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Scavenging effects of lotus seed extracts on reactive nitrogen species

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Abstract

The inhibitory effect of lotus (*Nelumbo nicifera*) seed extracts extracted with water (LSWE), ethyl acetate (LSEAE) and hexane (LSHE) on reactive nitrogen species, induced by DNA damage in macrophage RAW 264.7 cells, was investigated. The results showed that all extracts could inhibit nitric oxide accumulation in LPS-activated RAW 264.7 cells. The extracts in the range of 0.01–0.2 mg/ml showed a dose-dependent inhibitory effect on the accumulation of nitric oxide upon decomposition of sodium nitroprusside (SNP). The potency of inhibitory activity was in the order: LSEAE > LSWE > LSHE. The results of the comet assay indicated that the three extracts could inhibit DNA damage in macrophage RAW 264.7 cells induced by SNP. In addition, the three samples, at 0.2 mg/ml, showed 63%, 59%, and 38% inhibition of DNA damage in macrophage RAW 264.7 cells induced by peroxynitrite, respectively. All extracts tested were found to be potent peroxynitrite scavengers, capable of preventing the nitration of tyrosine. The data obtained suggest that lotus seed extracts might act as chemopreventers through reduction of excess amounts of nitric oxide.

Keywords: Lotus seed; Nitric oxide; Peroxynitrite; Single cell gel electrophoresis; Macrophage RAW 264.7

1. Introduction

Nitric oxide (NO), a potentially toxic gas with free radical properties (Tamir & Tannenbaum, 1996), is induced by stimulation of bacterial lipopolysaccharide (LPS) or interferon-γ (IFN-γ) to form stoichiometric amounts of L-citrulline from L-arginine in some cell lines, such as macrophages (Nathan & Xie, 1994). It has been recently discovered to act as a messenger molecule mediating various physiological functions (Moncada, Palmer, & Higgs, 1989). NO accounts for the activity of endothelium-derived relaxing factor, which is released from the endothelium and stimulates vasodilation by activating soluble guanylate cyclase in adjacent smooth muscle of blood vessels (Palmer, Ferrige, & Moncada, 1987).

NO has been reported to cause mutagenesis and deamination of DNA bases and, more importantly, to form carcinogenic N-nitroso compounds (Xie et al., 1997). Moreover, an important chemical property of NO is that it reacts rapidly and spontaneously with a superoxide anion ($O_2^{-\bullet}$) to form a peroxynitrite anion ($ONOO^-$), which is more toxic than $O_2^{-\bullet}$ or NO to biological systems by causing modification of proteins (e.g. 3-nitrotyrosine) (Van der Vliet, Eiserich, O'Neil, Halliwell, & Cross, 1995) or nucleic acids (e.g., 8-nitroguanine) (Yermilov et al., 1995). Therefore, studies on terminating the reaction between NO and O_2^- or scavenging reactive nitrogen species become important. Recent research has highlighted the potential role of the edible or herbal plants as contributors to the scavenging of reactive nitrogen.

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The Chinese herb "Lian-Zi", which is the seed of the plant *Nelumbo nicifera* (lotus), has been used for medicinal purposes as an astringent, tonic, and sedative in oriental countries. It has been indicated that lotus seed has an anti-aging effect and enhances immunity. In our previous study, lotus seed extracts exhibited a potential antioxidant activity on lipid peroxidation (Yen, Duh, & Su, 2005). However, the scavenging activity of lotus seed extracts on reactive nitrogen species is still unclear. Thus, the aim of this work is to investigate the scavenging and protective effects of lotus seed extracts (LSE) against reactive nitrogen, including sodium nitroprusside (SNP)- and peroxynitrite-induced cytotoxicity and DNA damage in macrophage RAW 264.7 cells.

