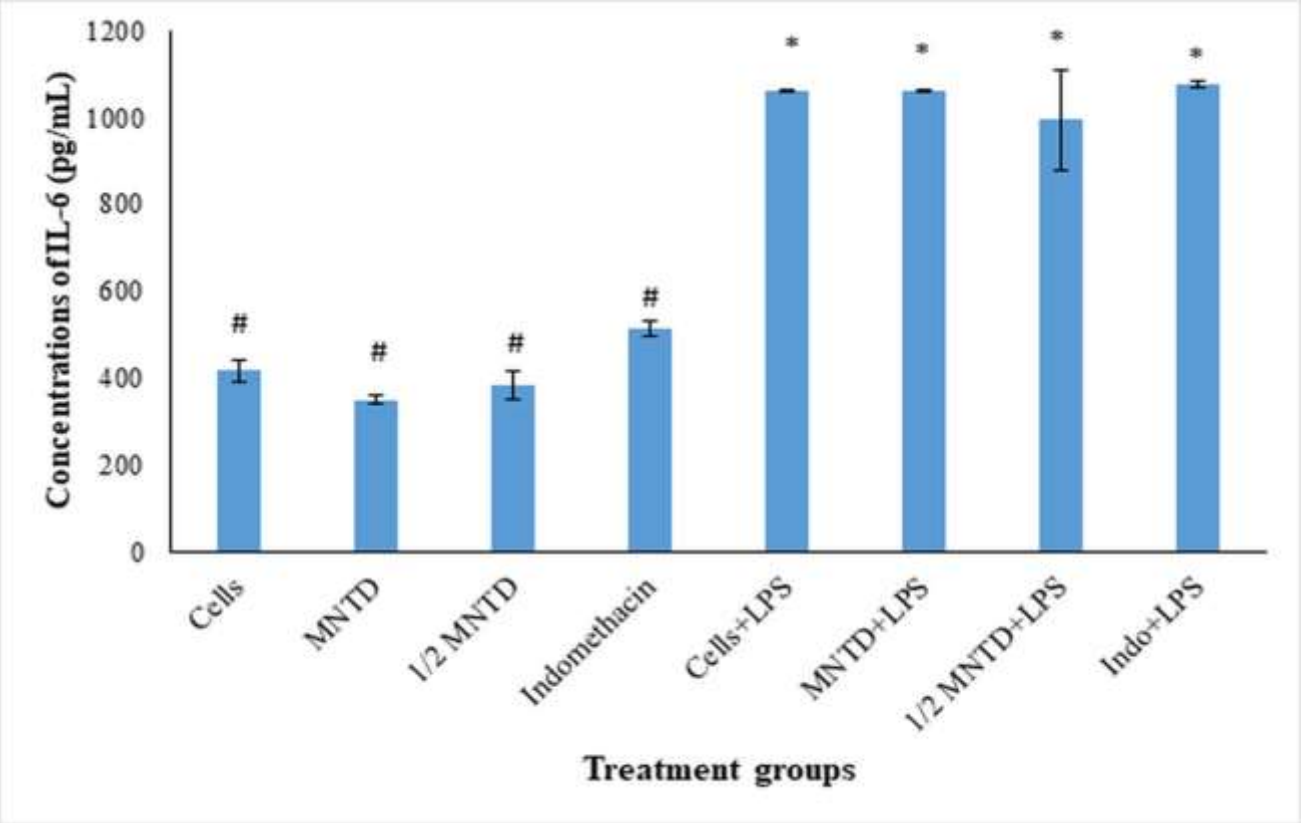


**Fig. 4.** The interleukin-6 (IL-6) level in LPS-stimulated BV2 cells upon treatment with chloroform extract of *Panax ginseng*. Bars indicate the means±standard deviation. '*' indicates that the treatment was significantly different from the untreated cells using one-way ANOVA followed by Tukey's HSD Multiple Comparison Test at $p< 0.05$. '#' denotes the treatment was significantly different from the LPS-stimulated cells using one-way ANOVA followed by Tukey's HSD Multiple Comparison Test at $p< 0.05$.

