Effect of polyphenolic compounds from *Coriandrum sativum* on H$_2$O$_2$-induced oxidative stress in human lymphocytes

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Abstract

Polyphenolic compounds are widely distributed in plants and known to be excellent antioxidants in vitro. They have the capacity to reduce free-radical formation by scavenging free radicals and protecting antioxidant defences. The present study evaluated the antioxidant potencies of polyphenolic compounds from a spice, *Coriandrum sativum* against hydrogen peroxide-induced oxidative damage in human lymphocytes. Pretreatment with polyphenolic rich fractions protected human lymphocytes against H$_2$O$_2$-induced oxidative damage. H$_2$O$_2$ treatment significantly decreased the activities of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-*S*-transferase and caused decreased glutathione content and increased thiobarbituric acid-reacting substances (TBARS). Treatment with polyphenolic fractions (50 µg/ml) increased the activities of antioxidant enzymes and glutathione content and reduced the levels of TBARS significantly. Observed reduction in the level of lipid peroxides showed a decreased tendency of peroxidative damage. We conclude that, under these experimental conditions, polyphenolic compounds effectively suppress hydrogen peroxide-induced oxidative stress.

1. Introduction

There is great interest in plant polyphenolic compounds because of their potential roles as cancer chemopreventive agents and chronic disease protectors. Their beneficial effect is considered to be mainly due to their antioxidant and chelating activities. One of the major groups of polyphenolic compounds is the flavonoids which occur widely in plant foods, such as fruits, vegetables, cereals and beverages (Anila & Vijayalakshmi, 2002; Bravo, 1998; Cook & Samman, 1996; Harborne, 1989; Lakenbrink, Lapczynski, Maiwald, & Engelhardt,

2000). There is an increasing interest in polyphenols due to their potentially positive effect against certain diseases, namely mainly some forms of cancer and coronary heart diseases. They can act as free radical-scavengers, neutralizing dangerous reactive oxygen species and metal ion chelators. Both of these activities are responsible for antioxidant properties. Flavonoids and other plant phenolics have been reported to have a in vitro antioxidant activities, even higher than the common antioxidants, vitamin C and E (Rice-Evans, Miller, & Paganga, 1997; Scott, Butler, Halliwell, & Aruoma, 1993). The antioxidant capacity of this diverse group of compounds depends on the individual structure and number of hydroxyl groups.

Antioxidants behave differently under various conditions (Decker, 1997). At physiological levels typical of

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healthy diet, they act as antioxidants, but at pharmacological doses, some of them act as prooxidants, stimulating the production of free radicals (Brown, Morrice, & Duthie, 1997). The best way to supplement antioxidant nutrients is to eat generous servings of fruits and vegetables, rich in antioxidants, such as polyphenols. However, intake is not always sufficient to meet the requirement. In such cases, drugs come to the rescue and natural drugs are preferable in this context to avoid side effects induced by synthetic drugs. Since dietary polyphenol has been reported to be inversely associated with lipid peroxidation cytotoxicity, an attempt was made to study the effects of polyphenolic fractions from *Coriandrum sativum* on activities of antioxidant enzymes in H_2O_2-treated human lymphocytes. Coriander (*Coriandrum sativum* Linn.) is an annual herb that belongs to the carrot family (Umbilliferae) and a native of the Mediterranean region. At present, it is extensively cultivated in Russia, central Europe, India, Turkey, Morocco, Argentina and the United States of America. The fruits are aromatic, bitter, anti-inflammatory, digestive aids, diuretics, and are useful in vitiated conditions of 'pitta' burning sensation, cough, bronchitis, vomitting, dyspepsia, diarrhoea, dysentery, gout, rheumatism, intermittent fevers and giddiness (Varier, 1994). Antiperoxidative effect of coriander seeds (*C. sativum*) was studied in rats administered a high fat diet (Chitra & Leelamma, 1999). Quercetin 3-glucuronide, isoquercitrin and rutin were separated and identified in coriander fruits by means of chromatography on cellulose columns (Kunzemann & Herrmann, 1977). But no detailed investigations were carried out on the antioxidant effect of polyphenolic compounds from coriander seeds. Since coriander seeds are one of the commonly used spices, we have made an attempt to evaluate the role of polyphenolic compounds from coriander seeds on oxidative damage induced by H_2O_2 in human lymphocytes.

