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Antioxidant Effects of Extracts from *Cassia tora* L. Prepared under Different Degrees of Roasting on the Oxidative Damage to Biomolecules

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The effects of water extracts from *Cassia tora* L. (WECT) treated with different degrees of roasting (unroasted and roasted at 150, 200, and 250 °C) on the oxidative damage to deoxyribose, DNA, and DNA base in vitro were investigated. It was found that WECT alone induced a slight strand breaking of DNA. In the presence of Fe^{3+}/H_2O_2, WECT accelerated the strand breaking of DNA at a concentration of 2 μg/mL; however, it decreased with increasing concentrations (>5 μg/mL) of WECT. WECT also accelerated the oxidation of deoxyribose induced by Fe^{3+}–EDTA/H_2O_2 at a concentration of 0.2 mg/mL but inhibited the oxidation of deoxyribose induced by Fe^{3+}–EDTA/H_2O_2/ascorbic acid. Furthermore, WECT accelerated the oxidation of 2′-deoxyguanosine (2′-dG) to form 8-OH-2′-dG induced by Fe^{3+}–EDTA/H_2O_2. The prooxidant action of WECT on the oxidation of 2′-dG was in the order of unroasted > roasted at 150 °C > roasted at 200 °C > roasted at 250 °C. The decrease in the prooxidant activity of the roasted sample might be due to the reduction in its anthraquinone glycoside content or the formation of antioxidant Maillard reaction products after roasting. Thus, WECT exhibited either a prooxidant or an antioxidant property in the model system that was dependent on the activities of the reducing metal ions, scavenging hydroxyl radical, and chelating ferrous ion.

Keywords: *Cassia tora L.; roasting; antioxidant; prooxidant; oxidative damage; DNA; anthraquinones*

INTRODUCTION

Polyphenolic compounds are widely distributed in higher plants. Several epidemiological studies have shown that an increased dietary intake of natural phenolic antioxidants correlates with reduced coronary heart disease (Hertog et al., 1993; Stampfer et al., 1993). These compounds have also been found to exhibit many health-related properties that are based on their antioxidant activities. These properties include anticancer, antiviral, and antiinflammatory activities, effects on capillary fragility, and an ability to inhibit human platelet aggregation (Benavente-Garcísa et al., 1997). However, besides, these beneficial effects, some phenolic compounds have been found to be mutagenic in vitro. The harmful effects were suspected to be the result of prooxidant rather than antioxidant action by these compounds (Popp and Schimmer, 1991; Sahu and Gray, 1994). Many studies have shown that several phenolic antioxidants can accelerate oxidative damage in vitro to DNA, proteins, and carbohydrates despite their antioxidant action on the lipids (Laughton et al., 1989). It is, therefore, important to characterize the effect of antioxidants on all biological molecules and not just lipids (Aruoma et al., 1993). Moreover, many methods, including deoxyribose assay, oxidative DNA damage, and bleomycin–iron-dependent DNA damage, have been used to evaluate the prooxidant activity of a sample in order to know if antioxidants can promote biological molecular damage (Aruoma, 1996).

The Chinese herb "Jue-ming-zi", which is the seed of the plant *Cassia tora* L. (*Leguminosae*), has been used as a laxative and a tonic; it is also popularly used as a health tea drink. The commercial products of *C. tora* include both unroasted and roasted samples, and the laxative effect was found to be higher in unroasted *C. tora* than in the roasted sample (Zhang et al., 1996). Kim et al. (1994) reported that methanol extracts from *Cassia tora* exhibited a strong antioxidant activity on the lipid peroxidation. Yen et al. (1998) have also indicated that the antioxidant activity of methanolic extracts from *C. tora* was higher than that of *C. occidentalis* and also identified an antioxidative compound, emodin, from *C. tora.* However, whether the extracts of *C. tora* possess a prooxidant action toward biological molecules remains unclear. The objective of this study was to further investigate the effects of water extracts from *C. tora* prepared under different degrees of roasting on the DNA damage in vitro.

MATERIALS AND METHODS

Materials. The seeds of *C. tora* L. were obtained from a local market at Taichung, Taiwan. Deoxyribose, ascorbic acid, 2′-deoxyguanosine (2′-dG), gallic acid, trolox, ϕX174 DNA, 5,5-

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dimethyl-1-pyrroline *N*-oxide (DMPO), mannitol, mercaptoethanol, and 8-hydroxy-2′-deoxyguanosine (8-OH-2′-dG) were purchased from the Sigma Chemical Co. (St. Louis, MO). Iron(II) chloride tetrahydrate, sodium dihydrogen phosphate, disodium hydrogen phosphate, thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were purchased from the E. Merck Co. (Darmstadt, Germany).

Sample Preparation. To obtain *C. tora* L. seeds with different degrees of roasting, washed and sun-dried samples were unroasted or roasted at 150, 200, and 250 °C (internal temperature) for 5 min using an automatic roasting machine. Each unroasted and roasted sample (20 g) was extracted with boiling water (200 mL) for 10 min, and the filtrate was freeze-dried. The water extracts of *C. tora* L. were named as WECT.

