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Antioxidant properties of lotus seed and its effect on DNA damage in human lymphocytes

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Abstract

The antioxidant properties of lotus seeds extracts and their effect on DNA damage in human lymphocytes were investigated. The results showed that boiling water extracts of lotus seeds (WELS) exhibited stronger antioxidant activity and that boiling water gave higher yields of extracts than other organic solvents. The WELS showed significant chelating binding on ferrous ions and marked interaction with hydrogen peroxide. Phenolic acids including caffeic acid, chlorogenic acid, *p*-hydroxybenzoic acid, gallic acid and large amounts of phenolic compounds, as found in WELS, were conjectured to be responsible for the antioxidant activity of WELS. The WELS showed neither changes on lipid peroxidation nor DNA damage in human lymphocytes with or without inducement by hydrogen peroxide.

Keywords: Lotus seed; DNA damage; Polyphenol; Single cell gel electrophoresis; Lymphocytes

1. Introduction

Oxygen free radicals, such as superoxide anion ($O_2^{-}\cdot$), hydroxyl radical (·OH) and hydrogen peroxide (H_2O_2) have been implicated in mediating various pathological processes, such as ischemia, inflammatory disease and atherosclerosis. The antioxidant enzymes-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX)-play an important role in scavenging oxidants and preventing cell injury. However, these defences are not perfect and, consequently, cellular macromolecules, including DNA, protein and lipid become oxidatively damaged (Bergendi, Benes, Durackova, & Ferencik, 1999). Thus the knowledge of antioxidants, that is, their antioxidant mechanism or contribution to the studied activity, is essential in the prevention of disease.

Several epidemiological studies have shown that increased dietary intake of natural phenolic antioxidants correlates with reduced coronary heart disease. In fact, phenolic compounds may act as antioxidants, with mechanisms involving both free radical-scavenging and metal chelation (Lien, Ren, Bui, & Wang, 1999). This is the reason for the current strong interest in natural antioxidants and their roles in human health and nutrition.

The Chinese herb 'Lian-Zi', which is the seed of the lotus (*Nelumbo nicifera*), has been used for medicinal purposes, such as an astringent, tonic, and sedative in oriental cultures. Recently, it has been indicated that lotus seed has an anti-aging effect and enhances immunity. In our previous study (Liou, Chen, & Yen, 1999), we found that the methanolic extracts from lotus seed exhibit a potential antioxidant activity against lipid peroxidation. However, the antioxidant properties of lotus seed extracts and their effect on DNA damage remain unclear. Therefore, the purpose of this study was to investigate the antioxidant properties of lotus seed extracts and the effect on DNA damage in human lymphocytes and to elucidate their possible mechanisms.

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2. Materials and methods

2.1. Materials

N-Lauroyl sarcosinate, ethidium bromidine (EtBr), Triton X-100, peroxidase, ferric chloride, linoleic acid, ferrozine, ascorbic acid, gallic acid, butylated hydroxyanisole (BHA), protocatechuic acid, caffeic acid, vanillic acid, ferulic acid, chlorogenic acid, quercetin, kaempferol, morine, naringenin, α-naphthoflavone, catechin and trypan blue were purchased from Sigma Co. Ltd. (St. Louis, MO, USA). Ammonium dihydrogen phosphate, sodium dihydrogen phosphate, disodium hydrogen phosphate, ortho-phosphoric acid, $NaHCO_3$, trichloracetic acid (TCA) and dimethyl sulfoxide (DMSO) were purchased from Merck Co. Ltd. (Darmstadt, Germany). Hydrogen peroxide (H_2O_2) was from Wako Co. Ltd. (Osaka, Japan). Blood samples were from healthy volunteers. Lotus seed was purchased from a local market in Taichung, Taiwan.

2.2. Sample preparation

The plumule of lotus seed was removed and ground into a fine powder with a mill (TECATOR CEMOTEC 1090 SAMPLE MILL). The powder was passed through an 80-mesh sieve, collected and sealed in a plastic bag, and then stored at 4 °C for further use.

