Artikel Asli/*Original Articles*

Anti-neuroinflammatory Effects of *Hibiscus sabdariffa* Linn. (Roselle) on Lipopolysaccharides-induced Microglia and Neuroblastoma Cells
(Kesan Anti-neuroinflammatori *Hibiscus sabdariffa* Linn. (Roselle) pada Aruhan Lipopolisakarida Sel Mikroglia dan Neuroblastoma)

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ABSTRACT

Hibiscus sabdariffa Linn. (roselle) is a polyphenol rich fruit. This study aimed to identify the neuroprotective effect of roselle on LPS-induced cell proliferation and nitric oxide-induced free radical in microglia and neuroblastoma cells. MTT assay was used to identify the appropriate concentration of roselle and LPS for microglia and neuroblastoma cells proliferation study. Griess assay were used to determine the level of nitric oxide accumulated based on the reaction of Griess to estimate the activity of iNOS in nitric oxide production. The results showed that roselle at the concentration of 50 µg/mL and 100 µg/mL and LPS at concentration of 1 µg/mL does not give cytotoxic effect towards microglia C8-B4 and neuroblastoma LN18 cells. The roselle treatment at 50 µg/mL and 100 µg/mL showed a protective effect on LPS-induced microglia C8-B4 cells. However, in neuroblastoma LN18 cells, no protective effect was seen on both 50 µg/mL and 100 µg/mL of roselle treatment following induction with 1 µg/mL of LPS. On the other hand, the production of nitric oxide (NO) was reduced when LPS-induced microglia C8-B4 cells were treated with 50 µg/mL of roselle. Treatment of roselle at concentration 100 µg/mL on LPS-induced neuroblastoma LN18 cells also reduced the production of nitric oxide. As a conclusion, roselle had the ability to give neuroprotective effect by the inhibition of LPS induction activity on microglia activation for normal and cancer cells at different concentrations.

Keywords: Hibiscus sabdariffa Linn.; neuroinflammation; neuroprotective; nitric oxide; anti-inflammatory

ABSTRAK

Hibiscus sabdariffa Linn. (roselle) adalah sejenis buah kaya polifenol. Kajian ini dijalankan untuk mengenalpasti kesan perlindungan neuron roselle ke atas sel mikroglia dan neuroblastoma yang teraruh proliferasi sel dan radikal bebas nitrik oksida (NO) oleh LPS. Asai MTT digunakan untuk mengenalpasti kepekatan roselle dan LPS yang paling sesuai untuk kajian proliferasi sel mikroglia dan neuroblastoma. Manakala, asai Griess digunakan untuk penentuan aras nitrik oksida yang terkumpul berdasarkan tindakbalas Griess supaya aktiviti iNOS dalam penghasilan nitrik oksida dapat dianggarkan. Hasil kajian menunjukkan bahawa roselle pada kepekatan 50 µg/mL dan 100 µg/mL dengan kepekatan LPS 1 µg/mL tidak menyebabkan kesan sitotoksik terhadap sel mikroglia C8-B4 dan neuroblastoma LN18. Rawatan roselle pada kepekatan 50 µg/mL dan 100 µg/mL juga berupaya memberi kesan perlindungan terhadap sel mikroglia C8-B4 teraruh LPS. Walau bagaimanapun, tiada kesan perlindungan didapati dalam sel neuroblastoma LN18 cells selepas pemberian roselle pada kepekatan yang sama dengan aruhan LPS 1 µg/mL. Selain itu, penghasilan NO didapati berkurang selepas sel mikroglia C8-B4 teraruh LPS dirawat dengan 50 µg/mL roselle. Malahan pemberian roselle pada kepekatan 100 µg/mL ke atas sel neuroblastoma LN18 teraruh LPS turut menyebabkan pengurangan penghasilan NO. Kesimpulannya, roselle mempunyai keupayaan untuk menyebabkan kesan perlindungan neuron melalui perencatan aktiviti aruhan LPS ke atas pengaktifan mikroglia untuk sel normal dan kanser pada kepekatan yang berbeza.

