http://dx.doi.org/10.5012/bkcs.2014.35.1.117

Chemistry Study on Protective Effect against ·OH-induced DNA Damage and Antioxidant Mechanism of *Cortex Magnoliae Officinalis*

Xican Li,[a,*] Qian Fang,[a] Jing Lin, Zhengpeng Yuan, Lu Han, and Yaoxiang Gao

School of Chinese Herbal Medicine, Guangzhou University of Chinese Medicine, Guangzhou 510006, China
[*]E-mail: lixican@126.com
Received August 30, 2013, Accepted October 14, 2013

As a Chinese herbal medicine used in East Asia for thousands years, *Cortex Magnoliae Officinalis* (CMO) was observed to possess a protective effect against ·OH-induced DNA damage in the study. To explore the mechanism, the antioxidant effects and chemical contents of five CMO extracts were determined by various methods. On the basis of mechanistic analysis, and correlation analysis between antioxidant effects & chemical contents, it can be concluded that CMO exhibits a protective effect against ·OH-induced DNA damage, and the effect can be attributed to the existence of phenolic compounds, especially magnolol and honokiol. They exert the protective effect *via* antioxidant mechanism which may be mediated *via* hydrogen atom transfer (HAT) and/or sequential electron proton transfer (SEPT). In the process, the phenolic –OH moiety in phenylpropanoids is oxidized to the stable quinine-like form and the stability of quinine-like can be ultimately responsible for the antioxidant.

Key Words : *Cortex Magnoliae Officinalis*, DNA damage, Antioxidant, Hydroxyl radical, Phenylpropanoid

Introduction

As we know, reactive oxygen species (ROS) are various forms of activated oxygen including free radicals and non-free-radical species. ROS, particularly hydroxyl radical (·OH) with high reactivity, can attack DNA to cause transient and subsequent stable damages which are closely associated with various biological consequences, including mutagenes, carcinogenesis, aging, premature senescence, hereditary and degenerative diseases. To prevent against DNA damage, cells have evolved elaborate DNA repair machinery, including enzymatic or non-enzymatic repairs. Enzymatic repair is generally considered as the major repair system of organisms and it has been widely investigated.[1]

Unlike enzymatic repair, fast non-enzymatic repair however is not well-known yet. Zheng and colleagues reported that it usually only needs several μs and is one billion times faster than the enzymatic one. It has been demonstrated to be accomplished by phenolics naturally occurring plants (especially Chinese herbal medicine).[1-3] The works of Zheng and colleagues, however, focused on the interaction of phytophenolic antioxidant and transient DNA radicals.[1] And little attention has been devoted to additional insights into the antioxidant mechanism of Chinese herbal medicine and the relevant phytophenols.

Recent studies have pointed out that *Cortex Magnoliae Officinalis* (CMO, Suppl. 1), a Chinese herbal medicine used in East Asia for over thousands years, presents anti-clastogenic[4] and antineoplastic potentials.[5] Therefore, we tried to use CMO as a reference to address the following problems: (*i*) Is the protective effect of phytophenol implicated with the other components in Chinese herbal medicine? (*ii*) How and why phytophenols exert the repair action on DNA damage?

As we know, two phenolic phenylpropanoids magnolol and honokiol are regarded as the bioactive compounds in CMO. Undoubtedly, the study will also enhance the understanding of the interaction between phenylpropanoid and DNA mediated by ROS, and will play an important role in biochemistry.

Experimental

Chemicals and Plant. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH·), pyrogallol, (±)-6-hydroxyl-2,5,7,8-tetramethlychromane-2-carboxylic acid (Trolox), and neocuproine were from Sigma-Aldrich Shanghai Trading Co. (Shanghai, China). 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) (ABTS) was obtained from Amresco Inc. (Solon, OH, USA). Uracil, cytosine, adenine, thymine, guanine, and DNA sodium salt (fish sperm) were purchased from the Aladdin Chemistry Co. (Shanghai, China). Methanol and water were of HPLC grade. All other reagents were of analytical grade. Dried *Cortex Magnoliae Officinalis* was obtained from Yanghe Pharmacy of Guangzhou University of Chinese Medicine (Guangzhou, China), and authenticated by Professor Shuhui Tan. A voucher specimen was deposited in our laboratory.