Effect of WECT on Strand Breaking of Supercoiled DNA. The effect of WECT on the strand breakage of supercoiled DNA, induced by hydrogen peroxide, was analyzed according to the method of Kobayashi et al. (1990) with a slight modification. To a reaction mixture (20 μL) in sodium phosphate buffer solution composed of 0.3 μg of DNA, 0.1 mM H_2O_2, and 20 μM $FeCl_2$ were added different concentrations of WECT, followed by incubation at 37 °C for 1 h. The reaction was stopped by the addition of 5 μL of 0.1 M EDTA containing 50% (w/v) sucrose and 0.1% bromophenol blue, and the reaction mixture was analyzed by means of agarose gel electrophoresis (0.7% agarose) with 40% mM Tris-HCl/5 mM sodium acetate/1 mM EDTA (pH 7.1) as the running buffer. The DNA image was analyzed using 1D Image Analysis software (Kodak Digital Science, Rochester, NY). All analyses were run in duplicate and averaged.

Effect of WECT on the Oxidation of 2′-Deoxyguanosine. The effect of WECT on the oxidation of 2′-deoxyguanosine to 8-hydroxy-2′-deoxyguanosine was assayed as stated in the previous report by Yen et al. (1997). The reaction mixture (0.7 mL) contained WECT (1.4 mg), 2′-dG (0.5 mM), and KH_2PO_4−K_2HPO_4 buffer (0.1 M, pH 7.4) and was initiated by a Fenton reaction model system [H_2O_2 (50 mM), $FeCl_3$ (1.3 mM), and EDTA (6.5 mM)] with or without additional ascorbic acid (15 mM). The entire mixture was incubated at 37 °C for 30 min, and incubation was terminated by placing the samples in an ice bath, followed by filtering through a 0.45-μm filter before use. The filtrate was analyzed by HPLC (Hitachi, Japan), using the LiChrosphere RP-18 column (150 mm × 4 mm, 5 μm) and a UV detector (measured at 254 nm). The column was equilibrated with 50 mM KH_2PO_4 (pH 4.6)/methanol (93.5:6.5, v/v) at a flow rate of 0.5 mL/min. 2′-dG and 8-OH-2′-dG were identified by comparing their retention times with those of known standards and were determined by peak areas from the chromatograms. The control sample was without WECT and ascorbic acid. Also, the effects of gallic acid and trolox at a concentration of 2 mg/mL on the oxidation of 2′-dG were also compared.

Effect of WECT on Deoxyribose Damage. To test whether WECT had the ability to accelerate the oxidative damage of deoxyribose, a Fenton reaction model system that contained $FeCl_3$−EDTA and H_2O_2 was used (Smith et al., 1992). The reaction mixture (3.5 mL), which contained WECT (0−3.5 mg), deoxyribose (3 mM), H_2O_2 (1 mM), KH_2PO_4−K_2HPO_4 buffer (20 mM, pH 7.4), $FeCl_3$ (50 μM), and EDTA (100 μM), was incubated at 37 °C for 1 h, with or without additional ascorbic acid (100 μM). The extent of deoxyribose degradation was measured using the TBA method. One milliliter of 1% TBA and 1 mL of 2.8% TCA were added to the mixture, which was then heated in a water bath at 100 °C for 20 min. The absorbance of the resulting solution was measured spectrophotometrically at 532 nm. The control sample was without WECT and ascorbic acid. Moreover, the effects of gallic acid, mannitol, and mercaptoethanol at concentrations of 0−1 mg/mL on deoxyribose damage were also compared.

Determination of the Reducing Power. The reducing power of the samples was determined according to the method reported by Oyaizu (1986). WECT (2.5 mL, 1−10 mg/mL) was added to sodium phosphate buffer (0.2 M, pH 6.6, 2.5 mL) containing potassium ferricyanide [$K_3Fe(CN_6)$] (1%, 2.5 mL). The reaction mixture was incubated at 50 °C for 20 min, at the end of which 2.5 mL of trichloroacetic acid (10%) was added, and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (5 mL) was mixed with distilled water (5 mL) and $FeCl_3$ (0.1%, 1 mL), and the absorbance was measured at 700 nm. An increase in absorbance of the reaction mixture was taken as a measure of the reducing power of WECT.

Scavenging Effect of WECT on the Hydroxyl Radical. The hydroxyl radical generated reacted rapidly with nitrone spin-trap DMPO. The resultant DMPO−OH adduct was detected by means of electron paramagnetic resonance (EPR) spectrometry using an EPR spectrometer (Bruker EMX-10/12, Karlsruhe, Germany) set under the following conditions: with a magnetic field of 3483.5 G, modulation amplitude of 1.25 G, and a time constant of 163.84 ms. The EPR spectrum was recorded 2.5 min after 0.2 mL of WECT (10 mg/mL) was mixed with H_2O_2 (10 mM, 0.2 mL), ferrous ammonium sulfate (10 mM, 0.2 mL), and DMPO (0.3 M, 0.2 mL) in a phosphate buffer solution (pH 7.2, 0.2 mL) (Yen and Chen, 1995). A smaller peak of a sample indicated a higher scavenging activity on the hydroxyl radical. The data were the mean value of duplicates.

Chelating Activity of WECT on Fe^{2+}. The chelating activity of WECT on Fe^{2+} was measured according to the method of Dinis et al. (1994). WECT (0−5 mg/mL) was reacted with $FeCl_2$ (2 mM, 0.2 mL) and ferrozione (5 mM, 0.2 mL) for 10 min, and the spectrophotometric absorbance was determined at 562 nm. A lower level of absorbance indicated a stronger chelating activity.