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 Soxhlet extraction and filtered; the filtrate was called *n*-hexanolic extracts of lotus seeds (HELS). The residues were extracted by acetone (100 ml) and ethylacetate (100 ml), respectively, followed by filtration and evaporation of the filtrate to dryness in a vacuum. The acetone extracts and ethylacetate extracts of lotus seeds were named AELS and EALS, respectively.

2.3. Chelating activity on ferrous ions

The chelating activity of extracts on Fe^{2+} was measured according to the method of Dinis, Madeira, and Almeida (1994). One millilitre of sample (0.1–0.6 mg/ml) was reacted with $FeCl_2$ (2 mM, 0.1 ml) and ferrozine (0.5 mM, 0.2 ml) for 10 min, and the spectrophotometric absorbance was determined at 562 nm.

2.4. Scavenging effect on hydrogen peroxide

The scavenging effect of extracts on hydrogen peroxide was measured using the method of Rinkus and Taylor (1990) with a minor modification. Phenol red (sodium salt) was prepared in 0.2 M potassium phosphate, pH 6.2, at a final concentration of 7.5 mM. Horseradish peroxide (HRPase) was prepared in 0.2 M potassium phosphate pH 6.2, at a final concentration of 0.5 mg/ml. One volume of HRPase solution and two volumes of phenol red solution were mixed. Sample was incubated with 0.4 ml H_2O_2 for 20 min with added (0.6 ml) HRPase/phenol red solution. The tubes were vortexed and then allowed to sit for 10 min, and then placed in an ice bath immediately. This reaction mixture was monitored at 610 nm.

2.5. Determination of antioxidant ability

The total antioxidant activity of extracts was measured using the TEAC assay, as described by Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993). The TEAC value is based on the ability of the antioxidant to scavenge the blue–green 2,2′-azinobis 3-ethylbenzothiazoine-6-sulfonate ($ABTS^{\cdot+}$) radical cation relative to the $ABTS^{\cdot+}$ scavenging ability of the water-soluble vitamin E analogue 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). $ABTS^{\cdot+}$ cation can be generated by the interaction of ABTS (100 M), H_2O_2 (50 μM), and peroxidase (4.4 U/ml). To measure antioxidant capacity, the extracts were mixed with an equal volume of ABTS, H_2O_2, peroxidase, and deionized water. Absorbance was monitored and addition of reactant was used to calculate the TEAC value. TEAC value was expressed as millimolar concentration of Trolox solution having the antioxidant equivalent to a 1000 ppm solution of the sample under investigation. The higher the TEAC value of the sample, the stronger was the antioxidant activity.

2.6. Determination of total phenolic compounds

The phenolic content of the extracts was determined according to the method of Taga, Miller, and Pratt (1984) and calculated using gallic acid as a standard. The extracts (100 mg) were dissolved in 5 ml of 0.3% HCl in methanol/water (60:40, v/v). The resulting solution (100 μl) was added to 2 ml of 2% Na_2CO_3. After 2 min, 50% Folin–Ciocalteu reagent (100 μl) was added to the mixture, which was then left for 30 min. Absorbance was measured at 750 nm using a spectrophotometer. Results were expressed as milligrammes of gallic acid equivalents (GAE) per gram.

2.7. Preparation of human lymphocytes

Human lymphocytes were isolated from fresh whole blood by adding blood to RPMI 1640, then underlaying it with Histopaque 1077 before centrifuging at 1600 rpm for 15 min. Lymphocytes were separated as a pink layer at the top of the Histopaque 1077. Lymphocytes were washed in RPMI 1640. Cell number and viability (Try-

pan blue exclusion) were determined using a Neubauer Improved Haemocytometer before treatment. Human lymphocytes were incubated at a density of 5×10^5/ml and viability was over 90%.

2.8. Cytotoxicity

Cell suspensions were incubated with different concentrations of compounds (the final concentration was 0.1–0.6 mg/ml) for 30 min at 37 °C in a dark incubator together with untreated control samples. Samples were then centrifuged at 800*g*, the lymphocytes were resuspended in RPMI 1640 and 0.4% trypan blue, and viable and dead cell were scored.