Preparation of Five Extracts from *Cortex Magnoliae Officinalis*. The dried and powdered plant material was extracted in sequence with petroleum ether (60-90), ethyl acetate, absolute ethanol, 95% ethanol and water by Soxhlet extractor to respectively prepare petroleum ether extract (PEM), ethyl acetate extract (EAM), absolute ethanol extract (AEM), 95% ethanol extract (95EM), and water extract

[a]These authors contributed equally to this work.

(WM) (Suppl. 2). These extracts were concentrated under reduced pressure to a constant weight. The dried extracts were then stored at 4 °C until used.

Protective Effect Against ·OH-induced DNA Damage. The experiment was conducted using our method.[6] Briefly, sample was dissolved in methanol to prepare the sample solution. Various amounts (5-20 μL) of sample solutions (0.4 mg/mL) were then separately taken into mini tubes. After evaporating the sample solution in tube to dryness, 450 μL phosphate buffer (0.2 M, pH 7.4) was brought to the sample residue. Then, 100 μL DNA (10.0 mg/mL), 75 μL H_2O_2 (33.6 mM), 50 μL $FeCl_3$ (0.3 mM) and 100 μL Na_2EDTA solutions (0.5 mM) were added. The reaction was initiated by mixing 75 μL ascorbic acid (12 mM) and the total volume of the reaction mixture was adjusted to 800 μL with buffer. After incubation in a water bath at 55 °C for 20 min, the reaction was terminated by 250 μL trichloroacetic acid (0.6 M). The colour was then developed by addition of 150 μL 2-thiobarbituric acid (TBA) (0.4 M, in 1.25% NaOH aqueous solution) and heated in an oven at 105 °C for 15 min. The mixture was cooled and absorbance was measured at 530 nm (Unico 2100, Shanghai, China). The percent of protection of DNA is expressed as follows:

$$\text{Protective effect \%} = \frac{A_0 - A}{A_0} \times 100\%$$

Where A_0 is the absorbance of the control without sample; and A is the absorbance of the reaction mixture with sample.

Superoxide ($\cdot O_2^-$) Radical-scavenging Assay. Superoxide ($\cdot O_2^-$) radical-scavenging was measured by our method.[7] Briefly, 0.4 mg/mL sample solution *x* μL (*x* = 0, 10, 20, 30, 40, and 50) was mixed with (580-*x*) μL Tris-HCl buffer (0.05 M, pH 7.4) containing EDTA (1 mM). After 20 μL pyrogallol (60 mM in 1 mM HCl) was added, the mixture was shaken rapidly at room temperature. The absorbance was immediately measured at 325 nm with a spectrophotometer (Unico 2100, Shanghai, China), against the Tris-HCl buffer as blank every 30 s for 5 min. The slope of the correlation of absorbance with time was calculated. The reaction mixture without sample was used as the control. The $\cdot O_2^-$ scavenging ability was calculated as:

$$\left(\frac{\Delta A_{325nm,control}}{T} - \frac{\Delta A_{325nm,sample}}{T}\right) / \frac{\Delta A_{325nm,control}}{T} \times 100\%$$

Here, $\Delta A_{325nm,\ control}$ is the increase in A_{325nm} of the mixture without the sample and $\Delta A_{325nm,\ sample}$ is that for the mixture with the sample; T = 5 min.

DPPH· Radical-scavenging Assay. The DPPH· radical-scavenging activity was determined as described.[8] Briefly, 1000 μL of DPPH· solution (0.1 mM) was mixed with 500 μL sample solution with various concentrations (in 95% ethanol). The mixture was kept at room temperature for 30 min, and then the absorbance was measured at 519 nm on a spectrophotometer, using 95% ethanol as the blank. The DPPH· inhibition percentages of the samples were calculated:

$$\text{Inhibition \%} = \frac{A_0 - A}{A_0} \times 100\%$$

Where A is the absorbance with samples; and A_0 is the absorbance without samples.