Determination of the Anthraquinone Content. The total anthraquinones, including free anthraquinones (anthraquinones aglycon) and bound anthraquinones (anthraquinones *O*-glycosides), were determined according to the method of Koshioka and Takino (1978). Powdered WECT (0.5 g) was extracted with 30 mL of ether for 24 h. The ether extract was separated by means of filtration, and the residue was further extracted with ether several times until all the free anthraquinones were extracted. The ether extracts were combined and preserved for estimation of the free anthraquinones (extract I). The amount that remained after the extraction of free anthraquinones was refluxed with 2 mL of 12 N HCl and 15 mL of glacial acetic acid in a water bath for 1 h. After the mixture was cooled, 30 mL of ether was added and refluxing was continued for 15 min. After cooling, the ether/acid mixture was filtered into a separatory funnel, and the marc was extracted with 50 mL of ether. The combined ether extracts were washed with distilled water to remove excess acid and used for estimation of the bound anthraquinones (extract II).

The ether extracts I and II were treated with a sufficient volume of aqueous 5% sodium hydroxide solution containing 2% ammonia until the ether layer became colorless. The alkaline extract was measured at 526 nm from a standard curve prepared from authentic emodin. The molar extinction coefficient of emodin (ϵ) was 3.43×10^3.

Statistical Analysis. All analyses were run in triplicate and averaged, except the experiments of strand breakage of supercoiled DNA and scavenging on hydroxyl radical. Statistical analyses were performed according to the SAS Institute (1985) 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.

RESULTS AND DISCUSSION

Effect of WECT on Strand Breakage of Supercoiled DNA. From the results shown in Figure 1, it can be seen that WECT alone induced the strand breaking of DNA, which was concentration dependent. At a concentration of 10 μg/μL, the strand breaking of DNA induced by WECT was in the following order: roasted at 150 °C (12%, lane 7) > roasted at 200 °C (11%, lane 10) > unroasted (9%, lane 4) > roasted at 250 °C (6%, lane 13). Anthraquinones have been reported as the

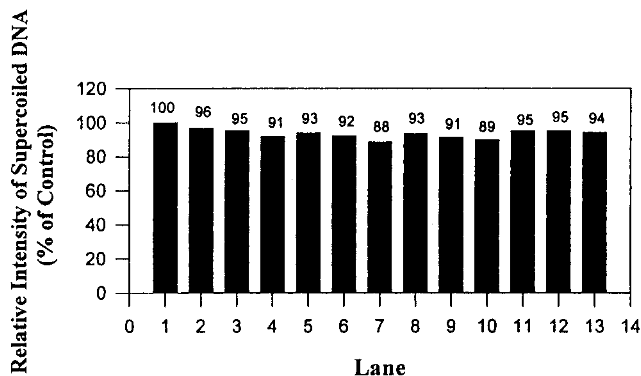

Figure 1. Effects of water extracts from *Cassia tora* L. (WECT) prepared under different degrees of roasting on DNA single-strand cleavage. The profiles of DNA relative intensity scanned from agarose gel electrophoretic patterns of Phage ϕX174 DNA. Phage ϕX174 DNA supercoiled DNA (0.1 μg) was incubated with unroasted WECT (2, 5, 10 μg/μL; lanes 2−4); 150 °C, WECT (2, 5, 10 μg/μL; lanes 5−7); 200 °C, WECT (2, 5, 10 μg/μL; lanes 8−10); and 250 °C, WECT (2, 5, 10 μg/μL; lanes 11−13). Lane 1 contained only DNA incubated without WECT.

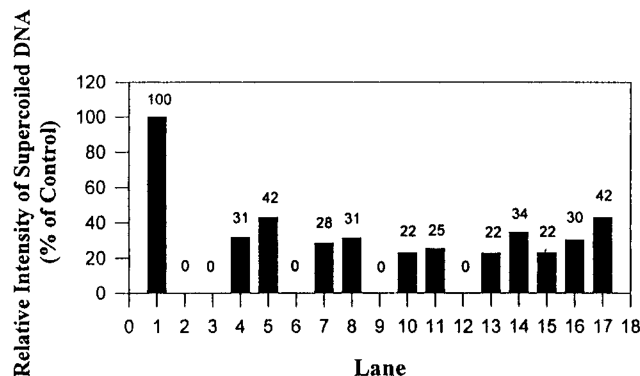

Figure 2. Effects of water extracts from *Cassia tora* L. (WECT) prepared under different degrees of roasting on DNA single-strand cleavage induced by Fe^{2+}/H_2O_2. The profile of DNA relative intensity scanned from agarose gel electrophoretic patterns of Phage ϕX174 DNA. Phage ϕX174 DNA supercoiled DNA (0.1 μg) was incubated with Fe^{2+}/H_2O_2 in the presence of unroasted WECT (2, 5, 10 μg/μL; lanes 3−5); 150 °C, WECT (2, 5, 10 μg/μL; lanes 6−8); 200 °C, WECT (2, 5, 10 μg/μL; lanes 9−11), 250 °C, WECT (2, 5, 10 μg/μL; lanes 12−14); and mannitol (2, 5, 10 μg/μL; lanes 15−17). Lanes 1 and 2 contained only DNA incubated without and with Fe^{2+}/H_2O_2.