2.9. Effect of lotus seed extracts on DNA damage induced by H_2O_2 in human lymphocyte

Lymphocytes were incubated with different concentrations of lotus seed extracts (or H_2O_2, or a combination of lotus seed extracts and H_2O_2) at 37 °C for 30 min in a dark incubator. After incubation, the lymphocytes were harvested by means of centrifugation at 800*g* for 5 min, and the cells were resuspended in LMA for the comet assay. The comet assay was performed under alkaline conditions following the methods of Yen, Hung, and Hsieh (2000). Lymphocytes were incubated with water extracts of lotus seed with or without hydrogen peroxide treatments at 37 °C for 30 min, then suspended in 75 ml 1% low melting point agarose in PBS, pH 7.4, and then immediately pipetted on to a frosted glass microscope slide precoated with a layer of 1% normal melting point agarose. After application of a third layer of 1% low melting point agarose, the slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM Na_2-EDTA, 10 mM Tris, 1% *N*-lauroyl sarcosineate, 1% Triton X-100, 10% DMSO, pH 10) for at least 1 h at 4 °C. The microscope slides were then placed in an electrophoresis tank, and the DNA was allowed to unwind for 20 min in freshly prepared alkaline electrophoresis buffer (1 mM EDTA, 0.3 N NaOH, pH 13). Electrophoresis was conducted at 4 °C for 20 min at 25 V and 300 mA. The slides were then neutralized with Tris buffer (0.4 M, pH 7.5) and stained with ethidium bromide. The slides were observed using a fluorescent microscope attached to a CCD camera and connected to a personal-computer-based image analysis system (Komet 3.0; Kinetic Imaging Ltd.). 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).

2.10. Determination of lipid peroxidation in cells

The TBARS generated were determined according to the method of Ramanathan, Das, and Tan (1994). Cell suspensions (1×10^6/ml) were incubated with extracts at 37 °C for 30 min and centrifuged at 1000 rpm. The cell pellet was washed twice with phosphate buffer saline and suspension in 0.1 N NaOH. The cell suspension was incubated with 10% TCA and 0.6 M thiobarbituric acid (TBA) in a boiling water bath for 10 min. Upon cooling, the absorbance was read at 532 nm, using a spectrophotometer. The protein content of the cells was determined by Bio-Rad protein assay kit. TBARS were expressed as malonaldehyde (MDA) equivalents, calculated by using the molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\,cm^{-1}$.

2.11. Phenolic acid analysis

The sample (1.0 g) was rinsed with 10 ml of methanol or distilled water into a bottle. For the separation of phenolics, a ternary solvent system with increasing hydrophobicity and changing pH was used. The solvents were 50 mM ammonium dihydrogen phosphate, pH 2.6 (solvent A), 0.2 mM orthophosphoric acid, pH 1.5 (solvent B) and 20% of solvent A in 80% acetonitrile (solvent C). The solvent gradient elution programme used was in the proportion of 100% A decreasing 92%, and 0% C increasing to 8% for 10 min, to 92% B decreasing to 20%, and 8% C increasing to 80% after a further 70 min. Analyses were performed using a LiChrospher RP-18 (250×4 mm, 5 μm) column. The flow rate was 1 ml/min and injection volume was 10 μl. Diode array detection was used for the identification of the compounds. Retention times and UV-Vis spectra of the peaks were compared with those of the standards. *p*-hydroxybenzoic acid was detected at 260 nm, gallic acid at 280 nm, hydroxycinnamic acids at 320 nm and flavonols at 340 nm.

2.12. 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. Yields of extracts

The extents of yields of extracts followed the order: boiling water (6.20%) > *n*-hexane (5.39%) > ethylacetate (0.87%) > acetone (0.80%). This indicates that boiling water can be effective in the extraction of lotus seed due to the heat treatment. This finding suggests that water-soluble components in lotus seed may easily be extracted by boiling water.

