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Concentration-dependence of prooxidant and antioxidant effects of aloin and aloe-emodin on DNA

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Abstract

To understand the toxic effects and conflicting reports of aloin and aloe-emodin, we investigated their antioxidant and prooxidant effects on free radical-induced DNA breaks over a wide range of concentrations. Aloin, at concentrations ranging from 1.25 to 2.5 mM, prevented ·OH-induced DNA breaks by 5–30% over control values. In contrast, addition of aloin at lower concentrations (8–300 μM) increased DNA damage by about 24–74%, indicating its prooxidant effect. Aloe-emodin showed little antioxidant effect at any tested concentration, but addition of higher concentrations (1.25–2.5 mM) of aloe-emodin had a prooxidant effect on DNA (about 29–35%). The greater reducing activity of aloin at low concentrations, reducing Fe^{3+} to Fe^{2+}, which enhanced the generation of ·OH from the Fenton reaction, may play a key role in its prooxidant effect on DNA. At higher concentrations, however, free radical-scavenging activity of aloin gradually predominated over its reducing power, resulting in the protection of DNA. Predomination of reducing power over scavenging activity resulted in the prooxidant effect of aloe-emodin at high concentrations. The relationship between structures and anti- or pro-oxidant effects of aloin and aloe-emodin is also discussed.

Keywords: Aloin; Aloe-emodin; Prooxidant effect; Antioxidant effect; DNA break; Reducing power

1. Introduction

Aloe has long been used for medicine, dietary supplements and cosmetic purposes. The Chinese have used the aloe's skin and the inner lining of its leaves to clear constipation due to accumulation of heat. *Aloe* extracts have been used to treat inflammation (Hutter et al., 1996), cancers (Yoshimoto, Kondoh, Isawa, & Hamuro, 1987) and AIDS (Kahlon et al., 1991). However, there are also researchers warning of possible allergic and toxic effects of aloe extracts for some patients (Avila, Rivero, Herrera, & Fraile, 1997; Briggs, 1995). Many active components have been isolated from *Aloe* species and studied for their biological activities. Among them, aloin and aloe-emodin (Fig. 1) have been identified as the main active components in *Aloe*. Yen, Duh, and Chuang (2000) investigated the antioxidant activity of aloe-emodin, which exhibited scavenging activity on free radicals. Aloe-emodin showed high inhibitory activity against peroxidation of linoleic acid catalyzed by soybean 15-lipoxygenase (Malterud, Farbrot, Huse, & Sund, 1993). In contrast, some toxic effects of aloe-emodin and aloin have also been established. Aloe-emodin and aloin had a disruptive effect on monolayer cell cultures (Reynolds & Dweck, 1999). Tests on human subjects exposed to UV radiation showed an allergic dermatitis after the application of *Aloe* extract (Dominguez-Soto, 1992). A recent study indicated that incubation of human skin fibroblasts with a certain dose of aloe-emdin, but not aloin, followed by irradiation with UV or visible light, resulted in photocytotoxicity,

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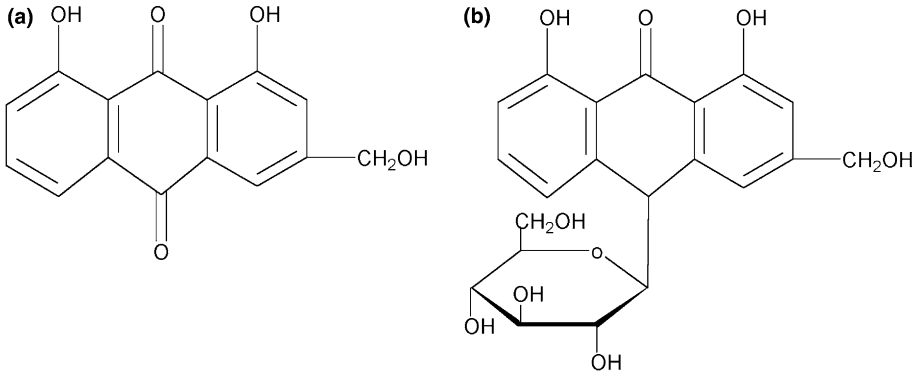

Fig. 1. Structure of aloe-emodin, 1,8-dihydroxy-3-(hydroxymethyl)-9,10-anthracenedione (a) and aloin, 10-glucopryanosyl-1,8-dihydroxy-3-(hydroxymethyl)-9(10*H*)-anthracenone (b).

accompanied by free radical-induced oxidative damage in cellular DNA (Wamer, Vath, & Falvey, 2003). The photoexcitation of aloe-emodin resulted in the formation of singlet oxygen ($^1O_2$).