ABST$^{\cdot+}$ Radical-scavenging Assay. The ABTS$^{\cdot+}$ scavenging activity was evaluated by the method.[9] The ABTS$^{\cdot+}$ was produced by mixing 200 μL ABTS diammonium salt (7.4 mM) with 200 μL potassium persulfate (2.6 mM). The mixture was kept in the dark at room temperature for 12 h to allow completion of radical generation, then diluted with 95% ethanol (about 1:50) so that its absorbance at 734 nm was 0.70 ± 0.02. To determine the scavenging activity, 1200 μL of ABTS$^{\cdot+}$ reagent was mixed with 30 μL sample solutions (1 mg/mL), the total volume of system was adjusted to 1500 uL with 95% ethanol, and the absorbance at 734 nm was measured 6 min after the initial mixing, using 95% ethanol as the blank. The percentage inhibition was calculated as:

$$\text{Inhibition \%} = \frac{A_0 - A}{A_0} \times 100\%$$

Where A_0 is the absorbance of the negative control without any samples, A is the absorbance of the mixture with samples.

Cu^{2+}-reducing Power Assay. The cupric ions (Cu^{2+}) reducing capacity was determined according to the method,[8] with minor modifications. Briefly, $CuSO_4$ aqueous solution (125 μL, 10 mmol/L), neocuproine ethanolic solution (125 μL, 7.5 mmol/L) and (750-*x*) μL CH_3COONH_4 buffer solution (0.1 mol/L, pH 7.5) were brought to test tubes with different volumes of samples (1 mg/mL, *x* = 30-150 μL). Then, the total volume was adjusted to 1000 μL with the buffer and mixed vigorously. Absorbance against a buffer blank was measured at 450 nm after 30 min (Unico 2100). The relative reducing power of the sample as compared with the maximum absorbance, was calculated by the formula:

$$\text{Relative reducing effect \%} = \frac{A - A_{min}}{A_{max} - A_{min}} \times 100\%$$

where, A_{max} is the maximum absorbance at 450 nm and A_{min} is the minimum absorbance in the test. A is the absorbance of sample.

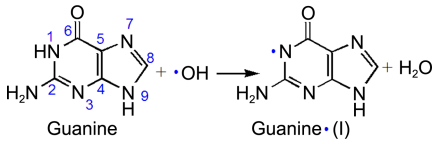
Determination of Chemical Contents. Five CMO extracts were determined for the chemical contents, including total phenolics, total sugars, magnolol, and honokiol. The total phenolics contents were determined by the Folin-Ciocalteu method,[10] and pyrogallol was used as the reference compound to establish the standard curve ($y = 0.08424+101.52994x$, R = 0.9996, Suppl. 3A). The total sugars contents were measured by phenol-sulfuric acid method,[10] in which glucose was used to act as standard to establish the standard curve ($y = 0.00543+39.09828x$, R = 0.9998, Suppl. 3B). The contents of magnolol and honokiol, however, were estimated by HPLC analysis which was performed on a Syltech P510 system (Los Angeles, California, USA), equipped with Diamonsil C_{18} (250 mm × 4.6 mm, 5 μm) (Dikma Co., Beijing, China). All samples were dissolved in methanol at 10 mg/mL and filtered through a 0.45 μm membrane. The mobile phase was methanol:acetonitrile:0.5% acetate acid (50:28:22) and the flow rate was 1.0 mL/min, injection volume was 10 μL, detection wavelength was 294 nm. Magnolol and honokiol

in CMO extracts were identified by the retention times and the peak areas were used to characterize the relative contents in the study.

Statistical Analysis. Each experiment was performed for three times, and the results were presented as mean ± standard deviations (SD). Based on the dose-response curve, the IC_{50} value was calculated in the study. Determination of significant differences between the mean IC_{50} values was performed using one-way ANOVA the T-test. The analysis was performed using SPSS software 13.0 (SPSS Inc., Chicago, IL) for windows. $P < 0.05$ was considered to be statistically significant. In the correlation graphs, the correlation coefficients (R values) were calculated by Origin 6.0 professional software.

Results and Discussion

As the most reactive ROS, hydroxyl radical (·OH) can easily attack various classes of DNA to firstly bring about transient DNA· radical.[1] For example, two latest reports pointed out that ·OH-induced guanine damage may produce a transient radical (I) (Eq. 1), mainly *via* a hydrogen atom abstraction mechanism not addition to C_8.[11,12]



(1)

In the process, radical (I) can further transfer into various carbon-centered radicals or nitrogen-centered radicals *via* tautomerization. Nevertheless, if these transient radicals cannot be non-enzymatically or enzymatically repaired in time, it will directly cause intrastrand cross-links and damage,[13] or transfer to various oxidative lesions[11] (Suppl. 4), to cause mutagenesis even cancer.