main active compounds in *C. tora* (Duke, 1992). They have a quinone structure that easily oxidizes to semiquinone radicals and produces reactive oxygens (Hartman and Goldstein, 1989). Emodin is one of the anthraquinones that does not produce reactive oxygens; however, its derivative, 2-hydroxyemodin, can produce reactive oxygens and cause the strand breaking of ϕX174 DNA (Kodama et al., 1987). Therefore, the strand breaking of DNA induced by WECT might be due to the oxidation of anthraquinones to its free-radical form and/or production of reactive oxygens from other derivatives. Hiramoto et al. (1993) reported that the Maillard reaction products, prepared by heating glucose and amino acid at 200 °C for 5 min, can induce the strand breaking of DNA. Thus, the strand breaking of DNA induced by WECT alone may also be due to the Maillard reaction products that were formed during the roasting process or boiling water extraction.

Since DNA breakage usually occurs in the presence of metal ions, the effect of WECT on DNA breakage was evaluated in the presence of metal ions. From the results shown in Figure 2, it can be seen that the supercoiled DNA was completely broken in the presence of Fe^{2+}/H_2O_2 (lane 2). DNA breaking was not inhibited by the addition of WECT at a concentration of 2 μg/μL (Figure 2, lanes 3, 6, 9, and 12), whereas 23% of DNA breakage was reduced by mannitol at a concentration of 2 μg/μL. However, WECT showed an inhibitory effect on DNA breaking at a higher concentration. The unroasted WECT exhibited the greatest inhibitory effect on DNA breakage at a concentration of 10 μg/μL. This means that WECT can through chelating Fe^{2+}, scavenging H_2O_2 and hydroxyl radicals, and reducing the oxidative damage to DNA by Fenton reaction.

Figure 3 shows the effect of WECT on strand breaking of DNA in the presence of Fe^{3+}/H_2O_2. From the figure, it can be seen that 49% of DNA breakage was observed in the presence Fe^{3+}/H_2O_2 alone (lane 2). However, DNA was completely broken with the addition of different concentrations of ascorbic acid (lanes 3−5), which might be because the ascorbic acid induced an increase in the generation of hydroxyl radicals by reducing Fe^{3+} to Fe^{2+}- and maintained the Fenton reaction. The strand breaking of DNA was accelerated by the addition of WECT,

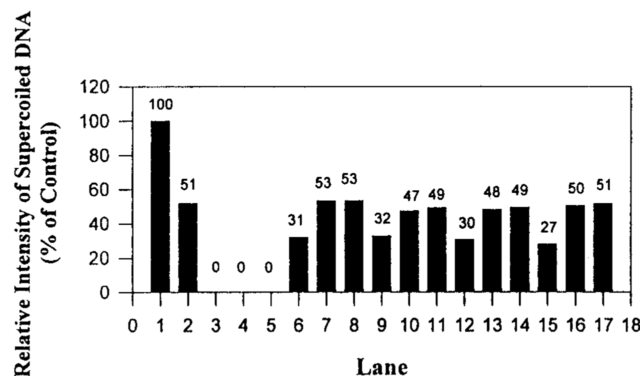

Figure 3. Effects of water extracts from *Cassia tora* L. (WECT) prepared under different degrees of roasting on DNA single-strand cleavage induced by Fe^{3+}/H_2O_2. The profile of DNA relative intensity scanned from agarose gel electrophoretic patterns of Phage ϕX174 DNA. Phage ϕX174 DNA supercoiled DNA (0.1 μg) was incubated with Fe^{3+}/H_2O_2 in the presence of ascorbic acid (lanes 3−5); unroasted WECT (2, 5, 10 μg/μL; lanes 6−8); 150 °C, WECT (2, 5, 10 μg/μL; lanes 9−11); 200 °C, WECT (2, 5, 10 μg/μL; lanes 12−14); 250 °C, WECT (2, 5, 10 μg/μL; lanes 15−17). Lanes 1 and 2 contained only DNA incubated without and with Fe^{3+}/H_2O_2.

especially at a lower concentration (2 μg/μL) (lanes 6, 9, 12, and 15), but this phenomenon was reduced at a higher concentration (5 or 10 μg/μL). WECT accelerated the DNA breakage in the Fe^{3+}/H_2O_2 system which might be because WECT reduced Fe^{3+} to Fe^{2+} and reacted with H_2O_2 to form the hydroxyl radicals. Many antioxidants, such as ascorbic acid, exhibit an antioxidant or a prooxidant property in different systems (Smith et al., 1992; Zhao and Jung, 1995). In the present study, WECT showed a prooxidant activity on DNA breakage at a lower concentration, but the prooxidant effect decreased at a higher concentration. This may also be related to the status of iron ions in the reaction system. In the reaction system, the additional iron ions will bind with DNA, since Shires (1982) demonstrated that ^{59}Fe can directly bind to DNA. Hence, the Fe^{3+} in DNA was reduced to Fe^{2+}, which produced hydroxyl radicals to attack DNA in the presence of reducing agents and H_2O_2. In this situation, the hydroxyl radical scavengers cannot inhibit DNA damage due to the "site-specific"

