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Research article

Anti-inflammatory potential of mung bean seed coat water extract in lipopolysaccharide-induced 3T3-L1 adipocytes

Norathee Buathong[a], Kasemsiri Chandarajoti[b,c], Sudathip Sae-tan[a,*]

[a] *Department of Food Science and Technology, Faculty of Agro–Industry, Kasetsart University, Bangkok 10900, Thailand*

[b] *Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand*

[c] *Drug Delivery System Excellence Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand*

Article Info

Abstract

Excessive lipid accumulation in adipocytes results in low-grade inflammation, which is a major cause of many chronic diseases including metabolic syndromes, hypertension, diabetes and cardiovascular diseases. The prolonged lipid accumulation causes the production of inflammatory enzymes, pro-inflammatory cytokines and free radicals. Many plant extracts rich in polyphenols have reported anti-inflammatory activities. In this study, mung bean seed coat, a by-product from the mung bean industry, was extracted with boiling water to obtain mung bean seed coat water extract (MSWE). The MSWE contained high amounts of polyphenols (mean ± SD, 4.39 ± 0.01 mg gallic acid equivalents/g of dry weight extract). The anti-inflammatory activity of MSWE was investigated in lipopolysaccharide-stimulated 3T3-L1 adipocytes. MSWE had 2,2 diphenyl-1-picrylhydrazyl scavenging activity (half maximal inhibitory concentration value of 234 µg/mL). Low doses of MSWE (6.25 µg/mL or 12.5 µg/mL) reduced intracellular reactive oxygen species. In addition, MSWE decreased cyclooxygenase 2 and increased inducible nitric oxide synthase mRNA expressions. In addition, MSWE reduced the mRNA expression of pro-inflammatory cytokines (interleukin (IL)-1β, IL-6 and tumor necrosis factor, TNF-α). Low doses of MSWE (6.25 µg/mL or 12.5 µg/mL) increased the mRNA expression of IL-10. In conclusion, MSWE exerted anti-inflammatory potential via the modulation of inflammatory enzymes and inflammatory cytokines as well by free radical scavenging activity. The results suggested that MSWE was beneficial in alleviating inflammatory diseases and would be suitable as a functional food.

* Corresponding author.
  E-mail address: fagists@ku.ac.th (S. Sae-tan)

Introduction

Low-grade inflammation in adipose tissue as a result from excessive fat accumulation leads to hypoxia, adipocyte cell death and the secretion of adipocytokines (Sanada et al., 2016) resulting in chronic diseases (Handschin and Spiegelman, 2008). Many detrimental stimuli have been used to study inflammation including lipopolysaccharide (LPS), an endotoxin derived from Gram-negative bacteria. Binding of LPS to toll-like receptor 4 (TLR4) activates the nuclear factor-κB (NF-κB) signaling pathway, which induces inflammatory enzymes including inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2) as well as pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α (Leyva-López et al., 2016). Reactive oxygen species (ROS) can be generated as a by-product of this cellular mechanism. When prolonged inflammation occurs, overproduction of ROS plays a vital role in the progression of inflammatory associated diseases (Griffith et al., 2009). iNOS is a synthase responsible for nitric oxide (NO) production; thus, iNOS expression and NO production are correlated (MacMicking et al., 1997). A high level of iNOS was reported to prevent infection and increase immune responses by elevating the NO level (MacMicking et al., 1997). Takeuchi et al. (2006) reported that rats with enterobacterial infection showed increased iNOS expression resulting in the NO production. The presence of NO prevented intestinal mucosa from enterobacterial infection (Takeuchi et al., 2006).

Mung bean is one of the important legumes widely consumed in many countries, especially in Asia and it is rich in protein with essential amino acids and considerable amounts of vitamins and minerals (Nair et al., 2013). Mung bean is a source for value-added edible products including noodles and desserts (Hou et al., 2019). However, the mung bean seed coat is typically discarded as waste in the mung bean industry. Nevertheless, the mung bean seed coat is rich in polyphenols especially flavonoids with 96% of flavonoids in mung bean, including vitexin and isovitexin, being in the seed coat (Cao et al., 2020). Many studies reported health benefits of mung bean, including as an antioxidant (Guo et al., 2012), lowering hypertension (Nakamura et al., 2016), anti-cancer (Handschin and Spiegelman, 2008), anti-diabetic and anti-inflammatory activity (Luo et al., 2016; Manikandaselvi et al., 2015; Saeting et al., 2021). In addition, mung bean protein inhibits angiotensin I-converting enzyme activity (Li et al., 2006). However, only a few studies have reported the health benefits of the mung bean seed coat (Li et al., 2016; Luo et al., 2016; Sae-tan et al., 2020). Seed coats of other beans have demonstrated health benefits. Faba bean seed coat showed bile acid-binding activity (Çalışkantürk Karataş et al., 2017). Black soybean seed coat showed the antioxidant activity against 2,2 diphenyl-1-picrylhydrazyl (DPPH) radicals and low-density lipoprotein (LDL) oxidation (Astadi et al., 2009). A recent study reported that mung bean seed coat water extract (MSWE) inhibited inflammation in LPS-induced RAW 264.7 macrophages via TAK1/IκBα/NF-κB (Sae-tan et al., 2020). However, reporting on the anti-inflammatory effects in adipocytes is unclear. Based on information on polyphenols and flavonoids in the mung bean seed coat (Li et al., 2016; Luo et al., 2016), the current study hypothesized that MSWE exerted anti-inflammatory effects in LPS-induced 3T3-L1 adipocytes.

