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Inhibitory effect of wheat bran feruloyl oligosaccharides on oxidative DNA damage in human lymphocytes

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Abstract

The present work assessed the protective effect of feruloyl oligosaccharides (FOs), the ferulic acid ester of oligosaccharides from wheat bran, against oxidative DNA damage in normal human peripheral blood lymphocytes induced by hydrogen peroxide (H_2O_2). The DNA damage was measured by using the single cell gel electrophoresis assay (comet assay). Lymphocytes were subjected to DNA damage by exposure to a range of H_2O_2 concentrations (10–200 μmol/l). H_2O_2, at a concentration of 200 μmol/l, resulted in nearly all cells being highly damaged. FOs showed no cytotoxicity and genotoxicity to normal human lymphocytes at the tested concentrations (10–500 μmol/l). In addition, DNA damage in human lymphocytes induced by 100 μmol/l H_2O_2 was inhibited by FOs in a concentration-dependent fashion with 91.1% inhibition of lymphocyte DNA damage at 500 μmol/l as compared with the control. The results suggest that water-soluble FOs from wheat bran are able to enhance the ability of human lymphocytes to resist H_2O_2 induced oxidative damage.

Keywords: Comet assay; Feruloyl oligosaccharides; Human lymphocytes; Wheat bran

1. Introduction

During normal oxidative metabolism in the human body, cells maintain a proper balance between the levels of free radicals, such as reactive oxygen species, and antioxidants to ensure the structural integrity of cellular components. If antioxidant defense mechanisms are overwhelmed by free radicals, oxidative stress occurs. Oxidative stress increases formation of superoxide anions, hydroxyl radicals and hydrogen peroxide, which can cause widespread damage to biological molecules leading to lipid peroxidation, protein oxidation, DNA base modification and so on. Oxidative damage has been known to play an important role in the etiology of several human diseases such as cancer, atherosclerosis, arthritis and diabetes (Finkel & Holbrook, 2000; Gey, 1990). As well as disease, oxidative stress is implicated in the mechanism of aging (Sohal, 2002).

Several epidemiological and experimental studies have shown that increased consumption of whole grain products has been associated with a reduced risk of cardiovascular diseases, cancer, and other chronic diseases (Jacobs, Pereira, Slavin, & Marquart, 2000; Kushi, Meyer, & Jacobs, 1997; Slavin, Jacobs, & Marquart, 1997). These positive physiological properties mainly have been ascribed to the phytochemicals including phenolic acids such as vanillic, *p*-coumaric, caffeic, chlorogenic, and, largely, ferulic acids (Andreasen, Landbo, Christensen, Hansen, & Meyer, 2001; Zieliński & Kozlowska, 2000). Phenolic compounds have strong *in vitro* and *in vivo* antioxidant activities associated with their ability to scavenge free radicals, break radical chain reactions, and chelate metals (Noguchi & Niki, 2000). They may inhibit oxidative damage, mutagenesis, and carcinogenesis, and thereby reduce the risk of chronic degenerative diseases associated with free radicals (Kelly, Xu, Alexander, & Loo, 2001). Cereal grains are rich in phenolic compounds, which are in both free and bound forms, and concentrated in the bran portion of cereal kernels (Lempereur, Rouau, & Abecassis, 1997). Among

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cereal phenolic compounds, free ferulic acid has been the most extensively investigated because of its physiological roles in antioxidant, antimicrobial, anti-inflammatory, antithrombotic, and anticancer activities and in the prevention of coronary disease, lowering cholesterol in serum and liver, and increasing sperm viability (Ou & Kwok, 2004). However, in cereals, significant levels of phenolic acids, predominately ferulic acid, are in an insoluble bound form, esterified to plant cell wall material (Moore, Cheng, Su, & Yu, 2006).

