Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2014; 39 (1) ; 30–36
doi: 10.5505/tjb.2014.65882

Research Article [Araştırma Makalesi]

Yayın tarihi 30 Mart, 2014 © TurkJBiochem.com
[Published online 30 March, 2014]

Amentoflavone protects against hydroxyl radical-induced DNA damage via antioxidant mechanism

[Amentoflavon antioksidan mekanizma ile hidroksil radikali ile indüklenmiş DNA hasarına karşı koruyucu etkiye sahiptir]*

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Registered: 29 July 2013; Accepted: 2 December 2013
[Kayıt Tarihi: 29 Temmuz 2013; Kabul Tarihi: 2 Aralık 2013]

ABSTRACT

Objective: Oxidative DNA damage is involved in mutation, cell death, carcinogenesis, and aging. Since biflavonoid amentoflavone exhibited beneficial effects on DNA, we therefore investigated its protection against •OH-induced DNA oxidative damage then discussed the mechanism. It will enhance the understanding of interaction between biflavonoid *vs* DNA mediated by free radicals.

Methods: The protective effect of amentoflavone against •OH-induced DNA damage was measured using our method. To explore the mechanism, it was further determined by •OH-induced bases damage, deoxyribose damage, and various antioxidant assays.

Results: Amentoflavone increased dose-dependently its protective percentages against •OH-induced damage on DNA, bases, and deoxyribose; The IC_{50} were 31.85±4.75, 198.75±33.53, 147.14±20.95, 75.15±10.52, 93.75±16.36, 167.69±13.90, and 137.95±19.86 μM, respectively for DNA, cytosine, uracil, adenine, thymine, guanine, and deoxyribose damages. Radical-scavenging assays suggested that amentoflavone could effectively scavenge $•O_2^-$, DPPH•, $ABTS•^+$ radicals (IC_{50} values were respectively 8.98±0.23, 432.25±84.05, 7.25±0.35 μM).

Conclusions: Based on the mechanistic analysis, it is concluded that amentoflavone can effectively protect against •OH-induced oxidative damage DNA (including base & deoxyribose moieties), via deoxynucleotide radical repairing, and reactive oxygen species (ROS) scavenging approaches which may be mediated by donating hydrogen atom (H•) and electron (*e*). Further analysis indicated that both scavenging and repairing approaches can be primarily attributed to its antioxidant mechanism which may ultimately arise from to the stability of its oxidized product semi-quinone form. Its protection against DNA damage may be generally responsible for the radioprotective and anti-inflammation effects.

Key Words: Amentoflavone, hydroxyl radical, DNA damage, antioxidant, mechanism

Conflict of Interest: The authors declare that there are no conflict of interests.

ÖZET

Amaç: Oksidatif DNA hasarı mutasyon oluşumu, hücre ölümü, kanserleşme ve yaşlanma ile ilişkilidir. Biflavonoid amentoflavon DNA üzerinde yararlı etkiler gösterdiği için, hidroksil ile uyarılmış oksidatif DNA hasarındaki koruyucu rolü ve mekanizması araştırılmıştır. Serbest radikal aracılı biflavonoid ve DNA etkileşiminin anlaşılması amaçlanmıştır.

Yöntem: Hidroksil ile uyarılmış DNA hasarına karşı amentoflavonun koruyucu etkisi kendi yöntemimiz kullanılarak çalışılmıştır. Ayrıca hidroksil ile uyarılmış baz ve deoksiriboz hasarı ve çeşitli antioksidan mekanizmalara bakılmıştır.

Bulgular: Amentoflavonun koruyucu etkisi doza bağımlı olarak hidroksil ile uyarılmış DNA, baz ve deoksiriboz hasarlarına karşı artmaktadır. DNA, sitozin, urasil, adenin, timin, guanin ve deoksiriboz hasarları için IC_{50} değerleri sırasıyla 31.85±4.75, 198.75±33.53, 147.14±20.95, 75.15±10.52, 93.75±16.36, 167.69±13.90, ve 137.95±19.86 μM olarak bulunmuştur. Radikal-temizleyici çalışmalar amentoflavonun $•O_2^-$, DPPH•, $ABTS•^+$ radikallerini etkili bir şekilde ortadan kaldırdığını göstermektedir (IC_{50} değerleri sırasıyla 8.98±0.23, 432.25±84.05, 7.25±0.35 μM).