Materials and Methods

Materials and chemicals

Mung bean seed coat was provided by Kittitat Co., Ltd. (Thailand). LPS from *Escherichia coli* O111:B4 was purchased from Sigma (Saint Louis, MO, USA). The Total RNA Mini Kit was purchased from GeneAid (New Taipei city, Taiwan). RevertAid First Strand cDNA synthesis kit was purchased from Thermo Fisher (Vilnus, Lithuania). SYBR Green Supermix (Biorad; Hercules, CA, USA). The enzyme-linked immunosorbent assay (ELISA) Mouse IL-6 kit was purchased from R&D systems (Minneapolis, MN, USA). All other chemicals were analytical grade and obtained from reputable suppliers.

Mung bean seed coat water extract preparation

Mung bean seed coat water extract (MSWE) was prepared according to Sae-tan et al. (2020). Briefly, 60 g of mung bean seed coat was boiled in deionized water for 30 min. Then, it was filtered through Whatman paper No.1. The filtrate was collected, concentrated and freeze-dried to obtain MSWE. The extract was stored in conical tubes and kept at -80°C until further analysis.

Determination of total phenolic content

Folin-Ciocalteu reagent was used to determine the total phenolic content (Sumczynski et al., 2015). Briefly, 500 μL of MSWE (0.1 mg/mL) was mixed with 2.5 mL of 0.2 M Folin-Ciocalteu reagent and equilibrated for 5 min. The mixture was

mixed with 2 mL of Na_2CO_3 (7.5%). The solution was mixed and kept in the dark for 30 min. The absorbance of the mixture was determined at a wavelength of 765 nm using an ultraviolet-visible spectrophotometer (CECIL 1011; Cecil Instruments Ltd.; Peterborough, UK). Gallic acid was used as a reference standard and the result was presented as milligrams of gallic acid equivalents (GAE) per gram of dry weight extract.

Determination of 2,2 diphenyl-1-picrylhydrazyl radical scavenging activity

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as described by Thuphairo et al. (2019). MSWE was dissolved in distilled water in the concentration range 31.125–500 μg/mL. Briefly, 20 μL of MSWE solution was mixed with 190 μL of freshly prepared DPPH. The mixture was kept in the dark at 37°C for 30 min. The absorbance was determined at a wavelength of 517 nm using a microplate reader (Tecan Infinite 200 Pro; Männedorf, Switzerland). Trolox was used as a reference standard and the antioxidant activity was expressed as % DPPH radical scavenging activity.

Identification of major flavonoids in mung bean seed coat water extract

MSWE was dissolved in deionized water and high performance liquid chromatography (HPLC) with a diode array detector (DAD) (Waters 600; Milford, MA, USA) was used to detect flavonoids in the MSWE according to Sae-tan et al. (2020). An analytical column C18 (4.6×250 mm, 5 μm; Waters Symmetry Column, Dublin, Ireland) was used and kept at 30°C. A gradient event of mobile phase solvent A (1% acetic acid in deionized water, volume per volume, v/v) and solvent B (1% acetic acid in methanol, v/v) was as follows: 10–35% B (10 min), 35–42% B (15 min), 42–75% B (10 min), 75% B (5 min), 75–10% B (5 min) and 10% B (5 min). The injection volume of each sample was 10 μL and the flow rate was 1 mL/min. Standard vitexin and isovitexin (25–100 parts per million) were used to prepare standard curves and to determine the concentration of vitexin and isovitexin in the MSWE.

Cell culture and differentiation

Murine 3T3-L1 preadipocytes were kindly provided from Assistant Professor Dr. Wasaporn Chanput (Department of Food Science and Technology, Kasetsart University).

3T3-L1 preadipocytes were cultured and differentiated as described in the American Type Culture Collection (ATCC) instruction. Briefly, the cells were grown in high glucose Dulbecco's modified Eagle's medium (DMEM) (4.5 g/L glucose) supplemented with 10% fetal bovine serum (FBS) and 1% non-essential amino acids at 37°C in a humidified 5% CO_2 atmosphere. One day after confluence (designated as day 0), the medium was discarded and replaced with differentiated medium (high glucose DMEM supplemented with 10% FBS, 1 μM dexamethazone, 0.5 mM 3-isobutyl-1-methyl-xanthine and 1μg/mL insulin) for 2 d. At day 3, the cells were grown in high glucose DMEM supplemented with only 10% FBS and 1 μg/mL insulin (post-differentiated medium) until day 9 and the medium was changed every 2 d (Alves et al., 2015).