Wheat bran as an important by-product of the cereal industry is produced worldwide in enormous quantities and recognized as a good source of dietary fiber. FOs, the ferulic acid ester of oligosaccharides, could be released either by microorganisms in the colon or from enzymatic hydrolysis of arabinoxylans present in wheat bran (Ishii, 1997; Kroon, Faulds, Ryden, Robertson, & Williamson, 1997). It has been reported that water-soluble FOs can be prepared from wheat bran insoluble dietary fiber by *Bacillus subtilis* xylanase treatments (Yuan, Wang, & Yao, 2006), which can stimulate the *in vitro* growth of *Bifidobacterium bifidum* (Yuan, Wang, & Yao, 2005a) and effectively protect normal rat erythrocytes against *in vitro* oxidative damage for the first time (Yuan, Wang, & Yao, 2005b). In *in vitro* models, FOs are more effective antioxidants towards low density lipoproteins oxidation and DPPH free radical scavengers than free ferulic acid (Katapodis et al., 2003; Ohta, Sembokum, Kuchii, Egashira, & Sanada, 1997; Ohta, Yamasaki, Egashira, & Sanada, 1994). In *in vivo* studies, wheat bran FOs are a suitable antioxidant for protection against oxidative damage due to diabetes (Ou et al., 2007). Also, it was reported that ferulic acid bound to arabinoxylans from wheat bran was more bioavailable in rats than the free compound by increasing the plasma elimination half-life period in the organism and decreasing the level of urinary excretion in 24 h (Rondini et al., 2004). Therefore, FOs might become a very attractive group of natural antioxidants that can be supplemented to the human diet.

Recently, a growing number of scientific concern is focusing on the effect of reactive oxygen species on DNA, and the protective effects of certain dietary antioxidants by employing the comet assay (Møller & Loft, 2006). It has been postulated that antioxidants, mainly of dietary origin, may scavenge free radicals and thus, limit the damage incurred by the DNA and therefore, protect against mutagenesis and cancer (Foti et al., 2005; Khanduja et al., 2006). The comet assay, also known as the single cell gel electrophoresis, has gained wide-spread popularity as a sensitive, simple, rapid and reliable method to measure directly genotoxic and cytotoxic effects of physical and chemical agents as well as kinetics of DNA repair in individual cells. The peripheral blood mononuclear cell is the most commonly examined cell type using the comet technique (Fairbairn, Olive, & O'Neill, 1995).

To the best of our knowledge, there is little literature on the potential effect of FOs from wheat bran on a cellular level. In particular, evidence for a protective role for these compounds against DNA damage in human cells is lacking. Therefore, in the present study, the protective effect of water-soluble FOs from wheat bran against oxidative DNA damage in human peripheral blood lymphocytes induced by peroxide hydrogen (H_2O_2) was investigated.

2. Materials and methods

2.1. Materials

FOs were from wheat bran insoluble dietary fiber treated with *Bacillus subtilis* xylanase. Trypan blue, ethidium bromide, Histopaque 1077 (Ficoll0 hypaque, density: 1077 g/l), RPMI 1640 medium, normal-melting point agarose and low-melting point agarose (electrophoresis grade) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Blood samples were from healthy volunteers. All other chemicals and solvents used were of analytical grade.

2.2. Isolation of human peripheral blood lymphocytes

Whole peripheral blood was collected by venepuncture from three healthy male non-smoking donors (not exceeding the age of 35 years) who had not been exposed to any known mutagens at least for six months prior to the experiment. Lymphocytes were isolated by centrifugation in a density gradient of Histopaque 1077. The fresh whole blood was diluted with phosphate buffered saline (PBS) at pH 7.4, and carefully layered over Histopaque 1077. After centrifugation at 400*g* for 30 min at room temperature, the upper layer was discarded and the opaque interface containing mononuclear cells at the top of the Histopaque 1077 was transferred into a clean centrifuge tube. After repeated washings of the lymphocytes with PBS, cells were again centrifuged at 250*g*, the resulting pellet was resuspended in 0.5 ml of PBS and finally washed with RPMI 1640 media. The number of lymphocytes was finally counted under a microscope with a Neubauer type haemocytometer and the viability of the isolated lymphocytes was assayed by the trypan blue exclusion test. The final concentration of the cells was adjusted to about 2×10^5 lymphocytes/ml by adding RPMI 1640 medium. The lymphocyte isolation was carried out in the dark to minimize DNA damage by exposure to light. The viability of these cells was about 96%.

2.3. Cytotoxicity assay

The viability of the cells was determined by the trypan blue exclusion assay according to the method described by Sliwinska, Blasiak, and Drzewoski (2006). Cell suspensions were incubated with different concentrations of FOs for 30 min at 37 °C in a dark incubator together with untreated control samples. Samples were centrifuged at 800g, the lymphocytes were resuspended in RPMI 1640, and an equal volume of 0.4% trypan blue was added to the cell suspension, and the percentage of viable cell was

evaluated under a field microscope. The assay was repeated three times.