***Determination of interleukin-10 (IL-10) level upon treatment***

Interleukin-10 (IL-10) is an anti-inflammatory cytokine in which it is released to mediate and suppress inflammation. It is an important component in the homeostasis and balancing of inflammatory processes. The level of IL-10 was also measured using Quantikine ELISA kit upon the treatment of various concentrations of chloroform extract of *P. ginseng* root culture. As exhibited in Figure 5, the untreated cells showed the production of IL-10 with concentration of 14837.96±197.44 pg/mL. The 0.1 µg/mL LPS stimulation caused the cells to produce 21186.34±414.24 pg/mL, which was an increment of 42 78% as compared to untreated cells. When the LPS-stimulated cells were treated with 25 µM of indomethacin, the concentration of IL-10 produced appeared to be 11533.56±236.99 pg/mL, a value that was equivalent to 83.69% reduction as compared to untreated LPS-stimulated cells. In contrary to indomethacin, the treatment using MNTD and ½ MNTD of chloroform extract of *P. ginseng* root culture showed a significant increase in the concentration of IL-10 by the cells up to 23206.02±350.82 pg/mL and 24033.56±566.39 pg/mL, respectively. This trend was equivalent to 9.53% and 13.44% increase in IL-10 level as compared to untreated LPS-stimulated cells.

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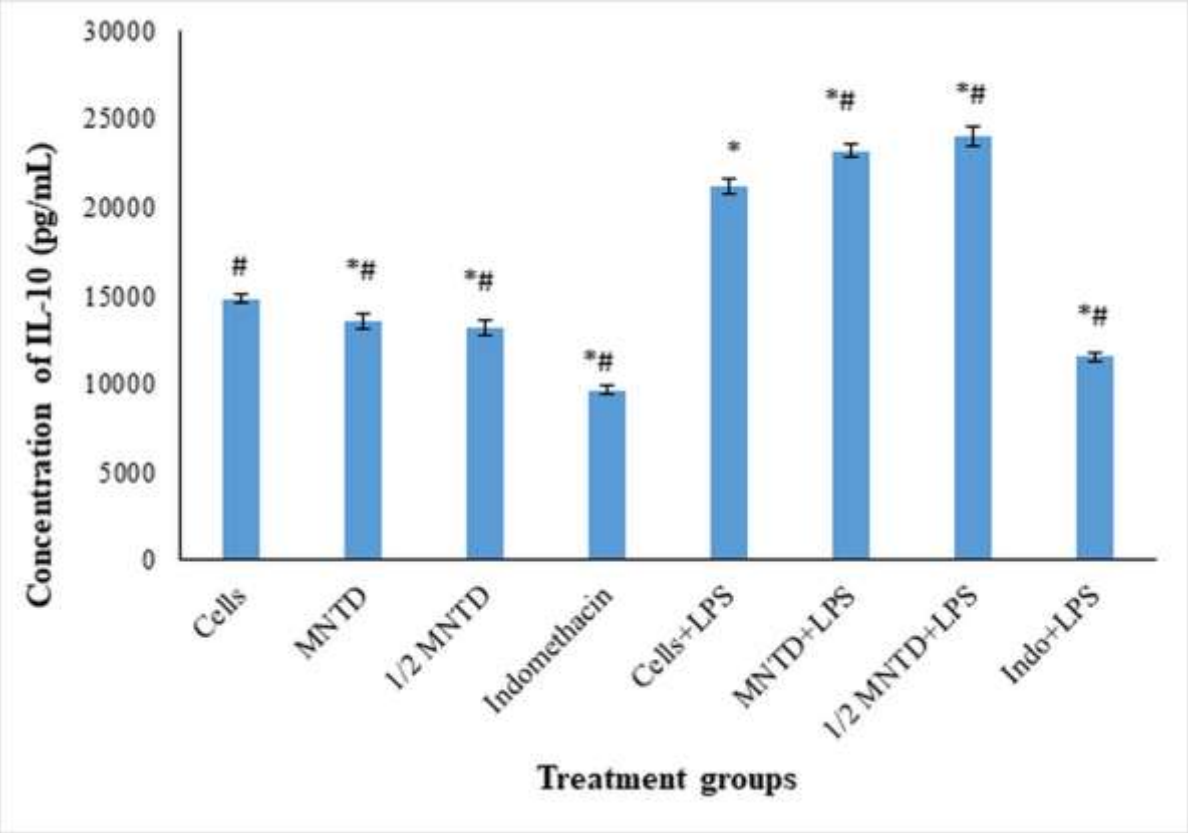


**Fig. 5.** The interleukin-10 (IL-10) level in LPS-stimulated BV2 cells upon treatment with chloroform extract of *Panax ginseng*. Bars indicate the means±standard deviation. '*' indicates that the treatment was significantly different from the untreated cells using one-way ANOVA followed by Tukey's HSD Multiple Comparison Test at $p< 0.05$. '#' denotes the treatment was significantly different from the LPS-stimulated cells using one-way ANOVA followed by Tukey's HSD Multiple Comparison Test at $p< 0.05$.