2. Materials and methods

2.1. Plant material

Coriander seeds (*C. sativum* Linn.) available from local market were used for the extraction of polyphenolic compounds.

2.2. Extraction of polyphenolic compounds

Dried coriander seeds (*C. sativum*) were powdered and extracted with 80% methanol, thrice (1:1, w/v), at room temperature (Petra, Britta, Macki, & Eckart, 1999). The combined extract was concentrated in a vacuum evaporator and the residue was dissolved in water and fractionated successively with hexane, benzene, ethyl acetate and *n*-butanol. On estimating the levels of polyphenolic compounds in each fraction using standard quercetin (Eskin, Hoehn, & Frenkel, 1978), the ethyl acetate fraction was found to have the higher polyphenolic content. This extract was evaporated in vacuum and used for further purification using column chromatography.

2.3. Chromatographic separation

The dark brown solid (15 g) was adsorbed on silica gel (20 g) and transferred to a column of silica gel (150 g) equilibrated with hexane. Elution was performed with hexane, hexane:chloroform (3:1), hexane:chloroform (1:1), hexane:chloroform (1:3), chloroform, chloroform:ethyl acetate (3:1), chloroform:ethyl acetate (1:1), chloroform:ethyl acetate (1:3), ethyl acetate, ethyl acetate:methanol (3:1), ethyl acetate:methanol (1:1), ethyl acetate:methanol (1:3) and methanol. The polyphenolic content was determined by the $TiCl_4$ method (Eskin et al., 1978). Chloroform:ethyl acetate [$CHCl_3$:EtAc] (3:1), ethyl acetate [EtAc] and ethyl acetate:methanol [EtAc:MeOH] (3:1) fractions obtained from the silica gel column showed higher polyphenolic contents (Chart 1) and were used for in vitro experiments. Stock solutions of the polyphenols were prepared in dimethyl sulphoxide (DMSO) and diluted in cell culture medium or phosphate buffered saline (PBS). The final concentration of DMSO was always 0.2% (v/v) (Zielinska, Gulden, & Seibert, 2003).

2.4. Isolation of lymphocytes

Human lymphocytes were isolated from fresh whole blood from healthy volunteers (25–35 years old) using Histopaque 1077. Briefly, anticoagulated blood was diluted with an equal volume of RPMI 1640 containing 10% FCS on ice for 30 min, underlaying it with Histopaque 1077 and centrifuging at $200g$ for 3 min at 4 °C. Lymphocytes were separated as a pink layer at the top of the Histopaque (Noroozi, Angerson, & Lean, 1998). Cells were washed with PBS and centrifuging at $200g$ for 3 min at 4 °C (two times). Cells were rinsed with PBS and diluted to 5×10^6 cells/ml.

2.5. Antioxidant pretreatment

Cells were incubated with different fractions of polyphenols isolated from *C. sativum* at a concentration of 50 μg/ml for 30 min at 37 °C, together with untreated control samples, which contained DMSO. These samples were then centrifuged at $200g$ for 3 min at 4 °C. After polyphenol treatment, cells were centrifuged and washed twice with PBS (0.01 M) at $200g$ for 3 min at 4 °C.

Dried, powdered coriander seeds (1kg)

↓

Methanol extract   (800* mg)

↓

Fractionation

n- Hexane	Benzene	Ethyl acetate	n- Butanol	Water
(75* mg)	(49.8* mg)	(397.5* mg)	(150* mg)	(130* mg)

↓

Silica gel column chromatography

H	H: B (1: 1)	B	B: C (1: 1)	C	C: E (3:1)	C: E (1:1)	C: E (1:3)	E	E: M (3:1)	E: M (1:1)	E: M (1:3)	M
(7.5*)	(7.5*)	(5*)	(10*)	(9*)	(75*)	(30*)	(15*)	(48*)	(90*)	(9.0*)	(20*)	(12.5*)

*Polyphenolic content is expressed in milligrame.