Sonuç: Amentoflavon hidroksil ile uyarılmış oksidatif DNA hasarına (baz ve deoksiriboz hasarları da dahil olmak üzere) karşı deoksinükleotid radikal tamiri ve reaktif oksijen türlerini ortadan kaldırıcı yaklaşımlar kullanarak koruyucu etkiye sahiptir. Bu etki muhtemelen hidrojen atomu (H•) ve elektron (*e*) vererek olmaktadır. Daha ileri analizler, radikalleri ortadan kaldırıcı ve tamir edici özelliğin semi-kinon formda olan okside ürününün dayanıklılığı ile ortaya çıkan antioksidan mekanizmalara dayandığını ortaya koymaktadır. DNA hasarına karşı koruyucu etkisi genellikle radyoprotektif ve anti-enflamatuar etkileri ile ilişkilidir.

Anahtar Kelimeler: Amentoflavon, hidroksil radikali, DNA hasarı, antioksidan, mekanizma

Çıkar çatışması: Çıkar çatışması bulunmamaktadır.

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 oxidatively damage DNA then lead to severe biological consequences including mutation, cell death, carcinogenesis, and aging [1].

Since natural flavonoid plays a critical role in human nutrition and health, therefore its beneficial effects on DNA have attracted increasing attention over the past decade [2].

As a typical biflavonoid naturally occurring in many plants, amentoflavone (Figure 1) has recently been reported to present radioprotective [3-4] and anti-inflammation [5-6] effects. According to free radical biology and medicine [7], we hypothesized that these effects of amentoflavone may be associated with protection against DNA damage induced by ROS.

Therefore, the aims of the present study were to investigate its protective effect against DNA oxidative damage then to further discuss the mechanism. Since amentoflavone is regarded as a typical biflavonoid, the study will obviously enhance the understanding of the interaction between chemical molecule and biomolecule (i.e. biflavonoid *vs* DNA) mediated by ROS, and will play an important role in the field of biochemistry.

Materials and Methods

Chemicals

Amentoflavone (CAS number: 1617-53-4, 98%) was obtained from BioBioPha Co., Ltd (Kunming, China). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH·), butylated hydroxyanisole (BHA), pyrogallol, (±)-6-hydroxyl-2,5,7,8-tetramethlychromane-2-carboxylic acid (Trolox) were from Sigma-Aldrich Shanghai Trading Co. (Shanghai, China). Deoxyribose, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) (ABTS diammonium salt), and GSH (glutathione) were 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). All other reagents were of analytical grade.

Protective effect against •OH-induced DNA damage

The experiment was conducted by a hydroxyl-scavenging assay based on DNA damage *in vitro* established in our laboratory [8]. Briefly, sample (amentoflavone) was dissolved in methanol at (0.74 mM). Various amounts (5-20 μL) of sample solutions were then separately taken into mini tubes. After evaporating the sample solution in tube to dryness, 400 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 (1.2 mM) and the total volume of the reaction mixture was adjusted to 750 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 against the buffer (as blank). The percent of protection of DNA is expressed as follows: Inhibition % = $(A_0\text{-}A)/A_0 \times 100\%$. Where A_0 is the absorbance of the control without sample; and A is the absorbance of the reaction mixture with sample.

Protective effect against •OH-induced bases damage

The base damage assays were similar to the above method. However, DNA sodium was replaced by five bases of DNA, including uracil, cytosine, adenine, thymine, and guanine. The product mixture was then analyzed by a HPLC system.

