Anti-inflammatory Effect of Tamarind Seed Coat Extract against LPS-Induced RAW264.7 Macrophages

(Kesan Anti-Keradangan Ekstrak Lapisan Biji Asam Jawa terhadap Penggalak LPS RAW264.7 Makrofaj)

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ABSTRACT

Inflammatory response is modulated by stimulated immune cells, and has a pivotal role in host defense system against various stimuli. In this study, we evaluated the anti-inflammatory property of tamarind seed coat extract (TSCE) in lipopolysaccharide (LPS)-induced RAW264.7 macrophages. Various concentrations of TSCE (10, 25, and 50 μg mL^{-1}) were applied and then stimulated with LPS (1 μg mL^{-1}) in RAW264.7 macrophages and the level of reactive oxygen species (ROS) and nitric oxide (NO) were measured. Besides, enzyme-linked immunosorbent assay (ELISA) was used to measure the level of pro-inflammatory cytokines. Our results showed that TSCE suppressed LPS-induced intracellular ROS production and suppressed the NO levels in a dose-dependent manner. Significantly, the anti-inflammatory activity was correlated with a lowered LPS-stimulated TNF-α and IL-1β pro-inflammatory cytokines. These results implied that TSCE possess potent anti-inflammatory activity, which supported new insights into the TSCE utilization to protect inflammation-related disorders.

Keywords: Interleukin-1β; reactive oxygen species; Tamarindus indica*; tumor necrosis factor alpha*

ABSTRAK

Tindak balas keradangan dimodulasi oleh sel-sel imun yang dirangsang dan memainkan peranan penting dalam sistem pertahanan perumah terhadap pelbagai rangsangan. Dalam kajian ini, kami menilai sifat anti-radang daripada ekstrak lapisan biji asam jawa (TSCE) pada lipopolisakarida (LPS) yang diaruh makrofaj RAW264.7. Pelbagai kepekatan TSCE (10, 25 dan 50 μg mL^{-1}) digunakan dan kemudian dirangsang dengan LPS (1 μg mL^{-1}) pada makrofaj RAW264.7 dan tahap spesies oksigen reaktif (ROS) dan nitrik oksida (NO) diukur. Selain itu, asai imunosorben berkait enzim (ELISA) digunakan untuk mengukur tahap sitokin pro-radang. Hasil kajian menunjukkan bahawa TSCE menindas pengeluaran intraselular ROS yang disebabkan oleh LPS serta menindas aras NO bergantung pada dos atau kepekatan. Aktiviti anti-radang berkorelasi dengan penurunan LPS yang merangsang sitokin pro-radang TNF-α dan IL-1β secara ketara. Hasil ini menunjukkan bahawa TSCE memiliki aktiviti anti-radang yang tinggi sekaligus menyokong pandangan baru tentang penggunaan TSCE dalam melindungi gangguan yang berkaitan dengan keradangan.

Kata kunci: Faktor nekrosis tumor alfa; interleukin-1β; spesies oksigen reaktif; Tamarindus indica

INTRODUCTION

Inflammation is a body's immune system response to harmful stimuli associated with immune cells, molecular negotiators, and inflammatory cytokines. The exposure of pathogen, radiation, extremely high or low temperatures and autoimmune processes induce an inflammation (Ferrero-Miliani et al. 2007; Medzhitov 2010). Chronic inflammatory responses are related to the progression and manifestation of various inflammatory-related diseases, including rheumatoid arthritis, septic syndrome, cardiovascular diseases, cancer and neurodegenerative diseases (Chen et al. 2018).

Lipopolysaccharide (LPS), a potent inflammatory stimulator, is a basic structure of gram-negative bacteria cell wall. It activates host's macrophages to secret inflammatory negotiators, such as nitrite oxide (NO) and pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β (IL-1β), and IL-6, and subsequently stimulate a cascade of inflammatory response. An excess of chronic inflammatory response triggers the development of various inflammation-related diseases (Chen et al. 2018; Duque & Descoteaux 2014). In addition, reactive oxygen species (ROS) production by phagocytic cells, for example, neutrophils, monocytes,

macrophages, and eosinophils is one of the major prominent negotiators in the inflammatory process. ROS stimulation acts as an important and damaging member in abnormal inflammatory diseases (Mittal et al. 2014). Therefore, regulation of macrophage activation is a positive approach to prevent inflammatory-related diseases including rheumatoid arthritis, cardiovascular diseases, cancer and neurodegenerative diseases (Chen et al. 2018). The anti-inflammatory agents which has the ability to reduce the generation of pro-inflammatory negotiators are an effective idea to modulate inflammatory-related diseases.