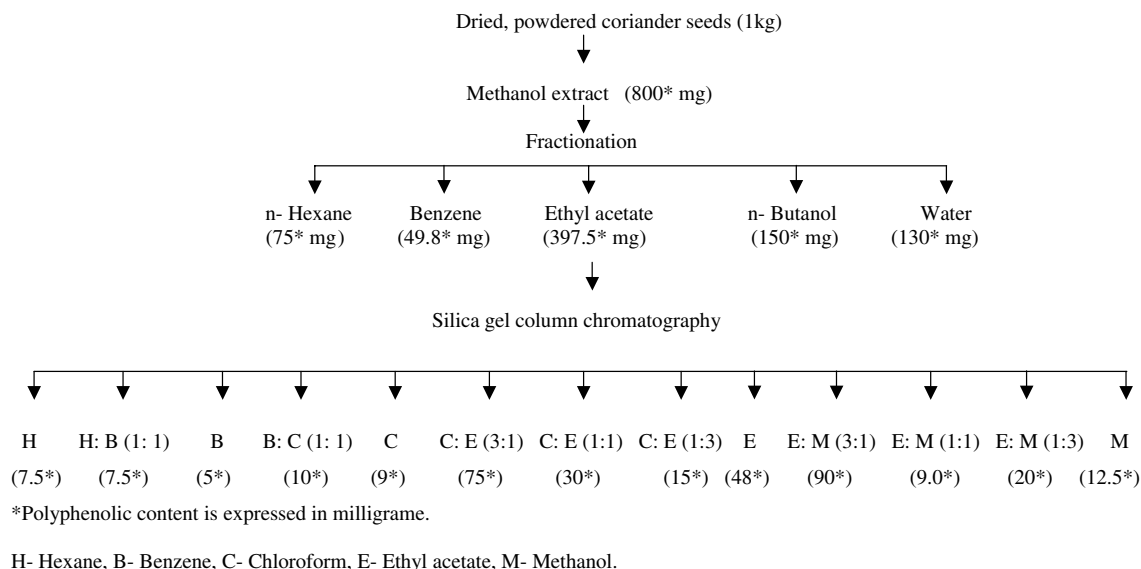H- Hexane, B- Benzene, C- Chloroform, E- Ethyl acetate, M- Methanol.

Chart 1. Polyphenolic content of different fractions from *Coriandrum sativum* Linn.

2.6. Oxygen radical treatment

Polyphenol-pretreated samples were suspended in PBS with 50 μM H_2O_2 for 5 min on ice in the dark. Samples were then centrifuged at 200g for 3 min at 4 °C. Control samples were treated with PBS alone without H_2O_2. After H_2O_2 treatment, cells were centrifuged and washed twice with PBS (0.01 M) at 200g for 3 min at 4 °C.

2.7. Experimental design

Lymphocytes were divided into six samples and each sample contained 5×10^6 cells/ml.

Sample I, treated with DMSO (normal control).
Sample II, treated with H_2O_2 (positive control).
Sample III, treated with H_2O_2 and quercetin.
Sample IV, treated with H_2O_2 and ethyl acetate fraction.
Sample V, treated with H_2O_2 and ethyl acetate:methanol (3:1) fraction.
Sample VI, treated with H_2O_2 and chloroform:ethyl acetate (3:1) fraction.

2.8. Biochemical assays

The biochemical assays included the determination of activities of enzymes involved in the antioxidant mechanism. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assessed on the basis of colour intensity of the chromogen produced by the reduction of nitro blue tetrazolium on addition of NADH, measured at 560 nm (Kakkar, Das, & Viswanathan, 1984). Catalase (EC 1.11.1.6) was assayed by noting the decrease in extinction at 240 nm, followed by the decomposition of H_2O_2 (Maechlay & Chance, 1954). Activities of glutathione peroxidase (Gpx, EC 1.11.1.9) (Paglia & Valentine, 1967) and glutathione reductase (GR, EC 1.6.4.2) (David & Richard, 1983) were measured by following the decrease in the absorbance due to oxidation of NADPH. Glutathione-S-transferase (GST, EC 2.5.1.18) activity was assayed by measuring the increment of absorbance at 340 nm due to the formation of 2,4-dinitrophenyl-S-glutathione from 1-chloro 2,4-dinitrobenzene (CDNB) and GSH (Habig, Pabst, & Jakoby, 1974). In addition, concentrations of thiobarbituric acid-reacting substances (TBARS) and reduced glutathione were estimated in human lymphocytes. Malondialdehyde was identified as the product of lipid peroxidation that reacts with thiobarbituric acid, forming a red-coloured compound absorbing at 535 nm (John & Steven, 1978). Glutathione reacts with an excess of alloxan to produce a substance which has an absorption spectrum maximum at 305 nm (Patterson & Lazarow, 1955). Protein content was estimated in samples after TCA precipitation. Protein reacts with Folin–Ciocalteau reagent to give a coloured complex which can be measured at 660 nm (Lowry, Rosebrough, Farr, & Randall, 1951).