In uracil and cytosine damage assay, sample amentoflavone was dissolved at 0.74 mM. Various amounts (0.0074-0.045 μmol) of sample solutions were then separately taken into mini tubes. After evaporating the sample solution in tube to dryness, 105 μL phosphate buffer (0.2 M, pH 7.4) solution containing uracil (0.70 μmol) and cytosine (0.71μmol), 5 μL $FeCl_2$ (12.5 mM) and 10 μL Na_2EDTA (20.0 mM) solutions were added. The reaction was initiated by mixing 7.5 μL H_2O_2 (1.3 M), and the total volume of the reaction mixture was adjusted to 165 μL with buffer. After incubation in a water bath at 55°C for 20 min, the mixture was cooled then measured using a HPLC system equipped with a UV 30 detector (Syltech P510 HPLC, Los Angeles, USA) and a Diamonsil C_{18} column (250 mm×4.6 mm, 5 μm, Dikma Ltd., China). The mobile phase consisted of methanol: 0.5% acetic acid solution (5:5, v/v) and was degassed. UV detection wavelength was 254 nm, flow rate was 0.5 mL/min, injection volume was 2 μL. The relative contents of bases (uracil and cytosine) were obtained on the basis of the peak area. The percent of protection was calculated as: Protective effect % = $(A\text{-}A_{damage})/(A_0\text{-}A_{damage}) \times 100\%$. Where A_0 is the peak area of base in buffer, A_{damage} is the peak area of base in reaction system without sample, and A is the peak area of base in reaction system with sample.

In the adenine and thymine damage assay, the above procedure was repeated, using the solution containing adenine (0.58 μmol) and thymine (0.63 μmol). In the guanine damage assay, however, its amount was modified as 0.14 μmol, and the mobile phase consisted of methanol: 0.5% acetic acid solution (4:1, v/v). The detection wavelength was 243 nm, flow rate was 0.5 mL/min, injection volume was 5 μL.

Protective effect against •OH-induced deoxyribose damage

To eliminate the strong solvent interference, the experiment was conducted using our method [9]. Briefly, sample amentoflavone was dissolved in methanol and various amounts of sample solution were separately taken into mini tubes. After evaporating the sample solution in tube to dryness, the sample residue was determined by the similar procedure to the DNA damage assay.

Superoxide ($\bullet O_2^-$) radical-scavenging assay

Measurement of superoxide anion ($\bullet O_2^-$) scavenging activity was based on our method [10]. Briefly, 0.74 mM amentoflavone 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 at 325 nm of the mixture was immediately measured (Unico 2100, Shanghai, China) against the Tris-HCl buffer as blank every 30 s for 5 min. The $\bullet O_2^-$ 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 increment in A_{325nm} of the mixture without the sample and $\Delta A_{325nm,\ sample}$ is that with the sample; T = 5 min. The experiment temperature was 37°C.

DPPH• radical-scavenging assay

The DPPH• radical-scavenging activity was determined as described [11]. Briefly, 60 µL of DPPH• solution (0.1 mM) was mixed with 540 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 (Unico 2100, Shanghai, China), using 95% ethanol as the blank. The DPPH•inhibition percentages of the samples were calculated: Inhibition % = $(A_0\text{-}A)/A_0 \times 100\%$. Where A is the absorbance with samples; and A_0 is the absorbance without samples. Trolox and BHA were used as the positive controls.

ABST•$^+$ radical-scavenging assay

The ABTS•$^+$ scavenging activity was evaluated by the method [12]. The ABTS•$^+$ was produced by mixing 350 µL ABTS diammonium salt (7.4 mM) with 350 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, 0.8 mL of ABTS•$^+$ reagent was mixed with sample solutions (final concentrations: 0.0074-0.037 µM), the total volume of system was adjusted to 1500 µL 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: Inhibition % = $(A_0\text{-}A)/A_0 \times 100\%$. Where A_0 is the absorbance of the negative control without any samples, A is the absorbance of the mixture with amentoflavone, Trolox or BHA.

Statistical analysis

Results are reported as the mean ± SD of three measurements, the IC_{50} values were calculated by linear regression analysis and one-way analysis of variance (ANOVA) were performed for comparison between groups. A p value of less than 0.05 was considered significant. All linear regression in this paper was analyzed by Origin 6.0 professional software.