Table 1
Antioxidant activity of lotus seed extracts (LSE)

LSE	Concentration (mg/ml)	Scavenging effect (%)	TEAC (mM)
LSWE[a]	0.05	46.0	0.21 ± 0.09[b]
	0.1	57.3	
	0.2	78.4	
LSHE	0.05	10.2	0.06 ± 0.01
	0.1	21.0	
	0.2	30.0	
LSEAE	0.05	30.8	0.14 ± 0.05
	0.1	53.0	
	0.2	71.7	

[a] LSWE: Water extract; LSHE: *n*-Hexane extract; LSEAE: EtOAC extract.
[b] TEAC is the millimolar concentration of a trolox solution having the antioxidant capacity equivalent to a solution of the substance under investigation. Results are means ± SD for $n = 3$.

As the antioxidant activity is dependent on the test system used to determine the lipid peroxidation, a single analytical assay may be inadequate. Both $ABTS^{\cdot+}$ assay and metal-chelating assay therefore were used for the determination of antioxidant activity of the extracts.

3.2. Antioxidant activity of extracts

Free radical-scavenging is a generally accepted mechanism for antioxidants to inhibit lipid oxidation. The antioxidant activity of lotus seed extracts extracted from various solvents was evaluated by means of TEAC assay, and the results are shown in Table 1. The scavenging activities against $ABTS^{\cdot+}$ radicals for water extracts of lotus seed (WELS), *n*-hexane extracts of lotus seed (HELS) and ethylacetate extracts of lotus seed (EALS), in the range of 0.05–0.2 mg/ml, ranged from 46.0–78.4, 10.2–30.0, and 30.8–71.7%, respectively. Apparently, the antioxidant activity of lotus seed extracts was found to be in the order: WELS > EALS > HELS. Of the three samples tested, WELS had the highest TEAC values (0.21 mM trolox equivalent). The results obtained suggest that the higher the polarity of lotus seed extracts, the stronger is the antioxidant activity. This finding also indicates that active components with water soluble characteristics might exist in lotus seed extracts. The lotus seeds are edible and are commonly prepared by means of cooking treatments. This is meaningful to consumers because lotus seed extracts prepared from boiling water still retain a strong antioxidant activity.

3.3. Chelating action on ferrous ion

Fig. 1 shows the chelating effect of lotus seed extracts on ferrous ions. The chelating effect increased with increasing concentration of extracts tested. The extracts of three samples tested, at 0.6 mg/ml, showed 57.4%, 37.3% and 9.6% chelating effect on ferrous ion for WELS, EALS and HELS, respectively. Lotus seed extracts exhibited chelating effect on ferrous ions, suggesting that they minimize the concentration of ferrous ions in the Fenton reaction. Thus, lotus seed extracts protection may be partially related to iron interaction. Of three samples tested, WELS showed the strongest chelating effect on ferrous ion. Trolox, BHA and ascorbic acid showed a negligible effect on Fe^{2+} binding. Iron is essential for life because it is required for oxygen transport, respiration, and the activity of many enzymes. However, iron is an extremely reactive metal and will catalyze oxidative changes in lipid, proteins, and other cellular components (Decker & Hultin, 1992). In addition, lipid peroxidation was induced by a Fenton reaction in which iron is a reactant. According to the data in Table 1, and Fig. 1, lotus seed extracts of various solvents decreased the amount of preformed peroxides and reacted with iron. However, from a toxicological point of view, water, as a solvent, is safer than organic solvent. In addition, WELS showed the highest antioxidant activity and the strongest chelating effect on irons. Thus, WELS was used in the following study.

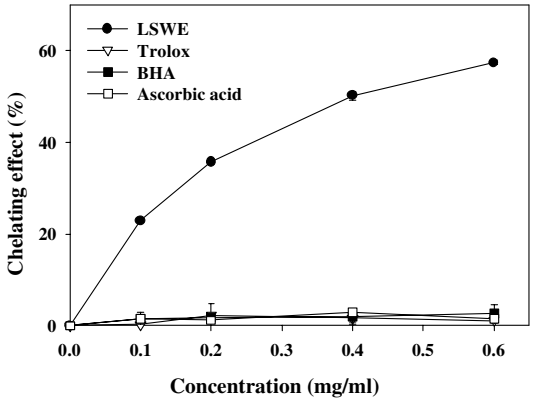

Fig. 1. Chelating effects of lotus seed water extracts on Fe^{2+}. Results are means ± SD for $n = 3$.

