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Protective Effect Against Hydroxyl Radical-induced DNA Damage and Antioxidant Mechanism of [6]-gingerol: A Chemical Study

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[6]-Gingerol is known as the major bioactive constituent of ginger. In the study, it was observed to effectively protect against •OH-induced DNA damage (IC_{50} 328.60 ± 24.41 μM). Antioxidant assays indicated that [6]-gingerol could efficiently scavenge various free radicals, including •OH radical (IC_{50} 70.39 ± 1.23 μM), $•O_2^-$ radical (IC_{50} 228.40 ± 9.20 μM), DPPH• radical (IC_{50} 27.35 ± 1.44 μM), and $ABTS^+$• radical (IC_{50} 2.53 ± 0.070 μM), and reduce Cu^{2+} ion (IC_{50} 11.97 ± 0.68 μM). In order to investigate the possible mechanism, the reaction product of [6]-gingerol and DPPH• radical was further measured using HPLC combined mass spectrometry. The product showed a molecular ion peak at *m/z* 316 $[M+Na]^+$, and diagnostic fragment loss (*m/z* 28) for quinone. On this basis, it can be concluded that: (*i*) [6]-gingerol can effectively protect against •OH-induced DNA damage; (*ii*) a possible mechanism for [6]-gingerol to protect against oxidative damage is •OH radical scavenging; (*iii*) [6]-gingerol scavenges •OH radical through hydrogen atom (H•) transfer (HAT) and sequential electron (***e***) proton transfer (SEPT) mechanisms; and (*iv*) both mechanisms make [6]-gingerol be oxidized to semi-quinone or quinone forms.

Key Words : [6]-Gingerol, Hydrogen atom transfer, Sequential electron proton transfer, Antioxidant mechanism, Hydroxyl radical

Introduction

As the most harmful reactive oxygen species (ROS), hydroxyl radical (•OH) can oxidatively damage DNA, lead to deleterious biological consequences, including genetic mutation,[1] carcinogenesis,[2] and cell death.[2] Therefore, it is critical to search for potential therapeutic agents for oxidative DNA damage. Since ginger has been demonstrated to possess beneficial effects on cells,[3] its relevant bioactive compounds had attracted considerable attention in recent years. As a major pungent principle of ginger, [6]-gingerol (Fig. 1) has therefore been intensively investigated for pharmacological and physiological activities.

Several studies suggested that [6]-gingerol can be responsible for the anti-inflammatory, anti-tumour and antioxidant activities of ginger. [6]-Gingerol can prevent against various cancers,[4,5] especially prostate cancer,[6] skin cancer,[7] colon cancer.[8] In addition, it has been observed to attenuate oxidative cell death[9] and prevent genotoxicity.[10]

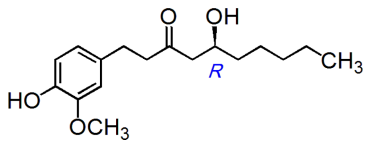

Figure 1. The chemical structure of [6]-gingerol.

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These effects are thought to be related to its protective effects against oxidative DNA damage and antioxidant ability.

Previously, Dugasani and colleagues have compared the antioxidant levels of four bioactive components in ginger (including [6]-gingerol).[11] Unfortunately, some experimental data are not reliable, including superoxide radical scavenging and hydroxyl radical scavenging assays. In hydroxyl radical scavenging assay, they used DMSO for sample solution preparation, and the sample solution was directly used for hydroxyl radical-scavenging assay. As mentioned in our previous report,[12] DMSO itself can scavenge •OH radical and bring about considerable interference. In fact, the reaction of DMSO with •OH had been recognized for over 30 years.[13,14] The reaction rate was calculated as 9×10^9 L mol^{-1} s^{-1}.[15] The product of •OH reaction with DMSO has been demonstrated to be $•CH_3$.[16] In addition, in xanthine-luminal-xanthine oxidase assay, since alkaline DMSO can generate superoxide anion radicals,[17,18] DMSO as the solvent undoubtedly cause interference with the determination of superoxide radical scavenging. In a word, it is necessary to reevaluate the antioxidant level of [6]-gingerol by a reliable method.

On the other hand, despite that there have been several computational studies for free radical-scavenging mechanisms[19] and some hydrogen atom transfer kinetic studies for DPPH• scavenging,[20] no mechanistic study based on oxidized product analysis has been reported. The present study, however, tried to use HPLC and MS to explain the

mechanism. The structure elucidation of mass spectrum can be used to interpret the antioxidant mechanism of [6]-gingerol. Obviously, the present study will provide important insights into the mechanisms underlying the antioxidant of [6]-gingerol.