Results and Discussion

As we know, hydroxyl radical (•OH) can be generated via Fenton reaction (Eq. 1) or radiation on water (Eq. 2 & 3) [13]:

$$Fe^{2+} + H_2O_2 \rightarrow HO\bullet + OH^- + Fe^{3+} \quad \text{Eq. } \mathbf{1}$$

$$H_2O + h\nu \rightarrow H_2O^+\bullet + e^- (\rightarrow e^-_{aq}) \quad \text{Eq. } \mathbf{2}$$

$$H_2O^+\bullet + H_2O \rightarrow HO\bullet + H_3O^+ \quad \text{Eq. } \mathbf{3}$$

In the study, however, hydroxyl radical was produced by Fenton reaction (Eq. **1**). As the most reactive ROS, hydroxyl radical can easily attack DNA to yield malondialdehyde (MDA) and a number of oxidative lesions (Supplementary file 1), which are related to various diseases and mutations. Especially, 2'-deoxyadenosine 5'-monophosphate (5'-dAMP) [14] has been considered as the important biomarkers monitoring oxidative damage to DNA (Supplementary file 1). MDA, however, could further combine 2-thiobarbituric acid (TBA) to produce thiobarbituric acid reactive substances (TBARS) with a maximum absorbance at 530 nm [8]. Hence, the value of A_{530nm} can quantitatively evaluate the extent of DNA damage and the decrease of A_{530nm} value indicates a protective effect against DNA damage. Our data indicated that amentoflavone increased its protective percentages in a dose-dependent manner (Supplementary file 2, Fig. S1). As listed in Table 1, its IC_{50} value (31.85±4.75 µM) was significantly lower (p<0.05) than the positive control caffeic acid (36.56±11.72 µM). Undoubtedly, amentoflavone can effectively protect against hydroxyl-induced DNA oxidative damage.

In order to explore the protective mechanism, we further evaluated the effect of amentoflavone on bases and deoxyribose damages. As seen in Supplementary file 3, all bases presented a maximum peak in buffer, and they were considerably reduced when attacked by •OH in our model. However, when amentoflavone was added, the peak area was partially restored. Based on the peak area (Supplementary file 3), the percent of

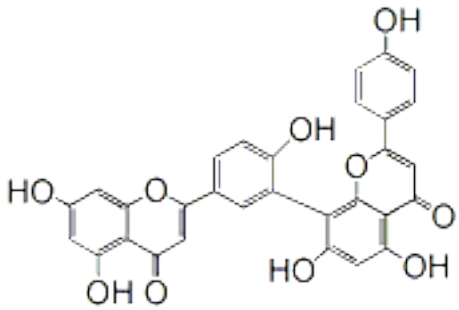

Figure 1. The structure of amentoflavone.

Table 1. The IC_{50} values of amentoflavone and positive controls (μM)

assay	amentoflavone	positive controls	
		caffeic acid	other
DNA	31.85±4.75*	36.56±11.72	
C	198.75±33.53*	253.05±33.00	
U	147.14±20.95*	314.61±47.44	
A	75.15±10.52*	227.94±20.33	
T	93.75±16.36*	586.44±40.17	
G	167.69±13.90*	140.72±27.05	
Deoxyribose	137.95±19.86*	--	403.78±143.50[b] 164.16±35.80[t]
$\bullet O_2^-$	8.98±0.23*	67.39±1.33	--
DPPH•	432.25±84.05*	--	85.33±18.56[b] 199.40±54.24[t]
ABTS•+	7.25±0.35*	--	6.05±0.22[b] 5.20±0.080[t]

IC_{50} value is defined as the concentration of 50% radical inhibition (or protection 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. * Significant difference compared to the controls significant difference compared to the controls (one-way ANOVA, $p < 0.05$). [b] For BHA (butylated hydroxyanisole). [t] For Trolox. --: Not detected. Their dose response curves were shown in Supplementary file 2.

protection was calculated and plotted as dose response curves (Supplementary file 2, Fig. S2). The percentages suggested that amentoflavone could effectively protect against hydroxyl radical-induced base damage and its IC_{50} values were calculated as 31.85±4.75, 198.75±33.53, 147.14±20.95, 75.15±10.52, 93.75±16.36, and 167.69±13.90 μM, respectively for C, U, A, T, and G. In the deoxyribose degradation assay, amentoflavone also increased its percentages concentration-dependently (Supplementary file 2, Fig. S3) and the IC_{50} value was calculated as 137.95±19.86 μM (Table 1). It suggests that amentoflavone can protect against hydroxyl radical-induced deoxyribose damage as well.