Table 1. Effects of Water Extracts from *C. tora* L. Prepared under Different Degrees of Roasting on the Oxidation of 2′-dG to 8-OH-2′-dG Induced by Fe^{3+}−EDTA/H_2O_2

sample[a]	8-OH-2′-dG, μg	
	without ascorbic acid	with ascorbic acid
control	0.42 ± 0.14[f] [b]	4.62 ± 0.84[c]
gallic acid	13.44 ± 0.70[a]	9.38 ± 0.70[a]
trolox	0.42 ± 0.14[f]	2.80 ± 0.56[d]
unroasted	9.52 ± 0.42[b]	7.14 ± 0.42[b]
roasted		
150 °C	7.56 ± 0.70[c]	6.72 ± 0.70[b]
200 °C	5.88 ± 0.70[d]	4.62 ± 0.84[c]
250 °C	1.96 ± 0.42[e]	2.80 ± 0.28[d]

[a] The concentration of water extracts from *C. tora* L., gallic acid, and trolox was 2 mg/mL. [b] Values in a column with different superscripts are significantly different at $P < 0.05$.

effect, which results from the fact that the half-life of the hydroxyl radical is very short and the distance between the hydroxyl radical and DNA is very small (Von Sonntag and Suchmann, 1994). Therefore, iron ions play an important role in the prooxidant action, and the site-specific DNA damage can be reduced when the iron ions in DNA are removed by the chelating agents. The iron ions bound to the chelating agent become a free form in an aqueous solution; thus, the produced hydroxyl radical can be scavenged.

In general, the antioxidants, such as ascorbic acid and gallic acid, exhibited prooxidant and antioxidant activities in a reaction system; this may be because the antioxidants have multiple properties, such as reducing power, chelating metal ions, and scavenging free radicals. Yen et al. (1997) indicated that tea extracts have prooxidant and antioxidant effects in different systems due to their ability to reduce metal ions and scavenge reactive oxygens. Since iron ions should be bound to the DNA in this system, the antioxidants induced prooxidant action at a lower concentration due to their reducing power. As the antioxidant concentration increased, iron ions in DNA were chelated by antioxidant and removed. The target of site-specific damage was changed to antioxidants; thus, the hydroxyl radicals generated can be scavenged by the antioxidants and, thus, reduce the DNA damage.

Effect of WECT on the Oxidation of 2′-dG. From the results shown in Figures 1−3, it was observed that WECT induced oxidative damage to DNA under certain conditions. In general, the role of reactive oxygen species on DNA damage could be revealed by determining the damaged DNA base, e.g., thymidineglycol, 5-hydroxymethyl-2′-deoxyuridine or 8-OH-2′-dG (Frenkel, 1992). The formation of 8-OH-2′-dG from 2′-dG in the presence of Fe^{3+}−EDTA/H_2O_2 was analyzed by means of HPLC. The retention times for 2′-dG and 8-OH-2′-dG were 10.85 and 15.73 min, respectively (data not shown). Incubation of ascorbic acid and Fe^{3+}−EDTA/H_2O_2 at 37 °C led to a marked oxidation of 2′-dG to 8-OH-2′-dG. As seen in the results shown in Table 1, the oxidation of 2′-dG was significantly accelerated ($P < 0.05$) by the addition of gallic acid or WECT at a concentration of 2 mg/mL to the Fe^{3+}−EDTA/H_2O_2 system. However, the prooxidant action of WECT decreased with the increase of the extent of roasting ($P < 0.05$). Trolox (2 mg/mL) showed no prooxidant activity in this reaction system ($P > 0.05$). The oxidation of 2′-dG was also accelerated by the addition of WECT to the Fe^{3+}−EDTA/H_2O_2/ascorbate system, but it was weaker than that without ascorbic acid.

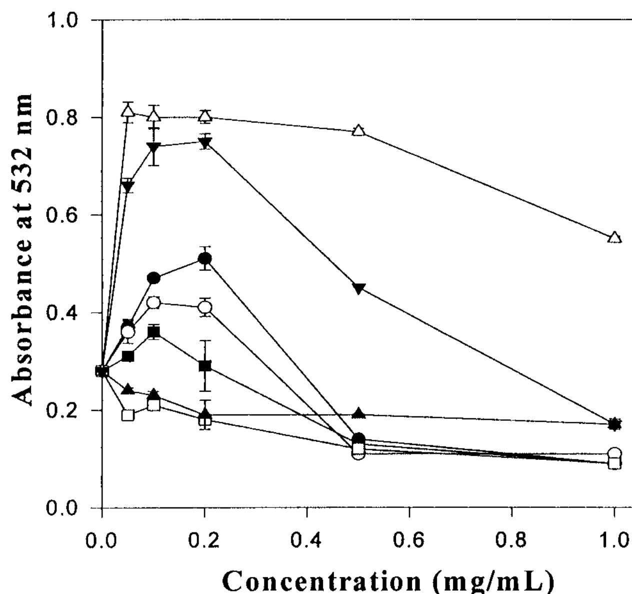

Figure 4. Effects of water extracts *Cassia tora* L. prepared under different roasting conditions on deoxyribose damage induced by Fe^{3+}-EDTA/H_2O_2. Unroasted (●); roasted at 150 °C, 5 min (○); roasted at 200 °C, 5 min (■); roasted at 250 °C, 5 min (□); mannitol (▲); gallic acid (△); mercaptoethanol (▼).