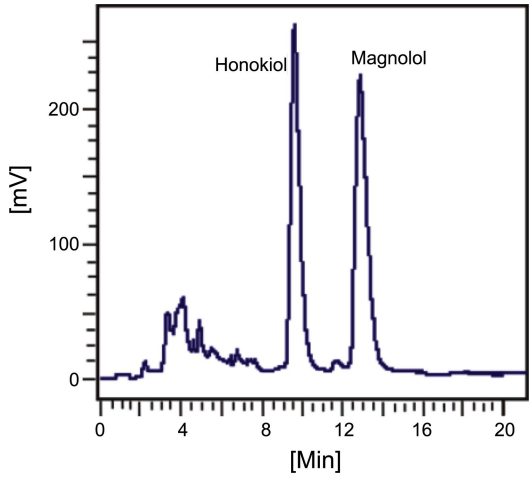


Figure 1. Typical HPLC profile of ethyl acetate extract from *Cortex Magnoliae Officinalis* (EAM).

In the study, however, DNA itself was used as the substrate for the investigation. Thus, MDA was accordingly generated in the reaction system as the product of ·OH radical and deoxyribose moiety. Since MDA can be readily detected at 530 nm using a spectrophotometer *via* combining TBA (Suppl. 5) without interference with oxidative lesions, it therefore can quantitatively evaluate the extent of DNA damage.[6] The dose response curves in Suppl. 6 Fig. S6.1 and IC_{50} values in Table 1 suggested that CMO could effectively

Table 1. The IC_{50} values of extracts from *Cortex Magnoliae Officinalis*, magnolol and the positive controls (μg/mL)

Assays	PEM	EAM	AEM	95EM	WM	Trolox	Caffeic acid	Magnolol
DNA damage assay	123.20 ± 9.85^{a}	153.81 ± 8.45^{b}	166.36 ± 9.91^{b}	127.78 ± 3.75^{a}	943.93 ± 1.36^{c}	147.89 ± 7.03^{b}	126.00 ± 4.04^{a}	N.D.
$\cdot O_2^-$ scavenging	621.65 ± 10.99^{d}	459.11 ± 6.12^{c}	666.28 ± 8.84^{e}	250.89 ± 4.48^{b}	1050.38 ± 20.01^{f}	256.24 ± 1.78^{b}	N.D.	$29.97\pm2.35^{a*}$
DPPH· scavenging	86.78 ± 1.57^{e}	29.02 ± 0.29^{d}	30.21 ± 1.93^{d}	29.31 ± 0.82^{d}	98.97 ± 5.42^{f}	5.48 ± 0.02^{b}	4.56 ± 0.02^{a}	$25.92\pm3.02^{c*}$
ABTS·$^{+}$ scavenging	24.67 ± 0.28^{e}	23.34 ± 0.17^{d}	32.39 ± 0.27^{f}	35.34 ± 0.49^{g}	109.71 ± 1.86^{h}	18.27 ± 0.16^{c}	12.63 ± 0.04^{b}	$0.85\pm0.12^{a*}$
Cu^{2+} reducing	79.55 ± 0.17^{c}	101.05 ± 1.72^{d}	99.79 ± 1.35^{d}	106.28 ± 1.80^{e}	217.50 ± 4.31^{f}	62.44 ± 0.96^{b}	16.29 ± 0.02^{a}	N.D.

IC_{50} value is defined as the concentration of 50% effect percentage and calculated by linear regression analysis and expressed as mean ± SD ($n = 3$). The linear regression was analyzed by Origin 6.0 professional software. Means values with different superscripts in the same row are significantly different ($p < 0.05$), while with same superscripts are not significantly different ($p < 0.05$). *The mean of IC_{50} values was cited from reference [23], and the SD was recalculated by our laboratory. N.D., not detected. PEM, petroleum ether extract from *Cortex Magnoliae Officinalis*; EAM, ethyl acetate extract from *Cortex Magnoliae Officinalis*; AEM, absolute ethanol extract from *Cortex Magnoliae Officinalis*; 95EM, 95% ethanol extract from *Cortex Magnoliae Officinalis*; WM, water extract from *Cortex Magnoliae Officinalis.* −: Below the detection limit.

