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Anti-inflammatory Activity of Soymilk and Fermented Soymilk Prepared with Lactic Acid Bacterium and Bifidobacterium

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ABSTRACT

In the present study, anti-inflammatory activity of the methanol extracts of soymilk and soymilk fermented with *Streptococcus salivarius* subsp. *thermophilus* CCRC 14805 alone or in conjunction with *Bificobacterium infantis* CCRC 14603 was evaluated. It was found that extracts of soymilk and the prepared fermented soymilk dose-dependently suppress nitric oxide (NO) production of lipopolysaccharide (LPS)-induced macrophages, with soymilk showing the highest inhibition ability followed by *S. thermophilus*-fermented soymilk. Extracts of soymilk and fermented soymilk did not affect the activity of inducible nitric oxide synthase (iNOS). The extracts of the fermented soymilk inhibited the protein expression of iNOS in LPS-induced macrophages, but soymilk did not. Moreover, these extracts did not show obvious inhibition on cyclooxygenase-2 (COX-2) protein expression, while all showed the inhibitory effect on LPS-induced pro-inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β and prostaglandin E_2 (PGE_2) produced by RAW 264.7 macrophages.

Key words: COX-2**,** fermented soymilk, iNOS, pro-inflammatory cytokines, lipopolysaccharide, RAW 264.7 macrophages

INTRODUCTION

Soybean is generally considered to be a food material with high nutritive quality. However, its undesirable bean odor and the presence of stachyose and raffinose, which contribute to flatulence, limit the broad application of soybean as a foodstuff[(1)]. To overcome these drawbacks and to develop a probiotic diet adjunct, a series of studies on the fermentation of soymilk, the water extract of soybean, with the probiotic culture of lactic acid bacteria and bifidobacteria have been conducted in our laboratory[(2-7)]. It was found that both the soymilk and the fermented soymilk exerted antioxidant activity[(7)] and antimutagenic effect against 4-nitroquinoline-N-oxide (4-NQO) and 3, 2'-dimethyl-4-amino-biphenyl (DMAB) [(5)]. In addition, fermented soymilk shows greater antioxidant and antimutagenic activities than those of the non-fermented soymilk. Moreover, fermentation was found to change the isoflavone distribution profile of soymilk[(4)]. The fermented soymilk contained a significantly higher content of the bioactive isoflavone aglycone than its unfermented counterpart. These findings suggest that soymilk, and especially fermented soymilk, may be considered as a potentially useful ingredient for the development of healthy foods.

In human beings, macrophages play an important role in the defense against infection and tumor formation. The production of nitric oxide (NO) and hydrogen peroxide (H_2O_2) by macrophages mediates the killing or growth inhibition of infected bacteria, fungi, parasites and tumor cells[(8)]. The development of several diseases such as cardiovascular disease, cancer, diabetes and renal disease have been linked with the excessive production of NO[(9,10)]. On the other hand, prostaglandin E_2 (PGE_2), a pleiotropic mediator can be produced at inflammatory sites by inducible cycloxygenase 2 (COX-2) which gives rise to pain, stiffness and swelling[(11)]. Elevating the level of COX-2 and PGE_2, encouraging malignant growth, is also associated with the occurrence of colon carcinoma[(12)]. Additionally, it has been reported that

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lipopolysaccharides (LPS) of the outer membrane of gram-negative bacteria may induce the response of local cells to secrete a high level of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and IL-6. These cytokines initiate and augment subsequent inflammatory sequences, leading to tissue destruction[13].

In the present study, macrophage cell line, RAW 264.7 cells were stimulated by LPS. The methanol extracts of soymilk and fermented soymilk prepared with lactic acid bacteria (*Streptococcus salivarius* subsp. *thermophilus* CCRC 14085) alone or in concert with bifidobacteria (*Bifidobacterium infantis* CCRC 14603) were evaluated for their effects on LPS-induced nitric oxide, IL-6, IL-1β, and PGE_2 production by RAW 264.4 macrophages. In addition, the protein expressions of inducible nitric oxide synthase (iNOS) and COX-2 by macrophages as influenced by these methanol extracts were also investigated.