Kata kunci: Hibiscus sabdariffa Linn.; neuroinflamasi; perlindungan neuron; nitrik oksida; anti-inflamatori

INTRODUCTION

Brain is one of the most important organs. It plays various important roles in learning, memory, cognitive and others. Several diseases are developed when there is abnormality in the brain function such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington and others. Normal brain aging occurred when there is a decline in brain performance as increase in age. This will cause decreasing in motor and cognitive performance. Brain aging also involve directly in neurodegenerative diseases such as AD. It is believed that brain aging is caused by inflammation process occurred in the brain (Eikelenboom et al. 1994; McGeer & McGeer 1995; Rogers 1995; Rogers et al. 1996).

Alzheimer disease (AD) is due to the accumulation of abnormal proteins in the brain such as β-amyloid (Aβ). The accumulation of Aβ will induce the inflammation process which is characterized by the activation of microglia cells. The activated microglia will release pro-inflammation molecules such as nitric oxide (NO), prostaglandin (PGE2), tumor necrosis factor (TNF-α), interleukins (IL-1, IL-6) and others (Takeuchi et al. 2006; Stone et al. 2009). Microglia cells are one of the resident cells in the brain and act as immune cells in the brain. It also involve in neuroinflammation process. Neuroinflammation plays important role in various types of neuropathology and neurodegenerative diseases (Gao & Hong 2008; Tambuyzer et al. 2008). There are several neuron and glia cells involved in neuroinflammation such as microglia and astrocyte. Neuroinflammation factors include excess production of NO, activation of oxide and production of glial cytokine (Stewart & Heales 2003). Neuroblastoma cells are one of brain cancer cell at stage IV.

Hibiscus sabdariffa Linn. (roselle) is a plant belongs to Malvaceae family (Cobley 1975). It has many benefits towards human health such as protective effect towards atherosclerosis (Chen et al. 2003; Chang et al. 2005; Kao et al. 2009), anticarcinogenic activities (Tseng et al. 1998; Chewonarin et al. 1999; Tseng et al. 2000; Chen et al. 2003) especially in leukaemia field (Chang et al. 2005; Hou et al. 2005), cyclooxygenase inhibitory activities (Christian et al. 2006). Anthocyanin is one of the active components in the roselle fruits (Tsai et al. 2002). Anthocyanin pigments give colour to fruits such as red, dark red and purple. It is believed that, anthocyanin can inhibit the inflammation process. Based on previous study by Joeng et al. (2013), anthocyanin from soy beans can inhibit pro-inflammatory mediators and cytokines production induced by lipopolysaccharide (LPS) such as NO, PGE2, TNF-α, and IL-1β without giving any cytotoxic effect. Thus, this study was aimed to identify the neuroprotective effect of roselle on LPS-induced cell proliferation and nitric oxide-induced free radical as a protective mechanism against neuroinflammation in microglia and neuroblastoma cells.

MATERIALS AND METHODS

ROSELLE AQUEOUS EXTRACT PREPARATION

Roselle extract preparation was done according to method by Akim et al. (2011). Roselle calyces were separated from seeds before washing and weighing. Next, distilled water was added in proportion of 100 g roselle calyces per 200 mL of distilled water. Solution was boiled at 60 to 70°C for 10 to 15 minutes. Boiled solution was filtered and then stored at -20°C. Freeze-dried process of roselle extract to powder form was done for three days using freeze-dryer. Roselle powder was weighed and stored in dark vacuumed container at -20°C.

REAGENTS AND CELL CULTURE

Cell microglia C8-B4 and neuroblastoma LN18 were purchased from ATCC and cultured in complete DMEM media culture at 5% CO_2, 37°C and 10% fetal bovine serum (FBS). Kuromanin chloride and LPS were purchased from Sigma Aldrich.

CELL VIABILITY TEST

Cell viability was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The C8-B4 microglia cells and LN18 neuroblastoma cells were seeded in triplicate at density of 5×10^4 cells/well on 96-well plate. Both cells were treated with roselle and LPS for 26 hours. MTT was added to each well and cells were incubated for 4 hours at 37°C. Next, dimethyl sulfoxide (DMSO) was added before the optical density was measured at 570 nm (Hwang et al. 2010).