Table 2. Chemical contents of extracts from *Cortex Magnoliae Officinalis*

extracts	PEM	EAM	AEM	95EM	WM
Total phenolics (mg Pyrogallol/g)	181.45 ± 8.65^{b}	181.58 ± 13.89^{b}	213.55 ± 2.76^{c}	221.11 ± 3.24^{d}	79.87 ± 0.89^{a}
Total sugars (mg Laminarin/g)	17.3 ± 1.32^{a}	78.14 ± 1.18^{c}	194.49 ± 1.25^{d}	213.74 ± 6.06^{e}	59.58 ± 1.23^{b}
Magnolol (peak area)	13154762 ± 118185^{c}	9337101 ± 164927^{b}	3740978 ± 200795^{a}	3106948 ± 414083^{a}	–
Honokiol (peak area)	10988728 ± 15770^{d}	8390810 ± 118180^{c}	4330666 ± 228731^{b}	2273514 ± 230011^{a}	–

Each value is expressed as mean ± SD ($n = 3$). Means values with different superscripts in the same row are significantly different ($p < 0.05$), while with same superscripts are not signifiacntly different ($p > 0.05$). PEM, petroleum ether extract from *Cortex Magnoliae Officinalis*; EAM, ethyl acetate extract from *Cortex Magnoliae Officinalis*; AEM, absolute ethanol extract from *Cortex Magnoliae Officinalis*; 95EM, 95% ethanol extract from *Cortex Magnoliae Officinalis*; WM, water extract from *Cortex Magnoliae Officinalis.* −: Below the detection limit.

Table 3. The R values between chemical contents and $1/IC_{50}$

Assays	Total phenolics	Magnolol	Honokiol	Total sugars
DNA damage assay	0.89	0.75	0.67	0.27
$\cdot O_2^-$ scavenging	0.65	0.15	0.15	0.61
DPPH· scavenging	0.76	0.63	0.56	0.71
$ABTS^{\cdot+}$ scavenging	0.69	0.87	0.90	–0.10
Cu^{2+} reducing	0.77	0.84	0.86	–0.01
Average	0.75	0.63	0.63	0.29

R, Correlation coefficient. $1/IC_{50}$, values were shown in Suppl. 6.

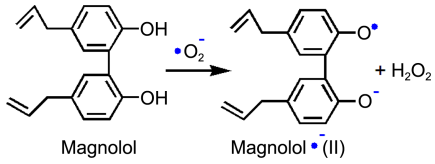
protect against hydroxyl-induced DNA oxidative damage.

In order to verify which chemical component in CMO can be responsible for the protective effect, we further determined the chemical contents, including total phenolics, total sugars, magnolol, and honokiol. The results were calculated and shown in Table 2, Figure 1, and Suppl. 7. On this basis, the correlation diagrams between chemical contents/peak areas and protective effects ($1/IC_{50}$ values, Suppl. 8 Fig. S8.1) were plotted to calculate the correlation coefficient (R value). As shown in Table 3, R values of total phenolics, magnolol, honokiol, and total sugars were respectively 0.89, 0.68, 0.67, and 0.27. Obviously, total sugars exhibited much lower R value than the others. It means that the protective effect of CMO can be attributed to the total phenolics not total sugars. Despite polysaccharide in CMO has also been reported to exhibit a ·OH radical-scavenging ability,[14] however, in terms of our previous study,[15] polysaccharide could not effectively exert the antioxidant action when it was used along with phenolics in plants. In fact, the antioxidant activity of polysaccharide in CMO was lower than that of phenolics, according to the IC_{50} values.[14] As seen Figure 1 or Suppl. 7, magnolol and honokiol presented two strong peaks in the HPLC profiles, it suggests that total phenolics in CMO mainly includes magnolol and honokiol, and that the protective effect of CMO can be mainly attributed to the existence of magnolol and honokiol.