2.9. Statistical analysis

The statistical analyses were performed with the statistical software SPSS/Windows (SPSS 10.0. LNK). The results were expressed as the means ± SEM to show variations in a group. Differences were considered significant at $p \leqslant 0.05$.

3. Results

There was a significant decrease in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-*S*-transferase (Figs. 1 and 2) in H_2O_2-treated lymphocytes when compared to normal control. But the activities of antioxidant enzymes were significantly increased in all polyphenol-treated samples when compared with H_2O_2 control and the decrease in activities induced by H_2O_2 was significantly restored to normal control levels. $CHCl_3$:EtAc (3:1) was the most active fraction among all polyphenolic fractions in bringing the activities of superoxide dismutase, catalase, glutathione reductase and glutathione-*S*-transferase towards normal level. Regarding the activity of glutathione peroxidase, polyphenolic-rich fractions from *C. sativum* showed significant increase and it was well comparable with the known antioxidant, quercetin. Concentration of glutathione (Fig. 2) showed significant decrease in H_2O_2 control when compared to normal control. There was a significant increase in concentration of glutathione in all polyphenol-treated samples compared with H_2O_2 control and it was almost restored to normal level. Maximum increase in glutathione content was observed in the chloroform:ethyl acetate (3:1) polyphenolic fraction when compared to the positive control. When compared with the normal control, there was a significant increase in the concentration of TBARS (Fig. 2) in the H_2O_2 control. Significant decrease in concentration of TBARS was shown by all polyphenol-treated samples when compared with the H_2O_2 control. Among the polyphenolic fractions, EtAc:MeOH (3:1) showed a better ameliorating effect.

4. Discussion

Many studies have examined the possible benefits of antioxidants in altering, reversing or forestalling the negative effects of oxidative stress. The antioxidants defence system of the body is composed of different antioxidant components. The antioxidant capacities of these antioxidant compounds depend on which free rad-
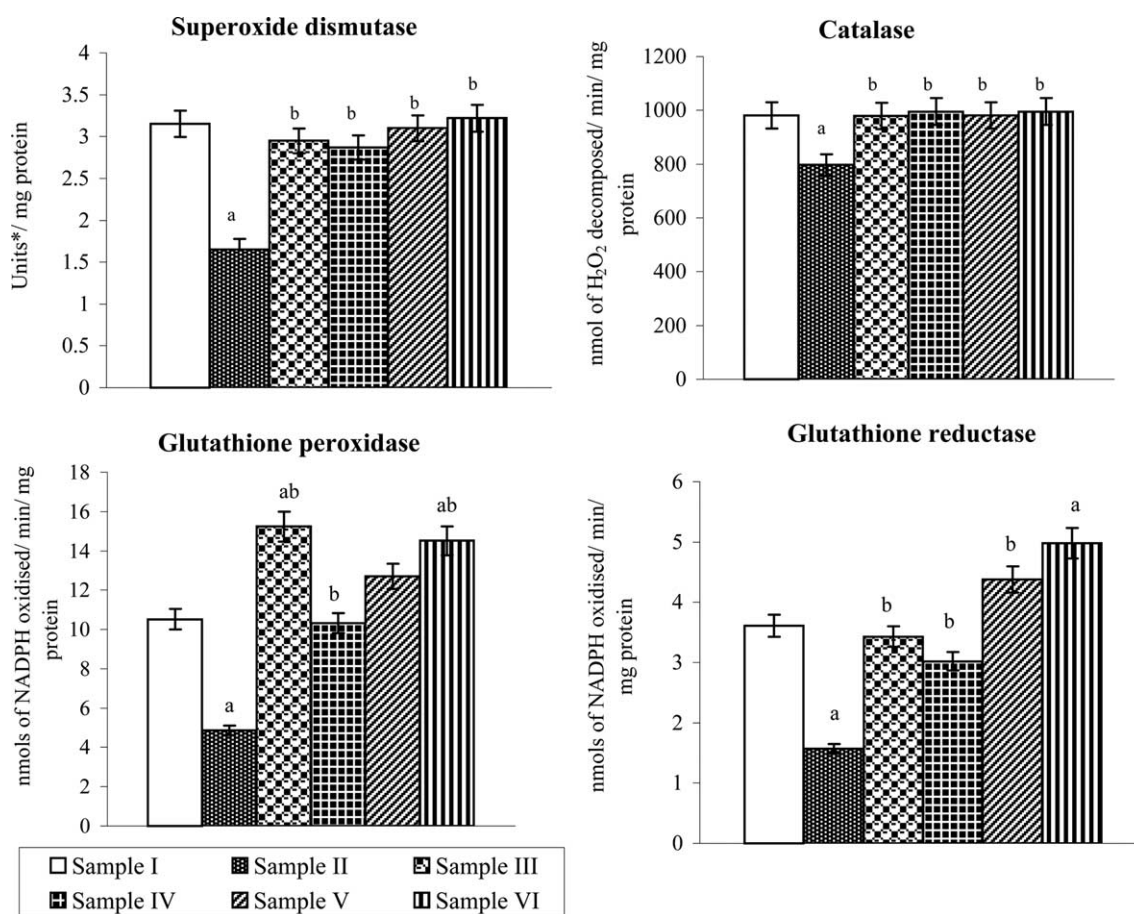