Experimental

Chemicals. [6]-Gingerol (CAS number: 519-34-6, 98%) was obtained from Weikeqi Biological Technology Co., Ltd (Chengdu, China). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH•), (±)-6-hydroxyl-2,5,7,8-tetramethlychromane-2-carboxylic acid (Trolox), and BHA (butylated hydroxyanisole) were from Sigma-Aldrich Shanghai Trading Co. (Shanghai, China). Deoxyribose and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) (ABTS diammonium salt) were obtained from Amresco Inc. (Solon, OH, USA). DNA sodium salt (fish sperm, 98%) was purchased from Aladdin Chemistry Co. (Shanghai, China). Water and methanol were of HPLC grade. All other reagents were of analytical grade.

Protective Effect Against •OH-induced DNA Damage. The experiment was conducted using the method developed by our laboratory.[21] Briefly, the sample was firstly dissolved in 95% ethanol at 2 mg/mL. The sample solution was then aliquoted into tubes. After evaporating the sample solutions in the tubes to dryness, 300 μL of phosphate buffer (0.2 M, pH 7.4) was added to the sample residue. Subsequently, 50 μL of DNA sodium (10 mg/mL), 75 μL of H_2O_2 (33.6 mM), 50 μL of $FeCl_3$ (3.2 mM) and 100 μL of Na_2EDTA (0.5 mM) solution were added. The reaction was initiated by adding 75 μL of ascorbic acid (1.2 mM). After incubation in a water bath at 55 °C for 20 min, the reaction was terminated by adding 250 μL of trichloroacetic acid (10%, w/w). The color was then developed by addition of 150 μL of TBA (5%, in 1.25% NaOH aqueous solution) and heating in an oven at 105 °C for 15 min. The mixture was cooled and the absorbance was measured at 530 nm against the buffer (as the blank). The inhibition percentage for •OH is expressed as follows:

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

Where A_0 is the absorbance at 530 nm of the control without sample, and A is the absorbance at 530 nm of the reaction mixture with sample.

Hydroxyl Radical (•OH) Scavenging Assay. The experiment of •OH radical-scavenging was conducted according to our method.[12] In brief, the sample ethanolic solution (1 mg/mL) was separately added into tubes. After evaporating the sample solutions in the tubes to dryness, 550 μL of phosphate buffer (0.2 M, pH 7.4) was added to the sample residue. Then, 50 μL glucose (2.8 mM), 50 μL Na_2EDTA (1 mM), 50 μL $FeCl_3$ (3.2 mM) and 50 μL H_2O_2 (2 mM) were added. The reaction was initiated by mixing 50 μL ascorbic acid (1.8 mM) and the total volume of the reaction mixture was adjusted to 800 μL with buffer. After incubation at 50 °C for 20 min, the reaction was terminated by 500 μL trichloroacetic acid (5%, w/w). The color was then developed by addition of 500 μL TBA (5%, 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 532 nm (Unico 2100, spectrophotometer, Shanghai, China) against the buffer (as blank). The hydroxyl radical scavenging activity was expressed as:

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

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

Superoxide Anion ($\bullet O_2^-$) Radical-scavenging Assay. Measurement of superoxide anion ($\bullet O_2^-$) scavenging activity was based on our method.[22] Briefly, the sample was dissolved in methanol at 2 mg/mL. The sample solution (x μL, where x = 0, 30, 60, 90, 120 and 150 μL) was mixed with 2960-x μL Tris-HCl buffer (0.05 mol/L, pH 7.4) containing Na_2EDTA (1 mmol/L). When 40 μL pyrogallol (60 mmol/L in 1 mmol/L HCl) was added, the mixture was shaken at room temperature immediately. The absorbance at 325 nm of the mixture was 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:

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

Here, $\Delta A_{325\text{nm},control}/T$ is the increase in $A_{325\text{nm}}$ of the mixture without the sample and $\Delta A_{325\text{nm},sample}/T$ 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.[23] Briefly, 500 μL of DPPH• solution (0.1 mM) was mixed with 250 μL sample 95% ethanol solution with various concentrations. The mixture was kept at room temperature for 30 min, and then the absorbance was measured at 519 nm against 95% ethanol (as 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; while A_0 is the absorbance without samples. Trolox and BHA were used as the positive controls.