3.4. Scavenging effect on hydrogen peroxide

Fig. 2 shows the scavenging effect of WELS on hydrogen peroxide. WELS scavenged hydrogen peroxide in a concentration-dependent manner. WELS, at a level of 1.0 mg/ml, showed a 44.2% scavenging effect on hydrogen peroxide, compared with the control. In addition, the scavenging effect of WELS on hydrogen peroxide was less than trolox and ascorbic acid. Ruch and Klauning (1989) reported that green tea diminished hydrogen peroxide concentration from 4 to 0.5 mM, exhibiting a strong scavenging effect on hydrogen peroxide. Hydrogen peroxide, which is a relatively stable oxidant, is one of the active oxygen species; however it can initiate lipid peroxidation or be toxic to cells because it generates hydroxyl radicals by Fenton reaction (Namiki, 1990). Therefore, the ability of WELS to

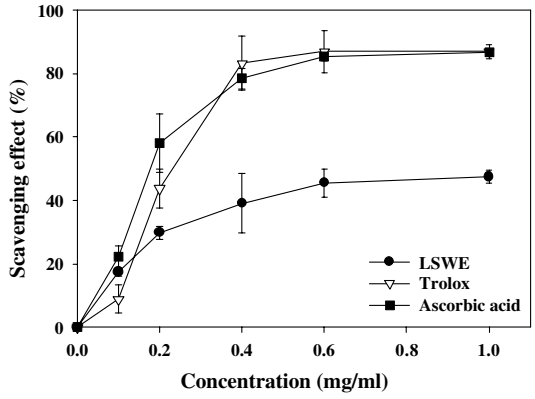

Fig. 2. Scavenging effects of lotus seed water extracts on hydrogen peroxide. Results are means ± SD for $n = 3$.

scavenge hydrogen peroxide may contribute to its inhibition of lipid peroxidation.

3.5. Phenolic acid and phenolic compounds in WELS

Several works have reported that polyphenols exhibit antioxidant activity, antimutagenic effect and anticarcinogenic action, attributed to scavenging of reactive oxygen species and chelating metal ions. In the present study, the total phenolic contents in WELS, EELS and HELS were 143.1, 28.5 and 25.7 mg/g of sample, respectively. This finding is in agreement with that reported by Moller, Madsen, Altonen, and Skibsted (1999), that the amounts of ploypheno1s in plants varied with various solvent extractions. In addition, WELS had a higher amount of total polyphenolic compounds than the other extracts. These results are in agreement with the finding that the higher the total polyphenol content, the stronger is the antioxidant activity (Table 1). In other words, the contents of polyphenolics in WELS are higher than EELS and HELS, which may be in part responsible for the fact that the antioxidant activity of WELS was stronger than HELS and EALS (Table 1).

Phenolic acids, have been proved to have antioxidant activity (Pekkarinen, Stockmann, Schwarz, Heinonen, & Hopia, 1999). To understand whether phenolic acids contribute to the antioxidant activity of WELS, it is necessary therefore to determine phenolic acids are present in lotus seed. The HPLC chromatogram of phenolic acid in WELS is shown in Fig. 3. The HPLC analysis of phenolic acid was successfully achieved as these compounds appeared at 11.21, 21.04, 22.81 and 24.28 min after the injection of the sample, respectively. Identification of these phenolic acids was done by comparing the retention times with authentic standards. Based on the retention times obtained, the phenolic compounds were proposed to be gallic acid, *p*-hydroxybenzoic acid, chlorogenic acid and caffeic acid. These phenolic acids in WELS are widely distributed in the plant kingdom. Some of these phenolic compounds are known to have physiological properties, such as antibacterial, anti-inflammatory, antiproliferative, antiatherosclerotic and antioxidative effects (Son & Lewis, 2002). Son and Lewis (2002) reported that caffeic acid and its analogues are potential natural antioxidants with multiple mechanisms, involving free radical-scavenging, metal ion chelation, and inhibitory actions on specific enzymes that induce free radical and lipid hydroperoxide formation. Pekkarinen et al. (1999) noted that antioxidant activities of phenolic acid are generally governed by their chemical structures. The activity improves as the number of hydroxyl group increase. In the present work, caffeic acid, chlorogenic acid with two OH groups, gallic acid with three OH groups and *p*-hydroxybenzoic acid with one OH group are found in the WELS, thus, suggesting that these phenolic acids contribute to the antioxidant activity of WELS.