NITRITE QUANTIFICATION

Nitric oxide (NO) secreted in microglial and neuroblastoma culture supernatants was measured by Griess reagent. After C8-B4 microglia and LN18 neuroblastoma cells were treated with roselle and LPS in culture dish, NO^{2-} concentration in culture supernatants were measured to assess NO production in both cells. One hundred µL of sample supernatants were mixed with 100 µL of Griess reagent on a 96-well plate and incubated at 25°C for 10 minutes. The absorbance at 570 nm was measured on a microplate reader. Sodium nitrite ($NaNO_2$) was used as the standard to calculate NO^{2-} concentration.

STATISTICAL ANALYSIS

Results were expressed as mean ± SEM. The data were analyzed by one-way ANOVA following the Dunnett post-hoc analysis by using SPSS program (version 20.0). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Effect of Roselle and LPS on C8-B4 and LN18 cell viability MTT assay was used to measure cell viability based on the quantity of MTT salt that had been reduced by functional mitochondria enzymes. The negative control ware microglia C8-B4 or neuroblastoma LN18 cells in growth media only. Based on results obtained from Figure 1, the cell viability of microglia C8-B4 treated with roselle shows the non-homogenous patterns which at concentrations of 6.25 µg/mL, 12.5 µg/mL and 25 µg/mL shows decreased in the percentage of cell viability that is not significant compared with negative control (95.18 ± 1.70%, 96.05 ± 1.46% and 93.29 ± 3.72%) while the percentage of cell viability of microglia C8-B4 treated with roselle at concentrations of 50 µg/mL and 100 µg/mL show a pattern of significant
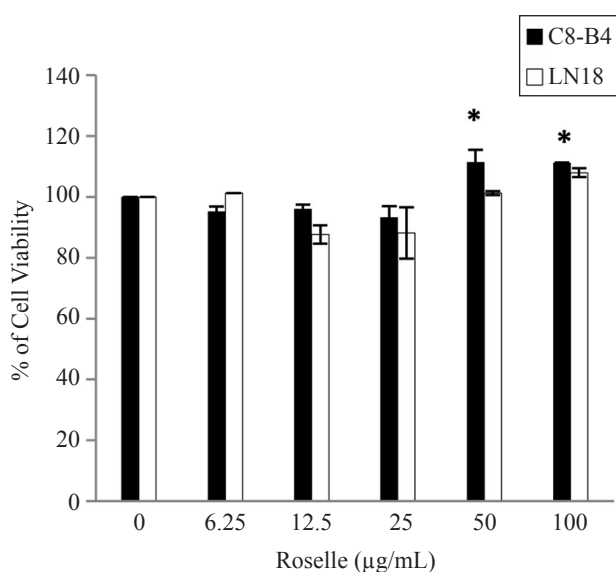
FIGURE 1. The effect of roselle on the cell viability of microglia C8-B4 and neuroblastoma LN18 after 2 hours incubation. Each data were expressed as the mean ± SEM (n = 3)
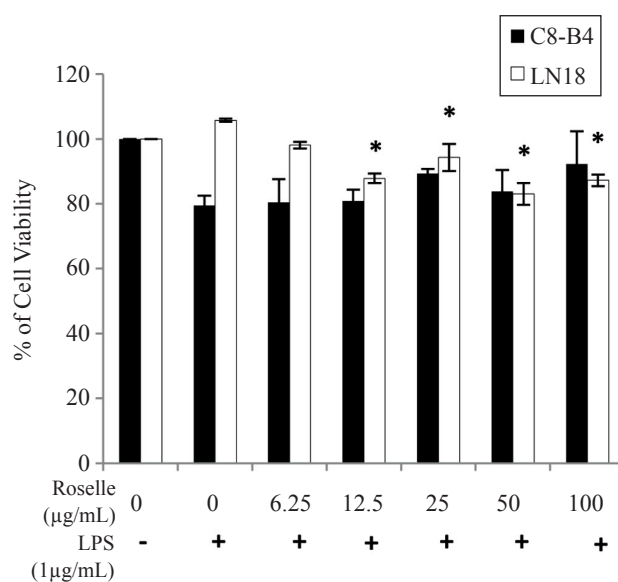

FIGURE 2. The effect of roselle on the cell viability of LPS-induced microglia C8-B4 and neuroblastoma LN18 after 26 hours incubation. Each data were expressed as the mean ± SEM (n = 3)

increase ($p < 0.05$) compared with negative control which are 111.44 ± 4.02% and 111.20 ± 0.13%.