## Discussion

### *Determination of maximum non-toxic dose (MNTD)*

The MNTD of chloroform extracts of *P. ginseng* root culture was comparatively low, which shows that the extract exerted high toxicity to BV2 microglia cells. This could be highly related to the chloroform extraction which primarily targeted the non-polar and less polar bioactive compounds from the *P. ginseng* root culture. Past studies showed that lipophilicity of non-polar compounds may cause accumulation of such compound in the cell, exerting its cytotoxicity by damaging the mitochondria with increased ROS formation, and this could possibly be the reason why non-polar extracts elicited high cytotoxicity (17,18). Meanwhile, a growth promoting effect at concentration below 24.3±5.5 µg/mL could be due to the presence of ginsenoside $Rh_2$ in the extract which upregulated the pituitary adenylate cyclase-activating polypeptide (PACAP) (19).

An increase in cytotoxicity when concentration of extract was beyond the MNTD could be due to the bioactive compounds present in the extract. Stereoisomer of the ginsenoside $Rg_3$ known as 20(S)-$Rg_3$ that could be possibly extracted using chloroform solvent was found to promote cell senescence and apoptosis via the p53 pathway, leading to increase cytotoxicity (20, 21). Metabolism of various less polar ginsenosides found in chloroform extract of *P. ginseng* root culture, such as $Rg_2$, $Rg_3$, $Rh_1$ and $Rh_2$ produce the metabolite known as compound K, which exerts anti-inflammatory and chemotherapeutic effects on various cell lines (22). However, overproduction of compound K led to endoplasmic reticular stress that ultimately

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caused cell apoptosis due to accumulation of intracellular calcium, which may be one of the causes of cytotoxic effect that have been observed on the extract (22).

***Determination of intracellular nitrosative stress upon treatment***

This study revealed that in non-activated state, the BV-2 possessed small amount of iNOS that catalyses the production of NO, which has homeostatic role in modulating ROS production and oxidative stresses (23). However, stimulation of BV2 microglia cells with LPS induces the production of pro-inflammatory cytokines such as TNF-α and IL-1β, which targets the pathway of c-Jun N-terminal kinase (JNK) and MAPK, leading to upregulation of mRNA coded for iNOS, thus significantly increases NO production (24). This condition was reduced with the presence of indomethacin most likely due to the fact that indomethacin exerts inhibitory properties in the production of prostaglandins and thromboxane by antagonistically binds to COX enzyme, downregulating the iNOS expression and thus reducing the NO level (25,26). Meanwhile, present study also showed that with the presence of chloroform extracts at both MNTD and 1/2MNTD, the NO level was reduced as compared to the LPS alone, though without any significant difference. This reduction could be due to the fact that *P. ginseng* might contain ginsenoside $Rh_1$ and metabolite compound K which exerted anti-depressive and anti-oxidative effect by inhibiting the increase in protein activation of COX-2 and iNOS, whereby iNOS actively catalyzed the conversion of arginine to citruline, producing NO as a by-product (27).

***Determination of tumor necrosis factor-α (TNF-α) level upon treatment***

Microglia cells produce low amount of TNF-α under unstimulated and non-activated state, and it was found that these homeostatic TNF-α functioned to strengthen synaptic plasticity and regulate cognition function (28, 29). This study also showed insignificant anti-inflammatory property of chloroform extract of *P. ginseng* root culture in terms of TNF-α. However, many past studies showed that *P. ginseng* extract is able to reduce the expression of TNF-α. The contradictory findings in this study could possibly be due to the absence or minute presence of the polar ginsenoside $Rb_1$, particularly in the *in vitro* root culture (30). $Rb_1$ is the major constituent in *P. ginseng* that modulates the production of TNF-α by suppressing MAPK and NF-κB signalling pathways (31,32). Besides that, the acidic polysaccharides such as panaxans and ginsans have immune-stimulatory effect in which they induce the expression of IL-12, TNF-α and MHC class II molecules (33,34).