Cytotoxicity assay

The cytotoxicity of the MSWE on 3T3-L1 was assessed using 3-(4,5-dimethylthiazol-2-yl)-1,5-diphenyltetrazolium bromide (MTT) assay (Zhang and de Mejia, 2020). 3T3-L1 preadipocytes were seeded at a density of 3×10^4 cells/well in a 96-well plate. The cells were induced for differentiation as mentioned above. After the cells had fully differentiated, they were grown in post-differentiated medium with the absence or presence of MSWE (3.125 μg/mL, 6.25 μg/mL, 12.5, 25 μg/mL) until day 9. On day 9, 10 μL of 5 mg/mL of MTT was added into each well and incubated at 37°C for 4 hr. Dimethyl sulfoxide was used to dissolve the formazan crystals. The cell viability was determined by recording the absorbance at 570 nm using a microplate reader (Tecan Infinite 200 Pro; Männedorf, Switzerland). The results were expressed as percentages of the control cells.

Determination of intracellular reactive oxygen species

Intracellular ROS was quantified using 2'-7'-dichlorodihydrofluorescein diacetate (DCFH-DA) according to the method of Khole et al. (2016). Briefly, 3T3-L1 preadipocytes were seeded at a density of 3×10^4 cells/well in a 96-black well plate. The cells were treated for RNA harvesting as mentioned above. After the cells had been stimulated with LPS, they were washed three times with phosphate buffer solution (PBS) 200 μL. Then, 10 μL of DCFH-DA (10 μM) was added in each well and incubated at 37°C for 30 min. After that, the cells were washed three times with PBS to remove excess dye; then, 100 μL of DMEM was added to each well. The fluorescence intensity was determined at an excitation

wavelength of 485 nm and an emission wavelength of 528 nm using a microplate reader (Tecan Infinite 200 Pro, Männedorf, Switzerland). The data were expressed in terms of the relative percentage of intracellular ROS.

RNA preparation and real-time polymerase chain reaction analysis

3T3-L1 preadipocytes were seeded at a density of 1×10^5 cells/well in a 12-well plate. The cells were induced for differentiation and fully differentiated 3T3-L1 adipocytes were incubated with post-differentiated medium in the absence or presence of MSWE (6.25 µg/mL, 12.5 µg/mL, 25 µg/mL) during days 3–9. On day 9, the cells were stimulated with LPS (1 µg/mL) for 1 hr (Zhang and de Mejia, 2020). The total RNA was isolated using the Total RNA Mini Kit. RNA concentration was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific 2000; Wilmington, DE, USA). The isolated RNA was used to synthesize cDNA using the RevertAid First Strand cDNA Synthesis Kit. Quantitative real-time PCR analysis was performed using SYBR Green Supermix with the CFX Manager Software CFX96 real-time system (Singapore) according to the manufacturer's protocol. The PCR reaction conditions for each cycle were: 5 min at 95°C followed by 40 cycles of 30 s at 95°C and 30 s at 61.3°C. The data were analyzed using the $2^{-\Delta\Delta Ct}$ model. Each value for the mRNA of analyzed genes was normalized to the mRNA for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer sequences are listed in Table 1.

Enzyme-linked immunosorbent assay

3T3-L1 preadipocytes were seeded at a density of 1×10^5 cells/well in a 12-well plate. The cells were treated for RNA harvesting as mentioned above. After the cells had been stimulated with LPS, the media were collected and kept at -20°C for further analysis. The level of IL-6 was determined using the ELISA Mouse IL-6 kit following the manufacturer's instructions.

Statistical analysis

All experiments were performed in biological triplicates. Data were presented as mean ± SD. Statistical differences were determined using one-way analysis of variance and Duncan's post-hoc test using the IBM SPSS Statistics version 28.0 software (Thaisoftup Co., Ltd.; Bangkok, Thailand). Statistical differences were considered significant at $p < 0.05$. The concentration of extract in milligrams per milliliter that inhibited 50% of the DPPH radicals (IC_{50}) was determined using GraphPad Prism 5 statistical package (GraphPad Software Inc; San Diego, CA, USA).

Results

Flavonoids identification and antioxidant activity of mung bean seed coat water extract

Inflammation leads to the development of many non-communicable diseases including cancers, cardiovascular disease, Alzheimer's disease and type 2 diabetes (Khan et al., 2016). Phenolic compounds have been reported to lower the risk of those diseases via antioxidant activity and anti-inflammatory activity (de Albuquerque et al., 2019). In the current study, mung bean seed coat was extracted with boiling water and investigated for anti-inflammatory activity in adipocytes. The results showed that the total phenolic content in MSWE was 4.39 ± 0.01 mg GAE/g dry weight. MSWE also exerted antioxidant activity reported as DPPH radical scavenging activity with an IC_{50} value of 234 µg/mL (Fig. 1). HPLC-DAD was used to identify the major flavonoids in MSWE. The results showed that the two major flavonoids in MSWE were vitexin and isovitexin (Fig. 2). These results