HPLC Analysis and Mass Spectrometry of Reaction Product of [6]-gingerol and DPPH• Radical. The product mixture of [6]-gingerol and DPPH• radical (10:3, mol/mol) was filtered using 0.45 μm filters then analyzed by a preparative HPLC system (Prominence LC-20A, Shimadzu, Japan), equipped with a Diamonsil C_{18} (250 mm × 4.6 mm, 5 μm) column (Dikma Co., Beijing, China). The mobile phase consisted of methanol-0.5% acetic acid (90:10, v: v) and the flow rate was 0.5 mL/min, injection volume was 27 μL,

detection wavelength was 225 nm. [6]-Gingerol and DPPH• radical were also comparatively measured under the same chromatographic conditions.

The reaction product isolated by HPLC was further analyzed by a microflex LT MALDI TOF-Q II (Bruker Daltonics, USA) mass spectrometer which was equipped with an electrospray ionisation (ESI) source and run in positive mode. The scan range was 50-3000 *m/z*. ESI parameters were optimised with direct infusion of dansylated amine mixture by an external syringe and set as follows: capillary, +4.5 kV; nebulizer pressure, 0.3 bar; dry gas flow, 4.0 L/min; dry gas temperature, 180 ºC. Argon was applied as the collision gas, and the collision energy was set to 10 eV to provide some structural information and to focus ion flux. High purity nitrogen was applied both as a nebulizer gas and a drying gas. The Q-TOF/MS parameters were optimised to the following: funnel 1 was 300.0 Vpp and 2 was 400.0 Vpp; hexapole Rf was 400.0 Vpp; quadrupole ion energy was 5.0 eV; collision Rf was 650.0 Vpp. The ion transfer time and prepulse storage time were set to 120 and 10 us, respectively.

$ABTS^{+}$• Radical-scavenging Assay. The $ABTS^{+}$• scavenging activity was evaluated by the method.[23] The $ABTS^{+}$• was produced by mixing 350 μL ABTS diammonium salt (7.4 mM) with 350 μL $K_2S_2O_8$ aqueous 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. Then, 0.6 mL diluted $ABTS^{+}$• reagents were brought to 150 μL sample ethanolic solutions (0.01 mg/mL). After incubation for 6 min, the absorbance at 734 nm was read on a spectrophotometer (Unico 2100, Shanghai, China). The percentage inhibition was calculated as:

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

Where A_0 is the absorbance of the control without any samples, A is the absorbance of the mixture with [6]-gingerol, Trolox, or BHA.

Cu^{2+}-reducing Power Assay. The cupric ions (Cu^{2+}) reducing power capacity was determined based on the method,[23] with a slight modification. In brief, 100 μL $CuSO_4$ aqueous solution (10 mM), 100 μL neocuproine ethanolic solution (7.5 mM) and 400 μL CH_3COONH_4 buffer solution (100 mM, pH 7.0) were brought to test tubes with different volumes of samples (0.1 mg/mL, 15-75 μL). Then, the total volume was adjusted to 800 μL with the buffer and mixed vigorously. Absorbance against a buffer blank was measured at 450 nm after 15 min. The relative reducing power of the sample as compared with the maximum absorbance, was calculated using the formula:

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

Here, A_{max} is the maximum absorbance in the test and A_{min} is the minimum absorbance in the test. A is the absorbance of sample.

Statistical Analysis. Each experiment was performed in triplicate and the data were recorded as mean ± SD (standard deviation). The IC_{50} value was defined as the final concentration of 50% radical inhibition (relative reducing power, or chelating effect). Statistical comparisons were made by one-way ANOVA to detect significant difference using SPSS 13.0 (SPSS Inc., Chicago, IL) for Windows. $P < 0.05$ was considered to be statistically significant.

Results and Discussion

In the present study, we used Fenton reaction (Equation 1) to produce •OH radicals for the investigation on the protective effect of [6]-gingerol against oxidative DNA damage.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \text{•}OH + OH^{-} \quad (1)$$

The data indicated that [6]-gingerol and the positive controls dose-dependently increased the protective effect against oxidative DNA damage at 0-200 μg/mL (Fig. 2). Based on the IC_{50} values (328.60 ± 24.41 and 690.76 ± 12.31 μM, respectively for [6]-gingerol and Trolox, Table 1), it can be inferred that [6]-gingerol presented 2.10 times higher protective effect than the standard antioxidant Trolox in our model. This is consistent with the previous study, in which [6]-gingerol was found to prevent UVB-induced ROS production and oxidative DNA damage.[24] Its protective effect against DNA oxidative damage may be primarily responsible for the pharmacological effects, including anti-inflammatory and anti-cancer effects.[4-8] In fact, oxidative DNA damage has been observed to play a key role in inflammation-related carcinogenesis.[25]