***Determination of interleukin-6 (IL-6) level upon treatment***

The present studies showed that homeostatic production of IL-6 cytokines by microglia cell promoted the differentiation of oligodendrocytes as well as regeneration of peripheral nerves, making it a potent neurotrophic factor (35,36).

An insignificant reduction of IL-6 concentration in LPS-stimulated cells treated with MNTD and ½ MNTD could be related to the less polar ginsenosides $Rg_5$ and $Rh_3$, which were also found to decrease the expression of IL-6 through the inhibition of NF-κB by upregulation of sirtuin 1 (SIRT 1) and enhanced phosphorylation with the 5'-adenosine monophosphate-activated protein kinase (AMPK) that inhibiting Akt and janus kinase 1 (JAK1), ultimately reducing IL-6 production (37). However, the reduction of IL-6 level upon treatment was rather insignificant. This could possibly be explained due to the absence or minute amount of polar ginsenoside $Rb_1$ and $Rg_1$ in the chloroform extract of *P. ginseng* root culture (38). $Rb_1$ and $Rg_1$ are major constituent in *P. ginseng* that reduces the expression of both TNF-α and IL-6. $Rb_1$ targets the NF-κB pathway whereas the $Rg_1$ supresses the production of IL-6 and TNF-α via the suppression of activator protein 1 and protein kinase A activity (38). Moreover, the suppression of TNF-α production may reduce

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the expression of IL-6 as production of IL-6 is often mediated by the presence of TNF-α (39).

***Determination of interleukin-10 (IL-10) level upon treatment***

The anti-inflammatory IL-10 plays an important role in modulating immune responses of microglia cells. IL-10 binds to its receptor and it downregulates the gene expression of pro-inflammatory cytokines, as well as the MHC Class II (40). Besides that, IL-10 were found to have anti-apoptotic effect via the activation of phosphatidylinositol-4,5-bisphosphate-3-kinase (PI3K)/Akt signalling pathway, and also activation of anti-apoptotic factors such as Bcl-2 (40).

The treatment using MNTD and ½ MNTD of chloroform extract of *P. ginseng* root culture showed a significant increase in the concentration of IL-10 by the cells up to 23206.02±350.82 pg/mL and 24033.56±566.39 pg/mL, respectively. This trend was equivalent to 9.53% and 13.44% increase in IL-10 level as compared to untreated LPS-stimulated cells. Chloroform extract of *P. ginseng* root culture at MNTD and ½ MNTD that containing ginsenoside $Rh_1$ have also showed a significant increase in the IL-10 levels. The extracts could have targeted the cyclic adenosine monophosphate (cAMP)-dependent protein kinase signalling pathway, ultimately induced the expression of both IL-10 and heme oxygenase-1 that mediated the anti-inflammatory responses (41). Previous studies also confirmed that ginsan present in *P. ginseng* was also capable in increasing the production of IL-12 and IL-10 by suppressing the TLR signalling pathway (42). Therefore, findings in the present study showed that chloroform extract of *P. ginseng* root culture has high potency in mediating the expression of anti-inflammatory cytokines.

Based on the findings of this study, the chloroform extract of *P. ginseng* root culture was found to be able to reduce the level of nitric oxide insignificantly and increase the level of interleukin-10 significantly as compared to the LPS-stimulated BV2 microglia cells without treatment. However, there were no observable significnt effect of the extract against the production of TNF-α and IL-10, comparing to the untreated LPS-stimulated BV2 microglia cells. Despite the inefficiency of chloroform extract of *P. ginseng* root culture in reducing the level of nitric oxide, tumour necrosis factor-α and interleukin-6, the elevation of anti-inflammatory cytokine (interleukin-10) showed the potential anti-neuroinflammatory properties of the extract in the model of this experiment. Further investigation should be conducted on other pro-inflammatory and anti-inflammatory mediators to verify the anti-neuroinflammatory properties of chloroform extract of *P. ginseng* root culture. Besides, fractionation and purification of the bioactive compounds found in chloroform root extract should be done to provide a greater insight in the study of its mechanism of action as well as its anti-neuroinflammatory properties, especially in the identification of potent bioactive compounds found in *in vitro* root culture. This allows the further development of chloroform extract of *P. ginseng* root culture as an alternative in the treatment of anti-neuroinflammation which curbs the neurodegeneration and ND with fewer undesirable side effects.

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The authors declare that they have no competing interests.

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