Table 1 Sequences of reverse transcriptase-polymerase chain reaction primers

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
COX2	ATGGTGGCTGTTTTGGTAGGCTGTG	ATGGTGGCTGTTTTGGTAGGCTGTG
iNOS	GAAGAAAACCCCTTGTGCTG	TGGTCAAACTCTTGGGGGTTC
IL-1β	GAAGGGCTGCTTCCAAACCT	GTTGTTCATCTCGGAGCCTG
IL-6	CCACTTCACAAGTCGGAAGCTTA	GCAAGTGCATCATCGTTGTTCATA
TNF-α	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGGGAG
IL-10	CACAAAGCAGCCTTGCAGAA	AGAGCAGGCAGCATAGCAGTG
GAPDH	AACGACCCCTTCATTGAC	TCCACGACATACTCAGCAC

COX2 = cyclooxygenase 2; *iNOS* = inducible nitric oxide synthase; *IL-6* = interleukin-6; *IL-1β* = interleukin-1β; *TNF-α* = tumor necrosis factor-α; *IL-10* = interleukin-10; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase

agreed with other studies that reported the presence of vitexin and isovitexin in mung bean seed coat (Cao et al., 2011; Sae-tan et al., 2020). These two isomers were reported to be abundant (> 96%) in mung bean seed coat rather than in the cotyledon and to be antioxidant compounds in mung bean (Cao et al., 2011). Vitexin was proposed as a bioactive compound for anti-inflammatory activity in RAW 264.7 macrophages (Sae-tan et al., 2020).
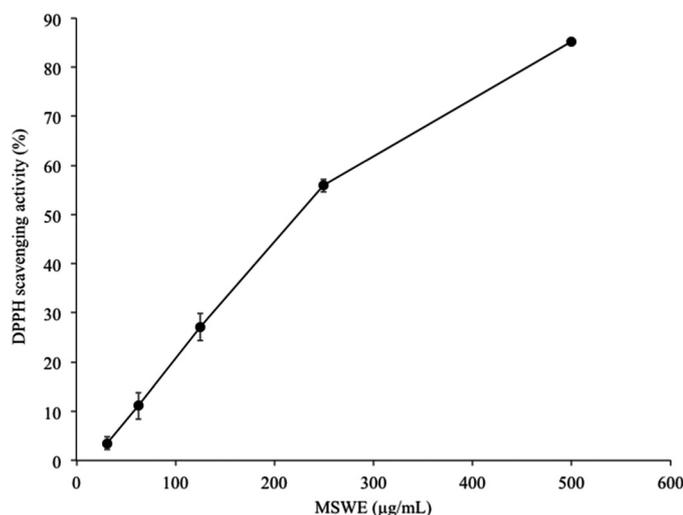

Fig. 1 Percentage of 2,2 Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of mung bean seed coat water extract (MSWE), where data are mean ± SD.
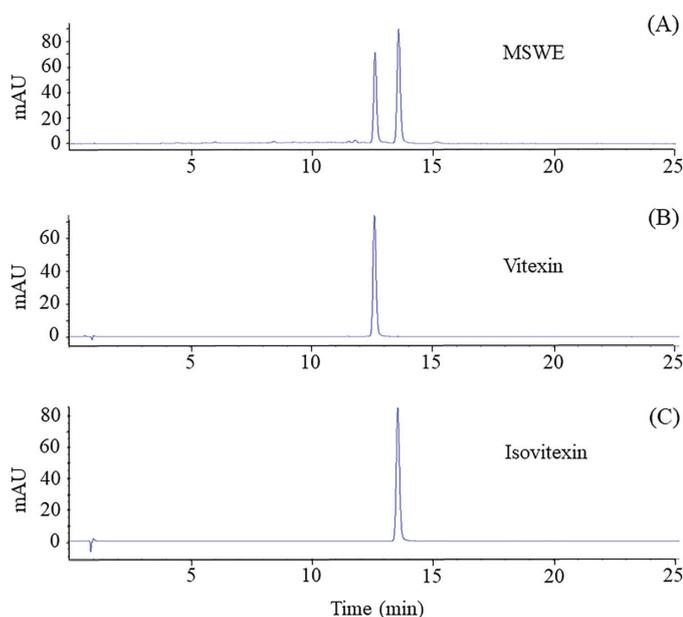

Fig. 2 Representative high performance liquid chromophotography-diode array detector chromatographic profiles (λ = 337 nm) of: (A) mung bean seed coat water extract; (B) standard vitexin (50 µg/mL); (C) standard isovitexin (µg/mL)

Cytotoxicity of mung bean seed coat water extract on 3T3–L1 adipocytes

The cytotoxicity of MSWE was determined on 3T3-L1 adipocytes. The results showed that cell viability values of 3T3-L1 adipocytes treated with MSWE at concentrations of 3.125–25 µg/mL were not significantly different to that of control cells (Fig. 3). Therefore, 6.25–25 µg/mL MSWE was used in the subsequent studies.