2.4. Genotoxicity of FOs toward normal human lymphocytes

Fresh isolated human lymphocytes were incubated with the different concentrations of FOs at 37 °C for 30 min in a dark incubator together with untreated control sample. Samples were then centrifuged at 800*g* for 3 min at 4 °C. After treatment, cells were centrifuged and washed twice with PBS at 800*g* for 3 min at 4 °C. The lymphocytes were resuspended in low-melting point agarose for the comet assay.

2.5. DNA damage exerted by H_2O_2 on normal human lymphocytes

DNA damage was induced *ex vivo* by exposing the fresh isolated human lymphocytes to a range of H_2O_2 concentrations (10–200 μmol/l) at 37 °C for 30 min in a dark incubator to choose a desired dose of H_2O_2 with significant DNA damage toward the tested cells. After incubation, the lymphocytes were harvested by centrifugation at 800*g* for 3 min at 4 °C and the cells were resuspended in low-melting point agar for the comet assay.

2.6. Effect of FOs on H_2O_2 induced DNA damage in human lymphocytes

Fresh isolated human lymphocytes were incubated with the different concentrations of FOs and 100 μmol/l H_2O_2 for 30 min at 37 °C in a dark incubator. Control samples were treated with PBS alone without H_2O_2. After incubation, cells were centrifuged and washed twice with PBS at 800*g* for 3 min at 4 °C. The lymphocytes were resuspended in low-melting point agarose for the comet assay.

2.7. Comet assay

The comet assay was carried out according to the procedure of Singh, McCoy, Tice, and Schneider (1998). The treated lymphocytes were suspended in 75 μl 1% low-melting point agarose in PBS, pH 7.4, and were spread onto microscope slides precoated with 1% normal-melting point agarose. After application of a third layer of 1% normal-melting point agarose, the slides were placed on ice for 5 min. After that, the slides were immersed in cold lysing solution (2.5 mol/l NaCl, 100 mmol/l Na_2–EDTA, 10 mmol/l Tris, 1% *N*-lauroyl sarcosineate, 1% Triton X-100, 10% dimethyl sulfoxide, pH 10) in the dark for at least 1 h at 4 °C. The microscope slides were then placed in a horizontal electrophoresis apparatus side by side, and the DNA was allowed to unwind for 20 min in freshly prepared alkaline electrophoresis buffer (1 mmol/l Na_2–EDTA, 300 mmol/l NaOH). Then electrophoresis was conducted at 25 V and 300 mA for 20 min. The slides were subsequently rinsed for 15 min with Tris–HCl buffer (0.4 mol/l, pH 7.5) at 4 °C in the dark. After neutralization, the gels were stained with 20 μg/ml ethidium bromide and kept in a moisture chamber in the dark at 4 °C until analysis.

2.8. Evaluation of DNA damage

The evaluation of DNA damage were carried out by the subjective visual score (Noroozi, Angerson, & Lean, 1998). For visualization of DNA, the slides were examined at an 1000× magnification using a 100 objective (oil immersion) on an Olympus BX60F5 fluorescence microscope (Olympus Optical Co. Ltd., Japan) with an excitation filter of 515–560 nm, and a barrier filer of 590 nm. Cells were assigned a score on a 5-point scale (range: 0–4) according to the amount of DNA in tail of the comet. Microscopic images revealed circular shapes (undamaged DNA) and 'comet' like shape, in which the DNA had migrated from the head to form a tail (damaged DNA). For each slide, one hundred nonoverlapping comets were randomly selected and assigned a score on a arbitrary scale of 0–4 (i.e., grade 0: no damage, <5%; grade 1: low damage, 5–25%; grade 2: medium damage, 25–45%; grade 3: high damage, 45–70%; grade 4: very high damage, >70%) based on perceived comet tail length migration and relative proportion of DNA in the comet tail. A total damage score for each slide was derived by multiplying the number of cells assigned to each grade of damage by the numeric of the grade and summing over all grades. Thus the total score for 100 comets could range from 0 (all undamaged) to 400 (all maximally damaged). In most cases, three separate experiments were conducted for each sample.