Previous works have shown that there are two approaches for natural phenolic antioxidant to non-enzymatically repair DNA oxidative damage: one is to fast repair the transient DNA· damage (*i.e.* repairing approach); one is to scavenge ROS (especially ·OH radicals) prior to DNA damage (*i.e.* ROS scavenging approach).[16,17] For the sustained DNA damage, however, the proportion of repairing approach was only about 40%,[17] so the ROS scavenging approach may be especially important.

To explore ROS scavenging approach of CMO, we further determined its $O_2^{\cdot-}$ radical-scavenging ability. To obtain more reliable data, five CMO extracts were measured using our method at pH 7.4.[7] The dose response curves (Suppl. 6) and IC_{50} values (Table 1) suggested an effective $O_2^{\cdot-}$ radical-scavenging ability of CMO. This also supports the hypothesis that ROS scavenging is one approach for CMO to protect DNA oxidative damage. It must be emphasized that the IC_{50} value of magnolol (29.97 ± 2.35 μg/mL) was much lower than five CMO extracts, it means that magnolol and its isomer honoloil may be the bioactive compounds.

Based on the previous mechanistic studies,[18] the reaction for magnolol to scavenge $O_2^{\cdot-}$ radical can proposed as Eq. (2). In the process, magnolol was thought to donate a hydrogen atom to $O_2^{\cdot-}$ radical *via* hydrogen atom transfer (HAT) mechanism; In addition, a single electron transfer and deprotonation would be expected to take place, this is so-called sequential electron proton transfer (SEPT) mechanism.[19]



(2)

To illustrate the possibilities of HAT and SEPT, we further measured the radical-scavenging effects of CMO on DPPH· and $ABTS^{\cdot+}$. The dose response curves in Suppl. 6 showed that CMO could effectively scavenge both DPPH· and $ABTS^{\cdot+}$ radicals. The IC_{50} values in Table 1, however, suggested that magnolol was of much higher radical-scavenging levels than five CMO extracts. Therefore, magnolol and honoloil may be regarded as two bioactive compounds in both radical-scavenging assays as well.

As suggested in earlier investigations,[20] DPPH· scavenging is a HAT process. Therefore, the DPPH·-scavenging of magnolol could be illustrated as Figure 2. In the process, phenolic –OH underwent homolysis to produce H· and magnolol· radical (III). (III) is actually a stable radical, as it can readily form a serial of resonance stabilized phenoxy radicals[21] (Suppl. 9), thus reaction would be easy to be initiated (Step 1). Meanwhile H· combined DPPH· to generate DPPH-H molecule, and (III) might transform into (IV) which could be further extracted H· by excess DPPH· to form stable quinine-like (VI). The product of honokiol was also thought to be transformed a similar quinine-like structure (Suppl. 10). Unlike DPPH· radical, $ABTS^{\cdot+}$ radical cation needs only an electron (***e***) to neutralize the positive charge. Therefore, $ABTS^{\cdot+}$ scavenging is an electron (***e***) transfer process.[22] In the reaction, magnolol was assumed to give an electron (***e***), accompanied by H^+ transfer. The elec-

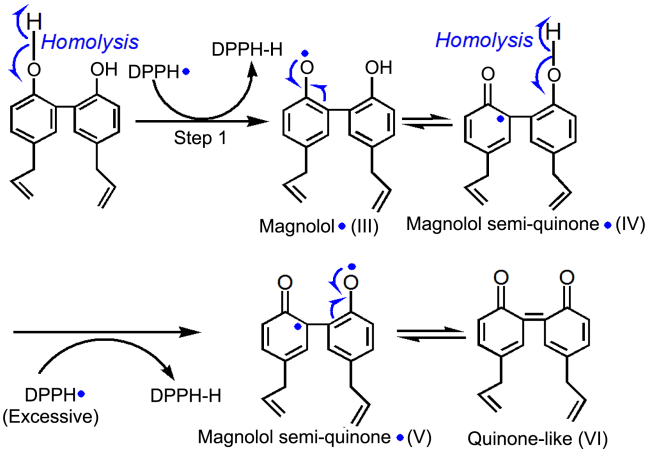
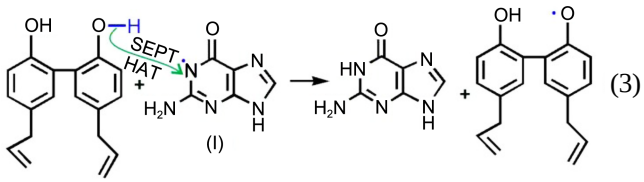