MATERIALS AND METHODS

I. *The Preparation of Soymilk and Fermented Soymilk*

Soymilk and fermented soymilk were prepared according to the procedures described by Wang *et al.*[3]. Essentially, whole soybeans were washed and soaked overnight in distilled water. After decanting the water, the soaked soybeans were mixed with distilled water 10 times their weight and then mixed in a blender for 3 min. The resultant slurry was then filtered through a double-layered cheesecloth to yield soymilk. Soymilk, after autoclaving, was then fermented with *S. thermophilus* BCRC 14085 or co-culture of *S. thermophilus* BCRC 14085 and *B. infantis* BCRC 14603 at 37°C for 32 h to prepare the fermented soymilk.

To investigate the effect on LPS-induced inflammation, soymilk and fermented soymilk were first freeze-dried and homogenized. Dry powder was extracted with 80% methanol by shaking (120 rpm) at 25°C for 2 h and filtered through Whatman No. 42 filter paper. The filtrate was taken to dryness under vacuum. The dried material was redissolved in dimethylsulfoxide (DMSO).

II. *Cell Culture*

The mouse macrophage cell line RAW 264.7 was obtained from Bioresource Collection and Research Center, Hsinchu, Taiwan. Cells were cultivated in Dulbecco's modified Eagle medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (Sigma Chemical Co) and maintained at 37°C in a humidified incubator that contained 5% CO_2. After washing twice with phosphate buffer saline solution (PBS, pH 7.4), cells were scrapped with a cell scrapper and suspended in PBS. Cell number was assessed by trypan blue dye exclusion on a hemocytometer.

III. *Cell Viability Assay-MTT*

Assay of cell viability was followed that described by Yen and Lai [14]. Cells were plated in 96-well dishes at 4,000 cells/well and allowed to grow for 24 h. Treatments with vehicle (DMSO), test samples of soymilk or fermented soymilk extract and/or LPS were carried out under a serum-free condition. After the removal of the supernatants from the plates, cell viability was determined with MTT assay kit purchased from R & D Systems (Techne Co., Minneapolis, MN, USA). In this assay, cell respiration as an indicator of cell viability was determined on the basis of mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide to formazan.

IV. *Measurement of NO Production*

The method described by Yen and Lai[14] with minor modification was followed to measure the NO production. The cells were first seeded in the 96-well culture dish at a density of *ca.* 8×10^4 cells/well. After 24 h of incubation, the adherent cells were washed 3 times with PBS. The cells were then incubated with the extract of soymilk or fermented soymilk (0-500 μg/mL) in the absence or presence of LPS (1 μg/mL). The medium was collected after 18 h of incubation. Nitrite concentration in the medium was determined as an indicator of NO by Griess reaction[15]. Briefly, 100 μL of culture supernatant or various amounts of sodium nitrite standard was mixed with an equal volume of Griess Reagent kit (Promega Co., Madison, WI, USA) in microtiter plate wells. After 5 min at room temperature, the optical density at 570 nm (OD_{570}) was measured by an ELISA microplate reader (MRX-reader; Dynex Technologies, Chantilly, VA, USA).

V. *Evaluation of iNOS Enzyme Activity*

Essentially, the procedure described by Chen *et al.*[16] was followed to evaluate the iNOS enzyme activity in the LPS-stimulated macrophage cells. Briefly, RAW 264.7 cells were cultured in a 10-cm culture dish at a density of *ca.* 4×10^6 cells/dish and stimulated with LPS (100 ng/mL) for 12 h. They were then harvested and plated in a 24-well culture dish and treated with various amounts of extract of soymilk or fermented soymilk for a further 12 h. The medium was then collected and assayed for nitrite.

VI. *Measurement of TNF-α, IL-1β and IL-6 Production*

In this study, RAW 264.7 cells were plated in a 96-well culture dish and treated with the extract of soymilk or fermented soymilk (0-500 μg/mL) in the presence of LPS (1μg/mL) for 24 h.

The cytokine ELISA kits (Mouse TNF-α kit, DouSet, DY410; Mouse IL-1β kit, DouSet, DY401; Mouse

IL-6 kit , DouSet, DY406) obtained from R & D Systems (Minneapolis, MN, USA) were used to determine the amount of TNF-α, IL-1β and IL-6 released to the culture media after LPS-stimulation. Briefly, the standard or sample was added to an ELISA well plate pre-coated with the specific monoclonal capture antibody. After 2 h of incubation at room temperature, polycolonal anti-TNF-α, anti-IL-1β or IL-6 antibody conjugated with streptavidin-horseradish peroxidase was added and incubated for 2 h at room temperature. Then, a substrate solution containing hydrogenperoxide (H_2O_2) and tetramethylbenzidine was added and allowed to react for 20 min. The level of cytokine expression was measured by an ELISA reader at 450 nm.