2.9. Statistical analysis

All analyses were run in triplicate and averaged. Results are expressed as means ± SD. Data were analyzed using the software STATISTICA 6.0. 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 of FOs

The cytotoxicity of FOs to normal human lymphocytes was evaluated by using trypan blue exclusion. As a whole, the observed cell viability was greater than 96% when FOs were incubated with cells at 37 °C for 30 min as shown in Table 1, and no significant difference ($P > 0.05$) in cell viability (%) was found at the tested concentrations of FOs ranging from 10 to 500 μmol/l, indicating that FOs showed no cytotoxicity to normal human lymphocytes under the tested concentrations. It has been reported that incubation of ferulic acid with normal human peripheral blood mononuclear cells for 18 h did not cause any appreciable cytotoxicity as analyzed by trypan blue exclusion method (Khanduja et al., 2006).

Table 1
Cytotoxicity of FOs toward normal human blood lymphocytes

Concentration (mmol/l)	Viability (%)[a]
0	99.1 ± 0.2
10	98.9 ± 0.6
20	98.3 ± 0.5
50	97.2 ± 1.1
100	98.7 ± 0.8
200	96.8 ± 1.4
500	98.5 ± 0.3

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

3.2. Genotoxicity of FOs toward normal human lymphocytes

Single cell gel electrophoresis (the comet assay) has been widely employed for the measurement of genotoxic and cytotoxic effects of physical and chemical agents on animal and human cells (Duthie & Collins, 1997). In the assay, under alkaline conditions, DNA loops containing breaks lose supercoiling, unwind, and are released from the nucleus to form a comet-like image with a brightly fluorescent head and a tail streaming away from it when viewed by fluorescence microscopy after gel electrophoresis (Fairbairn, Olive, & O'Neill, 1995). DNA strand breaks are thus visualized by the comet assay and can be quantified by image intensification and computer analysis or by visual grading (Singh et al., 1998). Generally cells with a high level of DNA damage exhibit increased comet parameters, which may be expressed as tail length, %DNA in the tail and tail moment (tail length × %DNA in the tail) or simply as high, medium and low damage (Faust et al., 2004). It has been reported that there was a close relation between the subjective visual score and the measurements of the percentage of DNA in the tail image analysis (Noroozi et al., 1998). In this study, the visual scoring for the comet method was used to evaluate DNA damage in human lymphocytes on the basis of their morphology and degree of damage. The genotoxicity of FOs toward normal human lymphocytes was assessed by using the comet assay. Table 2 showed the DNA damage in lymphocytes treated with different concentration of FOs at 37 °C for 30 min. The extent of DNA damage in the model system was expressed as the total score. The values of the total score of the tested cells treated with FOs at a concentration of 10–500 μmol/l were lower than 15, which was not significantly different ($P > 0.05$) from that of the control group. The results indicated that FOs at the tested concentrations showed no damage in normal human lymphocytes as compared with the control group.

3.3. DNA damage exerted by H_2O_2 on normal human lymphocytes

H_2O_2 is one of the principle reactive products of oxygen metabolism. H_2O_2 is rather stable in most culture media, but in the presence of cells, the H_2O_2 concentration diminishes quickly, depending on cell population density and cellular catalase contents. H_2O_2 can readily cross plasma and nuclear membranes. Although H_2O_2 produced in the normal metabolism of aerobic organisms is a relatively unreactive species, it is the primary precursor for the generation of hydroxyl radicals (via a transition metal-catalyzed Haber–Weiss reaction). Accumulation of H_2O_2 can have profound deleterious effects on cells through base modifications and strand breakage in genomic DNA, damage to lysosomal membranes, and the induction of apoptosis (Deutshch, 1998). The susceptibility of normal human lymphocytes to treatment with H_2O_2 was assessed. Fresh isolated cells were incubated with varying concentrations of H_2O_2 for 30 min at 37 °C, followed by the evaluation of DNA damage by the comet assay. Fig. 1A depicts the percentage of cells with different visual grades of DNA damage in normal human lymphocytes treated with varying concentrations of H_2O_2. The control group only showed very slight DNA damage, and the percentage of cells showing DNA damage increased with increasing H_2O_2 concentration. Obvious DNA damage was observed at a concentration greater than 50 μM H_2O_2. Statistically significant increase in DNA damage ($P < 0.05$) was observed at the range of 10–200 μmol/l when compared to the untreated samples. An increase in the concentrations of H_2O_2 to 200 μmol/l resulted in nearly all cells exhibited a tailed DNA, indicating extensive damage. As shown in Fig. 1B, the total DNA damage score of the control group was 13.8 using the visual scoring system. A significant increase of the total DNA damage scores was observed at the tested concentration range (10–200 μmol/l) of H_2O_2, and the total DNA damage score reached a maximum value of 360.6 from 10 to 200 μmol/l of H_2O_2. DNA damage showed a dose-dependent effect. H_2O_2 is believed to be one