Figure 2. The proposed reaction of magnolol with DPPH· radical.

tron (***e***) was then donated to ABTS·$^{+}$ to form stable ABTS molecule. Meanwhile magnolol· changed to the magnolol· radical (III), which could also be further converted to semi-quinone· radical (IV) and quinine-like form (VI) in excess ABTS·$^{+}$. Obviously, it was a SEPT mechanism. The mechanism was further confirmed by the Cu-reducing power assay which is actually donating electron (***e***) process. As shown in Suppl. 6 Fig. S6.5, five CMO extracts increased their percentages in a dose-dependent manner, the results apparently support the findings of antioxidant assays.

The mechanisms of HAT and SEPT, can also be used for the interpretation of DNA· radicals repairing approach for CMO to protect DNA oxidative damage. For example, a transient guanine radical (I) was repaired by magnolol maybe *via* the mechanism in Eq. (3). In the process, magnolol was thought to donate either hydrogen atom (H·) or electron (***e***) to (I) to yield magnolol· radical (III), and guanine molecule. Obviously, the proposed mechanism agrees with the previous studies.[1,2]



(3)

On this basis, we regard the repairing approach as a special ROS scavenging one, *via* which magnolol scavenge guanine· (I) instead of ROS. It is easy to imagine that magnolol· radical can also be transferred into semi-like (VI), if guanine radical (I) are excessive. In other words, both scavenging and repairing approaches of magnolol could be considered as antioxidant mechanism, and yield the stable oxidized product quinine-like form (VI).

Taken together, there are seemingly two different approaches (repairing approach & ROS scavenging approach) for these phenolic antioxidants to protect against ·OH-induced DNA damage, however, both approaches can be actually regarded as an antioxidant process, in which these phenolic antioxidants remove DNA radicals or ROS *via* HAT and SEPT.

It is worth mentioning the following. As the main resources of natural phenolic antioxidants, flavonoids and phenolic acids have been widely explored for the antioxidant mechanism and structure-activity relationship.[24] In comparison, since phenolic phenylpropanoids are relatively rare, their antioxidant mechanism has been seldom considered. The present study, however, for the first time reported its antioxidant mechanism, and structure-activity relationship (*i.e.* the reason why phenolic –OH in phenylpropanoids is essential for antioxidant ability, is the stability of its oxidized product quinone-like form).

Finally, in order to further identify whether total phenolics, magnolol and honokiol could be responsible for the antioxidant of CMO, we quantitatively analyzed the correlation coefficients (R values) between chemical contents and all antioxidant assays. As shown in Table 3, the average R values were 0.75, 0.63, 0.63, and 0.29, respectively for total phenolics, magnolol, honokiol, and total sugars. This clearly demonstrated that the protective and antioxidant effects of CMO should be attributed to total phenolics, especially magnolol and honokiol, indeed.

As mutagenic has been demonstrated to be directly associated with DNA oxidative damage,[25] thereby the anticlastogenic and antineoplastic potentials of CMO[4] are thought to arise from its protective effect against ·OH-induced DNA oxidative damage. For example, magnolol was proved to possess an inhibition of B(*a*)P-induced clastogenesis, B(*a*)P-induced carcinogenesis however was reported to be involved in ROS.[26]

Conclusion

In conclusion, *Cortex Magnoliae Officinalis* exhibits a protective effect against ·OH-induced DNA damage, which can be attributed to the existence of phenolic antioxidants, especially magnolol and honokiol. They exert the protective effect *via* antioxidant mechanism which may be mediated *via* hydrogen atom transfer (HAT) and/or sequential electron proton transfer (SEPT). In the process, the phenolic –OH moiety in phenylpropanoids is oxidized to the stable quinine-like form and the stability of quinine-like can be ultimately responsible for its antioxidant.

Acknowledgments. The publication cost of this paper was supported by the Korean Chemical Society.