Tamarindus indica Linn is known as traditional medicinal plants in Thailand and Asian. Their fruits, leaves and seeds have been reported to possesses anti-microbial, anti-inflammatory, hypoglycemic and anti-oxidant activities (Bhadoriya et al. 2011). Tamarind seed are known as a food industrial by-product and have been used as a coffee substitute (Vadivel & Pugalenthi 2010). The coat of seed is an abundant source of tannins and polyphenols which possesses anti-allergic and anti-microbial (Aengwanich et al. 2009; Tewtrakul et al. 2008), anti-oxidant (Nakchat et al. 2014; Sandesh et al. 2014; Suksomtip et al. 2010) and anti-inflammatory activities (Ameeramja & Perumal 2018; Komutarin et al. 2004). To date, the anti-inflammatory activity of tamarind seed coat extract (TSCE) in macrophages have not described. Our present study was therefore aimed to evaluate whether TSCE reduces proinflammatory negotiators in LPS-induced RAW264.7 macrophages.

MATERIALS AND METHODS

PREPARATION OF TAMARIND SEED COAT

The seeds of tamarind fruits were collected from Phetchabun, Thailand in May 2015. The seed coats were separated, and one kilograms of tamarind seed coat powder was then extracted using 70% ethanol in a Soxhlet apparatus for 72 h. After 72 h of incubation, tamarind seed coat extract (TSCE) was filtered with 0.45 μm filters, concentrated and dried under reduced pressure using a rotary vacuum evaporator. TSCE was then stored at 4 °C until use.

PHYTOCHEMICAL IDENTIFICATION OF TAMARIND SEED COAT EXTRACT

The phytochemical constituents such as phenolic compounds, flavonoids, proanthocyanidin and tannins in TSCE were quantified as our previous described (Choosri et al. 2017).

MINIMUM INHIBITION CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF TAMARIND SEED COAT EXTRACT

The MIC and MBC of TSCE were evaluated against gram-positive (*Staphylococcus aureus* ATCC29213 and *Streptococcus mutans* ATCC25175) and gram-negative (*Escherichia coli* ATCC25922). One hundred microliter of bacterial suspension (10^6 CFU mL^{-1}) were incubated with 5 mL of Muller Hinton broth. Afterthought, TSCE were added and incubated for 24 h at 37 °C.

CELL CULTURE AND REAGENTS

RAW264.7 cell was provided from the American Type Culture Collection (Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) containing 10% fetal bovine serum (FBS), 1% antibiotic (penicillin/streptomycin) and 1% L-glutamine (Gibco, USA) was used to maintain RAW264.7 cell in a 5% CO_2 incubator at 37 °C. Dexamethasone, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), dimethylsulfoxide and lipopolysaccharide (LPS) from *Escherichia coli* 055:B5 were provided from Sigma (St. Louis, MO, USA).

CYTOTOXICITY OF TAMARIND SEED COAT EXTRACT (TSCE) ON RAW264.7 CELLS

RAW264.7 macrophages (1×10^4 cells/well) were cultured into 96-well plate with the complete DMEM medium. After 24 h of incubation, TSCE at the concentration of 0, 1, 5, 10, 25, 50, 100, 250, 500, and 1000 μg mL^{-1} were then treated to the cells for 24 h. The RAW264.7 cells viability of TSCE-treated was measured by using a colorimetric MTT assay, and the optical density (OD) of each concentration was detected with a microplate reader at 570 nm (Bio-Tex Instruments, Inc., VT, USA).

MEASUREMENT OF NITRIC OXIDE (NO) LEVEL IN RAW264.7 CELLS

RAW264.7 macrophages (1×10^5 cells/well) were incubated with TSCE (10, 25, and 50 μg mL^{-1}) in with or without 1 μg mL^{-1} of LPS for 24 h. Next, Griess reagent was used to evaluate a nitrite level in the culture medium of RAW264.7 macrophages. Concisely, 100 μL of Griess reagent was mixed with an equivalent volume of media. The OD of the nitrite-containing samples was assessed with a microplate reader at 540 nm (Bio-Tex Instruments, Inc., VT, USA). A standard curve was marked from sodium nitrite solution.

MEASUREMENT OF INTRACELLULAR REACTIVE OXYGEN SPECIES (ROS) PRODUCTION IN RAW264.7 CELLS

The oxidation of DCFH-DA was used for determining the intracellular ROS production in RAW264.7 cells. In brief, RAW264.7 cells (5×10^4 cells/well) were grown in 96-well plate and then treated with TSCE (10, 25, and 50 μg mL^{-1}) in the presence or absence of LPS for 24 h. Then, 50 μM of DCFH-DA was mixed and incubated for 1 h, at 37 °C in dark condition. DCF fluorescence was

detected at an excitation wavelength of 485 nm and an emission wavelength of 530 nm using a fluorescence microplate reader (Bio-Tex Instruments, Inc., VT, USA).