2. Materials and methods

2.1. Materials

N-Lauroyl sarcosinate, ethyl bromide (EtBr), Triton X-100, L-tyrosine, lipopolysaccharide (LPS), MTT and 3-nitrotyrosine were purchased from Sigma Co. Ltd. (St Louis, MO, USA). Sodium chloride, disodium hydrogen phosphate and dimethyl sulfoxide (DMSO) were purchased from Merck Co. Ltd. (Darmstadt, Germany). Hydrogen peroxide (H_2O_2) was purchased from Wako Co. Ltd. (Japan). L-Glutamine, PSN antibiotic mixture (penicillin–streptomycinn–neomycin), Dubeccos' Modified Eagle Medium (DMEM), fetus bovine serum (FBS), trypsin–EDTA (T/E), penicillin–streptomycin, low melting point agarose (LMA) and normal melting point agarose (NMA) were purchased from Gibco Ltd. (Grand Island, NY, USA). Lotus seed was purchased from a local market in Taichung, Taiwan.

2.2. Sample extraction

The samples (10 g) were extracted with boiling deionized water (100 ml) for 15 min. The extracts were filtered through Whatman No. 2 filter paper, and the filtrates were freeze-dried to powder. The water extracts of lotus seeds were named WELS. As for organic solvent extraction, the samples (10 g) were extracted with 100 ml *n*-hexane by the Soxhlet method and filtered; the filtrate was called *n*-hexanolic extract of lotus seeds (HELS). The residue was extracted with acetone (100 ml) and ethylacetate (100 ml), respectively, followed by filtration and evaporation of the filtrate to dryness in a vacuum. The ethyl acetate extracts of lotus seeds were named LSEAE.

2.3. Synthesis of peroxynitrite

Peroxynitrite was synthesized essentially as described by Kato et al. (1997). Briefly, an ice-cold solution of 0.6 M HCl, 0.7 M H_2O_2 (10 ml) was simultaneously added to a well-stirred, cooled (4 °C) solution of 0.6 M $NaNO_2$ (10 ml), immediately followed by the addition of 1.5 M NaOH (20 ml). The synthetic solution in a 50 ml capped centrifuge tube was frozen at −20 °C. Peroxynitrite formed a yellow top layer due to freeze fractionation, which was retained for further studies. The top layer typically contained 150–200 mM peroxynitrite, as determined by UV-absorbance spectroscopy at 302 nm in 1.2 M NaOH ($\varepsilon_{302\ nm} = 1670\ M^{-1}\ cm^{-1}$).

2.4. Cell culture

The murine macrophage cell line RAW 264.7 was obtained from the Culture Collection and Research Center (CCRC, Hsinchu, Taiwan). RAW 264.7 cells were cultured in 25 or 75 cm^2 plastic flasks with Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% heat inactivated fetal calf serum and antibiotics (Gibco/BRL Life Technologies, Eggenstein, Germany). These cells were activated with 1 μg/ml of LPS (*Escherichia coli*, Serotype 0.55:B5) and cultured for 20 h at 37 °C in an atmosphere of 5% CO_2.

2.5. Nitrite assay

The nitrite assay was measured according to the method of Dirsch, Stuppner, and Volmar (1998). The cells were seeded in 96 well plates (8×10^4 cells/200 μl), cultured for two days and then incubated with or without LPS in the absence or presence of LSE in various concentrations for 20 h. As a parameter of NO synthesis nitrite concentration was assessed in the supernatant of macrophages RAW 264.7 by the Griess reaction. Briefly, 100 μl of cell culture supernatant were removed and combined with (i) 90 μl 1% sulfanilamide in 5% H_3PO_4 and (ii) 90 μl 0.1% *N*-(1-naphthyl) ethylenediamine dihydrochloride in H_2O in a 96 well plate, followed by spectrophotometric measurement at 570 nm, using a SPECTRA microplate reader (SLT-Labinstruments).

2.6. Cell viability

Cell respiration, an indicator of cell viability, was determined by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan. After removing the supernatants from the plate for nitrite determination the cells were incubated at 37 °C with MTT (0.5 mg/ml) for 45 min. The medium was aspirated and the cells were solubilized in DMSO (250 μl) for at least 2 h in the dark. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm.

2.7. Nitrite oxide scavenging

The scavenging effect of extracts on nitric oxide was measured according to the method of Marcocci, Maguire, Droy-Lefaix, and Packer (1994). Four ml of lotus seed extract solutions of different concentrations, were then added, in the test tubes, to 1 ml of sodium nitroprusside solution (25 mM) and the tubes incubated at 37 °C for 3 h. An aliquot (0.5 ml) of the incubation solution was removed and diluted with 0.3 ml Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 570 nm and referred to the absorbance of standard solutions of sodium nitrite treated in the same way with Griess reagent.