Guillot et al. (1996) reported that caffeic acid and its thermal degradation products accelerated the oxidation of 2′-dG to 8-OH-2′-dG in the presence of Fe^{3+}−EDTA. They also attributed this result to the reduction of Fe^{3+}. Therefore, the prooxidant activity of WECT might be due to the reducing power or the oxidation of its components. The pharmacological effect of *C. tora* is mainly due to its anthraquinone compounds, e.g., chrysophanol, emodin, etc. Although anthraquinones have many pharmacological effects, they produce superoxide anions after oxidation (Hartman and Goldstein, 1989). Therefore, besides the reducing power, the prooxidant activity of WECT may also be due to the partial oxidation of anthraquinones in the presence of ferrous ion, which produces reactive oxygens and induces the oxidation of 2′-dG.

Effect of WECT on Deoxyribose Damage. The deoxyribose assay has been used to assess the prooxidant properties of the samples (Halliwell et al., 1987). The effect of WECT on the oxidative damage of deoxyribose induced by Fe^{3+}−EDTA/H_2O_2 is shown in Figure 4. No prooxidant activity was found in water extracts from *C. tora* roasted at 250 °C and mannitol. WECT prepared from the samples roasted at 150 and 200 °C accelerated the oxidative damage of deoxyribose at a concentration of 0.2 mg/mL, but its prooxidant activity was weaker than that of gallic acid and mercaptoethanol. The prooxidant activity of these samples was in the order of gallic acid > mercaptoethanol > unroasted > 150 °C roasted > 200 °C roasted at a concentration of 0.2 mg/mL ($P < 0.05$). The prooxidant activity of all the WECT samples gradually increased with increasing concentration up to 0.2 mg/mL, while all the samples exhibited antioxidant activity at a concentration of 0.5 mg/mL. Gallic acid and mercaptoethanol also showed similar results.

Smith et al. (1992) reported that several flavonoids, such as morin, quercetin, myricetin, and rosemary extracts, accelerated the generation of hydroxyl radicals and stimulated damage to deoxyribose and DNA in the Fe^{3+}−EDTA/H_2O_2 system. However, flavonoid-induced

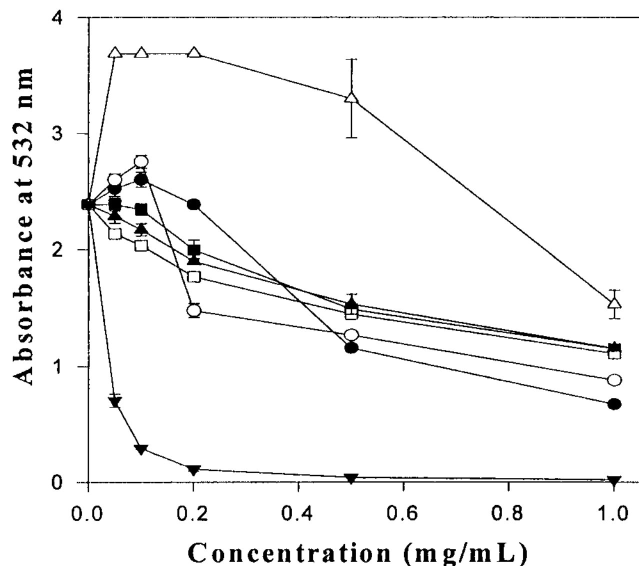

Figure 5. Effect of water extracts from *Cassia tora* L. prepared under different roasting conditions on deoxyribose damage induced by Fe^{3+}–EDTA/H_2O_2/ascorbic acid. Unroasted (●); roasted at 150 °C, 5 min (○); roasted at 200 °C, 5 min (■); roasted at 250 °C, 5 min (■); mannitol (▲); gallic acid (△); mercaptoethanol (▼).

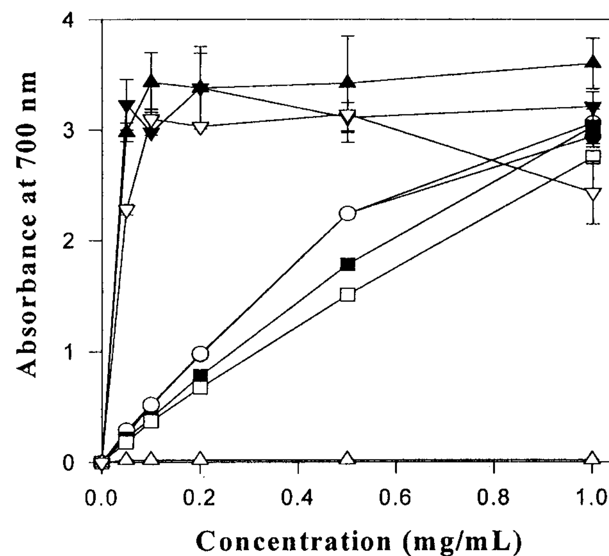

Figure 6. Reducing power of water extracts from *Cassia tora* L. prepared with different roasting conditions. *Reducing power: a high absorbance indicated a high reducing power. Unroasted (●); roasted at 150 °C, 5 min (○); roasted at 200 °C, 5 min (■); roasted at 250 °C, 5 min (□); ascorbic acid (▲); mannitol (△); gallic acid (▼); mercaptoethanol (▽).

damage can be reduced by adding albumin. Aruoma et al. (1993) indicated that gallic acid and its derivatives had prooxidant activity in the deoxyribose assay and bleomycin-dependent DNA damage system. They indicated that the prooxidant activity was caused when the reducing substances accelerated the production of hydroxyl radical in the Fenton reaction. Mercaptoethanol is a strong reducing agent and has shown a prooxidant action in the present study. Therefore, the prooxidant activity of WECT (unroasted, 150 °C roasted, and 200 °C roasted) at concentrations lower than 0.2 mg/mL may result from the reduction of Fe^{3+} to Fe^{2+}.