MEASUREMENT OF TNF-α AND IL-1β LEVEL IN RAW264.7 CELLS

RAW264.7 cells (1×10^5 cells/well) were maintained in 96-well plate and incubated for 24 h. TSCE (10, 25, and 50 µg mL^{-1}) and dexamethasone (1 µg mL^{-1}) were treated in the presence or absence of LPS for 24 h in RAW264.7 cells. After 24 h of incubation, TNF-α and IL-1β level in RAW264.7 cells cultured medium were measured by using ELISA method.

STATISTICAL ANALYSIS

The results were shown as mean ± standard error of mean (SEM). ANOVA was applied for measuring the differences of the mean values between four groups with Tukey post hoc test and $p < 0.05$ was used to be statistically significant.

RESULTS

PHYTOCHEMICAL PROFILE OF TAMARIND SEED COAT EXTRACT (TSCE)

As shown in Table 1, the preliminary phytochemical screening of TSCE showed the presence of phenolic compound, flavonoid, proanthocyanidin and tannin contents. Total phenolic compounds concentration was 26.43±0.56 mg of gallic acid equal per 100 g of dry extract. The concentration of total flavonoids was 3.54±0.06 mg of quercetin equal per 100 g. The content of proanthocyanidin was 1.43±0.08 whereas tannin content was showed at 21.49±1.49% pyrogallol equal, respectively. Our results suggested that TSCE presented a high concentration of phenolic compounds and tannin contents.

MIC AND MBC OF TAMARIND SEED COAT EXTRACT

Minimum inhibition concentration (MIC) values of TSCE was measured as an assessment of antimicrobial activity against pathogenic bacteria. The antimicrobial activity of TSCE were determined against two pathogenic bacterial strains, gram-positive (*Staphylococcus aureus* ATCC29213 and *Streptococcus mutans* ATCC25175) and gram-negative (*Escherichia coli* ATCC25922). MIC values as evaluated by the broth dilution method was 64 µg mL^{-1} for *S. aureus* as the most susceptible bacteria, while *E. coli* was more resistant because their growth was inhibited in the concentration more than 1204 µg mL^{-1} (Table 2).

CYTOTOXICITY EFFECT OF TSCE ON RAW264.7 CELLS

In order to evaluate the cytotoxicity of TSCE on macrophages, RAW264.7 cells were incubated with TSCE at concentrations 0-1,000 µg mL^{-1} for 24 h. As shown in Figure 1, 1-50 µg mL^{-1} of TSCE did not affect RAW264.7 cells viability after 24 h treatment. The percentage of cell viability was significantly decreased after administration with 75-1000 µg mL^{-1} TSCE in a concentration-dependent manner when compared to the untreated group. IC$_{50}$ of 24 h was 76.28 ± 0.09 µg mL^{-1} (Figure 1(A)).

TSCE DECREASED THE ROS PRODUCTION IN LPS-INDICED RAW264.7 CELLS

Reactive oxygen species (ROS) is a key molecule that plays an important role in the progression of the inflammatory disorders (Mittal et al. 2014). We evaluated whether TSCE could reduce intracellular ROS which are noted to stimulate inflammatory process. As shown in Figure 1B, LPS caused a significant increased ($p < 0.001$) in the production of intracellular ROS in RAW264.7 macrophages compare to the untreated control. TSCE at the concentration of 25 and 50 µg mL^{-1} significantly decreased the percentage of LPS-induced intracellular ROS generation in RAW264.7 cells in a concentration-dependent manner compared to the untreated control group. While dexamethasone treatment did not decrease the percentage of LPS-induced intracellular ROS production in RAW264.7 cells (Figure 1(B)).

TSCE INHIBITS LPS-INDUCED NITRIC OXIDE (NO) LEVEL IN RAW264.7 CELLS

NO is a key signaling molecules that plays a major role in the pathogenesis of inflammation (Sharma et al. 2007). We investigated whether TSCE could reduce the NO level which are known to stimulate the progression of inflammatory process. As shown in Figure 2(A), LPS caused a significantly increased nitrite level, as an index of NO in cultured medium of RAW264.7 macrophages ($p < 0.001$) compared to the untreated control group. Interestingly, TSCE at the concentration of 10, 25 and 50 µg mL^{-1} caused a significant diminish ($p < 0.001$) the nitrite content in LPS-induced RAW264.7 macrophages (Figure 2(A)) and also increased % NO inhibition with IC$_{50}$ value of 24 h were 32.85 ± 7.79 µg/mL (Figure 2(B)) when compared to the untreated group. Dexamethasone (1 µg mL^{-1}) as a positive drug significantly decreased the nitrite content in LPS-induced RAW264.7 macrophages.