2.8. Analysis of DNA damage (Comet assay)

Cells were incubated with different concentrations of extracts (the final concentration was 0.01–0.2 mg/ml) for 2 h at 37 °C in a dark incubator together with untreated control samples. Samples were then centrifuged at 800–900 rpm, the RAW 264.7 cells were resuspended in low melting point agarose for comet analysis.

Sodium nitroprusside (SNP) or peroxynitrite-induced DNA damage was estimated using single cell gel electrophoresis (SCGE or comet assay) (Singh, McCoy, Tice, & Schneider, 1988). Briefly, fully frosted slides were covered with 0.5% NMA as the first layer, a mixture of cell suspension and 0.5% of LMA as the second layer, and finally with 0.5% of LMA (without cell) as the third layer. After solidification at 4 °C, all slides were immersed in the lysing buffer (2.5 M of NaCl, 100 mM of EDTA, pH 10, with freshly added 1% Triton X-100 and 10% DMSO) at 4 °C for 1 h, and the slides were then placed in a horizontal electrophoresis tank. The tank was filled with freshly prepared electrophoresis solution (300 mM NaOH, 1 mM EDTA, pH 10, 4 °C), and the slides were left in the solution for 20 min to allow DNA unwinding and expression of alkali labile damage before electrophoresis. Electrophoresis was then conducted at 4 °C for 20 min, using 25 V and 300 mA. After electrophoresis, the slides were neutralized in neutralization buffer, stained with ethyl bromide, and kept in a humidified air-tight container and examined using a fluorescence microscope. Images of 100 randomly selected cells from each slide were analyzed. The degree of DNA damage was expressed by tail moment value. Tail Moment = (Tail length × Tail % DNA/100).

2.9. Measurement of tyrosine nitration

The tyrosine nitration was measured according to the method of Deiana et al. (1999). A stock concentration (10 mM) of L-tyrosine was prepared in 10 ml by adding 8 ml of water to 250 μl of 10% (w/v) KOH, followed by 250 μl of a 5% phosphoric acid solution with 1.5 ml of water. Tyrosine solution (0.1 ml), together with 10 μl of the extracts (0.01–0.2 mg/ml), was added to a plastic test tube containing 0.88 ml of buffer (500 mM K_2HPO_4/KH_2PO_4 pH 7.4) and incubated in a water bath at 37 °C for 15 min. Then, peroxynitrite (10 μl) was added to achieve a final concentration of 1 mM, and the tubes vortexed for 15 s and incubated for a further 15 min. The pH was measured after the addition of peroxynitrite and found to be 7.4–7.5. Measurement of 3-nitrotyrosine was performed using a LiChrospher RP-18 (150 × 4 mm, 5 μm) column. The eluant was 500 mM K_2HPO_4/H_3PO_4, pH 3.01, with 20% methanol (v/v) at a flow rate of 1 ml/min and UV detector set at 274 nm. Peak heights of 3-nitrotyrosine were measured and their concentrations calculated from a standard curve.

2.10. Statistical analysis

All analyses were run in triplicate and averaged. Statistical analyses were performed according to the SAS Institute User's Guide. Analyses of variance were performed using the ANOVA procedure. Significant differences ($P < 0.05$) between the means were determined using Duncan's multiple range test.

3. Results and discussion

3.1. Cytotoxicity

Table 1 shows the cytotoxicity of lotus seed extracts on RAW 264.7 cells in the presence of LPS measured by MTT assay. The viability of all the LPS-activated

Table 1
The cytotoxicity of lotus seed extracts on RAW 264.7 cells in the presence of LPS measured by the MTT assay

Concentration (mg/ml)	Cell viability (%)[a]		
	LSWE[b]	LSHE	LSEAE
0	100	100	100
0.01	97 ± 0.7	100 ± 6.6	96 ± 9.7
0.05	90 ± 5.5	98 ± 8.7	99 ± 7.8
0.1	92 ± 1.9	97 ± 6.0	107 ± 9.7
0.2	96 ± 1.2	108 ± 5.4	95 ± 8.1

[a] Values are percentages relative to control value (100%). Results are means ± SD for $n \geqslant 3$.