These conflicting reports on aloe-emodin and aloin may be due to different treated doses or conditions. Excessive doses or a prolonged use of aloe extracts may cause nephritis, gastritis, vomiting and diarrhoea (Capasso et al., 1998). The active components seem to have different effects at different concentrations. It is necessary to study the mechanism of antioxidant and prooxidant effects of these ingredients over a wide range of concentrations and under different conditions.

This paper aims at an investigation of the antioxidant and prooxidant potential of aloe-emodin and aloin to determine the conditions and factors that have led to contradictory observations. Antioxidant activity was measured using a chemiluminescence method. The enhancement or inhibition, by aloin and aloe-emodin, of free radical-induced DNA damage without treatment by UV irradiation was used for evaluating their concentration-dependent anti- or pro-oxidant effects. To investigate the mechanism of prooxidant effect, reducing power of these compounds on iron ions were also determined.

2. Materials and methods

2.1. Materials

Aloe-emodin and aloin were obtained from Sigma (St. Louis, MO, USA). The commercial samples were subjected to further purification and were identified, by HPLC, to have a purity of 99% before use. Epigallocatechin-3-gallate (EGCG, Sigma Co.) was kindly provided by Prof. Shengrong Sheng from the Department of Tea Science of Zhejiang University. Selected compounds were dissolved in dimethyl sulfoxide (DMSO). Plasmid pBR322 DNA was purchased from Takara Biomedicals (Japan).

2.2. Free radical-scavenging activity assay

Free radical-scavenging activity was assayed by a chemiluminescence method (Olinescu & Kummerow, 2001). The reaction mixture contained 50 μl of luminol (1 mM), 700 μl of 50 mM carbonic acid buffered saline solution (pH 10.2, containing 0.1 mM EDTA), and 10 μl of tested sample or DMSO (in control experiment). 10 μl of 50 mM H_2O_2 were added to trigger the chemiluminescence reaction. Luminescence was counted every second (expressed as "Counts/s") on a BPCL Model Ultra Weak Chemiluminescence Analyzer (Institute of Biophysics, Academia Sinica, Beijing, China) at 37 °C. The amounts of luminosity (total counts) were integrated. Free radical-scavenging activities (%) were calculated by the following equation

$$\text{Scavenging activity}\,(\%) = [(CL_{control} - CL_0) - (CL_{sample} - CL_0)]/(CL_{control} - CL_0),$$

where $CL_{control}$ is the luminosity of control, CL_0 is the luminosity of background and CL_{sample} is the luminosity of test samples.

2.3. Measurements of reducing power

Reducing power on iron ion was measured according to the method of Oyaizu (1986) as described by Yen et al. (2000). 0.5 ml of sample was added to 0.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and the mixture was incubated at 50 °C for 20 min; 0.5 ml of 10% trichloroacetic acid was added, and then the mixture was centrifuged at 3000*g* for 10 min. The upper layer of the solution (1.0 ml) was mixed with 1 ml of distilled water and 0.2 ml of 0.1% ferric chloride ($FeCl_3$), and the absorbance was measured at 700 nm. Higher absorbance indicated greater reducing power.

2.4. Assay for testing the effects of aloin, aloe-emodin and EGCG on free radical-induced DNA strand breaks

To study the effects of samples on DNA, the reaction was conducted in an Eppendorf tube at a total volume of 12 μl containing 0.5 μg pBR322 DNA in 3 μl of 50 mM phosphate buffer (pH 7.4), 3 μl of 2 mM $FeSO_4$ and 2 μl of tested samples at various concentrations. Then, 4 μl of 30% H_2O_2 were added and the mixture was incubated at 37 °C for 1 h (Yeung et al., 2002). The mixture was subjected to 0.8% agarose gel electrophoresis. DNA bands (supercoiled, linear, and open circular) were stained with ethidium bromide and quantified by scanning the intensity of bands with Quantity One programme (version 4.2.3, BioRad Co.). Evaluations of antioxidant or prooxidant effects on DNA were based on the increase or loss percentage of supercoiled monomer, compared with the control value. To avoid the effects of photoexcitation of samples, experiments were done in the dark.