Fig. 1. Effect of polyphenolic compounds from *Coriandrum sativum* on activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. Values are expressed as means ± SEM, for *n* = 6. (a) Groups II to VI are compared to group I at $p \leqslant 0.05$. (b) Groups III to VI are compared to group II at $p \leqslant 0.05$.
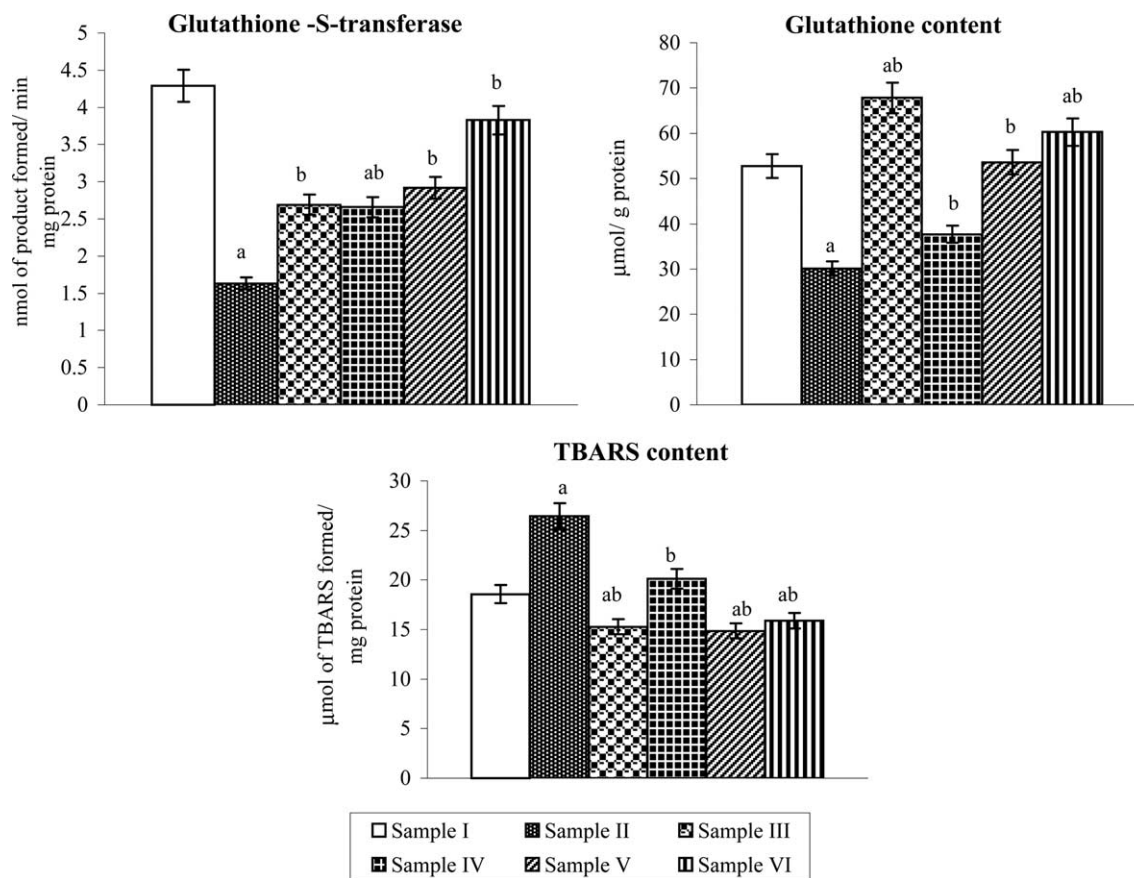
Fig. 2. Effect of polyphenolic compounds from *Coriandrum sativum* on activity of glutathione-*S*-transferase and the levels of reduced glutathione and TBARS. Values are expressed as means ± SEM, for $n = 6$. (a) Groups II to VI are compared to group I at $p \leqslant 0.05$. (b) Groups III to VI are compared to group II at $p \leqslant 0.05$.