VII. *Measurement of PGE_2 Production*

Macrophage cells were placed in a 96-well dish and incubated with extract of soymilk or fermented soymilk (0-500 μg/mL) in the presence or absence of LPS (1 μg/mL) for 18 h. One hundred microliters of culture medium supernatant was collected for the detection of PGE_2 concentration by a PGE_2 ELISA kit (Cayman Chemical, Ann Arbor, MI, USA).

VIII. *iNOS and COX-2 Protein Expression Analysis*

RAW 264.7 cells (4×10^6 cells/ 10 cm dish) were co-incubated with or without the extract of soymilk or fermented soymilk in the presence or absence of LPS (1 μg/mL) for 18 h. Plates were then scraped, cells were washed, harvested and homogenized by sonication. The crude cell lysates were cleared by centrifugation at 15,000 ×g for 30 min at 4°C. The total protein concentrations of lysates were measured by protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of protein of each cell lysate were subjected to 8% SDS-polyacrylamide gel eletrophoresis and transferred to nitrocellusose membranes (Bio-Rad Laboratories, Hercules, CA, USA) in a transfer chamber (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were blocked with 5% dried low-fat milk in Tris-buffered saline-Tween (0.05%) at room temperature for 1 h, the blot was incubated with antibodies against iNOS , COX-2 or β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in PBS-0.05% Tween (PBST) for 1 h followed by three washes with TBST (25 mM tris-HCl, 150 mM NaCl, 0.3% Tween, pH 7.4) 15 min each. The blots were then probed with goat anti-rabbit secondary antibodies conjugated with horseradish peroxidase and visualized using a diaminobenzidine (Amresco Inc., Solon, OH, USA) and ECL detection system (Amersham Pharmacia Biotech., Buckinghamshire, UK), respectively for COX-2 and iNOS.

IX. *Statistical Analysis*

Mean and standard deviation were calculated from the data obtained from the triplicate experiments. Means were analyzed and compared using Duncan's multiple range test method in SAS, version 8 (SAS Institute, Gary, NC, USA).

RESULTS

I. *The Effect of Soymilk and Fermented Soymilk Extracts on the Viability of RAW264.7 Cells*

In the preliminary study, the viability of RAW 264.7 cells after 24 h of treatment with various amounts of the extracts of soymilk or fermented soymilk (0-500 μg/mL) in the presence or absence of LPS (1 μg/mL) was measured with MTT assay. It was found that, in the absence of LPS, the extract of soymilk or fermented soymilk at all the dosage examined did not exert cytotoxicity on RAW264.7 cells (data not shown). The viability of all the LPS-activated cells treated with or without soymilk extract or fermented soymilk extract at different concentrations showed no significant difference ($P > 0.05$) (data not shown). Therefore, the anti-inflammatory test was investigated at dosage ranging from 1 to 500 μg/mL.

II. *The Effect of Soymilk and Fermented Soymilk Extracts on NO Production by LPS-activated Macrophages*

NO synthesized by iNOS was considered as a mediator of inflammation. In the present study, RAW264.7 macrophages were stimulated with LPS (1 μg/mL) for 18 h. NO in the supernatant was measured as an accumulation of its stable metabolite, nitrite, with the Griess reaction[17].

After 18 h of stimulation, LPS-only-treated cells produced *ca*. 22.54 μM nitrite compared to 0.35 μM nitrite produced by the unstimulated cells. As shown in Figure 1, the LPS-activated nitrite production was reduced significantly ($P < 0.05$) by incubation with the extract of soymilk or fermented soymilk at all the concentrations examined. Among the various extracts tested, soymilk extract exhibited the strongest suppression effect. Relative to the NO production of the LPS-only-treated cells (control) which was designed as 100%, these cells exhibited a NO production of 17% and 32-40%, respectively, of the control in presence of soymilk extract and fermented soymilk at a concentration of 500 μg /mL. This implied that at a concentration of 500 μg/mL, the soymilk extract reduced NO production by 83% compared to 60-68% noted with the fermented soymilk extract.