Previous work has shown that there are two approaches for natural phenolic antioxidants to protect against oxidative DNA damage: one is to scavenge the •OH radical prior to DNA damage; and the other is to prevent the DNA radicals resulting from •OH radical attack.[26]

To further confirm whether the protective effect of [6]-gingerol against oxidative DNA damage is relevant to its

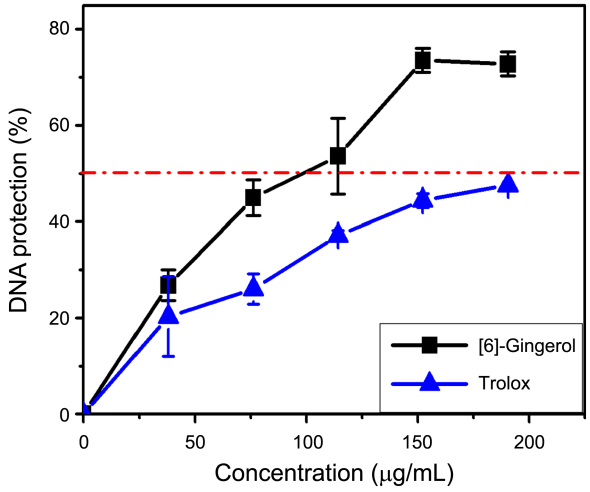

Figure 2. The protective effect against •OH radical-induced DNA damage of [6]-gingerol and positive control Trolox (Each value is expressed as mean ± SD, $n = 3$).

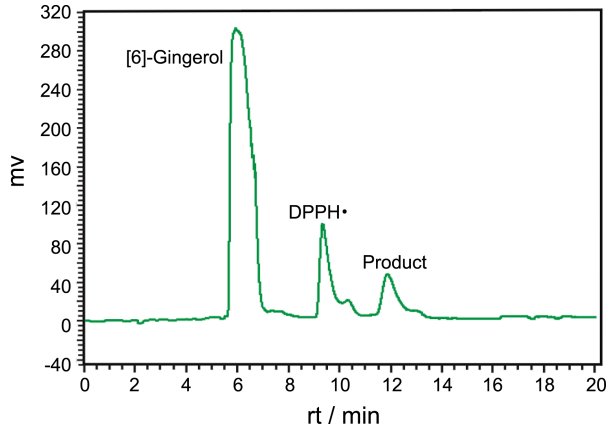

Figure 3. Typical HPLC chromatogram of the reaction product of [6]-gingerol with DPPH• (1,1-Diphenyl-2-picrylhydrazyl radical).

radical-scavenging ability, we then determined the radical-scavenging abilities of [6]-gingerol on •OH and $•O_2^-$. In the •OH radical-scavenging assay (Suppl. Fig. S1), [6]-gingerol could effectively scavenge •OH radicals at 0-22.5 μg/mL (0-77.7 μm) and its IC_{50} value was calculated as 70.39 ± 1.23; In the $•O_2^-$ radical-scavenging assay (Suppl. Fig. S2), [6]-gingerol along with the positive controls almost linearly increased the $•O_2^-$ radical-scavenging percentages, and the IC_{50} value of [6]-gingerol was 228.40 ± 9.20 μM (Table 1). These data clearly suggest that ROS-scavenging (especially •OH-scavenging) is one possible mechanism for [6]-gingerol to protect against oxidative DNA damage.

To explore the possible mechanism for [6]-gingerol to scavenge ROS, we further explored the radical-scavenging effect on DPPH• and $ABTS^+$• radicals.

The DPPH assay revealed that [6]-gingerol possessed a concentration-dependent effect at 0-10 μg/mL (0-34.01 μM, Suppl. Fig. S3), and the IC_{50} was calculated as 27.35 ± 1.44 μM (Table 1). It means that [6]-gingerol can effectively eliminate DPPH• radical.

In order to further explore the mechanism for [6]-gingerol to scavenge DPPH• radical, the reaction product of [6]-gingerol with DPPH• was measured using HPLC (Fig. 3). The HPLC profile clearly indicated a peak as the product at 11.98 min (retention time).

When the product was further analyzed by mass spectrometry, it gave a molecular ion peak at *m/z* 316 [M+Na^+] which was obviously one less than reactant [6]-gingerol at *m/z* 317 [M+Na^+]. It indicated a hydrogen atom transfer of [6]-gingerol to product. In addition, fragment units of *m/z* 302.30, 274.27, and 246.24 in the product were also observed. Obviously, these fragment units showed a loss of C=O (*m/z* 28) which is regarded as the characteristic diagnostic fragment loss for quinone (Fig. 4(a)). On the other hand, the reactant [6]-gingerol, however, did not exhibit the characteristic losses at *m/z* 28 (Fig. 4(b)). In a word, the hydrogen atom has been shown to be transferred from [6]-gingerol to DPPH•, and [6]-gingerol has been oxidized to semi-quinone or quinone by DPPH•.