Table 2
Genotoxicity of FOs toward normal human blood lymphocytes in the assay[a]

Concentration of FOs (mmol/l)	Percentage of cells showing DNA damage					Total score (out of 400)
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	
0	88.5 ± 2.1	10.2 ± 1.9	0.5 ± 0.3	0.6 ± 0.3	0.2 ± 0.2	13.8 ± 2.4
10	89.1 ± 1.8	9.9 ± 1.1	0.3 ± 0.1	0.5 ± 0.3	0.2 ± 0.2	12.8 ± 2.1
20	87.9 ± 0.9	11.0 ± 1.5	0.4 ± 0.2	0.5 ± 0.2	0.2 ± 0.1	14.1 ± 1.9
50	90.1 ± 1.7	8.7 ± 0.8	0.2 ± 0.2	0.8 ± 0.1	0.2 ± 0.1	12.3 ± 1.8
100	90.4 ± 2.3	8.5 ± 1.4	0.6 ± 0.1	0.4 ± 0.1	0.1 ± 0.1	11.3 ± 2.9
200	88.9 ± 0.8	10.1 ± 1.1	0.7 ± 0.1	0.3 ± 0.2	0 ± 0.0	12.4 ± 1.5
500	89.5 ± 1.4	9.8 ± 0.6	0.5 ± 0.1	0.2 ± 0.1	0 ± 0.0	11.4 ± 1.7

[a] Mean ± SD, $n = 3$ repeat experiments.