In a word, amentoflavone could effectively protect the oxidative damage of DNA (including base moiety and deoxyribose moiety).

Previous works indicated that there are two approaches for natural phenolic antioxidant to protect DNA oxidative damage: one is to scavenge ROS (especially •OH radicals) prior to DNA damage; one is to fast repair the deoxynucleotide radical cations damaged by free radicals [15-16]. To explore whether the protective effect of amentoflavone was associated to ROS scavenging, we further determined its $\bullet O_2^-$ radical-scavenging ability. In the assay, amentoflavone presented a good concentration-dependent manner (Supplementary file 2, Fig. S4) and its IC_{50} values was 8.98±0.23 μM (Table 1). The fact that amentoflavone could effectively scavenge $\bullet O_2^-$ radical, suggests ROS scavenging as one approach for amentoflavone to protect DNA oxidative damage.

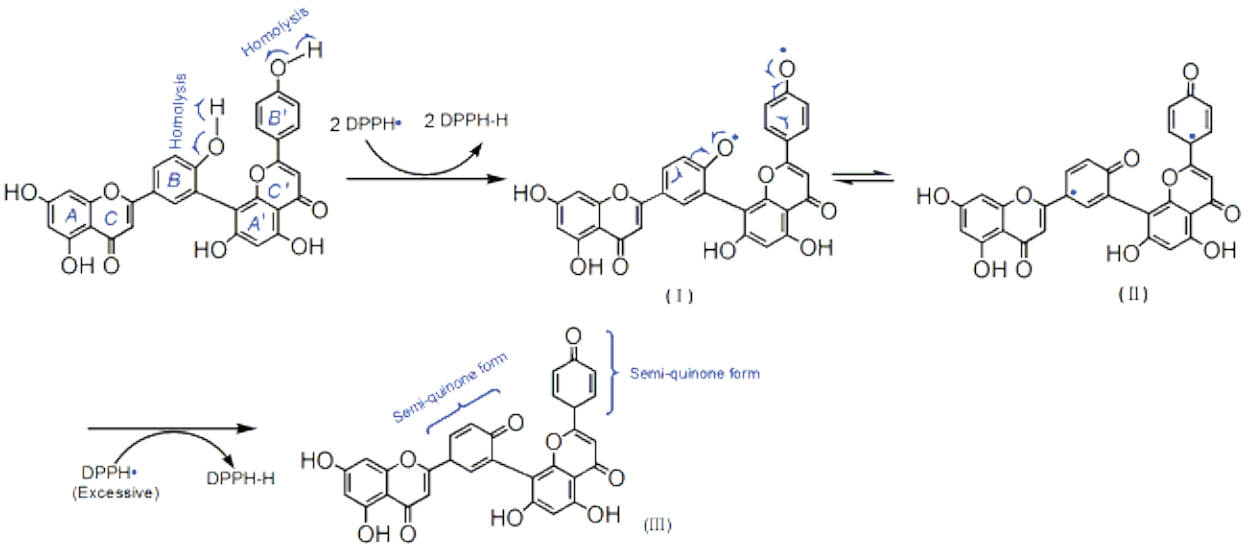

Figure 2. The proposed reaction of amentoflavone with DPPH·

To verify whether amentoflavone can scavenge free radicals, we further measured its radical-scavenging on DPPH• and $ABTS^{\cdot+}$. The dose response curves in Supplementary file 2, Fig. S5 and S6 indicated an effective inhibition on DPPH• and $ABTS^{\bullet+}$ radicals of amentoflavone and the IC_{50} values were respectively 432.25±84.05 and 7.25±0.35 μM (Table 1). Earlier investigations have shown that DPPH• may be scavenged by an antioxidant through donation of hydrogen atom (H·) to form a stable DPPH-H molecule [17]. In amentoflavone molecule, however, since the *B* (*B'*) ring is more reactive than *A* (*A'*) or *C* (*C'*) ring [18], therefore, phenolic –OH in *B* (*B'*) ring underwent homolysis prior to either the *A* (*A'*) or *C* (*C'*) ring to produce H· and amentoflavone· radical (Ⅰ). H· then combined DPPH· to generate DPPH-H molecule and the amentoflavone· radical might transform into the semi-quinone· radical (Ⅱ), which could be further extracted H· by excess DPPH• to form the stable semi-quinone (Ⅲ) (Figure 2).