Based on the above data, and previous report which DPPH•

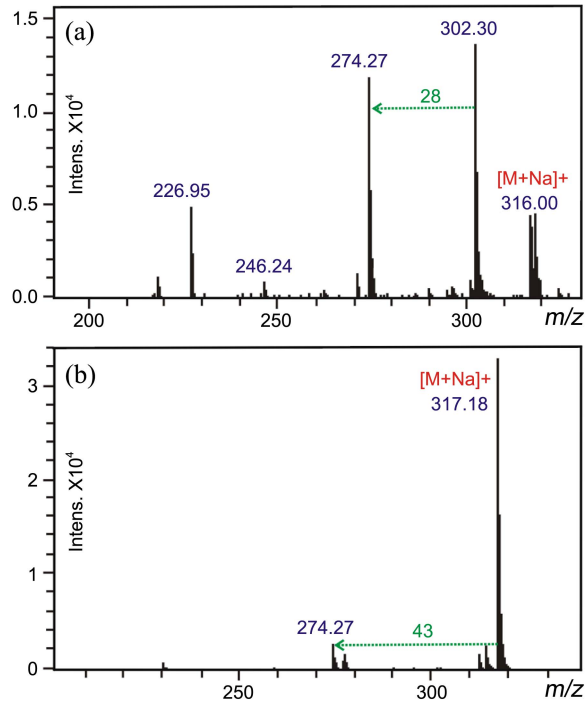

Figure 4 Mass spectra of the reaction product [6]-gingerol with DPPH• (a), and [6]-gingerol (b).

may be scavenged through hydrogen atom (H•) transfer (HAT) to form DPPH-H molecule,[27] the proposed reaction of [6]-gingerol with DPPH• can be illustrated in Figure 5. As shown in Figure 5, the reaction of [6]-gingerol with DPPH• was thought to yield a phenoxyl radical (I), which can be converted to semi-quinone (II). Semi-quinone (II), however, possessed various resonances formula, *e.g.* semi-quinone (III), semi-quinone (IV), and phenoxyl radical (I) which is some extent stable a stable species. Thus, step 1 for [6]-gingerol to transfer a hydrogen atom is easily to be initiated. Under the conditions of excessive DPPH•, however, semi-quinone (IV) may be further be extracted hydrogen atom to

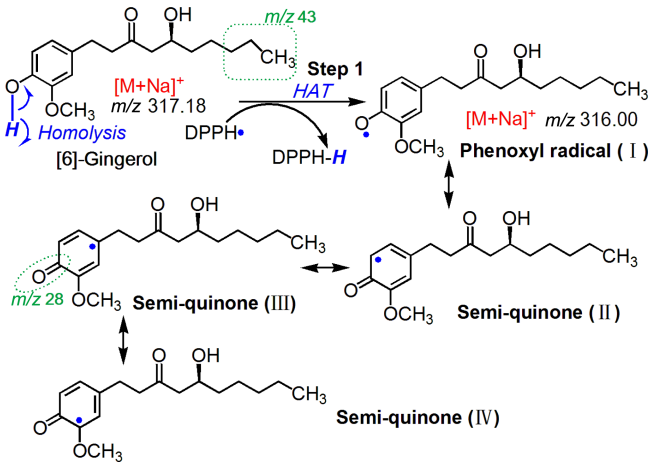

Figure 5. The proposed reaction of [6]-gingerol with DPPH• *via* HAT (hydrogen atom transfer) mechanism based on mass spectrometry analysis.

produce benzoquinone. However, since the *ortho*-position of –OH is –OCH_3 not –OH, the hydrogen extraction isn't so easy, therefore, the yield of benzoquinone is very less, and the peak at *m/z* 246.24 is very low in MS spectrometry (Fig. 4(a)).