The decrease in prooxidant activity at higher concentrations might have resulted from competition between the antioxidative and prooxidative action. Zhao and Jung (1995) indicated that ascorbic acid accelerated oxidative damage in deoxyribose, but it decreased damage at higher concentrations. This occurs because ascorbic acid can reduce Fe^{3+} to Fe^{2+} and stimulate the formation of hydroxyl radicals at lower concentrations. Yen et al. (1997) also indicated that tea extracts might act as antioxidants or prooxidants, depending on their ability to reduce iron and scavenge the hydroxyl radicals. Thus, WECT may have a strong scavenging activity on the hydroxyl radicals at higher concentrations and thus reduce its prooxidant activity.

To evaluate the inhibitory effect of WECT on hydroxyl radicals produced by the Fenton reaction, an Fe^{3+}–EDTA/H_2O_2/ascorbic acid system was used to generate hydroxyl radical to induce oxidative damage in deoxyribose. From the results shown in Figure 5, it can be seen that the deoxyribose damage increased by 5.5 times with the addition of 100 μM ascorbic acid in the system when compared with the control group (Figure 4). When unroasted or 150 °C roasted WECT was added into the reaction system, it induced deoxyribose damage at concentrations lower than 0.2 mg/mL ($P < 0.05$) but inhibited the damage at higher concentrations. Also, they showed a 63–71% inhibitory effect on deoxyribose damage at a concentration of 1 mg/mL; the inhibition was higher than that caused by gallic acid ($P < 0.05$). Gallic acid had a prooxidant action at concentrations lower than 1 mg/mL. However, WECT prepared by roasting at 200 and 250 °C did not cause any degradation of deoxyribose in the system.

As shown in Figure 4, mercaptoethanol exhibited prooxidant action, but this action decreased at higher concentrations; however, it showed antioxidant activity through scavenging of hydroxyl radicals in this reaction system. Zhao and Jung (1995) pointed out that the reaction rate of mercaptoethanol and hydroxyl radical is 6.74×10^9 M^{-1} s^{-1}; thus, it can inhibit the Fenton reaction induced by ascorbic acid. It can be predicted that the prooxidant activity of samples will decrease when its scavenging activity on hydroxyl radicals is greater than the formation of hydroxyl radicals in the Fenton reaction accelerated by reducing substances.

Reducing Power of WECT. From the above results, it is indicated that WECT showed prooxidant activity induced by metal ions, which resulted in the oxidative damage in the biomolecules. Some researchers (Aruoma et al., 1993; Zhao and Jung, 1995) have reported that the prooxidant activity of some antioxidants was mainly due to reducing Fe^{3+}, which caused the process of the Fenton reaction. Therefore, the reducing power of WECT was evaluated here in order to understand its role in prooxidant action. From the results shown in Figure 6, WECT showed the reducing power in the order of unroasted = 150 °C roasted > 200 °C roasted > 250 °C roasted at concentrations of 0.1–0.5 mg/mL ($P < 0.05$). This trend is similar to the prooxidant activity of WECT. However, both the reducing power and the prooxidant activity of WECT were lower than that of ascorbic acid and gallic acid ($P < 0.05$). In addition, mannitol had no reducing power, and it also did not exhibit any prooxidant activity. Thus, the prooxidant activity of WECT should be related to its reducing power.

Scavenging Activity of WECT on the Hydroxyl Radical. The effects of WECT on the intensity of the DMPO–OH adduct was determined using an EPR spectrometer. From the results shown in Table 2, the

Table 2. Effects of Water Extracts from *C. tora* L. Prepared under Different Degrees of Roasting on the EPR Spectrum Signal Intensity of DMPO−OH Spin Adducts

sample	concn, mg/mL	rel EPR signal intensity[a]
unroasted	0	100
	0.2	43.95
	1	17.65
	4	11.87
	10	2.76
roasted		
150 °C, 5 min	10	5.03
200 °C, 5 min	10	6.91
250 °C, 5 min	10	12.20

[a] The data were the mean value of duplicates. Relative EPR signal intensity (%) $=\{[h\Delta H^2(\text{sample})/h\Delta H^2(\text{dpph})]/[h\Delta H^2(\text{control})/h\Delta H^2(\text{dpph})]\}100$. h = height of the peak. ΔH = width of the peak.

intensity of the DMPO−OH adduct decreased with the increase in the concentration of extracts of unroasted *C. tora*. At the same concentration (10 mg/mL), the scavenging effect of WECT on hydroxyl radicals was in the order of unroasted > 150 °C roasted > 200 °C roasted > 250 °C roasted. This trend is also in agreement with the above result that the antioxidant activity of the extracts of unroasted samples was greater than that of roasted samples. The scavenging activity of WECT on hydroxyl radicals also increased with an increase in the concentration. Thus, the scavenging activity of WECT on the hydroxyl radical was greater than its prooxidant activity at higher concentrations, which thus resulted in its antioxidant activity.