3.6. Cytotoxicity of extracts

The cytotoxicity of WELS toward human lymphocytes, induced at 37 °C for 30 min, is shown in Table 2.

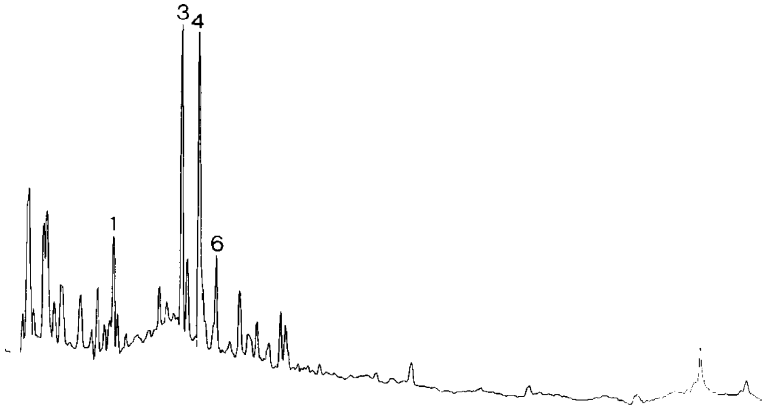

Fig. 3. HPLC chromatograms of flavonoids and phenolic compounds in the aqueous extract. 1. Gallic acid; 3. *p*-hydroxybenzoic acid; 4. Chlorogenic acid; 6. Caffeic acid from the lotus seed.

Table 2
Cytotoxicity of lotus seed water extracts toward human blood lymphocytes

Concentration (mg/ml)	Viability (%)[a]
0	98.9 ± 0.1
0.1	98.1 ± 1.3
0.2	96.8 ± 1.3
0.4	98.8 ± 0.7
0.6	98.1 ± 0.2

[a] The viability was [nonstained cells/(stained + nonstained cells)] × 100%. The high viability% indicated high live cells. Results are means ± SD for $n \geqslant 3$.

As a whole, the cell viability was greater than 90% at the concentrations tested, indicating no cytotoxicity toward human lymphocytes.

3.7. Effect of WELS on DNA damage

The comet assay is called 'single-cell gel electrophoresis assay', which is a sensitive method for detecting DNA strand breaks. Therefore, an increasing number of laboratories have began to use this extremely versatile assay to detect DNA damage. In the present work, the comet assay was used to determine the effect of WELS on DNA damage. Fig. 4a shows the DNA damage in human lymphocytes treated with different concentrations of WELS at 37 °C for 30 min and measured by means of comet assay. The degree of DNA damage in the model tested was expressed as Tail Moment. The values of DNA tail moment (TM) of the treated cells treated with WELS at a concentration of 0.1–0.6 mg/ml were the range 3–5, which was not significantly different ($P > 0.05$) from that of the control group. This result indicated that WELS showed no damage in human lymphocytes. In addition, Bechoua et al. (1999) have reported that glyceraldehyde-3-phosphate dehydrogenase and haem were inactive or damaged by hydrogen peroxide, which is a strong oxidant; consequently, cell death occurred. In the present study, the effect of WELS on DNA damage in human lymphocytes, induced by H_2O_2, was investigated. The DNA damage in human lymphocytes may be induced by 50 μM H_2O_2, the values of the tail moment of which were 25–27. However, the DNA damage in human lymphocytes induced by H_2O_2 (25 μM) was decreased by the addition of WELS at a concentration of 0.1–0.6 mg/ml (Fig. 4b). The inhibition was concentration-dependent. The WELS, at 0.6 mg/ml, showed 61% inhibition of DNA damage in human lymphocytes induced by H_2O_2 (25 μM), compared with the control group. As stated earlier, WELS exhibited a scavenging effect on hydrogen peroxide. Thus, the scavenging effect of WELS on hydrogen peroxide is conjectured to contribute to their inhibition of DNA damage in human lymphocytes.