[b] LSWE: Water extracts; LSHE: *n*-Hexane extracts; LSEAE: EtOAC extracts.

cells treated, with or without extracts, at different concentrations (0–0.2 mg/ml) was ≧90%. This result indicates that the extracts extracted with the different solvents show no cytotoxicity to RAW 264.7 cells.

3.2. Lotus seed extracts suppress NO production by LPS-activated macrophages

The inhibitory activities of lotus seed extracts toward LPS-induced NO production in RAW 264.7 are shown in Fig. 1. LSWE, LSHE and LSEAE showed dose-dependent inhibition of NO accumulation in LPS-activated (1 μg/ml) RAW 264.7 cells. Among the extracts, LSWE had a stronger inhibitory effect than LSEAE and LSHE. Obviously, the LPS-activated nitrite production was significantly reduced by incubation with lotus seed extracts at various concentrations. This observation indicates that some compounds in the plants contribute to the inhibition of NO production. This finding is somewhat in agreement with the report of Kim, Murakami, Nakamura, and Ohigashi (1998) who showed that some edible Japanese plants at 200 μg/ml had a 90% inhibitory effect on NO production in LPS-activated RAW 264.7 cells.

3.3. Scavenging effect on NO

NO is a defence molecule with cytotoxic, microbiocidal, and microbiostatic activities; however, large amounts of NO, peroxynitrite and other reactive nitrogen oxide species are considered to be potentially cytotoxic and capable of injuring the surrounding cells. In the present study, the scavenging effect of lotus seed extracts on NO was investigated. Sodium nitroprusside (SNP) is known to decompose in PBS solution and then produce NO. Nitric oxide, under aerobic conditions, reacts with oxygen to form nitrate and nitrite, which can be determined using Griess reagent (Marcocci et al., 1994). As shown in Fig. 2, the extracts in the range of 0.01–0.2 mg/ml generated a dose-dependent inhibition on nitrite production. In particular, the nitrite levels of the control, LSWE, LSHE and LSEAE at a concentration of 0.2 mg/ml were 12.2, 19.2, and 8.85 M, respectively, indicating that the extracts showed scavenging activity on NO. Of the three samples tested, LSEAE had a better scavenging activity on NO than did LSWE and LSHE.

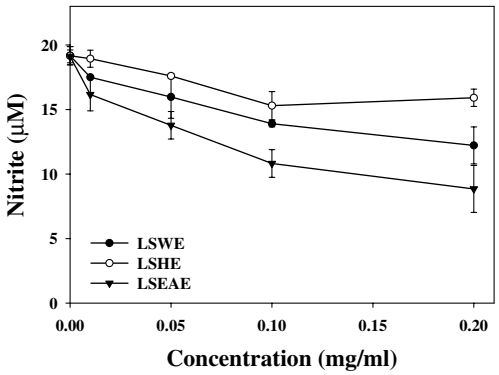

Fig. 2. Scavenging effects of lotus seed extracts on nitric oxide, generated with 5 mM SNP during incubation. Results are means ± SD for $n = 3$. (LSWE: Water extract; SHE: *n*-Hexane extract; LSEAE: EtOAC extract).

3.4. Effect of extracts on macrophage DNA damage

The effect of the three lotus seed extracts on DNA damage in RAW 264.7 cells is shown in Fig. 3. When RAW 264.7 cells were treated with extracts in the range 0.01–0.2 mg/ml and incubated at 37 °C for 2 h, the Tail moment was in the range of 3.0–5.0. No significant difference was found between the control and the sample, indicating that lotus seed extracts did not induce DNA damage in RAW 264.7 cells.

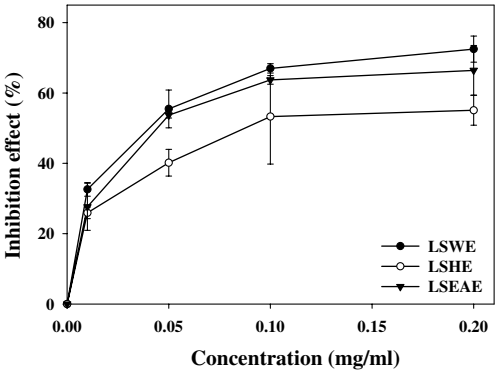

Fig. 1. Inhibitory activities of lotus seed extracts toward LPS induced NO generation in macrophage RAW 264.7 cells. (LSWE: Water extract; LSHE: *n*-Hexane extract; LSEAE: EtOAC extract).