TSCE SUPPRESSED LPS-INDUCED TNF-α AND IL-1β PRODUCTION IN RAW264.7 CELLS

As shown in Figures 3(A) and 4(A), LPS caused a significantly increased the secretion of IL-1β and

TNF-α in RAW264.7 macrophages. Significantly, TSCE at the concentration of 10, 25, and 50 µg mL^{-1} caused a significant reduction of IL-1β secretion ($p < 0.001$) (Figure 3(A)) when compared to the untreated group and increased % IL-1β inhibition with IC$_{50}$ value of 24 h were 28.33 ± 3.77 (Figure 3(B)). In addition, TSCE at the concentration of 10, 25, and 50 µg mL^{-1} significantly decreased the release of TNF-α ($p<0.001$) (Figure 4(A)) in LPS-induced RAW264.7 macrophages when compared to the untreated group and enhanced % TNF-α inhibition with IC$_{50}$ value of 24 h were 53.49 ± 9.04 µg mL^{-1} (Figure 4(B)). Dexamethasone (1 µg mL^{-1}) significantly decreased the IL-1β and TNF-α level in LPS-induced RAW264.7 macrophages (Figures 3 and 4). We further confirmed that TSCE decrease the level of inflammatory mediators (NO, ROS, IL-1β, and TNF-α) was not due to chemically activated cytotoxicity. The results presented that cell viability of LPS in with or without of 10, 25, and 50 µg mL^{-1} of TSCE as well as dexamethasone (1 µg mL^{-1}) was not significantly decreased (Figure 5). Accordingly, this result indicated that TSCE could decrease the LPS-induced inflammatory response in RAW264.7 cells by inhibiting the production of proinflammatory mediators.

TABLE 1. Phytochemical screening of phenolic compound, flavonoid, proanthocyanidin, and tannin in tamarind seed coat extract (TSCE)

	Total phenolic compound (g GAE/100 g dry extract)	Flavonoid content (g quercetin/100 g dry extract)	Proanthocyanidin (A500) in 100 µg mL^{-1}	Tannin (% pyrogallol equivalent)
TSCE	26.43±0.56	3.54±0.06	1.43±0.08	21.49±1.49

Values express in mean ± SEM from three observations

TABLE 2. Antibacterial activity of tamarind seed coat extract (TSCE)

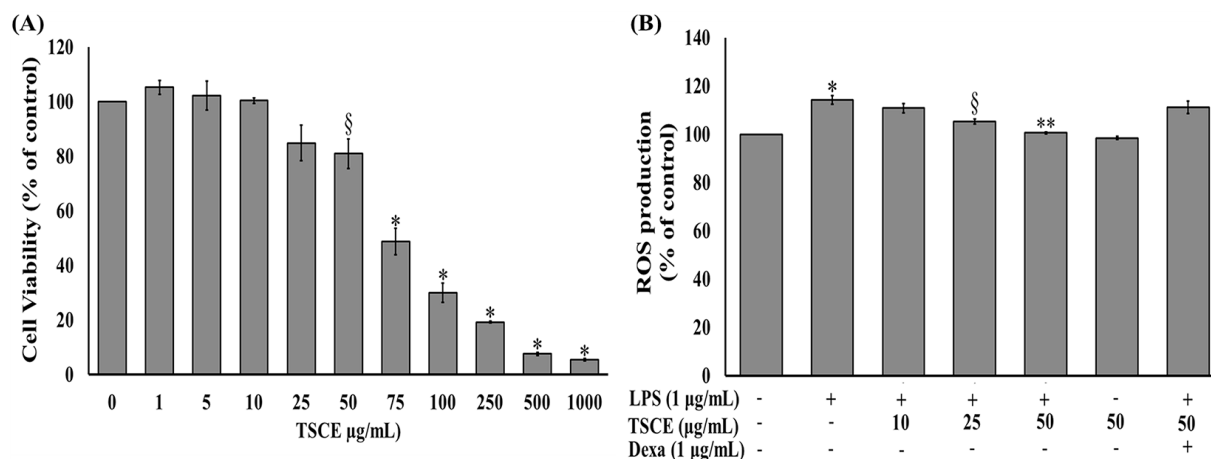| Bacterial strains	Minimal inhibitory concentration (µg mL^{-1})	Minimal bactericidal concentration (µg mL^{-1})
Staphylococcus aureus ATCC29213	64	>1204
Streptococcus mutans ATCC25175	128	>1024
Escherichia coli ATCC25922	>1204	>1204