III. *The Effect of Soymilk and Fermented Soymilk Extracts on iNOS Enzyme Activity*

To examine whether or not the extract of soymilk or fermented soymilk inhibited the activity of iNOS thus leading to the reduced production of NO observed, NO

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production of the LPS activated-RAW264.7 cells in presence of various amount of test samples was determined. As shown in Table 1, the amount of nitrite detected in LPS-activated cells in the presence or absence of extract, regardless of concentration, showed no significant difference ($P > 0.05$) implying that the synthesis of NO by iNOS was not altered. This observation suggested that the reduced NO production in RAW 264.7 macrophages (Figure 1) is not due to the direct inhibition of iNOS activity by the extract of soymilk or fermented soymilk.

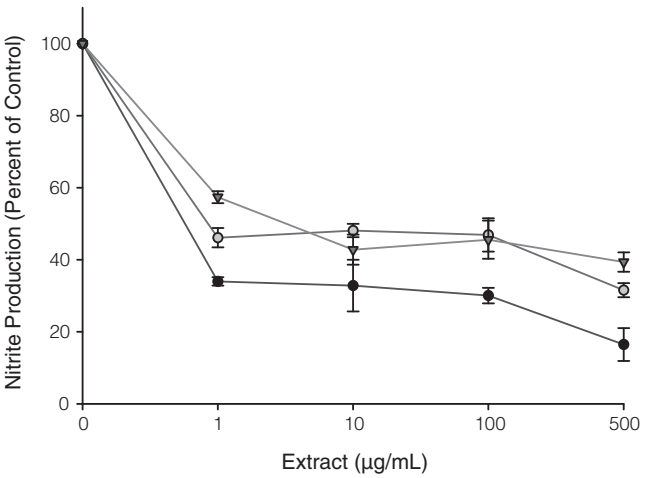

Figure 1. Effect of soymilk or fermented soymilk extract on LPS-induced NO production in RAW 264.7 cells. ●: soymilk; ○: *S. thermophilus*-fermented soymilk; ▼: *S. thermophilus* + *B. infantis*-fermented soymilk. RAW 264.7 cells were treated with soymilk or fermented soymilk extract (0 to 500 μg/mL) in the presence of l μg/mL LPS for 18 h. NO production was then determined by measuring the formation of nitrite. The nitrite level of the control and LPS-only-treated group was 0.35 ± 0.24 μM and 22.54 ± 0.66 μM, respectively. Data are presented as means ± SD (n = 3). The nitrite production of LPS-only-treated group is regarded as 100% and the test groups are expressed as the relative values to the LPS-only-treated group. All points are significantly different from LPS-only treated group ($P < 0.05$).

Table 1. Effect of soymilk and fermented soymilk extracts on iNOS enzyme activity in RAW 264.7 cells

LPS induction of cells[a]	Treatment with extract (μg/mL)	Nitrite (μM)[b]
None	DMSO (control)	0.78 ± 0.10
LPS (100 ng/mL)	DMSO (control)	5.97 ± 0.49*
	Soymilk	
	1	6.63 ± 0.08*
	10	6.15 ± 0.26*
	100	6.47 ± 0.50*
	500	6.26 ± 0.46*
	Fermented soymilk with *S. thermophlilus*	
	1	6.45 ± 0.50*
	10	6.03 ± 0.34*
	100	6.55 ± 0.30*
	500	6.61 ± 0.55*
	Fermented soymilk with *S. thermophilus and B. infantis*	
	1	6.38 ± 1.23*
	10	5.67 ± 0.65*
	100	6.79 ± 1.19*
	500	6.06 ± 0.23*

[a]RAW 264.7 cells were activated with LPS (1 μg/mL) for 12 h and the cells were washed with fresh medium. The cells were harvested and plated into a 24-well plate and incubated with soymilk or fermented soymilk for further 12 h.

[b]The amount of nitrite in the supernatant was assayed by the Griess reaction as described in the section of Materials and Methods. The values are expressed as mean ± SD (n = 3).

*No significant difference from that with LPS alone ($P > 0.05$).