2.5. Statistical analysis

Data from chemiluminescence assays were processed using Microcal Origin 6.0 software (Microcal Software, Inc., Northampton, MA, USA). All the experiments were performed three times and average values were used.

3. Results and discussion

3.1. Free radical-scavenging effects of aloin and aloe-emodin

Chemiluminescence assay is a widely used method for investigating free radical-scavenging effects of compounds (Zhao, Yu, Wang, Liang, & Hu, 2003). Oxygen free radicals were produced by the reaction between hydrogen peroxide and luminol as described in the method section. As shown in Fig. 2, aloin scavenged free radicals in a dose dependent manner as did EGCG, a polyphenolic compound well known for its antioxidant property. EGCG showed stronger free radical-scavenging activity than aloin or aloe-emodin at all tested concentrations (scavenging 85% of free radicals at 0.24 mM). However, aloin exhibited significant scavenging effect only at high concentrations (0.9–2.2 mM). At concentrations ranging from 15 μM to 0.5 mM, scavenging activity of aloin was less than 6%. Aloe-emodin, showed a lower free radical-scavenging activity (0.3–4%) at all concentrations, than aloin and EGCG. As seen in Fig. 1, the structure difference between aloin and aloe-emodin was that a ketone group at the position C-10 of aloe-emodin is replaced by a glycoside in aloin. It was reported that glucose and glucan exhibited some degree

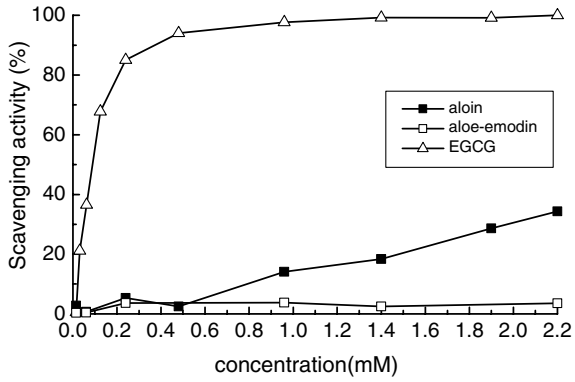

Fig. 2. Free radical-scavenging effects of aloin, aloe-emodin and EGCG. H_2O_2 was added to generate luminol chemiluminescence, and its inhibition by (■) aloin, (□) aloe-emodin, (△) EGCG at various concentrations was measured. Average values of three experiments are shown.

of free radical-scavenging activity, due to abstraction of the anomeric hydrogen from the reducing end (Tsiapali et al., 2001). We speculated that the free radical-scavenging activity of aloin may be partially due to the abstraction of the anomeric hydrogen from the C-10 glucoside, and its scavenging activity increased with increase of concentration. Moreover, anthrone, which lacks a ketone group at the position C-10, showed stronger free radical-scavenging activity than did aloe-emodin (Yen et al., 2000), indicating that the ketone group at C-10 in aloe-emodin lowered its scavenging activity.

3.2. Reducing power on iron ion

In a Fenton reaction, Fe^{2+} reacts with H_2O_2, resulting in the production of hydroxyl radical, which is considered to be the most harmful radical to biomolecules (Meneghini, 1997). Fe^{2+} is oxidized to Fe^{3+} in the Fenton reaction. By many reductants, such as ascorbic acid, the oxidized forms of iron ions can be reduced to reduced forms (Fe^{2+}), which can enhance the generation of hydroxyl radicals. To elucidate the role of reducing power in prooxidant effects, activities of aloin, aloe-emodin and EGCG on reduction of Fe^{3+} to Fe^{2+} were measured as shown in Fig. 3. Aloin and EGCG exhibited greater reducing power than did aloe-emodin at all concentrations. The absorbances, at 700 nm, of aloin and EGCG were above 1.0 when the concentration was above 0.5 mM. It was observed that aloin had remarkable reducing power at concentrations ranging from 0.1 to 0.625 mM. However, aloe-emodin showed an absorbance below 0.06 at the concentration of 0.5 mM. It only exhibited some degree of reducing power at concentrations above 1.25 mM, indicating that aloe-emodin had weak reducing power on iron ions.