FIGURE 1. Effect of the tamarind seed coat extract (TSCE) on cytotoxicity and intracellular ROS production in RAW264.7 macrophages. (A) RAW264.7 cells were treated with 0, 1, 5, 10, 25, 50, 100, 250, 500, and 1000 μg mL^{-1} of TSCE for 24 h. The RAW264.7 cells viability of TSCE-treated was measured by using a colorimetric MTT assay. (B) TSCE decreased the ROS production in LPS-induced RAW264.7 cells. Cells were treated with TSCE (10, 25, and 50 μg mL^{-1}) in the presence or absence of LPS for 24 h. Data are presented as mean ± S.E.M. for four independent experiments. *p < 0.05 compared to the 0 μg mL^{-1}. *p < 0.001 compared to the 0 μg mL^{-1}. §p < 0.001 compared to the untreated group. §p < 0.05 compared to the LPS group. **p < 0.001 compared to the LPS group
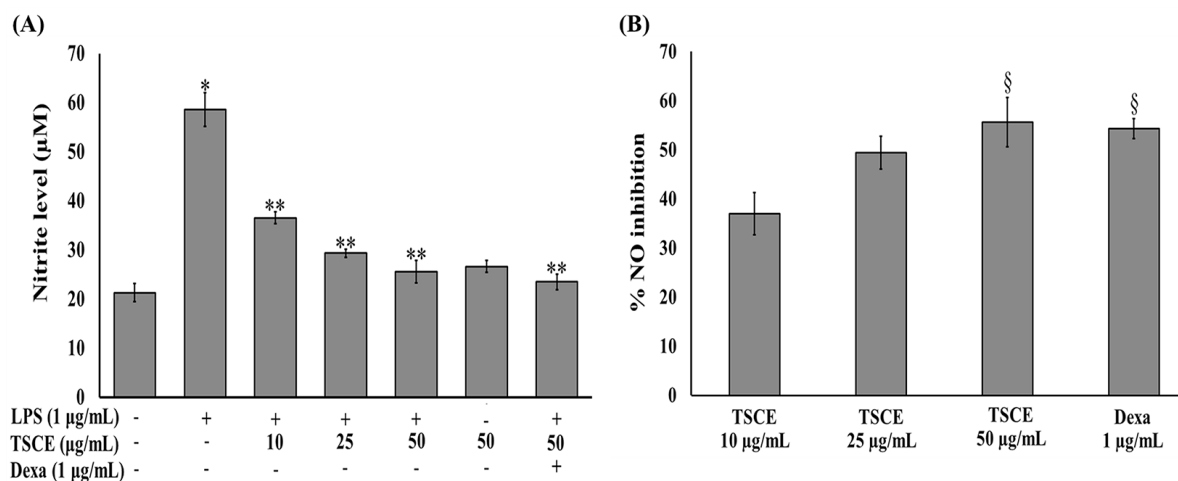

FIGURE 2. Tamarind seed coat extract (TSCE) inhibits LPS-induced NO production (A) and increases % NO inhibition (B) in RAW264.7 macrophages. RAW264.7 macrophages were received TSCE (10, 25, and 50 μg mL^{-1}) in the presence or absence of LPS for 24 h. Data are presented as mean ± S.E.M. for four independent experiments. *p < 0.001 compared to the untreated control group. **p < 0.001 compared to the LPS group. §p < 0.05 compared to the 10 μg mL^{-1} of TSCE
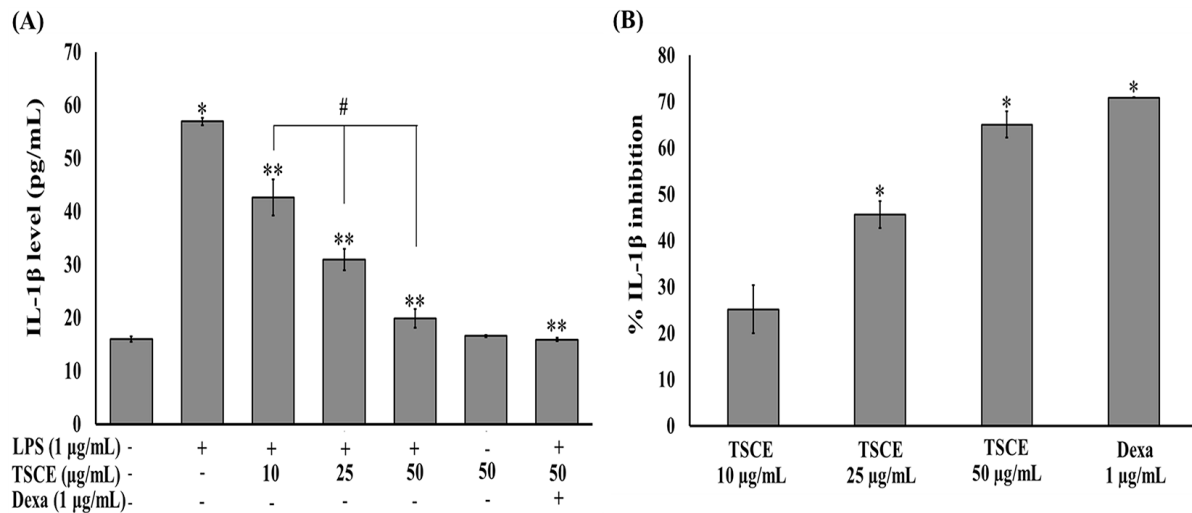
(A)