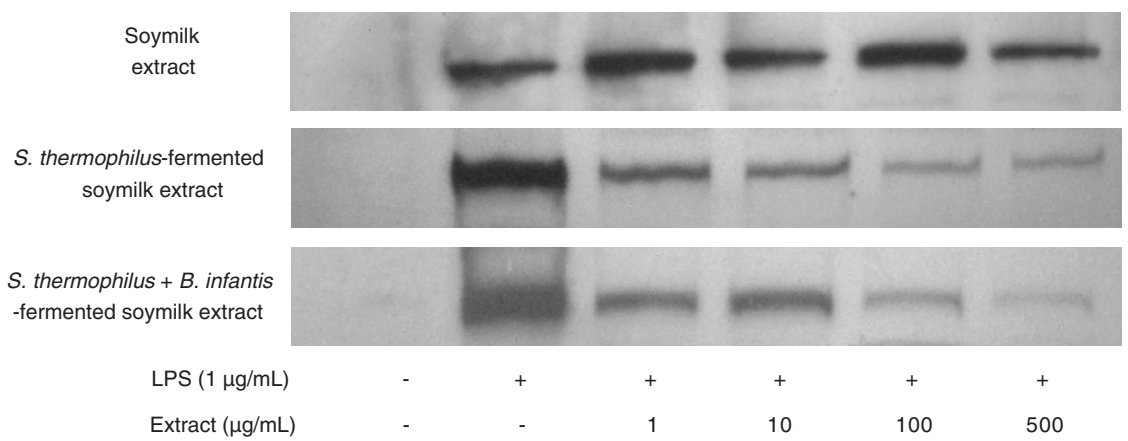

Figure 2. Effect of soymilk, *S. thermophilus*-fermented soymilk and *S. thermophilus* + *B. infantis*-fermented soymilk extracts on iNOS protein expression in LPS-induced RAW 264.7 cells. The RAW 264.7 macrophages were incubated with extract of soymilk or fermented soymilk (0 to 500 μg/mL) in the presence or absence of LPS (l μg/mL) for 24 h. Proteins (50 μg) from each sample were resolved on 8% SDS-PAGE, and western blotting analysis was performed.

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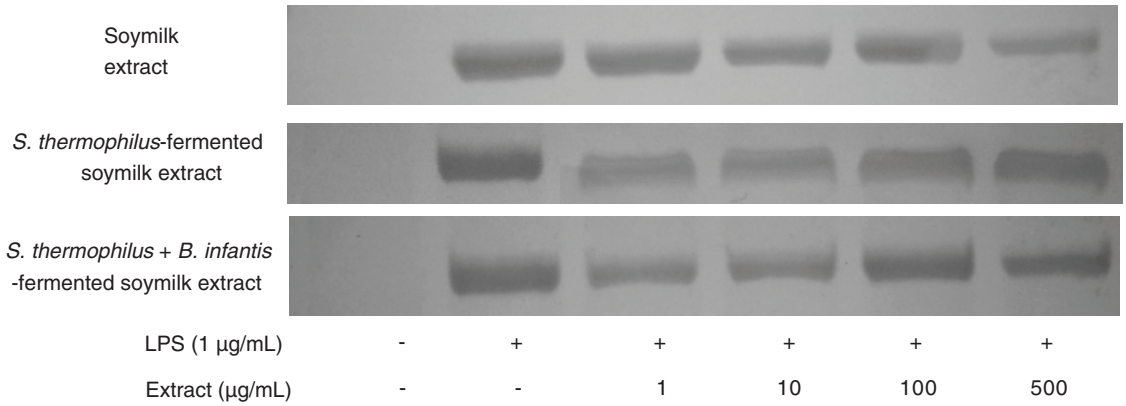

Figure 3. Effect of soymilk, *S. thermophilus*-fermented soymilk and *S. thermophilus* + *B. infantis*-fermented soymilk extracts on COX-2 protein expression in LPS-induced RAW 264.7 cells. The RAW 264.7 macrophages were incubated with extracts of soymilk or fermented soymilk (0 to 500 μg/mL) in the presence or absence of LPS (l μg/mL) for 24 h. Proteins (50 μg) from each sample were resolved on 8% SDS-PAGE, and western blotting was performed.

IV. *The Effect of Soymilk and Fermented Soymilk Extracts on the LPS-induced Expressions of iNOS and COX-2 Proteins*

In addition to inhibit iNOS activity, the suppression of protein expression of iNOS may be an alternative explanation of the reduced NO production. To examine this possibility, the iNOS expression of LPS activated-RAW 264.7 cells in the absence or presence of the extract of soymilk or fermented soymilk was measured by means of Western blot analysis. Figure 2 shows the protein levels in treated and untreated macrophage cells. Compared to the unactivated control and LPS control, an effect of dose-dependent reduction of fermented soymilk extract on the expression inhibition of iNOS protein was noted. On the other hand, no distinct difference in iNOS protein expression was observed in presence of soymilk extract.