For LN18, the cell viability also showed non-homogenous pattern which is at 6.25 µg/mL showed an increase in the percentage of cell viability which is not significant compared to the negative control (101.21 ± 0.02%). At a concentration of 12.5 µg/mL and 25 µg/mL, the percentage of cell viability was the same pattern of decline which is not significant compared to the negative control (87.62 ± 3.04% and 88.14 ± 8.41%). In contrast, the treatment of roselle at 50 µg/mL and 100 µg/mL, there was an increase in the percentage of cell viability and it is not significant compared to the negative control (101.19 ± 0.61% and 107.96 ± 1.47%). The percentage of cell viability of negative control for C8-B4 microglia cells and neuroblastoma LN18 was 100%.

The result of MTT assay for the LPS-induced microglia cells C8-B4 treated with roselle showed non-homogenous pattern as seen in Figure 2. At a concentration of 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL showed the percentage of cell viability is not significant compared with the positive control (80.41 ± 7.23%, 80.90 ± 3.47%, 89.34 ± 1.42%, 83.84 ± 6.60% and 92.30 ± 10.07%). The percentage of cell viability for LPS-induced neuroblastoma LN18 also showed non-homogenous pattern. At a concentration of 6.25 µg/mL roselle treatment showed a decrease in the percentage of cell viability which is not significant compared with the positive control (98.08 ± 1.0%). At a concentration of 12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL shows decreased the percentage of cell viability significantly ($p < 0.05$) compared with the positive control (87.86 ± 1.47%, 94.27 ± 4.14%, 83.01 ± 3:34%, and 87.22 ± 1.84%). The percentage of cell viability negative control for C8-B4 microglia cells and neuroblastoma LN18 was 100% and the percentage of positive control for cell

viability C8-B4 is 79.45 ± 3.00% and for LN18 cells was 105.76 ± 0.5%.

EFFECTS OF ROSELLE ON LPS-INDUCED NO IN C8-B4 AND LN18 CELLS

Griess assay was used to measure the accumulation of nitrite. Kuromanin chloride was used as positive control. The concentration of kuromanin chloride used was 50 µg/mL. The negative control was untreated cells. After 26 hours of incubation with roselle and LPS, Griess assay was conducted and nitrite accumulate in the media were counted relatively to positive control. In Table 1, the percentage of nitrite accumulated by microglia cells C8-B4 as shown by the negative control (0 µg/mL without LPS) is almost the same (104.17 ± 0.00%) in microglia cells C8-B4, which only with complete DMEM culture medium. For the positive control (0 µg/mL with LPS) has been a slight decrease in the accumulation of nitrite in insignificant compared to the negative control by 4%.

Treatment of roselle in C8-B4 microglia cells at concentrations of 50 µg/mL induced by LPS shows the percentage reduction of nitrite accumulated significantly compared to the positive control to 4.17 ± 0.00%. At a concentration of 100 µg/mL of roselle treatment towards C8-B4 microglia cells showed significantly increased nitrite accumulation compared with the positive control to 195.83 ± 0.00%. Significant differences compared to the percentage of nitrite of positive control ($p < 0.05$) were found to occur at concentrations of 50 µg/mL and 100 µg/mL according to one-way ANOVA. As for percentage of nitrite accumulated by microglia cells treated kuromanin chloride at concentrations of 50 µg/mL, result showed percentage was higher than the percentage of nitrite

TABLE 1. The concentration and percentage of nitrite accumulated on LPS-induced microglia C8-B4 treated with roselle and kuromanin chloride for 26 hours

Roselle concentration (μg/mL)	LPS (1μg/mL)	Concentration of nitrite accumulated (μM)	Percentage of nitrite accumulated (%)
0	-	1.645	104.17 ± 0.00
0	+	1.579	100.00 ± 0.00
50	+	0.066	4.17 ± 0.00 *
100	+	3.092	195.83 ± 0.00 *
Kuromanin chloride (50 μg/mL)	+	0.25641	16.24 ± 0.00*

Data were expressed as mean ± SEM

accumulated by C8-B4 microglia cells treated roselle at concentrations of 50 μg/mL by 16.24%.