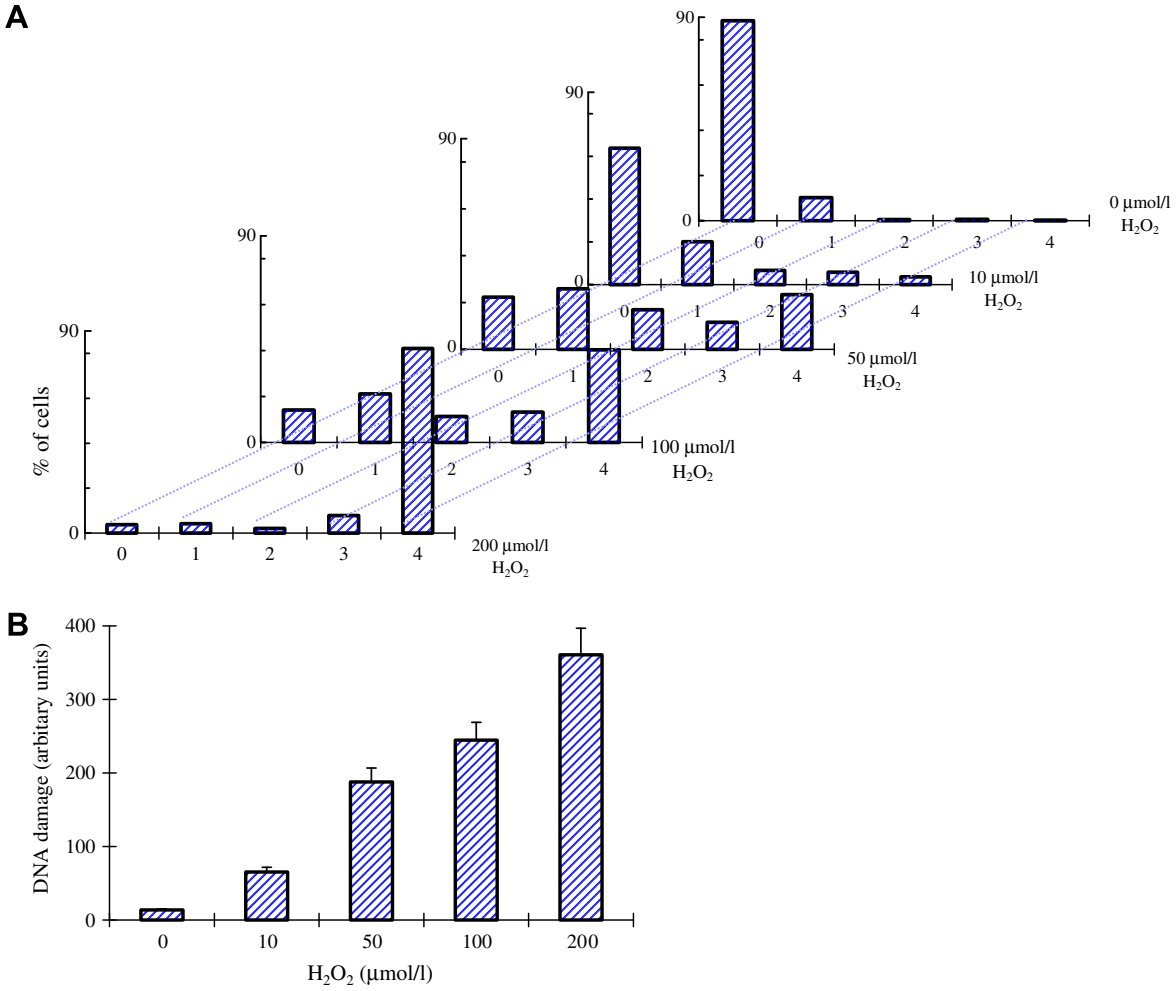

Fig. 1. DNA damage in normal human lymphocytes treated with varying concentrations of H_2O_2. (A) The percentage of cells with different visual grades of DNA damage. (B) The total DNA damage scores.

of the most potent cause of DNA damage, chromosomal alterations and gene mutations by generating hydroxyl radicals (**.**OH) close to the DNA molecule (Lazarová, Lábaj, Eckl, & Slameňová, 2006). It has been reported that the concentration of H_2O_2 used in human studies to induce DNA damage have ranged from 1 µmol/L up to 500 µmol/l (Riso, Pinder, Santangelo, & Porrini, 1999). Generally, a high proportion of studies use H_2O_2 concentrations between 50 and 100 µmol/l (Tomasetti, Littarru, Stocker, & Alleva, 1999; Wu et al., 2004). Therefore, based on the obtained results, it was decided that 100 µmol/l would be the most suitable concentration of H_2O_2 to induce *ex vivo* DNA damage in the following study even though 10 and 50 µmol/l H_2O_2 induced significant increases in DNA damage compared to the untreated samples.

3.4. Effect of FOs on H_2O_2 induced DNA damage in human lymphocytes

Polyphenolic compounds exert a variety of physiological effects *in vitro* including antioxidative, immunomodulatory and antigenotoxic effects (Bub et al., 2003). Dietary antioxidants including polyphenolic compounds are thought to decrease endogenous damage to DNA caused by oxygen-free radicals liberated during normal respiration (Duthie, Ma, Ross, & Collins, 1996). A study by Jeon et al. (2006) suggested that methanol extracts of rice hulls possess significant ROS scavenging and metal chelating activities and protective effect against oxidative DNA damage in human lymphocytes. Several studies have been reported the antioxidant activity of FOs. They are the potential sources of antioxidants with a capacity of inhibiting the peroxidation of low density lipoproteins in the presence of Cu^{2+} (Katapodis et al., 2003; Ohta et al., 1997) and protecting normal rat erythrocytes against oxidative damage mediated by peroxyl free radicals (Yuan et al., 2005b). FOs with higher molecular masses exhibited stronger antioxidant activities in the microsomal lipid peroxidation system in the presence of CCl_4 (Ohta et al., 1994). It is well known that human peripheral blood mononuclear cells are considered to be easily susceptible to oxidative stress because these cells lack peroxidase activity, and H_2O_2 which can diffuse through the cell membranes and other organelles react with different targets in the cell, trigger