COX-2 is an inducible enzyme which may be induced in macrophages in response to many stimuli and was activated at the site of the inflammation[11-18]. As shown in Figure 3, the induction of COX-2 protein was evident in the macrophage cells stimulated with LPS (1 μg/mL) for 24 h. However, no visible alternation in the COX-2 protein expression was noted in the LPS activated-cells co-treated with the extract of soymilk or fermented soymilk.

V. *The Effect of Soymilk and Fermented Soymilk Extracts on LPS-induced TNF-α, IL-6, IL-1β and PGE_2 Production*

In the present study, the potential effects of soymilk and fermented soymilk extracts on the production of proinflammatory cytokines such as TNF-α, IL-6, IL-1β and PGE_2 were determined. The macrophage cells were incubated with the extract of soymilk or fermented soymilk (0, 1, 10, 100, 500 μg/mL) in the presence or absence of LPS (1 μg/mL) for 24 h, and the cytokine levels were determined in the culture media by ELISA.

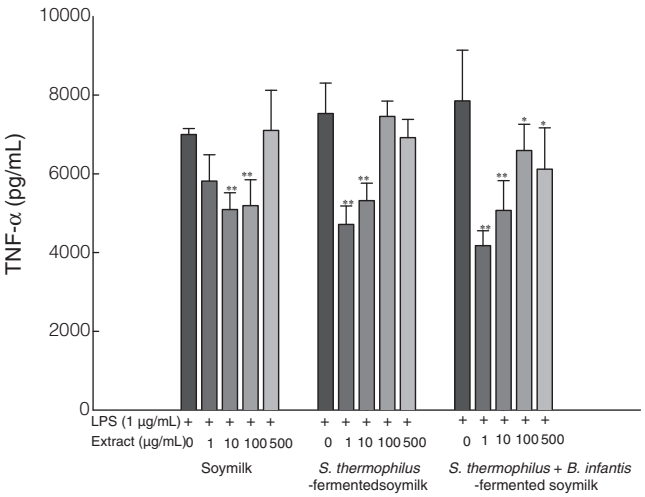

Figure 4. Effect of soymilk and fermented soymilk extracts on LPS-induced TNF−α production in RAW 264.7 cells. RAW 264.7 cells were treated with soymilk or fermented soymilk extract (0 to 500 μg/mL) in the presence of l μg/mL LPS for 24 h. The values are presented as means ± SD (n = 3). * $P < 0.05$, ** $P < 0.01$ indicate significant differences from that with LPS alone.

As shown in Figures 4, 5, 6 and 7, respectively, the level of TNF-α, IL-6, IL-1β and PGE_2 in the LPS-induced cells was significantly decreased by treatment with certain levels of the extracts of soymilk, regardless of fermentation. Furthermore, the fermented soymilk extract showed a dose-dependent reduction effect on IL-1β production (Figure 6) while a similar dose-dependent reduction effect on the level of IL-6 was also noted with the soymilk extract (Figure 5).

Additionally, the extracts of soymilk and fermented soymilk, the levels of 0-500 μg/mL, were also noted to exhibit a reduction effect on PGE_2, the most important inflammatory product of COX-2 activity (Figure 7).

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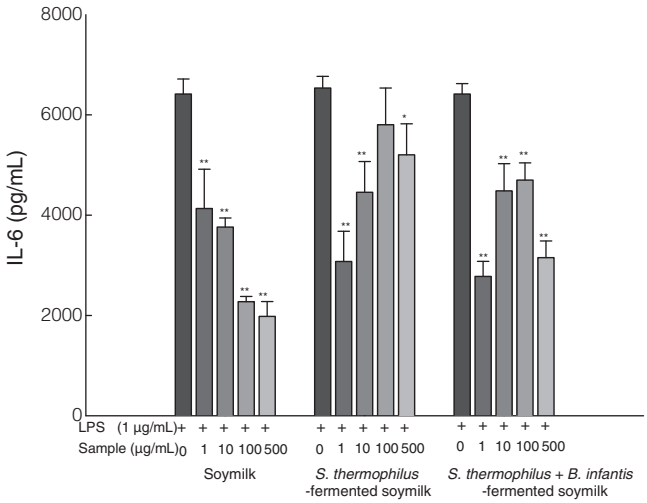

Figure 5. Effect of soymilk and fermented soymilk extracts on LPS-induced IL-6 production in RAW 264.7 macrophages. RAW 264.7 cells were incubated with soymilk or fermented soymilk extract (0 to 500 μg/mL) in the presence of l μg/mL LPS for 24 h. The values are presented as means ± SD (n = 3). * $P < 0.05$, ** $P < 0.01$ indicate significant differences from that with LPS alone.