Unlike DPPH· radical, $ABTS^{\bullet+}$ radical cation needs only an electron (***e***) to neutralize the positive charge. Therefore, $ABTS^{\bullet+}$ scavenging is an electron (***e***) transfer process [19]. In the reaction, amentoflavone produced electron (***e***) and H^+ cation. The electron (***e***) was then donated to $ABTS^{\bullet+}$ to form stable ABTS molecule. Meanwhile, amentoflavone changed to the amentoflavone• radical (Ⅰ), which could also be converted to semi-quinone· radical (Ⅱ) and semi-quinone (Ⅲ) in excess $ABTS^{\bullet+}$ (Figure 3).

The fact that amentoflavone could effectively scavenge both DPPH• and $ABTS^{+}$• radicals, implies that amentoflavone exerts ROS scavenging action by donating hydrogen atom (H•) and electron (***e***).

As shown in Supplementary file 4, amentoflavone contains large π-π conjugative systems, in which carbonyl groups (C=O) can greatly withdraw electron from *B* (*B'*) ring to enhance the acidity of phenolic –OH groups [20]. Therefore, under alkaline cellular environment, the acidity may predominate over the chemical action of amentoflavone, and phenolic –OH may firstly ionize to yield H^+ ion and subsequently donate electron (***e***) to form amentoflavone· (Ⅰ). It is the possible mechanism for amentoflavone to donate electron (***e***).

Under neutral or acidic cellular environment, however, the acidity cannot predominate over its chemical action.

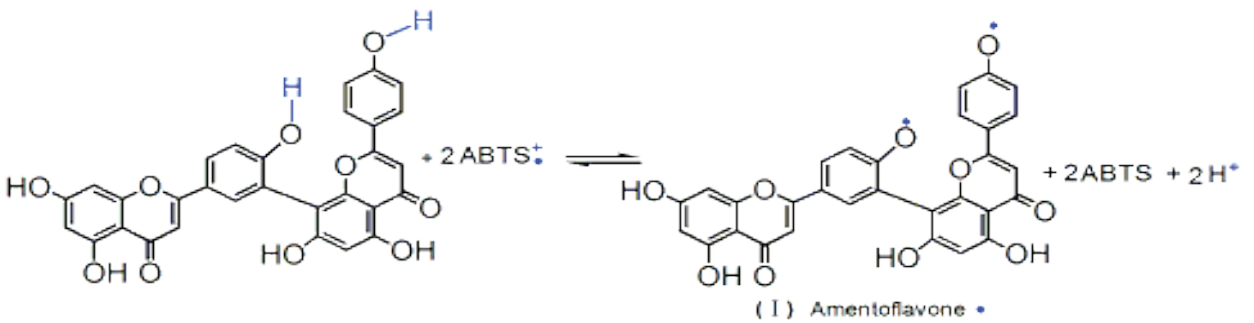

Figure 3. The proposed reaction of amentoflavone with $ABTS^{+}$·

As illustrated in Supplementary file 5, the rapid and direct attack of ROS may cause homolysis of amentoflavone to generate amentoflavone• (Ⅰ), and hydrogen atom (•H) which can instantly combine •OH to give rise to stable H_2O molecule. It is considered as the possible mechanism for amentoflavone to donate hydrogen atom (•H).

The mechanisms of donating hydrogen atom (H•) and electron (***e***), however, can also be used for the interpretation of another approach for amentoflavone to protect DNA oxidative damage, i.e. deoxynucleotide radicals repairing (Supplementary file 6).