In Table 2, the percentage of nitrite accumulated by LN18 neuroblastoma cells indicated by the negative control (0 μg/mL without LPS) is almost the same (111.11 ± 0.00%), which is only given complete DMEM culture medium. For the positive control (0 μg/mL with LPS) has been a slight decrease in the accumulation of nitrite in insignificant compared to the negative control by 11%. Treatment of neuroblastoma cells LN18 with roselle at concentrations of 50 μg/mL induced by LPS showed a significant percentage of the accumulated nitrite compared to the positive control ($p < 0.05$) to 255.56 ± 0.00%.

At a concentration of 100 μg/mL roselle treatment on neuroblastoma cells LN18 nitrite accumulation decreased significantly compared to the positive control ($p < 0.05$) to 0%. Significant differences compared to the percentage of nitrite positive control ($p < 0.05$) were found to occur at concentrations of 50 μg/mL and 100 μg/mL according to one-way ANOVA. Percentage of nitrite accumulated by neuroblastoma cells treated LN18 kuromanin chloride at concentrations of 50 μg/mL of 1906.89 ± 0.20% is significantly higher than the percentage of nitrite accumulated by LN18 treated neuroblastoma cells at concentrations rosellele 50 μg/mL ($p < 0.05$).

TABLE 2. The concentration and percentage of nitrite accumulated on LPS-induced neuroblastoma LN18 treated with roselle and kuromanin chloride for 26 hours

Roselle concentration (μg/mL)	LPS (1μg/mL)	Concentration of nitrite accumulated (μM)	Percentage of nitrite accumulated (%)
0	-	0.5	111.11 ± 0.00
0	+	0.45	100.00 ± 0.00
50	+	1.15	255.56 ± 0.00 *
100	+	0	0.00 ± 0.00*
Kuromanin chloride (50 μg/mL)	+	8.581	1906.89 ± 0.20*

Data were expressed as mean ± SEM

DISCUSSION

Hibiscus sabdariffa Linn. or locally known as roselle contains a high content of polyphenols, especially anthocyanin (Tseng et al. 2000; Hussein et al. 2010). There are a several studies which have been conducted to determine the biological activity found in roselle. The discovery has reported that roselle possess anti-cyclooxygenase activity, anti-hypertensive, chemoprotective effect and others (Tseng et al. 1998; Chewonarin et al. 1999; Tseng et al. 2000; Chen et al. 2003; Chang et al. 2005; Kao et al. 2009). According to Tseng et al. (2000) roselle has anti-inflammatory effects. But the clear mechanism of roselle anti-inflammatory effects in the brain are still lacking. Therefore, in this study, the effects of anti-inflammatory of roselle were conducted. Concentrations of roselle compound used were in range between 6.25 μg/mL to 100 μg/mL.

C8-B4 microglia cells and neuroblastoma LN18 were stimulated with LPS at a concentration of 1 μg/mL used as a cell model to study the effects and mechanism of anti-inflammatory of roselle. LPS concentrations of 1 μg/mL were tested and showed no cytotoxic effects on cells C8-B4 microglia and neuroblastoma LN18. Microglia cells C8-B4 is an in vitro model that is suitable for this study because LPS stimulation has been known to cause significant immune responses in cells and subsequently cause the production of inflammatory mediators such as inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2) and nitric oxide (NO). Inflammatory mediators can be used as biomarkers of inflammation process and allows observation of roselle anti-inflammatory properties.