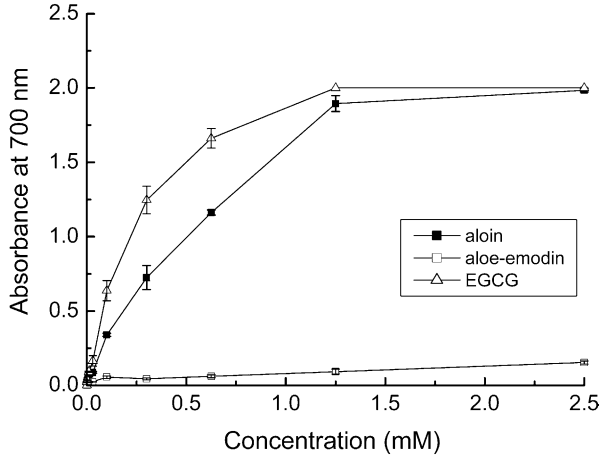

Fig. 3. Reducing power of aloin, aloe-emodin, and EGCG on iron ion. Values are means of three experiments ± standard deviations (mean ± SD). Higher absorbance at 700 nm indicated greater reducing power.

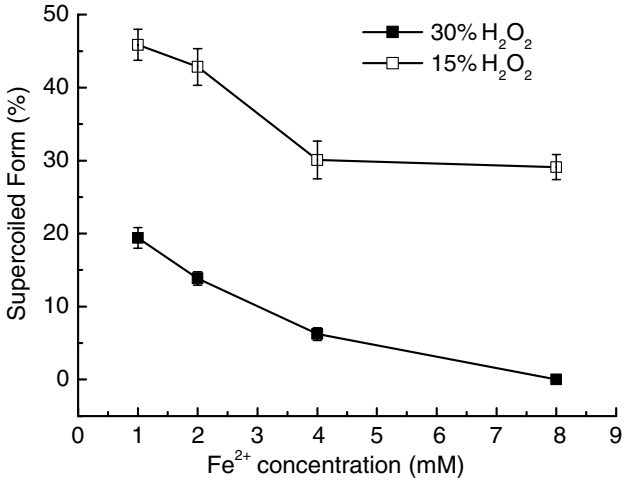

Fig. 4. DNA damage induced by ·OH generated from the Fenton reaction, depending on the concentrations of Fe^{2+} and H_2O_2. The degree of DNA damage was expressed as percentage of supercoiled form of DNA bands in an agarose gel electrophoresis assay: ■, 30% of H_2O_2; □, 15% of H_2O_2. Experiments were carried out in darkness. Average values of triplicate experiments are shown.

3.3. Effects of aloin, aloe-emodin and EGCG on free radical-induced DNA strand breaks

Antioxidant and prooxidant effects of aloin and aloe-emodin were investigated using a free radical-induced plasmid pBR322 DNA breaks system in vitro. With the attack of ·OH generated from the Fenton reaction, supercoiled plasmid DNA was broken into three forms, including supercoiled (SC), open circular (OC) and linear form (Linear). The degree of DNA damage was represented by the percentage of SC form in plasmid DNA bands, and the antioxidant or prooxidant effect of tested samples was presented by the ratio of SC percentage of tested sample to that of the control (DNA treated with $FeSO_4$ and H_2O_2), as shown in Fig. 8. Fig. 4 shows the concentration effects of Fe^{2+} and H_2O_2 on ·OH-induced DNA damage. Percentage of supercoiled form in plasmid decreased with the increase of Fe^{2+} concentration when the concentration of H_2O_2 was kept at 15% or 30%, indicating that free radical-induced DNA damage was dependent on the concentration of iron ion. Moreover, the degree of DNA damage was enhanced when the concentration of H_2O_2 reached 30%, from 15% (Fig. 4). Therefore, free radical-induced DNA damage was concentration-dependent on Fe^{2+} and H_2O_2. In order to obtain a clear separation of different forms of DNA on gels, 2 mM Fe^{2+} and 30% H_2O_2 were used in the following experiments.