icals or oxidants are produced in the body. The antioxidant enzyme system plays an important role in the defence of cells against oxidative insults. The study examined the ameliorating effect of polyphenolic rich fractions, isolated from *C. sativum*, on oxidative stress induced by H_2O_2 in human lymphocytes. The levels of glutathione and TBARS approached the normal control in all polyphenol-treated lymphocytes exposed to H_2O_2. Restoration of TBARS to nearly normal levels by these polyphenolic fractions may be due to an enhancement of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-*S*-transferase.

In the present study, H_2O_2 has been shown to induce oxidative stress in human lymphocytes. H_2O_2 treatment significantly decreased the activity of antioxidant enzymes in human lymphocytes. Our result is in agreement with several reports. Aherne and O'Brien (1999) showed that pre-treatment with 10–200 μM concentrations of both quercetin and rutin reduced DNA damage induced in Caco-2 and Hep G2 cells by a subsequent exposure to H_2O_2. Glei, Liegibel, Ebert, Bohm, and Pool-Zobel (2002) reported that β-carotene reduces H_2O_2-induced genetic damage in human lymphocytes when physiolog-

ical concentrations of water-soluble β-carotene and lycopene were incubated with lymphocytes and then treated with H_2O_2. Another report suggested that dietary flavonols protect diabetic human lymphocytes against oxidative damage to DNA (Lean et al., 1999). Docosahexaenoic acid protects human lymphocytes against H_2O_2-induced oxidative stress (Bechoua et al., 1999). Greenrod and Fenech (2003) reported that alcoholic and phenolic components of wine, such as catechin or caffeic acid are protective against DNA damaging and cytotoxic effects of H_2O_2 and γ-radiation in plasma or whole blood. Flavonoids can reduce macrophage oxidative stress by inhibition of cellular oxygenases or by activating cellular antioxidants (such as the glutathione system) (Fuhrman & Aviram, 2001). Excess hydrogen peroxide, induced by cadmium in A2780 ovarian cells, was significantly suppressed by isovitexin, a flavonoid in rice (Lin, Chen, Lee, & Lin, 2002). Quercetin, in a concentration range of 10–100 μM, was able to protect rat C6 glioma cells from cytotoxicity and lipid peroxidation (Zielinska et al., 2003).

It was observed, in the present study, that H_2O_2-treated lymphocytes showed significant elevation in the TBARS content. Polyphenols from *C. sativum* effectively

lowered the levels of lipid peroxides in H_2O_2-treated lymphocytes. Maximum reduction in TBARS contents were observed in both ethyl acetate:methanol (3:1) and chloroform:ethyl acetate (3:1) polyphenolic fractions. Reports by Halliwell and Chirico (1993) showed that polyphenols from red wine significantly decreased MDA levels in erythrocytes treated with H_2O_2. The mixture of flavonoids found in red wine lowered MDA levels and LDH leakage, maintained a higher reduced/oxidized glutathione ratio, and increased catalase/SOD and glutathione peroxidase/superoxide dismutase ratios, and glutathione reductase and glutathione-*S*-transferase activities (Roig, Cascon, Arola, Blade, & Salvado, 2002). Fruit juice, with cyanidin glycosides and epigallo-catechin gallate as major polyphenolic ingredients, decreased plasma malondialdehyde with time during juice interventions (Bub et al., 2003). Silymarin, catechin and phenolic acids had an inhibitory action against lipid peroxidation in human platelets, as reported by Koch and Loffler (1985). Flavonoids from aerial parts of *Achyrocline satureioides* (Lam) D.C. (Asteracea) significantly decreased bromobenzene-induced elevated levels of TBARS and also significantly elevated the depleted levels of liver glutathione (Kadarian et al., 2002). Reports showed that TBARS in the gastric mucosa, an index of lipid peroxidation were increased by ethanol injury, but the increase was inhibited by the administration of 200 mg/kg of quercetin, a natural flavone, through decrease of reactive O_2 metabolites (Martin et al., 1998). Pignol et al. (1988) reported antilipoperoxidative properties of flavonoids purified from *Ginko biloba* extract.