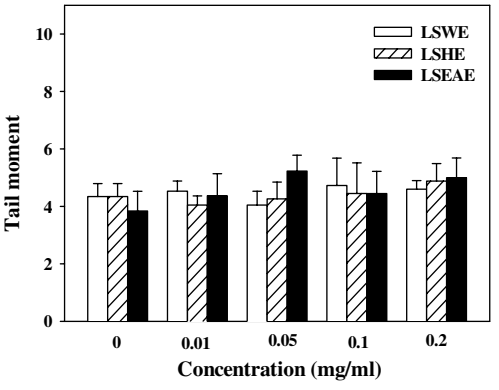

Fig. 3. Effect of lotus seed extract on DNA damage in macrophage RAW 264.7 cells. Results are means ± SD for $n \geqslant 3$. (LSWE: Water extract; LSHE: *n*-Hexane extract; LSEAE: EtOAC extract).

3.5. Effect of extracts on SNP-mediated macrophage DNA damage

The effect of the three lotus seed extracts on SNP-mediated macrophape DNA damage is shown in Fig. 4. The Tail moment value of the control was 27.3; however, it decreased with increasing concentration of the extracts. This result showed that SNP-mediated macrophage DNA damage was effectively inhibited by lotus seed extracts at all concentrations (0.01–0.2 mg/ml), and LSEAE, at 0.2 mg/ml, showed a better inhibitory effect than did LSWE and LSHE. These trends were also in agreement with the result that lotus seed extracts can directly scavenge NO generated from an in vitro acellular system, and NO scavenging ability of LSEAE was greater than that of other extracts (Fig. 2). Thus, the scavenging effects of lotus seed extracts on NO seemed to contribute to their inhibition of SNP-mediated DNA damage in macrophage.

Plant extracts and plant polyphenols have been found to modulate the production of NO in various in vitro systems (Kim, Cheon, Kim, Kim, & Kim, 1999). Their modulating mechanisms could be summarized in three ways: scavenging NO radicals, inhibiting the enzyme activity of nitric oxide synthase (NOS), and regulating the gene expression of NOS. Most plant extracts rich in polyphenols are found to suppress NO production. Chiang et al. (2004) indicated that plant extracts of *Bidens pilosa* could serve as a good source of caffeoylquinic acid derivatives, such as chlorogenic acid and exhibited significant antioxidant activity and an inhibitory effect on NO production in LPS/IFN-γ-activated macrophages. Complex mixtures of polyphenols, such as Ginkgo biloba extract (Egb761) have been reported to inhibit NO production in macrophages (Kobuchi, Droy-Lefaix, Christen, & Packer, 1997). Indeed, gallic acid and caffeic acid also cause similar effects (Ogiwara et al., 2003; Wang & Mazza, 2002). As stated earlier, extracts from lotus seed showed a potent antioxidant activity, and the main active compounds were identified as caffeic acid, chlorogenic acid, and gallic acid (Yen et al., 2005). Pekkarinen, Stockmann, Schwarz, Heinonen, and Hopia (1999) noted that antioxidant activities of phenolic acids are generally governed by their chemical structures. The activity improved as the number of hydroxyl group increased. Caffeic acid, chlorogenic acid with two OH groups and gallic acid with three OH groups were found in the lotus seed extracts, suggesting that these phenolic acids at least partly contributed to the NO scavenging activity of the lotus seed extracts.