References

1. Zheng, R. L.; Shi, Y. M.; Jia, Z. J.; Zhao, C. Y.; Zhang, Q.; Tan, X. R. *Chem. Soc. Rev.* **2010**, *39*, 2827-2834.
2. Shi, Y.; Kang, J.; Lin, W.; Fan, P.; Jia, Z.; Yao, S.; Wang, W.; Zheng, R. *Biochim. Biophys. Acta* **1999**, *1472*, 279-289.
3. Shi, Y. M.; Wang, W. F.; Huang, C. Y.; Jia, Z. J.; Yao, S. D.; Zheng, R. L. *Mutagenesis* **2007**, *23*, 19-26.
4. Saito, J.; Fukushima, H.; Nagase, H. *Biol. Pharm. Bull.* **2009**, *32*, 1209-1214.
5. Kapoor, S. *Liver Int.* **2009**, *29*, 951-952.
6. Li, X. C.; Mai, W.; Wang, L.; Han, W. *Anal. Biochem.* **2013**, *438*, 29-31.
7. Li, X. C. *J. Agr. Food Chem.* **2012**, *60*, 6418-6424.
8. Li, X. C.; Lin, J.; Han, W.; Mai, W. Q.; Wang, L.; Li, Q.; Chen, D. *Molecules* **2012**, *17*, 13457-13472.
9. Li, X. C.; Wu, X.; Huang, L. *Molecules* **2009**, *14*, 5349-5361.
10. Li, X. C.; Lin, J.; Gao, Y.; Han, W.; Chen, D. *Chem. Cent. J.* **2012**, *6*, 140.
11. Kumar, A.; Pottiboyina, V.; Sevilla, M. D. *J. Phys. Chem. B* **2011**, *115*, 15129-15137.
12. Galano, A.; Alvarez-Idaboy, J. R. *Org. Lett.* **2009**, *11*, 5114-5117.
13. Rokhlenko, Y.; Geacintov, N. E.; Shafirovich, V. *J. Am. Chem. Soc.* **2012**, *134*, 4955-4962.
14. Deng, F.; Zhang, F. Q.; Zhang, J. R.; Ma, Y.; Tang, G. Z.; Liu, C. F.; Lai, P. H. *J. Anhui Agri. Sci.* **2011**, *36*, 303-304.
15. Li, X. C.; Chen, D.; Mai, Y.; Wen, B.; Wang, X. *Nat. Prod. Res.* **2012**, *26*, 1050-1053.
16. Zheng, R. L., Huang Z. Y. Free Radical Biology; Higher Education Press: Beijing, 2007; p 143.
17. Anderson, R. F.; Fisher, L. J.; Hara, Y.; Harris, T.; Mak, W. B.; Melton, L. D.; Packer, J. E. *Carcinogenesis* **2001**, *22*, 1189-1193.

18. Jovanovic, S. V.; Steenken, S.; Simic, M. G.; Hara, Y. *J. Am. Chem. Soc*. **1995**, *117*, 9881-9888.
19. Iuga C, Alvarez-Idaboy J. R.; Vivier-Bunge A. *J. Phys. Chem. B* **2011**, *115*, 12234-12246.
20. Bondet, V.; Brand-Williams, W.; Berset, C. *LWT-Food Sci. Technol.* **1997**, *30*, 609-615.
21. Srinivasan, M.; Sudheer, A. R.; Menon, V. P. *J. Clin. Biochem. Nutr.* **2007**, *40*, 92-100.
22. Aliaga, C.; Lissi, E. A. *Int. J. Chem. Kinet.* **1998**, *30*, 565-570.
23. Li, X. C.; Chen, C. *Int. Res. J. Pure & Appl. Chem.* **2012**, *2*, 68-76.
24. Cai, Y. Z.; Sun, M.; Xing, J.; Luo, Q.; Corke, H. *Life Sci*. **2006**, *78*, 2872-2888.
25. Demple, B.; Halbrook, J. *Nature* **1983**, *304*, 466-468.
26. Hvastkovs, E. G.; So, M.; Krishnan, S.; Bajrami, B.; Tarun, M.; Jansson, I.; Schenkman, J. B.; Rusling, J. F. *Anal. Chem.* **2007**, *79*, 1897-1906.