Effects of mung bean seed coat water extract on intracellular reactive oxygen species in lipopolysaccharide-stimulated 3T3-L1 adipocytes

Prolonged inflammation results in overproduction of ROS, which worsens inflammation of the cells (Khan et al., 2016). In the current study, intracellular ROS was determined and LPS was able to stimulate 3T3-L1 cells (Fig. 4), thus producing a significantly higher level of intracellular ROS than the control cells. When MSWE was pre-treated without LPS stimulation, the intracellular ROS level produced in the 3T3-L1 cells was not significantly different to that of the control cells. However, when LPS-stimulated 3T3-L1 cells pretreated with 6.25 µg/mL or 12.5 µg/mL MSWE, the intracellular ROS significantly reduced, whereas 25 µg/mL MSWE treatment was unable to reduce the level of the intracellular ROS.
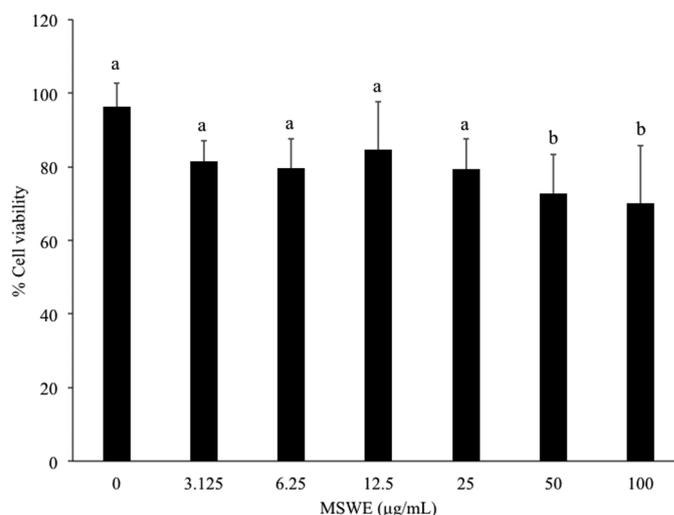

Fig. 3 Cytotoxicity of mung bean seed coat water extract (MSWE) on survival of 3T3-L1 adipocytes at different concentrations, where bars represent mean ± SD ($n = 3$) and different letters above bars indicate significant ($p < 0.05$) difference.
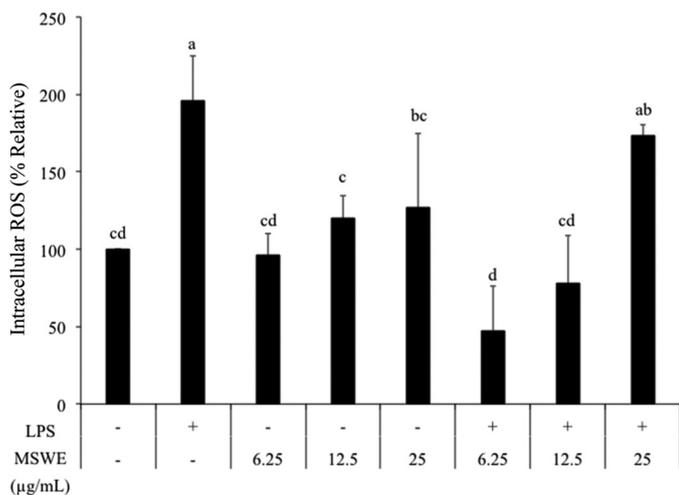
Fig. 4 Intracellular reactive oxygen species (ROS) of lipopolysaccharide (LPS)-stimulated 3T3-L1 adipocytes with or without mung bean seed coat water extract (MSWE), where bars represent mean ± SD ($n = 3$) and different lowercase letters above bars indicate significant ($p < 0.05$) difference.

Effects of mung bean seed coat water extract on mRNA expression of cyclooxygenase 2 and inducible nitric oxide synthase in lipopolysaccharide-stimulated 3T3-L1 adipocytes

LPS-stimulated 3T3-L1 adipocytes showed an elevated mRNA expression of inflammatory enzymes consisting of COX2 and iNOS compared to the control cells (Fig. 5). Treatment with 6.25 μg/mL or 12.5 μg/mL MSWE prior to LPS stimulation tended to suppress COX2 mRNA expression but the concentration of MSWE was inadequate to significantly

suppress the inflammation in LPS-stimulated 3T3-L1 adipocytes. However, the higher concentration of MSWE at 25 μg/mL was able to significantly downregulate a level of COX2, showing restoration of COX2 similar to the control cells (Fig. 5A). Furthermore, the effects of pretreatment with 6.25 μg/mL or 12.5 μg/mL MSWE in LPS-stimulated 3T3-L1 cells on iNOS mRNA expression were similar to the COX2 mRNA expression results. Interestingly, MSWE at 25 μg/mL produced a significant elevation of iNOS mRNA expression (Fig. 5B).