(B)

FIGURE 3. Tamarind seed coat extract (TSCE) inhibits LPS-induced IL-1β level (A) and increases % IL-1β inhibition (B) in RAW264.7 macrophages. RAW264.7 macrophages were administered TSCE (10, 25, and 50 μg mL^{-1}) in the presence or absence of LPS for 24 h. Data are presented as mean ± S.E.M. for four independent experiments. #p< 0.05 difference between treatment groups. *p< 0.001 compared to the untreated control group. **p< 0.001 compared to the LPS group. *p< 0.001 compared to the 10 μg mL^{-1} of TSCE

(A)

(B)

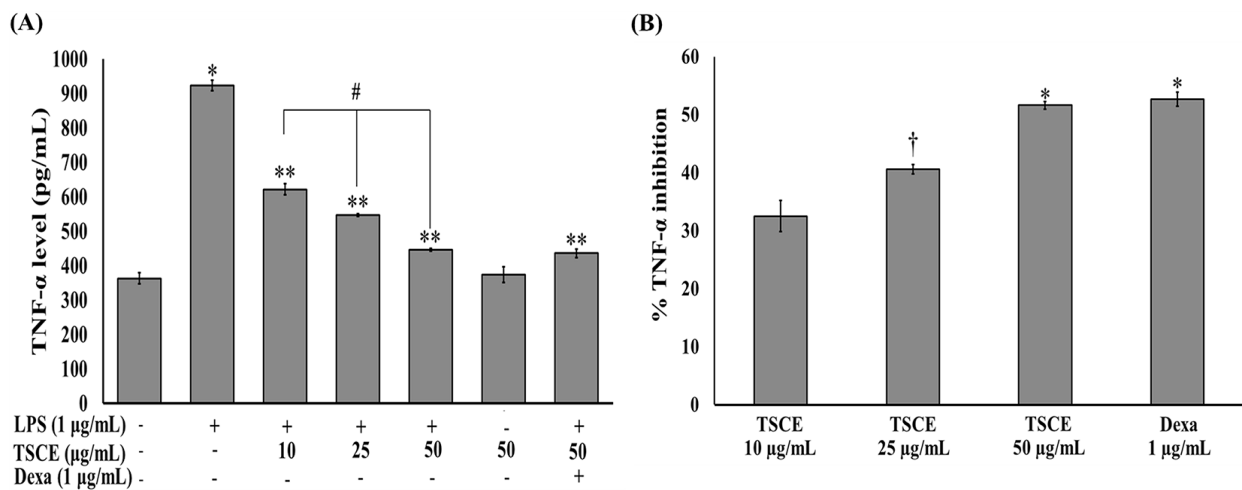FIGURE 4. Tamarind seed coat extract (TSCE) inhibits LPS-induced TNF-α level (A) and increases % TNF-α inhibition (B) in RAW264.7 macrophages. RAW264.7 macrophages were administered TSCE (10, 25, and 50 μg mL^{-1}) in the presence or absence of LPS for 24 h. Data are presented as mean ± S.E.M. for four independent experiments. #p< 0.05 difference between treatment groups. +p< 0.001 compared to the untreated control group. **p< 0.001 compared to the LPS group. *p< 0.01 compared to the 10 μg mL^{-1} of TSCE. *p< 0.001 compared to the 10 μg mL^{-1} of TSCE
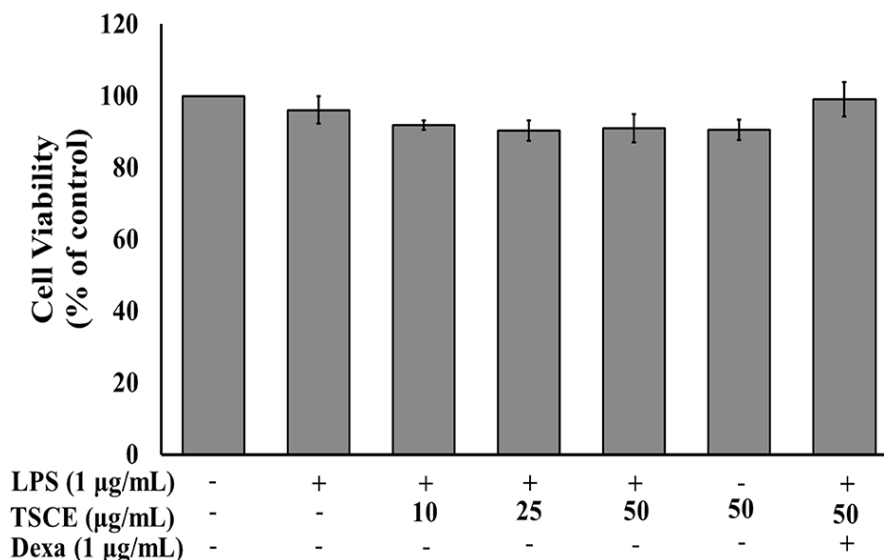
FIGURE 5. Effect of TSCE (10, 25, and 50 µg mL^{-1}) in the presence or absence of LPS on cytotoxicity in RAW264.7 macrophages. Cytotoxicity was evaluated by MTT assay. Data are presented as mean ± S.E.M. for four independent experiments