As shown in Supplementary file 6, amentoflavone was thought to donate either hydrogen atom (H·) or electron (***e***) to 2′-deoxyadenosine-5′-monophosphate radical (dAMP• radical, a typical deoxynucleotide radical) to yield amentoflavone· radical (Ⅰ), and dAMP molecule. Here dAMP• radical has been repaired and converted into a stable dAMP molecule without cytotoxicity. Obviously, the proposed mechanism agrees with the previous study [21]. On this basis, we regard the repairing approach as a special free radical-scavenging one, via which amentoflavone scavenge deoxynucleotide radical instead of ROS. It is easy to imagine that amentoflavone· radical can also be transferred into semi-quinone (Ⅲ), if deoxynucleotide radicals are excessive. In other words, both scavenging and repairing approaches of amentoflavone could be considered as radical-scavenging (i.e. antioxidant) mechanism, and both approaches could yield the final oxidized product semi-quinone (Ⅲ). As illustrated in Figure 2, semi-quinone (Ⅲ) is actually a stable form bearing a large π-π conjugation, and undoubtedly arises from the oxidation of phenolic -OH moiety in the amentoflavone molecule. It means that the antioxidant of amentoflavone results from phenolic -OH moiety. Of course, it is identical with many previous studies on structure-activity relationships of phenolic antioxidants. However, none of the previous literatures pointed out the fundamental reason behind the structure-activity relationship. Here we used the reaction of amentoflavone with DPPH• (Fig. 2) to clarify that, the reason why phenolic -OH group is essential for antioxidant of ability phenolic compound, is the stability of its oxidized product (i.e. semi-quinone form).

As mentioned in Eq. 2&3, radiation on water in cell can also be regarded as another resource of hydroxyl radical [13]. Similarly, hydroxyl radicals, once generated, can also induce DNA oxidation to give rise to various oxidative lesions which is related to cancers. Thus, ultraviolet radiation was proved to cause several human cancers [22-23], especially skin cancer [24] and breast cancer [22-25]. In the study, however, amentoflavone was observed to be a treating agent for the carcinogenesis [26-27]. Therefore, it can be induced that the anti-cancer effect of amentoflavone could be partly attributed to its protective effect against DNA oxidative damage and antioxidant mechanism. It is also consistent with the previous findings that tumor promotion of carcinogen was positively relevant to its prooxidant and ROS generation [28]. Since amentoflavone could efficiently scavenge excessive ROS which are well known to cause aging, and amentoflavone could be used for the treatment of skin aging induced by UV irradiation [3-4], hence it can be inferred that its pharmacological effect on skin aging is assumed to be via antioxidant mechanism.

Finally, anti-inflammatory effects of amentoflavone are also thought to be mainly attributed to its antioxidant ability. As we know, inflammation is mediated by ROS and its metabolites [29-30]. Amentoflavone, however, could scavenge ROS and itself was oxidized to semi-quinone. It undoubtedly is regarded as one possible mechanism for its anti-inflammation.

Conclusions

In conclusion, amentoflavone can effectively protect against hydroxyl-induced oxidative damage DNA (including base & deoxyribose moieties) via deoxynucleotide radical repairing approach, and ROS scavenging approach which may be mediated by donating hydrogen atom (H•) and electron (***e***). However, both scavenging and repairing approaches can result in its oxidation to a semi-quinone by free radicals as well. Therefore, the protective effect of amentoflavone can be primarily attributed to its antioxidant. From a structure-activity relationship viewpoint, its antioxidant ability may arise from the phenolic -OH moiety, and ultimately to the stability of its oxidized product semi-quinone form. Its protective effect against hydroxyl-induced DNA damage may be mainly responsible for the radioprotective and anti-inflammation effects.

Acknowledgements

This work was supported by the National Nature Science Foundation of China (81273896). In this work, XL designed the study and wrote the manuscript. LW and WH performed the data of antioxidant. WM tested the protective effect and checked the manuscript. DC revised the manuscript. All authors read and approved the final manuscript.

Conflict of Interest: The authors declare that there are no conflict of interests.

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