Yang and Schaich (1996) noted that lipid hydroperoxides and malondialdehyde could induce DNA damage. Cell injury was caused by free radicals, which are derived from lipid peroxidation (Halliwell, 1991). Halliwell and Guettteridge (1998) reported that large amounts of malondialdehyde are formed during lipid peroxidation, and they react with DNA to produce MDA-DNA adducts, which cause the cells' mutagenicity. The effect of WELS on changes of lipid peroxidation in human lymphocytes was also investigated. The effects of WELS on the production of lipid hydroperoxide in human lymphocytes are shown in Table 3. The TBARS

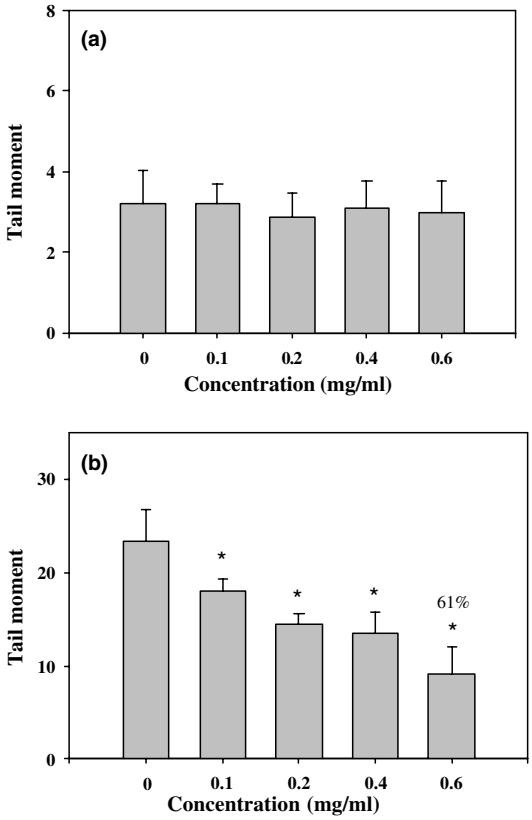

Fig. 4. Effect of lotus seed water extract on H_2O_2 induced DNA damage in human blood lymphocytes treated without (a) or with (b) H_2O_2. Inhibition of DNA damage is shown as percentage. Results are means ± SD for $n \geqslant 3$. *$p < 0.05$ when compared with the control.

Table 3
Effects of lotus seed water extracts on the production of lipid hydroperoxide in human blood lymphocytes

Concentration (mg/ml)	TBARS (nmol/mg protein)[a]
0	0.27 ± 0.07[ab]
0.1	0.28 ± 0.05[a]
0.2	0.27 ± 0.13[a]
0.4	0.29 ± 0.05[a]
0.6	0.29 ± 0.10[a]

[a] The concentration of the lipid peroxides was calculated by using a molar absorption coefficient of 1.56×10^5 $M^{-1}cm^{-1}$.

[b] Values are means ± SD of triplicate determinations. Values in a column with the same superscripts were not significantly different ($p > 0.05$).

values in human lymphocytes showed no significant change ($P > 0.05$) treated with WELS at a concentration of 0–0.6 mg/ml, compared with the control group, indicating that WELS did not induce lipid peroxidation in human lymphocytes.

In conclusion, antioxidant activity of lotus seeds extracts might have been in part due to their free radicals'-scavenging action and the chelating ability on ferrous ions. In addition, the phenolic acids, including caffeic acid, chlorogenic acid, *p*-hydroxybenzoic acid, gallic acid and a large amount of phenolic compounds, as found in WELS, seem to make contributions to the antioxidant activity of WELS, although further studies are required to reveal whether they contain other antioxidant constituents. As for the safety assessment, WELS neither induces lipid peroxidation nor cytotoxicity toward human lymphocytes. In addition, WELS showed, not only no DNA damage in human lymphocytes, but also significant inhibitory effect against DNA damage in human lymphocytes induced by hydrogen peroxide, which might be attributed to the interaction with hydrogen peroxide.

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