Reactive oxygen species (ROS), such as superoxide anions ($O_2^{\bullet-}$) and H_2O_2, are produced throughout cells during normal aerobic metabolism. The intracellular concentration of ROS is a consequence of both their production and their removal by various antioxidants. A major component of the antioxidant system in mammalian cells consists of three enzymes, namely, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These enzymes work in concert to detoxify $O_2^{\bullet-}$ and H_2O_2 in cells. Our results indicated that pretreatment of polyphenols from *C. sativum* caused an increase in the activity of antioxidant enzymes in H_2O_2-treated lymphocytes. Recent studies on the antioxidant properties of flavonoids reveal their stimulatory action on antioxidative enzymes (Miyake, Yamamoto, Tsujihara, & Osawa, 1998; Nagata, Takekoshi, Takagi, Honma, & Watanabe, 1999). Some flavonoids exert a stimulatory action on transcription and gene expression of certain antioxidant enzymes (Rohrdanz, Ohler, Tran-Thi, & Kahl, 2002).

Highly significant activities of superoxide dismutase and catalase were correlated with the reduction of lipid peroxides. Lang, Deak, Muzes, Pronai, and Feher (1993) reported that in vitro incubation of lymphocytes or erythrocytes with silymarin, at a concentration corresponding to the usual therapeutic dose, markedly increased the SOD expression of lymphocytes. Silymarin treatment, at a concentration achievable by in vitro treatment (10 μg/ml), significantly increased the SOD activity of erythrocytes and lymphocytes of patients with liver disease (Muzes et al., 1991). Another report showed that both in vitro and in vivo incubation of three hepato protective antioxidants (silymarin, cyanid-anol-3 and 4-amino5-imidazole) increased the expression and activity of SOD in lymphocytes of patients with alcoholic cirrhosis (Feher, Lang, Nekam, Muzes, & Deak, 1988).

Increase in activities of GPx, glutathione reductase (GRd) and glutathione-*S*-transferase (GST) were observed in samples treated with polyphenols from *C. sativum*. GPx catalyses the reduction of peroxides using reduced glutathione and converting into oxidised glutathione. Hence increased activities of the enzymes, GPx and GRd, were correlated with increased glutathione levels. This fact also demonstrated the antiperoxidative effect of polyphenols. It was reported that oral feeding of a polyphenolic fraction isolated from green tea in drinking water caused an increase in the activities of GPx and superoxide dismutase in mice (Khan, Lees, Douthwaite, Carrier, & Corder, 2002). Garcinol, isolated from *Garcinia indica* fruits has been reported to significantly elevate GST activities in rats, which might suggest its possible chemopreventive ability on colon tumirogenesis (Tanaka et al., 2000). La Casa, Villegas, Alarcon-de-la-Lastra, Motilva, and Martin (2000) reported that rutin, a natural flavone, at all tested doses, induced a significant increase in glutathione peroxidase activity and exerted a protective and antioxidant effect against ethanol-induced gastric lesions.

Treatment with three flavonoids, patuletin, nepetin, and axillarin, isolated from the *n*-butanol extract of *Inula britannica* (Asteraceae), significantly improved the decreased activities of antioxidant enzymes, such as superoxide dismutase, glutathione-peroxidase, and glutathione reductase, and also attenuated significant drops in glutathione induced by glutamate in primary cultured neurons (Kim et al., 2002). Higher levels of the antioxidant enzymes have been correlated with decreased susceptibility to cell damage (Werts & Gould, 1986). It can be concluded from the above observations that polyphenols from *C. sativum* act as antiperoxidative agents and warrant further study as promising drugs for prevention of oxidative damage in living systems.

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