Effects of mung bean seed coat water extract on mRNA expression of IL-6, IL-1β, TNFα and IL-10 in lipopolysaccharide-stimulated 3T3-L1 adipocytes

Inflammation results in the increasing pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α and the decreasing anti-inflammatory cytokines such as IL-10 (Kowalska et al., 2019). The current results showed that LPS-stimulated 3T3-L1 cells had an elevated mRNA expression of IL-6, IL-1β and TNF-α compared to that of control cells (Fig 6). Pretreatment with MSWE (6.25 μg/mL, 12.5 μg/mL or 25 μg/mL) prior to LPS stimulation significantly reduced the IL-6 mRNA levels compared to the LPS-stimulated 3T3-L1 cells without MSWE treatment, and the level was restored to the same level as in the control cells (Fig. 6A). Treatment with 6.25 μg/mL or 12.5 μg/mL of MSWE reduced IL-1β expression, whereas 25 μg/mL MSWE significantly reduced IL-1β in LPS-stimulated 3T3-L1 cells (Fig. 6B). All MSWE treatments had an inhibitory effect on the TNF-α mRNA level in LPS-stimulated 3T3-L1 cells
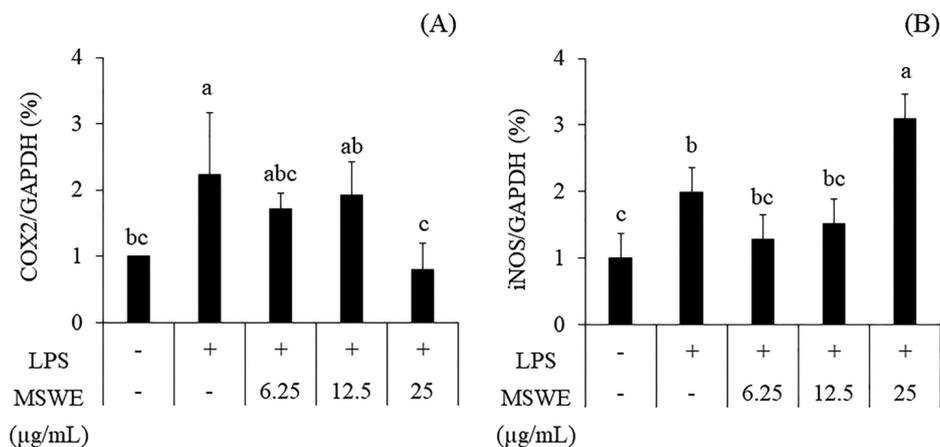

Fig. 5 Relative mRNA expression of: (A) cyclooxygenase 2 (COX2); (B) inducible nitric oxide synthase (iNOS) in lipopolysaccharide (LPS)-stimulated 3T3-L1 adipocytes with or without mung bean seed coat water extract (MSWE), where bars represent mean ± SD ($n = 3$); different lowercase letters above bars indicate significant ($p < 0.05$) difference; GAPDH = glyceraldehyde-3-phosphate dehydrogenase

(Fig. 6C). In contrast, LPS stimulation in 3T3-L1 cells reduced anti-inflammatory cytokines, as shown with the reduction of IL-10 mRNA expression compared to the control cells (Fig. 6D). Treatment with 6.25 µg/mL or 12.5 µg/mL of MSWE in LPS-stimulated 3T3-L1 cells significantly restored the IL-10 mRNA level compared to the control cells. Surprisingly, treatment with 25 µg/mL of MSWE significantly reduced the IL-10 mRNA level in the LPS-stimulated 3T3-L1 cells.

Effect of mung bean seed coat water extract on IL-6 level in lipopolysaccharide-stimulated 3T3-L1 adipocytes

According to the increased IL-6 mRNA expression mentioned above, the level of IL-6 produced in LPS-stimulated 3T3-L1 cells was observed. The results demonstrated that LPS-stimulated 3T3-L1 cells significantly increased the IL-6 level compared to the control cells (48.5%), as shown in Fig. 7. Treatment with 6.25 µg/mL or 12.5 µg/mL MSWE barely affected the level of IL-6 compared to LPS-stimulated 3T3-L1 cells. However, 25 µg/mL of MSWE significantly decreased the level of IL-6 (25.0%) compared to LPS-stimulated 3T3-L1 cells.
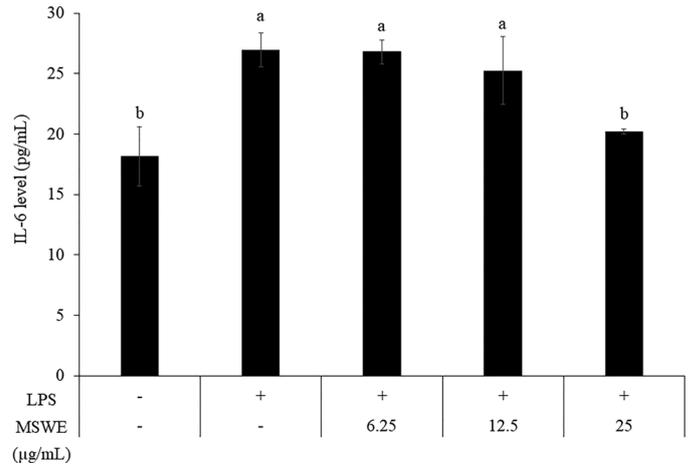