Besides DPPH• scavenging, $ABTS^{+}$• scavenging has also been used for investigation on the antioxidant mechanism of [6]-gingerol in the study. As seen in Suppl. Fig. S4, [6]-gingerol linearly (R=0.99925) increased its $ABTS^{+}$• scavenging percentages at 0-1.33 μg/mL (0-4.55 μM) and its IC_{50} was 2.53 ± 0.070 μM (Table 1). As we know, $ABTS^{+}$• scavenging is an electron (e) transfer process.[28] In the process, ***e*** transfer is always accompanied by deprotonation, so it is termed a sequential electron proton transfer (SEPT) mechanism,[29] or proton coupled electron-transfer (PCET) mechanism,[30] sequential proton loss single electron transfer (SPLET).[31] The SEPT mechanism for [6]-gingerol to scavenge $ABTS^{+}$• was proposed as described in Figure 6. Through SEPT mechanism, [6]-gingerol might change to phenoxyl radical (I). The SEPT mechanism is also supported by Cu^{2+} assay, in which [6]-gingerol increased the reducing percentage in a dose-dependent manner (Suppl. Fig. S5) with IC_{50} value being 11.97 ± 0.68 μM (Table 1). As we know, reducing reactions are actually an electron (***e***) transfer process. Similarly, phenoxyl radical (I) was thought to be further converted into semi-quinones, even quinone under excess $ABTS^{+}$• radicals or Cu^{2+} ions.

The fact that [6]-gingerol could effectively scavenge both $ABTS^{\bullet+}$ and DPPH• radicals, suggests the process of [6]-gingerol scavenging of ROS (especially •OH radicals) in cells would be mediated *via* HAT and SEPT mechanisms.

As shown in Eq. (1), the generation of •OH radicals may

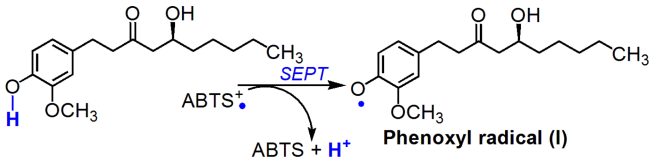

Figure 6. The proposed reaction of [6]-gingerol with $ABTS^{+}$• *via* SEPT (sequential electron proton transfer) mechanism.

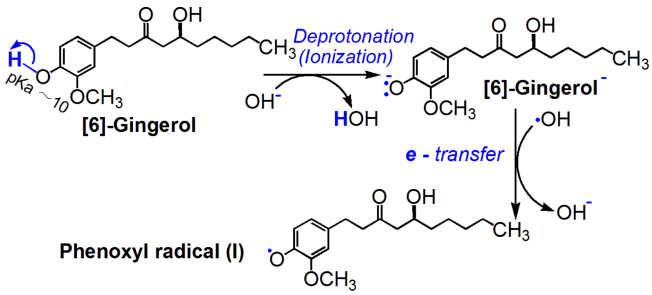

Figure 7. The proposed reaction for [6]-gingerol to scavenge •OH *via* SEPT (sequential electron proton transfer) mechanism.

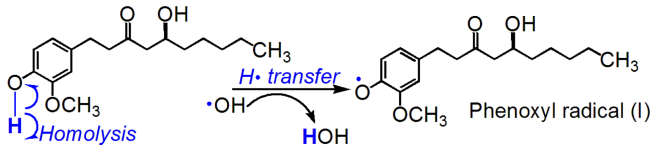

Figure 8. The proposed reaction for [6]-gingerol to scavenge •OH *via* HAT (hydrogen atom transfer) mechanism.

also bring about equal OH^{-} ions in cells. Therefore, at high levels of •OH radicals, massive OH^{-} ions could alkalize the cellular environment. Under alkaline conditions, however, the acidity may predominate over the chemical action of [6]-gingerol. As a phenol, [6]-gingerol presents a weak acidity (pK_a ~10).[32] Thus, [6]-gingerol might easily ionize to yield H^{+} ions, and [6]-$gingerol^{-}$ anion which could further donate an electron (***e***) to •OH radicals to form phenoxyl radical (I) (Fig. 7), even semi-quinones and benzoquinone. This is a possible mechanism for [6]-gingerol to directly scavenge •OH *via* the SEPT mechanism. The mechanism is similar to that of *trans*-resveratrol toward •OH radicals.[33]

However, at low levels of •OH radicals, the cellular environment was almost neutral and the acidity could not predominate over the chemical action of [6]-gingerol. The rapid and direct attack of •OH radicals may cause homolysis of [6]-gingerol to generate phenoxyl radical (I) and hydrogen atom (•H). Hydrogen atom, however, would immediately be donated to •OH to form the stable H_2O molecule (Fig. 8).