DISCUSSION

Plants and their extracts containing phenolics, flavonoids or tannin were extensively used as traditional drugs to restrain and treat inflammatory diseases in Asian for a long time (Wiart 2007). Natural phenolics, flavonoids or tannin have been noted to display anti-inflammatory activities *in vitro* (Park et al. 2014; Sergent et al. 2010) and *in vivo* (Alinejhad et al. 2016; Jayaraman et al. 2012). It has been indicated that natural extract could develop as new anti-inflammatory agents. The seed coat of tamarind has been described to protect fluoride-stimulated pulmonary inflammation and fibrosis (Ameeramja & Perumal 2018). However, the effects of tamarind seed coat extract in macrophages have not been studied. The aimed of this study was to evaluate the preliminary studies on anti-inflammatory capabilities of tamarind seed coat extract (TSCE) in LPS-induced RAW264.7 macrophages. The findings from our study provide an evidence of anti-inflammatory of TSCE effectiveness in the RAW264.7 cell culture model.

The phytochemical evaluation of tamarind seed extract has been reported on the presence of many active components including procyanidin B2, catechin, rutin, embelin, arecatannin B1, D-threo-isocitric acid and galactosyl glycerol (Sundaram et al. 2015). Procyanidin, catechin, rutin and embelin have been revealed to be an effective anti-oxidant and anti-inflammatory properties, which are capable of inhibiting oxidative stress (Babu

& Liu 2008; Martinez-Micaelo et al. 2012; Schaible et al. 2013). Moreover, polyphenols and tannins display antioxidant, antibacterial, and anti-inflammatory action (Tungmunnithum et al. 2018). Our results on phytochemical screening reported that TSCE contained the presence of tannins, flavonoids, proanthocyanidin and polyphenols which is consistent with previous reports (Bhadoriya et al. 2018; Nakchat et al. 2014a).

The gram-positive strains of *S. aureus* ATCC29213 was more sensitive to the activity of TSCE but it was not effective against the gram-negative strain of *E. coli* ATCC25922. The MIC of TSCE was also investigated and its value was 64 µg mL^{-1} for strains *S. aureus* ATCC29213 which is consistent with previous reports (Lima et al. 2017). However, gram-negative are more resistant with MIC values of >1204 µg mL^{-1} for *E. coli* ATCC25922. Antibacterial properties of plant extracts in gram-negative are more resistant than gram-positive might due to the presence of the chemical composition of cell wall. Gram-negative membrane causes rigidity and inhibits the bioactive compounds penetration into the bacteria.