Fig. 7 IL-6 level released from lipopolysaccharide (LPS)-stimulated 3T3-L1 adipocytes with or without mung bean seed coat water extract (MSWE), where bars represent mean ± SD ($n = 3$) and different lowercase letters above bars indicate significant ($p < 0.05$) difference.
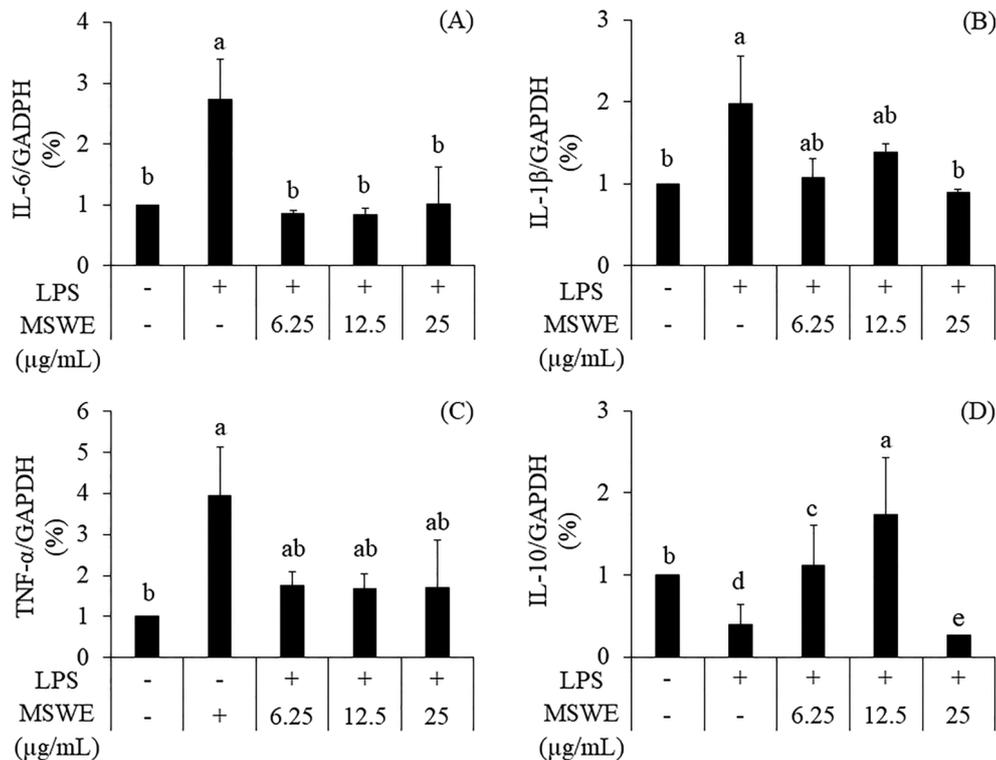

Fig. 6 Relative mRNA expression of (A) IL-6; (B) IL-1β; (C) TNF-α; (D) IL-10 in lipopolysaccharide (LPS)-stimulated 3T3-L1 adipocytes with or without mung bean seed coat water extract (MSWE), where bars represent mean ± SD ($n = 3$); different lowercase letters above bars indicate significant ($p < 0.05$) difference; GAPDH = glyceraldehyde-3-phosphate dehydrogenase

Discussion

The demand for plant-based functional food is economically growing due to the increasing numbers of consumers who prefer the fewer side effects from natural products compared to some modern medicines (Ahn et al., 2020). The current study investigated the effect of MSWE in attenuating inflammation in LPS-stimulated 3T3-L1 cells.

The current results showed that mung bean seed coat extracted with boiling water had anti-inflammatory effects on inflamed 3T3-L1 adipocytes using LPS as a stimulus. The results showed that the MSWE exerted antioxidant activity and modulated inflammatory enzymes and inflammatory cytokines at transcriptional and translational levels in the LPS-stimulated 3T3-L1 adipocytes. Other studies have reported that vitexin and isovitexin were major components contributing to the anti-inflammatory effects of mung bean (Lee et al., 2011; Zhang et al., 2013; Sae-tan et al., 2020). Vitexin and isovitexin have been identified in MSWE indicating that boiling in water was able to extract bioactive compounds from the mung bean seed coat, similar to the extraction from the whole seed of mung beans (Saeting et al., 2021). The current results indicated similar effects of MSWE in scavenging DPPH radicals.