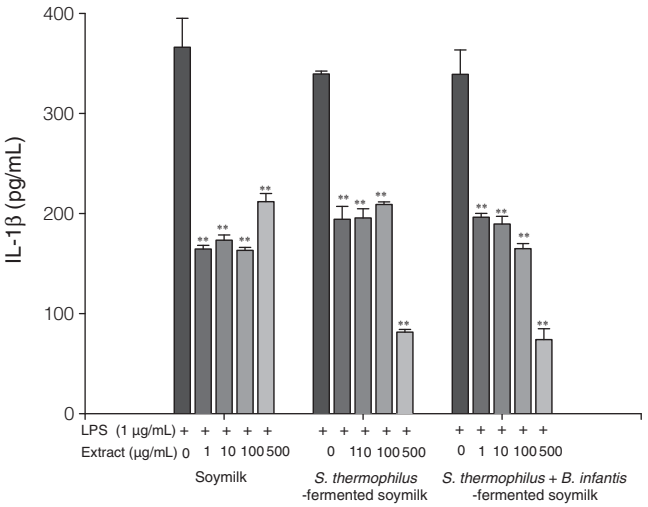

Figure 6. Effect of soymilk and fermented soymilk extracts on LPS-induced IL-1β production in RAW 264.7 macrophages. RAW 264.7 cells were incubated with soymilk or fermented soymilk extract (0 to 500 μg/mL) in the presence of l μg/mL LPS for 24 h. The values are presented as means ± SD (n = 3). * $P < 0.05$, ** $P < 0.01$ indicate significant differences from that with LPS alone.

DISCUSSION

This study was performed to elucidate the effect of methanol extracts of soymilk and fermented soymilk on the production of inflammatory mediators in macrophages.

NO is synthesized from L-arginine by NOS in various animal cells and tissues. It is a defense molecule with cytostatic, cytotoxic, microsidal and microstatic activities in the immune system[19]. Macrophages can greatly increase their production under pathological conditions while the formation of peroxynitrite ($ONOO^-$) from the interaction of NO with superoxide may lead to chronic pathological condition[20] and cause DNA damage[21,22]. It was found that the extracts of soymilk and fermented soymilk could reduce the nitrite production significantly (Figure 1). Sheu *et al.*[23] indicated that food and phytochemicals can exert NO-suppressing effect via three routes: (1) direct scavenging of NO; (2) the inhibition of iNOS catalytic activity; (3) and the suppression of iNOS expression. We observed that all the extracts examined did not alter the iNOS activity (Table 1) in the LPS-treated cells while the fermented soymilk extracts dose-dependently reduced the expression of iNOS protein as revealed by Western blotting (Figure 2). On the other hand, no distinct difference in iNOS protein expression was observed in LPS-induced cells treated with or without soymilk extract (Figure 2). Therefore, it is suggested that direct scavenging of NO and the suppression of the iNOS enzyme expression led to the reduced production of NO in the LPS-induced cells treated with the extract of fermented soymilk while inhibition of NO production may be attributed to its direct scavenging of NO by soymilk extract (Table 1).

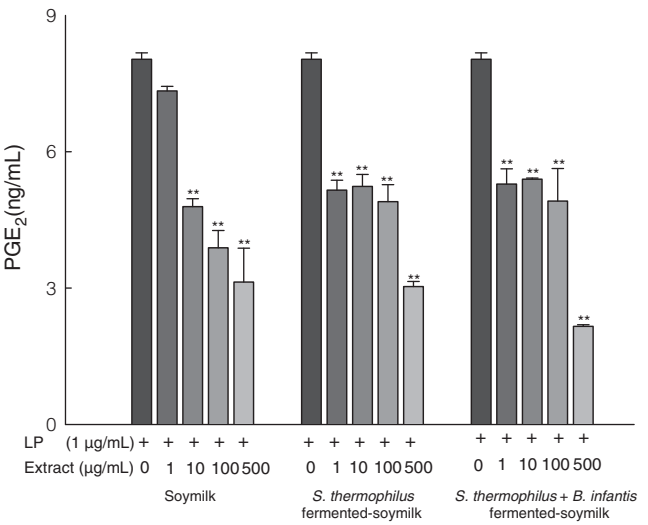

Figure 7. Effect of soymilk and fermented soymilk extracts on LPS-induced PGE_2 production in RAW 264.7 macrophages. RAW 264.7 cells were incubated with soymilk or fermented soymilk extracts (0 to 500 μg/mL) in the presence of l μg/mL LPS for 24 h. The values are presented as means ± SD (n = 3). ** $P < 0.01$ indicates significant difference from that with LPS alone.