LPS could be stimulated RAW264.7 macrophages and then secreted enormous amounts of the inflammatory negotiators, including ROS, NO and the pro-inflammatory cytokines, such as TNF-α and IL-1β (Sukketsiri et al. 2019). These inflammatory negotiators play pivotal functions in the development of inflammatory diseases (Duque &

Descoteaux 2014). The suppression of LPS-encouraged inflammatory negotiators is noted as an ameliorative approach for the protection of inflammation (Sukketsiri et al. 2019). Hence, ROS, NO, TNF-α, and IL-1β were selected to determine the anti-inflammatory property of TSCE in this study. Furthermore, the anti-inflammatory activities of TSCE are demonstrated quantitatively using the IC_{50} value, which is the TSCE concentration desired to restrict 50% generation of these LPS-triggered inflammatory negotiators. The IC_{50} value was accessed from the graph of the percentage of inhibited inflammatory negotiators and the TSCE concentration. Hence, the IC_{50} value for the TSCE in this experiment on the suppression of the inflammatory negotiators could be used to determined anti-inflammatory properties of the TSCE.

ROS are important for host defense mechanism and are generated by phagocytes in reaction to microbial and inflammatory stimuli (Leavy 2014). ROS are crucial signaling molecules that show a significant function in the advancement of inflammatory disorders. An excessive of ROS causes the stimulation of pro-inflammatory cytokines (Bryan et al. 2012; Schieber & Chandel 2014). Furthermore, ROS is also a secondary messenger augmented in response to LPS-induced inflammation (Sukketsiri et al. 2019). Accordingly, modulation of ROS is an essential target for approving the machinery of inflammatory stimulation. We found that TSCE could significantly attenuated the production of ROS in LPS-induced RAW264.7 macrophages which was in line with the previous reports (Ameeramja et al. 2016; Nakchat et al. 2014b). This effect of TSCE might due to its components, for example, phenolic, procyanidins, flavonoids and tannin which have reported to possesses antioxidant by elevated the non-enzymatic and enzymatic antioxidant system (Ameeramja et al. 2016; Nakchat et al. 2014a, 2014b).

In the inflammation process, NO is known as a pro-inflammatory negotiator that promotes inflammation due to excessive generation in abnormal conditions (Sharma et al. 2007). Thus, natural compounds able to be suppressing the generation of NO would be significant in restraining the occurrence of inflammatory disorder. TSCE has been reported to decrease NO level in fluoride-stimulated pulmonary inflammation in rats' model (Ameeramja & Perumal 2018). In this experiment, our results positively showed that TSCE significantly attenuated the level of NO secretion in LPS-encouraged RAW264.7 macrophages. Interestingly, TSCE are seemingly powerful with $IC_{50} = 32.85 \pm 7.79$ µg mL^{-1} in the suppression of NO generation in LPS-encouraged RAW264.7 macrophages. It might be postulated that the phenolic and tannin compounds at high concentrations in the TSCE might responsible for the strong inhibitory activity against NO generation. TSCE is considered as a promising functional food product by suppressing the generation of NO.

It has been noted that TNF-α and IL-1β pro-inflammatory cytokines are in associated with numerous inflammatory diseases (Chen et al. 2018; Duque & Descoteaux 2014). TNF-α is an important cytokine that activates macrophages through enhancing other pro-inflammatory cytokines in the progression of inflammation (Duque & Descoteaux 2014). IL-1β acts as an activator to enhance the secretion of inflammatory proteins during inflammation (Chen et al. 2018). Consequently, inhibitors of TNF-α and IL-1β have also been suggested as patterns for the anti-inflammatory drugs development. In this study, we measured the properties of TSCE on the TNF-α and IL-1β secretion in LPS-encouraged RAW264.7 macrophages. We found that TSCE considerably attenuated the release of both TNF-α and IL-1β. Significantly, TSCE is capable in lowering the TNF-α secretion with $IC_{50} = 53.49 \pm 9.04$ µg mL^{-1} in LPS-encouraged RAW264.7 macrophages. Additionally, TSCE is show in inhibiting the IL-1β secretion with $IC_{50} = 28.33 \pm 3.77$ µg mL^{-1} in LPS-encouraged RAW264.7 macrophages. Interestingly, TSCE showed higher suppression in lowering the secretion of IL-1β ($IC_{50} = 28.33 \pm 3.77$ µg mL^{-1}) than TNF-α ($IC_{50} = 53.49 \pm 9.04$ µg mL^{-1}) in this study model. Hence, our study suggested that TSCE could decrease the inflammatory response by diminishing the generation of proinflammatory cytokines IL-1β and TNF-α.

CONCLUSION

TSCE provided a potent anti-inflammatory property though the reduction of ROS, NO, IL-1β and TNF-α. Based on our results, we indicate TSCE might be a good candidate to develop as an anti-inflammatory drug; however, further investigations on the specific molecular mechanisms responsible for the anti-inflammatory effect of TSCE are still needed to clarify.

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