3.6. Effect of extracts on peroxynitrite induced macrophage DNA damage

The effect of extracts on peroxynitrite-induced DNA damage in macrophage is shown in Fig. 5. The result showed that DNA damage in RAW 264.7 cells, induced by peroxynitrite, was significantly reduced by addition of extracts at the concentrations of 0.01–0.2 mg/ml. The Tail moment values of the control was 24.5. The Tail moment value of LSWE, LSHE and LSEAE, at the concentration of 0.2 mg/ml were 9.5, 16.4, and 10.1, respectively. In other words, LSWE, LSHE and LSEAE showed 63%, 38%, and 59% inhibition of DNA damage in RAW 264.7, respectively, compared with the control. Peroxynitrite is a reactive oxidant and has the ability to cause DNA damage (Aruoma, Whiteman, England, & Halliwell, 1997). In addition, peroxynitrite serves as the injurious agent in cerebral injury and myocardial ischemia (Dawson, Dawson, Bartley, Uhl, & Snyder, 1993; Matheis et al., 1992), and it may contribute to atherosclerosis through oxidation of LDL within the arterial walls (Darley-Usmar, Hogg, Oleary, Wilson, & Moncada, 1992).

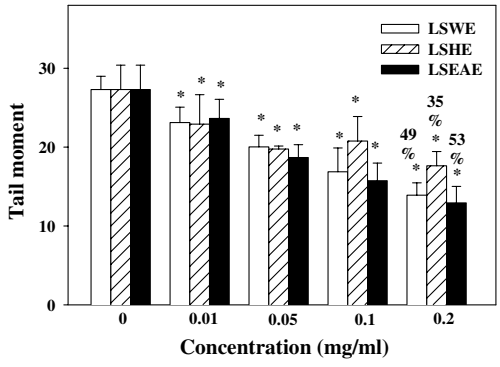

Fig. 4. Inhibitory effects of lotus seed extract on sodium nitroprusside (SNP)-induced DNA damage in macrophage RAW 264.7 cells. Inhibition of DNA damage is shown as a percentage. Results are means ± SD for $n \geqslant 3$. $^{*}p < 0.05$ when compared with the control. LSWE: Water extract; LSHE: *n*-Hexane extract; LSEAE: EtOAC extract.

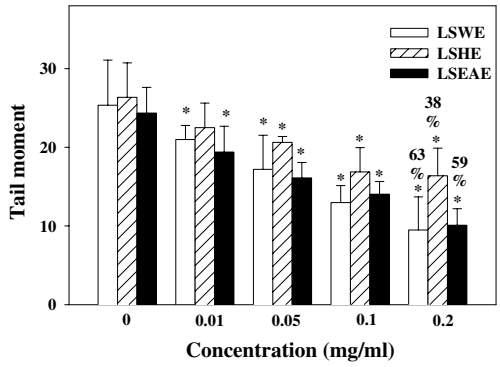

Fig. 5. Inhibitory effects of lotus seed extract on peroxynitrite-induced DNA damage in macrophage RAW 264.7 cells. Inhibition of DNA damage is shown as a percentage. Results are means ± SD for $n \geqslant 3$. $^{*}p < 0.05$ when compared with the control. LSWE: Water extract; LSHE: *n*-Hexane extract; LSEAE: EtOAC extract.

In our previous studies (Yen et al., 2005), chlorogenic acid was determined as a prominent compound of the extracts and has been reported to scavenge peroxynitrite (Grace, Salgo, & Pryor, 1998). In addition, Ohehime, Yoshie, Aurioi, and Gilibert (1998) reported that flavonoids, such as catechin, cyanidin, epicatechin, epicatechin gallate, epigallocatechin gallate, myricetin, and rutin, at 0.5 mM same concentration as that of peroxynitrite (0.5 mM), inhibited strand breakage by >80%. These results revealed the inhibitory effect on DNA damage in RAW 264.7 due to scavenging of peroxynitrite.