With the catalyzing activity of COX-2, arachidonic acid was converted to prostaglandin H_2, the precursor of a variety of biologically active inflammatory mediators such as thromboxane A2, PGE_2 and prostacyclin[24]. Prostaglandins, especially PGE_2, influence cell proliferation, tumor growth and suppress the immune response to malignant cells[12]. It has also been reported that many diseases such as rheumatoid arthritis and pulmonary fibrosis are caused by an over-production of

inflammatory mediators[25,26]. Therefore, these inflammatory diseases may be suppressed or prevented if the over-production of these inflammatory mediators was suppressed.

In the present study, we noted that the extract of soymilk or fermented soymilk did not exert a distinct effect on the COX-2 protein expression (Figure 3), although it reduced the PGE_2 production in the LPS-induced cells (Figure 7). Whether or not this is due to the inhibition of COX-2 enzyme activity by soymilk extract in RAW 214.7 cells remained to be explored further.

TNF-α, IL-6 and IL-1 are cytokines, which play important roles in inflammatory lesions[27]. Laskin and Pendino[28] indicated that inflammatory stimuli, such as LPS, induced cytokines in the process of macrophage activation, which mediate tissue response in different phases of inflammation in a sequential and concerted manner. The inhibition of cytokine production or function serves as a key mechanism in the control of inflammation[29]. Despite the variations in the dose-response effect observed, it has generally been found that the methanol extracts of soymilk and fermented soymilk reduced the production of TNF-α, IL-6, IL-1 and PGE_2 in the LPS-induced cells (Table 1, Figures 4-7).

Various investigators have suggested that the activation of nuclear factor-kappa B ($NF\text{-}_kB$) induces gene transcription of multiple proinflammatory mediators, including COX-2, TNF-α, IL-6 and others, while the degradation of I_kB is a critical process for $NF\text{-}_kB$ activation[30-33]. Among these investigators, Hseu *et al*.[30] reported that the fermented culture broth of *Antrodia camphorate* inhibited LPS induction of cytokines, iNOS and COX-2 expression by blocking $NF\text{-}_kB$ activation. Murakami *et al*.[32] observed that genistein attenuated I_kB degradation thus suppressing iNOS and COX-2 gene expression. Examining the expression of I_kB in the future will be helpful to further elucidate the pathway followed by the extracts of soymilk and fermented soymilk to suppress the expression of the proinflammatory mediators observed in the present study.

In the present study, extracts of soymilk and fermented soymilk, which contain different constituents, were examined. These constituents might demonstrate positive or negative immune-modulatory effects on cytokine secretion and retard the dose- related effect on the production of cytokines. This may thus led to the failure of the extracts to show a dose-dependent reduction effect on the level of some of the cytokines examined (Figures 4-7). Similar phenomenon was also reported by Lin and Tang[35] who investigated the effects of fruits and vegetable juices on the production of IL-2, IL-4 and IL-5.

The components of soybean and soymilk such as isoflavones, soy protein and saponin have been previously reported to elicit immune response[34]. However, fermentation can change components of these compounds[3,4]. For example, an increased aglycone with a decrease of glucoside isoflavones content was noted in soymilk after fermentation with lactic acid bacteria and/or bifidobacterium[4]. Additionally, the fermented soymilk examined in the present study contained components of lactic acid bacteria or bifidobacterium cells and peptides formed during the fermentation, which have been reported to affect the production of cytokines[36-40]. Therefore, the variation in the immune response observed with the extracts of soymilk and fermented soymilk may be attributed to their difference in composition and merit further investigation.

CONCLUSIONS

Previously, soymilk and fermented soymilk have been found to possess antioxidant and antimutagenic activity[5,7]. The suppression of the production of inflammatory mediators such as NO, PGE_2, TNF-α, IL-6 and Il-1β in macrophages, was observed in the present study. These results further demonstrated soymilk and fermented soymilk functional properties as a dietary adjunct. It is suggested that the regular intake of soymilk milk or fermented soymilk may provide protection against cardiovascular diseases and chronic inflammatory diseases.

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