Microglia is one of the brain's cell that acts as a first protective, and protect the central nervous system when there is a stimulus or other foreign material. Activation of microglia is caused by external stimuli such as LPS, interferon gamma (IFN-γ) and others. In addition, microglia cells C8-B4 were selected because microglia cells involved in the pathophysiology of various neurodegenerative diseases in the event of activation such as AD, PD, multiple sclerosis and others. LN18 neuroblastoma cells were selected because neuroblastoma cells are one of the brain cancer cells used as an in vitro model for neuroprotective study when induced by LPS.

MTT assay used to study the cytotoxic effects of roselle on C8-B4 microglia cells and neuroblastoma LN18. For MTT assay, results showed no cytotoxic effects on both cell C8-B4 microglia and neuroblastoma LN18 at roselle concentration of 50 μg/mL and 100 μg/mL which means it is dose independent. An increase of viability of C8-B4 microglia cells treated with roselle at concentrations of 50 μg/mL and 100 μg/mL were observed compared to the negative control (0 μg/mL). For LN18 neuroblastoma, the cell viability at treatment concentrations of roselle 50 μg/mL and 100 μg/mL, and showed increase cell viability compared to the negative control (0 μg/mL), although not significantly different statistically. Microglia cells C8-B4 recorded cell viability higher than LN18 neuroblastoma

cells at concentrations of roselle 12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL. This shows that the treatment roselle of C8-B4 microglia cells are more potent than in neuroblastoma cells LN18.

Effect of roselle on cell viability of LPS-induced microglia cells C8-B4 showed an increased in the percentage of cell viability at concentrations of 6.25 µg/mL to 100 µg/mL as compared to positive control (LPS-induced cell without roselle treatment) unsignificantly. For LPS-induced LN18 neuroblastoma cells, the percentage of cell viability showed a pattern that is not homogenous. The percentage of cell viability of the positive control was higher than the negative control, showing a difference of 5%. For roselle treatment of neuroblastoma LN18 cells induced at concentrations of 6.25 µg/mL to 100 µg/mL showed an independent decrease pattern when compared with negative controls. The percentage of LPS-induced microglia cell viability when treated with roselle show a lower percentage than the percentage of LPS-induced neuroblastoma LN18 cell viability at concentrations of 0 µg/mL, 6.25 µg/mL, 12.5 µg/mL and 25 µg/mL. At a concentration of roselle treatment 50 µg/mL and 100 µg/mL, the percentage of cell viability of LPS-induced microglia C8-B4 was higher than the percentage of cell viability of LPS-induced neuroblastoma LN18. This proves that the treatment roselle on microglia cells LPS-induced C8-B4 is more potent than roselle treatment on LPS-induced LN18 neuroblastoma cells.

Griess assay was used to study the effect of roselle in the inhibition of nitric oxide release in vitro. Roselle able to reduce the accumulation of nitric oxide stimulated by LPS at a concentration of 50 µg/mL for microglia cells C8-B4. According to Kao et al. (2009), roselle extract can reduce nitrite secretion in LPS-induced cells. While at concentration of roselle treatment 100 µg/mL on LPS-induced microglia cells C8-B4 may contribute to increased levels of oxidative stress in the form of nitric oxide. The release of nitrite accumulated in microglia C8-B4 cells at concentration of roselle treatment 50 µg/mL and 100 µg/mL are different. At a concentration of roselle 100 µg/mL treatment of C8-B4 microglia cells shown drastic increased in the percentage of nitric accumulation. This is likely due to ability of roselle to enhanced oxidative stress in higher concentration on microglia cells C8-B4.

Activation of microglia involved in the inflammatory process of neurons due to the production of bioactive molecules such as nitric oxide (Nakashima et al. 1995). According to Zielasek et al. (1992), cultured microglia cells will secrete nitric oxide when stimulated by IFN-γ or LPS. Nitric oxide and inflammatory molecules identified as inflammation molecules and is produced by nitric oxide synthase (NOS). Oxidative stress can occur if there is excessive production of nitric oxide through a combination of nitric oxide and superoxide (O^{2-}) to produce peroxynitric ($ONOO^-$), which is highly toxic (Blough & Zafiriou 1985). This will result in neurotoxicity to occur. Peroxynitric will attack neurons and glial cells (Pryor & Squadrito 1995).