Table 1. The IC_{50} values of [6]-gingerol, Trolox, and BHA in various assays (μM)

Assays	[6]-Gingerol	Positive controls		Ratio
		Trolox	BHA	
DNA protection	328.60 ± 24.41^{a}	690.76 ± 12.31^{b}	N.D.	2.10
•OH scavenging	70.39 ± 1.23^{a}	93.00 ± 1.35^{b}	124.62 ± 3.68^{c}	1.32
$•O_2^{-}$ scavenging	228.40 ± 9.20^{a}	226.54 ± 6.35^{a}	358.97 ± 11.41^{b}	0.99
DPPH• scavenging	27.35 ± 1.44^{a}	29.26 ± 0.59^{a}	34.71 ± 0.81^{b}	1.07
$ABTS•^{+}$ scavenging	2.53 ± 0.070^{a}	7.36 ± 0.43^{c}	3.41 ± 0.10^{b}	2.91
Cu^{2+} reducing	11.97 ± 0.68^{a}	36.83 ± 1.43^{c}	23.16 ± 0.21^{b}	3.08
Average				1.91

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 positive control is Sodium citrate. Ratio = $IC_{50,Trolox}$: $IC_{50,[6]\text{-gingerol}}$. The dose response curves of [6]-gingerol in antioxidant assay were shown in Supplemental 1. N.D., not determined. BHA, butylated hydroxyanisole.

Meanwhile, phenoxyl radical (I) changed into semi-quinones. This may be the HAT mechanism for [6]-gingerol to directly scavenge •OH. This mechanism agrees with the previous findings that the dopamine reaction towards •OH is mainly *via* HAT at physiological pH 7.4.[28]

To quantitatively evaluate the relative antioxidant level of [6]-gingerol, the ratio value was defined as $IC_{50,Trolox}/IC_{50,[6]\text{-gingerol}}$. As shown in Table 1, the ratio values of oxidative DNA damage, •OH-scavenging, $\cdot O_2^-$ scavenging, DPPH• scavenging, $ABTS^{+}$• scavenging, and Cu^{2+}-reducing were 2.10, 1.32, 0.99, 1.07, 2.91, and 3.08, respectively. The average ratio value was calculated as 1.91 (Table 1). It implies that [6]-gingerol had 1.91 times higher the total antioxidant capacity than the standard antioxidant Trolox.

It must be emphasized that the IC_{50} values of [6]-gingerol in our $\cdot O_2^-$ and •OH scavenging assays were respectively 228.40 ± 9.20 and 70.39 ± 1.23 μM, while they were calculated as 4.05 and 4.62 μM respectively in the previous literature.[11] Undoubtedly, there is a considerably difference. However, the IC_{50} values in DPPH scavenging assay are generally identical: As shown in Table 1, it was listed as 27.35 ± 1.44 μM in our study, while the previous literature shown as 26.3 μM.[11] It clearly indicated that DMSO used as a solvent in the previous work indeed brought about considerable interference with the $\cdot O_2^-$ and •OH scavenging assays.[11]

Conclusion

Based on the above discussion, it can be concluded that: (*i*) as the major bioactive constituent of ginger, [6]-gingerol can effectively protect against •OH-induced DNA damage; (*ii*) a possible mechanism for [6]-gingerol to protect against oxidative damage is •OH radical scavenging; (*iii*) [6]-gingerol scavenges •OH radicals possibly through hydrogen atom (H•) transfer (HAT) and sequential electron (***e***) proton transfer (SEPT) mechanisms; and (*iv*) radical scavenging makes [6]-gingerol be oxidized to semi-quinone or quinone forms.

Supplementary Materials: Dose response curves of antioxidant assays of [6]-gingerol and positive controls.

Conflict of Interest Statement. The authors confirm that there are no conflicts of interest.