lipid peroxidation, as well as the oxidation of protein and DNA (Khanduja et al., 2006). Therefore, in the present study, the effect of FOs on DNA damage in normal human lymphocytes induced by 100 μmol/l H_2O_2 was determined by the comet assay. Fig. 2A shows the percentage of cells with different visual grades of DNA damage in normal human lymphocytes treated with different concentrations of FOs (10–500 μmol/l). The control group showed very high DNA damage, and the percentage of undamaged DNA in the tested cells was obviously increased with increasing FOs concentration when compared with the untreated samples. An increase in the concentrations of FOs to 500 μmol/l resulted in 84.9% of cells maintained the circular shapes of DNA in normal human blood lymphocytes, indicating a very slight DNA damage. As shown in Fig. 2B, the total DNA damage score of the control group was 244.5 using the visual scoring system. A significant ($P < 0.05$) decrease of the total DNA damage scores was observed at the tested concentration range (10–500 μmol/l) of FOs, and the total DNA damage score lowered a minimum value of 21.8 from 10 to 500 μmol/l of FOs. FOs inhibited DNA damage in lymphocytes induced by H_2O_2 in a concentration-dependent manner with 91.1% inhibition of DNA damage at a concentration of 500 μmol/l as compared with the control group. The results indicated that FOs could be able to enhance efficiently the ability of normal human lymphocytes to resist H_2O_2 induced oxidative damage under *ex vivo* condition. A study by Khanduja et al. (2006) demonstrated that ferulic acid significantly exhibited DNA protective effect in normal human peripheral blood mononuclear cells exposed to induced oxidative stress. Ferulic acid also was an effective scavenger of superoxide anion radical produced by interaction of the tumor promoter benzoyl peroxide with murine peritoneal macrophages *in vitro* (Kaul & Khanduja, 1999).

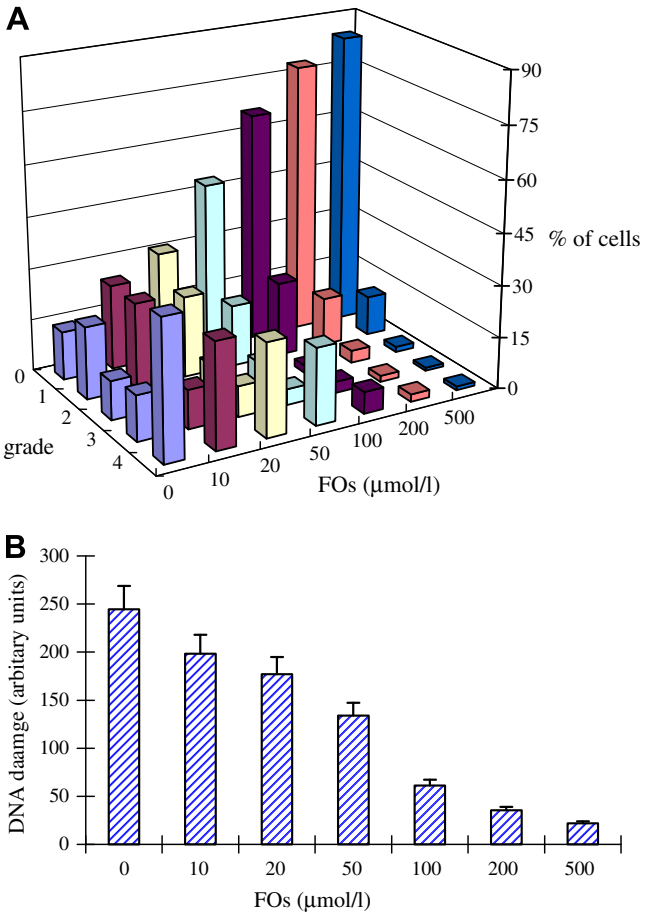

Fig. 2. Effect of FOs at different concentrations on the DNA damage in normal human lymphocytes induced by 100 μmol/l H_2O_2. (A) The percentage of cells with different visual grades of DNA damage. (B) The total DNA damage scores.