3.7. Effect of extracts on inhibitory activity of tyrosine nitration

Tyrosine, when exposed to peroxynitrite at pH 7.0, undergoes nitration to form 3-nitrotyrosine. Thus, the ability to inhibit 3-nitrotyrosine formation provides a useful assay to screen various compounds for their ability to scavenge peroxynitrite and the nitrating species derived from it (Pannala, Rice-Evans, Halliwell, & Singh, 1997). Fig. 6 shows the ability of the extracts to decrease peroxynitrite-mediated tyrosine nitration. The extracts were incubated with tyrosine prior to the addition of peroxynitrite, followed by determination of 3-nitrotyrosine formation. Results indicated that LSWE showed a dose-dependent 3-nitrotyrosine formation. At higher concentration (0.2 mg/ml) of the extracts, the percentage inhibitions of 3-nitrotyrosine formation were 29.0%, 21.0% and 8.0% for LSWE, LSEAE and LSHE, respectively, compared with the control. This result implies that all the extracts derived from various solvents were potent scavengers of peroxynitrite due to their ability to prevent the nitration of tyrosine. Pannala et al. (1997) noted that ECG, EGCG and gallic acid are the most effective peroxynitrite scavengers in the catechin polyphenolic series. In addition, quercetin, rutin, catechin and EGC have been proven to show peroxynitrite-scavenging ability, which consequently inhibited tyrosine formation (Haenen, Paguay, Korthouwer, & Bast, 1997). The greater the number of sites available for nitration, the higher was the antioxidant activity with respect to peroxynitrite. Ascorbic acid and α-tocopherol have been shown to inhibit nitration in some human studies and animal models (Mirvish, 1996), a suggested mechanism being by preferential reaction with nitrite or nitrite-derived species such as the nitrosyl cation (NO^+). Inhibition of nitration by the hydroxycinnamates, caffeic acid, ferulic acid, coffee (rich in caffeic acid esters) and tea (rich in catechin/gallates) in vivo has also been demonstrated (Paquay et al., 2000). For example, ferulic acid, a disubstituted monohydroxycinnamate, decreases urinary *N*-nitrosoproline levels by 14–45% in humans, suggesting its potential for nitration inhibition in vivo. In addition, the related dihydroxycinnamate, chlorogenic acid (an ester of caffeic acid and quinic acid), suppresses *N*-nitrosation of a model amine in vitro (Kono, Shibata, Kodama, & Sawa, 1995). The absorbed fraction of chlorogenic acid and caffeic acid and their metabolites might induce biological effects in the blood circulation (Olthof, Hollman, & Katan, 2000). Inhibition of peroxynitrite-mediated tyrosine nitration in vitro by chlorogenic acid and caffeic acid might therefore protect against cardiovascular disease (Pannala et al., 1998). The fraction of chlorogenic acid that escapes absorption is present throughout the whole gastrointestinal tract, where it might induce biological effects. Chlorogenic acid and caffeic acid are antioxidants, and they might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds (Kono et al., 1995). In our previous study (Yen et al., 2005), certain polyphenolic compounds, such as chlorogenic acid, caffeic acid and gallic acid, were found in lotus seed, which were thought to reduce tyrosine nitration as a result of scavenging peroxynitrite. Although the reactivity in vitro does not always reflect the physiological functions, the present finding, that LSWE has an extraordinarily high potential for reacting with peroxinitrite, suggests that LSWE might be a potent dietary component, for defence against peroxinitrite.

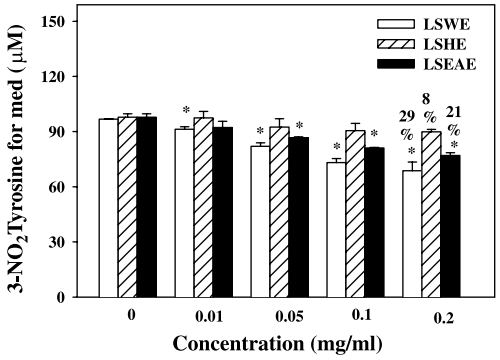

Fig. 6. Effects of lotus seed extracts on tyrosine nitration by peroxynitrite. Inhibitory effect is shown as a percentage. Results are means ± SD for $n \geqslant 3$. $^*p < 0.05$ when compared with the control. LSWE: Water extract; LSHE: *n*-Hexane extract; LSEAE: EtOAC extract.

In summary, we have demonstrated that the lotus seed extracts suppress NO production (LPS-activated and SNP-induced) in RAW 264.7 macrophages. LSWE showed better inhibitory effect on peroxynitrite-induced macrophage DNA damage than LSEAE or LSHE. In addition, lotus seed extracts were able to scavenge peroxynitrite, resulting in inhibition of tyrosine nitration. However, the underlying mechanism for inhibition of NO and peroxynitrite by lotus seed extracts needs further clarification.

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