Inflammatory response is a normal physiological defense; however, a long duration of inflammation leads to cellular injury by excessive generation of ROS (Khan et al., 2016). Excessive ROS can non-specifically damage many macromolecules including lipids, proteins and DNA in living cells, which subsequently contributes to several chronic diseases (Handschin and Spiegelman, 2008). An antioxidant agent not only demonstrates its scavenging activity but can also behave as a prooxidant. For example, epigallocatechin-3-gallate (EGCG), a well-known antioxidant, which contains a polyphenol chemical structure similar to vitexin and isovitexin, produces a prooxidant effect (Ouyang et al., 2020). EGCG at low dose acts an effective antioxidant, while at a high dose, EGCG acts as a prooxidant. EGCG at high doses (1–100 μM) was reported for its susceptibility to degradation via autooxidation, thus autogenerating ROS, suggesting that EGCG is capable of being both an antioxidant and a prooxidant depending on the concentration and the concentration of EGCG was reported to be a key factor in its activity (Ouyang et al., 2020). The current study showed that low doses (6.25 μg/mL or 12.5 μg/mL) of MSWE treatment in LPS-stimulated 3T3-L1 cells reduced intracellular ROS, while 25 μg/mL of MSWE increased ROS production. Chemical structures containing polyphenols found in EGCG, vitexin and isovitexin suggested a prooxidant effect at the high concentration of MSWE, with subsequent synergistic effect on ROS production; thus the ROS scavenging activity of MSWE was hindered at a high dose level. Hou et al. (2019) reported that the flavonoids found in MSWE reduced iNOS. Similarly, a phenomenon of the unexpected iNOS expression at 25 μg/mL of MSWE was a result of the expected prooxidant effect. Lowering iNOS by the two flavonoids presented in MSWE occurred at 6.25 μg/mL and at 12.5 μg/mL. However, the MSWE treatment at 25 μg/mL significantly increased iNOS expression due to the prooxidant activity of the polyphenols in the MSWE.

COX2 is a dominant inflammatory enzyme causing inflammation (Rouzer and Marnett, 2009). In an unstimulated cell, COX2 is present at a low level; however, when cell is stimulated by cytokines, chemokines or growth factors, COX2 expression quickly elevates (Peng et al., 2006; Rouzer and Marnett, 2009). LPS stimulated inflammation in 3T3-L1 cells as shown by the increase in COX2 expression. The current results showed that MSWE treatment can significantly decrease COX2 expression at 25 μg/mL whereas low concentrations (6.25 μg/mL or 12.5 μg/mL) produced no such effect. These results were consistent with other studies (Zhang et al., 2013; Sae-tan et al., 2020), demonstrating that whole seed mung bean extract significantly decreased COX2 expression at both the mRNA and protein levels in LPS-stimulated macrophages. In addition, Takeuchi et al. (2006) reported that COX inhibition was causally associated with the upregulation of iNOS, suggesting that the increased iNOS mRNA expression by the 25 μg/mL MSWE treatment in the current study may possibly have been due to the reduction of COX2 mRNA expression.

Inflammation stimulated by LPS results in an increase in pro-inflammatory cytokines (IL-6, IL-1β and TNF-α) and a decrease in anti-inflammatory cytokines (IL-10) (Chirumbolo et al., 2014). Many polyphenols and flavonoids have been reported to have anti-inflammatory effects. For example, catechins were reported to decrease the mRNA expression of IL-1α, IL-1β and IL-6, while increasing the mRNA expression of IL-4 and IL-10 in TNF-α-induced 3T3-L1 cells (Cheng et al., 2019). Vitexin produced anti-inflammatory effects in septic encephalopathy (SE)-induced mice by suppressing MCP-1, IL-6, IL-8 and TNF-α and regulating IL-10 in the mRNA expression in the SE-induced mice (Cao et al., 2020). The current study showed that MSWE treatment was able to decrease the mRNA expression of IL-6, IL-1β and TNF-α as well as IL-6 protein expression. However, only low doses of MSWE treatment increased the expression of IL-10 in LPS-stimulated 3T3-L1 adipocytes. The high dose of the MSWE treatment that had a reverse effect in regulating IL-10 was

potentially related to the prooxidant effect, which was observed with the iNOS results. All results from the current study were consistent with other studies that reported similar anti-inflammatory effects of mung bean extract in LPS-stimulated RAW 264.7 and J774 macrophages (Lee et al., 2011; Zhang et al., 2013; Sae-tan et al., 2020). The unexpected effects at 25 μg/mL of MSWE compared to lower doses (6.25 μg/mL and 12.5 μg/mL) were related to the increased ROS production and the mRNA expression of iNOS. Thus, MSWE at a high dose was unable to alleviate inflammation in LPS-stimulated 3T3-L1 adipocytes. A recent study reported that the anti-inflammatory effects of MSWE in RAW 264.7 macrophages were associated with the inhibition of TAK1/NF-κB/IκBα (Sae-tan et al., 2020) and the anti-inflammatory effects of vitexin were reported to be through the suppression of NF-κB p65 (Cao et al., 2020). Collectively, the modulation of inflammatory enzymes and inflammatory cytokines by MSWE treatment potentially inhibits NF-κB, although further investigation is needed.

The current study showed that mung bean seed coat water extract produced anti-inflammatory effects in LPS-stimulated 3T3-L1 adipocytes. The effects were potentially associated with three possible mechanisms: (1) scavenging free radicals (2) modulating inflammatory enzymes and (3) modulating inflammatory cytokines. The current results suggested that MSWE is beneficial in alleviating inflammatory diseases. In conclusion, mung bean seed coat, a by-product of the mung bean industry, is of interest for its potential use as a functional food. Further studies are needed to explore other modes of action of mung bean seed coat extract for functional food ingredient development.

Conflict of Interests

The authors declare that there are no conflicts of interests.

Acknowledgements

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