There are two ways for phenolic antioxidant to protect DNA from lesion by reactive oxygen species (ROS), either via scavenging ROS prior to their attacking DNA or fast repair of damage DNA induced by ROS, and these two ways complement each other (Shi et al., 1999). The main mechanism of action of phenolic antioxidants is considered to be the scavenging of free radicals by donating the phenolic hydrogen atom (Bakalbassis, Lithoxoidous, & Vafiadis, 2003). The ferulic acid moiety in FOs possess distinct structural motifs that can possibly contribute to the free radical-scavenging capability of the compounds. The presence of electron donating groups on the benzene ring (3-methoxy and more importantly 4-hydroxyl) of ferulic acid gives the additional resonance structures of the resulting phenoxyl radical, contributing to the stability of this intermediate or even terminating free radical chain reactions, and the carboxylic acid group in ferulic acid with adjacent unsaturated C=C double bond can provide additional attack sites for free radicals (Graf, 1998). It is suggested that the obvious protective effect of FOs against DNA damaged in normal lymphocytes induced by H_2O_2 might be related to scavenging activity against free radicals, thereby reducing the impact of free radical attack DNA in cells.

In addition, FOs also contain hydrophilic oligosaccharides that are beneficial for immunology, and they are a nonionic chemical species that may pass through cell membranes with a high density of inner negative charges more easily than the negative free phenolic compounds (Ou et al., 2007). It is suggested that the inhibitory effect of FOs on H_2O_2 induced oxidative DNA damage in normal lymphocytes might be also related to the activity of the oligosaccharides moiety in FOs. In recent years, several oligosaccharides have been reported to have low adverse effects, and exhibit many biological and physiological activities, which has attracted great interest (Yuan, Song, Li, Li, & Dai, 2006). Some pentaglucosides had high immunological activity and scavenging ability toward superoxide anions (Huang, Liu, Mei, & Wang, 2005). Agarooligosaccharides exerted no cytotoxic effects on human liver cell, and could permeate across the cell membrane to act as an efficient antioxidant in cells, thereby, showing the potential scavenging effects on intracellular oxidative damage directly induced by H_2O_2 (Chen & Yan, 2005). Acidic xylooligosac-

charide showed superoxide anion radical-scavenging activity, which could contribute, in part, to its suppressive activities on stress-induced mouse gastritis (Yoshino, Higashi, & Koga, 2006). Some synthetic oligosaccharides exhibited strong free radical-scavenging activity and administration of synthetic oligosaccharides could prevent the formation of lipid peroxidation by overall enhancement of tissue enzymatic and nonenzymatic antioxidant defenses in aged mice (Li, Shi, Wang, Wang, & Le, 2007). The carboxylated chitooligosaccharides exerted a considerable reactive oxygen-free radical-scavenging effect in cell systems, thereby protected radical-mediated oxidation of cellular biomolecules (Rajapakse, Kim, Mendisa, & Kim, 2007). Carrageenan oligosaccharides exhibited scavenging activity of reactive oxygen-free radicals, and exerted their antitumor effect by promoting the activity of the immune system, and showed effective protection against H_2O_2 induced intracellular oxidative damage on rat lymphocytes (Yuan et al., 2005; Yuan et al., 2006).

A major component of the antioxidant system in mammalian cells consists of the three enzymes, namely, superoxide dismutase, catalase, and glutathione peroxidase. These enzymes work in concert to detoxify ROS such as superoxide anion and H_2O_2 in cells. Higher levels of the antioxidant enzymes have been correlated with decreased susceptibility to cell damage (Yuan, Song, Zhang, et al., 2006). Mou, Jiang, and Guan (2003) found that the oral administration of κ-carrageenan oligosaccharides was advantageous to promote the activities of superoxide dismutase and catalase in mouse. The diabetic rats fed FOs showed an effective protection against oxidative stress by restoring the antioxidant enzyme capacity via increasing the activities of superoxide dismutase and glutathione peroxidase and decreasing xanthine oxidase and malondiadehyde content (Ou et al., 2007). The protection of FOs against oxidative DNA damage in normal lymphocytes induced by H_2O_2 might be also due to the improvement in the activities of intracellular antioxidant enzymes involved in the antioxidant mechanism, which will be further investigated.

In conclusion, the results presented in this paper showed water-soluble FOs from wheat bran insoluble dietary fiber had no cytotoxicity and genotoxicity to normal human blood lymphocytes at the tested concentrations and condition, and exhibited significant protective effect against DNA damage in cells induced by H_2O_2 under *ex vivo* conditions, which might be related to not only the antioxidant capacity of ferulic acid moiety but also the activity of hydrophilic oligosaccharides moiety. The precise mechanism on inhibition effect of FOs against oxidative DNA damage in human lymphocytes is needed to further investigate.

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