Based on the accumulation of nitrite by LPS-induced neuroblastoma LN18 cells it was shown that an increase of nitrite accumulated when treated with roselle at concentrations of 50 µg/mL while the treatment of roselle at 100 µg/mL, it showed a drastically decline of nitrite accumulation. At a concentration of 50 µg/mL roselle treatment of LPS-induced neuroblastoma LN18 cells contribute to increased levels of oxidative stress in the form of nitric oxide. Production of NO is associated with the onset and maintenance of inflammation (Brown & Bal-Price 2003). At a concentration of 50 µg/mL of roselle treatment on LPS-induced LN18 neuroblastoma cells, the process of initiation and maintenance of inflammation begins. It can be seen through the increase of nitrite accumulated when induced by LPS 1 µg/mL. At a concentration of 100 µg/mL of roselle treatment on LPS-induced LN18 neuroblastoma cells, the percentage of accumulated nitrite can be seen. This indicates that, concentration of 100 µg/mL of roselle is able to reduce the production of nitric oxide.

Kuromanin chloride or other name is cyanidin-3-O-glucoside at concentration of 50 µg/mL is used as a positive control in Griess assay. Kuromanin chloride is one of anthocyanins found in roselle (Mahadevan et al. 2009). Anthocyanins or anthocyanin extracts have been reported to have anti-inflammatory activity by inhibiting the expression and biological activity of several pro-inflammatory cytokines in vitro by suppressing NF-κB through down regulation of MAPK pathway (Wang et al. 1999; Pergola et al. 2006). Previous studies have shown that anthocyanin able to inhibit the expression of COX-2 in RAW 264 cells or inhibit the expression of LPS-induced iNOS protein and mRNA in mouse J774 macrophages (Hou et al. 2005).

According to previous studies, anthocyanin isolated from soybeans can inhibit pro-inflammatory mediator such as PGE2, NO and cytokines in LPS-induced BV2 microglia cells without significant cytotoxic effect (Jeong et al. 2013). Besides that, it also noted that anthocyanin could reduce the excess regulation of expression iNOS, COX-2, TNF-α and IL-β in LPS-induced BV2 microglia cells. Based on MTT assay performed by Jeong et al. (2013), anthocyanin concentrations of 20-100 µg/mL did not give cytotoxic effect to BV2 microglia cells after induced with LPS at 0.5 µg/mL. Therefore, the concentration of chloride kuromanin 50 µg/mL was selected as the positive control for this study.

The results obtained showed that roselle showed anti-inflammatory effect much more potent than the positive control (kuromanin chloride) for LPS-induced microglia C8-B4 cells and neuroblastoma LN18. This can be seen from the percentage of nitrite accumulated is lower when C8-B4 microglia cells treated with roselle at concentrations of 50 µg/mL which is 4% compared to the percentage of nitrite accumulated for C8-B4 microglia cells treated with kuromanin chloride at a concentration of 50 µg/mL which is 15.59%. Percentage of nitrite accumulated for LN18 neuroblastoma cells treated with roselle and induced by

LPS showed that at concentrations of 50 µg/mL is more potent as an anti-inflammatory against kuromanin chloride at a concentration of 50 µg/mL. Based on previous studies in microglia cells, the pathway which involved in LPS stimulation is the activation of inflammatory signal NF-κB and MAPK which involved in the production of iNOS. LPS does not give direct effect on neurons, but neuronal damage is caused by the activation of microglia (Dutta et al. 2008). Limitations of this study include the lower concentration of roselle treatment and slow rate growth of both cells.

CONCLUSION

This study shows that roselle can provide anti-inflammatory effects on microglia C8-B4 and neuroblastoma LN18 cells by reducing the production of nitric oxide at certain concentrations. Roselle is not cytotoxic on both cell lines. Therefore, roselle can give neuroprotective effect. However, to determine roselle mechanism of action on anti-inflammatory effect, further research should be conducted.

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