Chelating Effect of WECT. Metal ions play an important role in the acceleration of either lipid peroxidation or DNA damage. The DNA breakage induced by iron ions occurs as a result of the effect of site-specific damage, where iron ions first bind to DNA and then react with H_2O_2 to generate hydroxyl radicals which attack DNA (Chevion, 1988). In studies on the oxidative damage to DNA, deoxyribose, and 2′-deoxyguanosine, the additional iron ions were found to bind with DNA and cause prooxidant action in the presence of reducing agents. However, at higher concentrations of WECT, the iron ions were chelated by WECT from DNA, which resulted in a decrease of prooxidant action. From the results shown in Figure 7, WECT had a chelating effect on ferrous ion, and this chelating ability linearly increased with an increase in the WECT concentration of greater than 0.5 mg/mL ($P < 0.05$). When the DNA was damaged due to the site-specific effect, the hydroxyl radical scavengers could not carry out their functions. Thus, the decreased prooxidant action cannot only be attributed to the scavenging hydroxyl radical but also to the chelating effect of WECT.

Change in the Total Anthraquinones of WECT with Different Degrees of Roasting. Anthraquinones have been reported to be the main active components in *C. tora*, including aloe−emodin, anthrone, aurantiobtusin, chrysophanic acid, emodin, obtusifolin, physcion, rhein, etc. (Duke, 1992). Thus, the content of anthraquinones in WECT was determined to understand their role in antioxidant and prooxidant actions. From the results shown in Table 3, the total content of anthraquinones in WECT was in the order of unroasted (88.2 mg/g) > 150 °C roasted (70.7 mg/g) > 200 °C roasted (26.9 mg/g) > 250 °C roasted (14.9 mg/g) ($P < 0.05$). The above result indicates that anthraquinones were degraded by thermal treatment. The data also

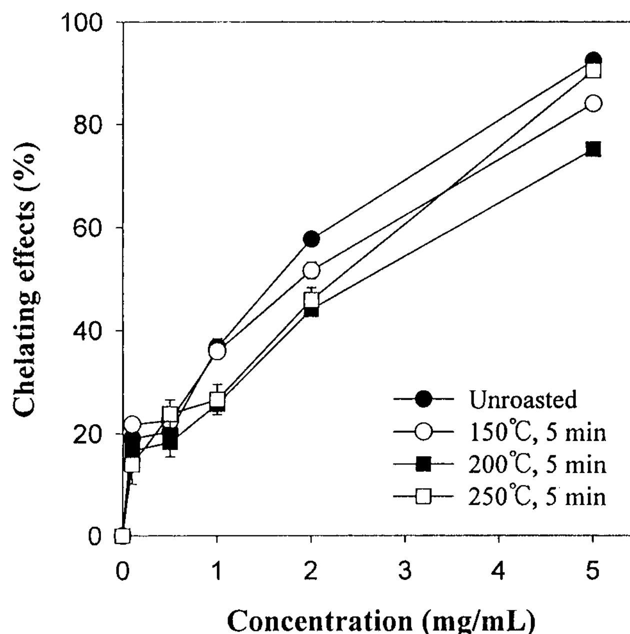

Figure 7. Binding effect of water extracts from *Cassia tora* L. prepared under different roasting conditions on Fe^{2+}.

Table 3. Contents of Anthraquinones in Water Extract from *C. tora* L. Prepared under Different Degrees of Roasting

sample	free, mg/g	bound, mg/g	total, mg/g
unroasted	0.89 ± 0.05^{a} [a]	87.35 ± 0.45^{a}	88.24^{a}
roasted			
150 °C, 5 min	0.78 ± 0.08^{ab}	69.90 ± 0.02^{b}	70.68^{b}
200 °C, 5 min	0.65 ± 0.06^{bc}	26.25 ± 1.27^{c}	26.90^{c}
250 °C, 5 min	0.54 ± 0.10^{c}	14.37 ± 0.98^{d}	14.91^{d}

[a] Values in a column with different superscripts are significantly different at $P < 0.05$.

indicate that most of the anthraquinones in WECT are in a bound form and contain glycosides. This is in agreement with the studies of Fairbirn and Moss (1970), who reported that the anthraquinones in *Cassia* plants are partly free but mostly glycosides (with aglycon occurring usually as a reduced form, e.g., anthrones). Malterud et al. (1993) reported that anthrones (reduced form) have better antioxidant activity than do anthraquinones on lipid peroxidation and free-radical scavenging. However, anthrones have reducing power and are easily oxidized to anthraquinones, which causes anthrones to have prooxidant activity. Huang et al. (1992) indicated that some anthraquinones, e.g., emodin, have the properties of immunosupression, vessel relaxation, and reduction of blood lipid. They suggested that the mechanism might be due to the formation of free radicals and hydrogen peroxide during the oxidation of emodin to semiquinone. Thus, anthraquinones may cause the prooxidant action of *C. tora*, but it may also have physiological effect.

Conclusions. Based on the results of the present study, *C. tora* extracts showed a prooxidant action in the presence of the induction of transitional metals and caused oxidative damage of biomolecules. The prooxidant activity of *C. tora* extracts found at low concentrations might have been due to its reducing power and the binding effect on metal ions. However, the *C. tora* extracts demonstrated antioxidant activity as the concentration increased. Therefore, when *C. tora* extracts are used as dietary antioxidants, their antioxidant

activity is dependent on the reaction system and the concentration.

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