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References

1. Hashizume, O.; Shimizu, A.; Yokota, M.; Sugiyama, A.; Nakada, K.; Miyoshi, H.; Itami, M.; Ohira, M.; Nagase, H.; Takenaga, K. *Proc. Natl. Acad. Sci.* **2012**, *109*, 10528-10533.
2. Bhattacharjee, S.; Deterding, L. J.; Chatterjee, S.; Jiang, J.; Ehrenshaft, M.; Lardinois, O.; Ramirez, D. C.; Tomer, K. B.; Mason, R. P. *Free Radic. Biol. Med.* **2011**, *50*, 1536-1545.
3. Butt, M. S.; Sultan, M. T. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 383-393.
4. Surh, Y. J. *Food Chem. Toxicol*. **2002**, *8*, 1091-1097.
5. Bode, A. M.; Ma, W. Y.; Surh, Y. H.; Dong, Z. G. *Cancer Res.* **2001**, *61*, 850.
6. Shukla, Y.; Prasad, S.; Tripathi, C.; Singh, M.; George, J.; Kalra, N. *Mol. Nutr. Food Res.* **2007**, *12*, 1492-1502.
7. Park, K. K.; Chun, K. S.; Lee, J. M.; Lee, S. S.; Surh, Y. J. *Cancer Letters* **1998**, *19*, 139-144.
8. Lin, C. B.; Lin, C. C.; Tsay, G. J. *Evid. Based Complement Alternat. Med*. **2012**, *7*, 1-7.
9. Lee, C.; Park, G. H.; Kim, C. Y.; Jang, J. H. *Food Chem. Toxicol.* **2011**, *6*, 1261-1269.
10. Yang, G.; Zhong, L.; Jiang, L.; Geng, C.; Cao, J.; Sun, X.; Chen, M.; Ma, Y. *Phytother Res*. **2011**, *10*, 1480-1485.
11. Swrnalatha, D.; Mallikarjuna, R. P.; Vishna, D. N.; Madhu, K. B.; Satyanarayana, T.; Jayaveera, N. K. *J. Ethnopharmacol.* **2010**, *127*, 515-520.
12. Li, X. C. *Food Chem.* **2013**, *3*, 2083-2088.
13. Doroshow, J. H. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 4514-4518.
14. Repine, J. E.; Pfenninger, O. W.; Talmage, D. W.; Berger, E.M.; Pettijohn, D. E. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 1001-1003.
15. Nakai, K.; Kadiiska, M. B.; Jiang, J. J.; Stadler, K.; Mason, R. P. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 4616-4621.
16. Chan, J.; Fujiwara, T.; Brennan, P.; McNeil, M.; Turco, S. J.; Sibille, J. C.; Snapper, M.; Aisen, P.; Bloom, B. R. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 2453-2457.
17. Qiao, X. L.; Chen, S. M.; Tan, L.; Zheng, H.; Ding, Y. D.; Ping, Z. H. *Magn. Reson. Chem.* **2001**, *39*, 207-211.
18. Hyland, K.; Voisin, E.; Banoun, H.; Auclair, C. *Anal. Biochem.* **1983**, *2*, 280-287.
19. Luga, C.; Alvarez-Idaboy, J. R.; Russo, N. *J. Org. Chem.* **2012**, *8*, 3868-3877.
20. Boudier, A.; Tournebize, J.; Bartosz, G.; Bengueddour, R.; Leroy, P. *Anal. Chim. Acta* **2012**, *20*, 97-116.
21. Li, X. C.; Mai, W. Q.; Wang, L.; Han, W. J. *Anal. Biochem.* **2013**, *438*, 29-31.
22. Li, X. C. *J. Agric. Food Chem*. **2012**, *25*, 6418-6424.
23. Li, X. C.; Fang, Q.; Lin, J.; Yuan, Z. P.; Han, L.; Gao, Y. X. *Bull. Korean Chem. Soc*. **2014**, *35*, 117-122.
24. Kim, J. K.; Kim, Y.; Na, K. M.; Surh, Y. J.; Kim, T. Y. *Free Radic. Res*. **2007**, *5*, 603-614.
25. Ohnishi, S.; Ma, N.; Thanan, R.; Pinlaor, S.; Hammam, O.; Murata, M.; Kawanishi, S. *Oxid. Med. Cell. Longev.* **2013**, *2013*, 387014.
26. Zheng, R. L., Huang, Z. Y. *Free Radical Biology*; Higher Education Press: Beijing, 2007; p 143.
27. Bondet, V.; Berset, C. *LWT-Food Sci. Technol.* **1997**, *30*, 609-615.
28. Aliaga, C.; Lissi, E. A. *Int. J. Chem. Kinet.* **1998**, *30*, 565-570.
29. Iuga, C.; Alvarez-Idaboy, J. R.; Vivier-Bunge, A. *J. Phys. Chem. B* **2011**, *115*, 12234-12246.
30. Nguyen, T. X.; Grampp, G.; Yurkovskaya, A. V.; Lukzen, N. *J. Phys. Chem. A* **2013**, *33*, 7655-7660.
31. López-Munguía, A.; Hernandez-Romero, Y.; Pedraza-Chaverri, J.; Miranda-Molina, A.; Regla, I.; Martinez, A.; Castillo, E. *PLoS. One* **2011**, *6*, e20115.
32. Graham Solomons, T. W.; Fryhle, C. B. *Organic Chemistry*; Chemical Industry Press: Beijing, 2003; p 1008.
33. Iuga, C.; Alvarez-Idaboy, J.R.; Russo, N. *J. Org. Chem.* **2012**, *77*, 3868-3877.