Effects of aloin on the free radical-induced DNA damage were investigated as shown in Fig. 5. The percentage of SC form in plasmid DNA decreased by 42.4% under the treatment of ·OH generated from the Fenton reaction (Lane 2), compared with plasmid DNA control (Lane 1). However, there was no significant difference of SC form between plasmid DNA and DNA treated with H_2O_2 alone (Lane 3) or treated with

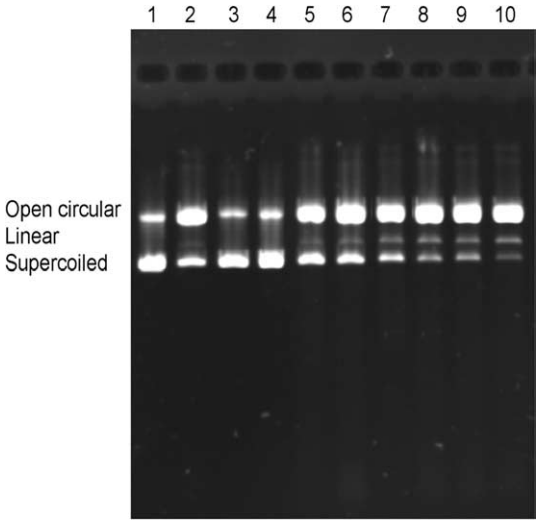

Fig. 5. Agarose gel electrophoretic patterns of plasmid DNA breaks by ·OH generated from Fenton reaction in the presence of aloin. 0.5 μg of pBR322 DNA was incubated at 37 °C for 1 h with 2 mM $FeSO_4$, 30% H_2O_2 and the following additives: Lane 1, no addition (plasmid DNA); Lane 2, $FeSO_4$ and H_2O_2 (DNA damage control); Lane 3, H_2O_2 treatment alone; Lane 4, Fe^{2+} treatment alone; Lanes 5–10, $FeSO_4$ and H_2O_2 in the presence of aloin with concentrations of 2.5 mM, 1.25 mM, 0.3 mM, 70 μM, 16 μM, and 8 μM, respectively.

Fe^{2+} alone (Lane 4). Results in Figs. 5 and 8 show that aloin at concentrations from 1.25 to 2.5 mM (Lanes 6 and 5 in Fig. 5) prevented ·OH-induced DNA breaks by 5–30% over control value (Lane 2 in Fig. 5, DNA treated with Fe^{2+} and H_2O_2), indicating an antioxidant effect. In contrast, addition of aloin with lower concentrations (300–8 μM, as shown in Lanes 7–10 in Fig. 5) markedly enhanced DNA damage. The prooxidant ef-

fect was clearly revealed by 24–74% decrease in the SC form of DNA, compared with the control value.

Aloe-emodin showed no significant antioxidant effect at any tested concentrations, as seen in Figs. 5 and 8. This result corresponds well with chemiluminescence assays on its free radical-scavenging ability. However, addition of aloe-emodin at high concentrations (1.25–2.5 mM) had a prooxidant effect on DNA by about 29–35% (Lanes 6 and 5 in Fig. 6). EGCG at 1.25 mM, the standard antioxidant, also showed a prooxidant effect on DNA by about 51% (Lane 5 in Fig. 7). The SC form in plasmid DNA was smeared at the treatment of EGCG (2.5 mM) with a 100% prooxidant effect (data not shown). However, at lower concentrations, from 0.5 to 70 μM, the antioxidant activity of EGCG gradually dominated over its prooxidant activity and exhibited an antioxidant effect (Lanes 8–11 in Fig. 7). With further decrease of concentration of EGCG (<0.5 μM), neither antioxidant nor prooxidant effects were observed on DNA (Lane 12 in Fig. 7).

Because of the effects of irradiation by UV and visible light, all the experiments were carried out in the dark. Moreover, we also investigated the actions of aloin and aloe-emodin on plasmid DNA in visible light. However, there were no prooxidant or antioxidant effects of aloin and aloe-emodin on DNA in the light observed in our present work (data not shown). This result was different from that of Wamer et al. (2003), who reported that incubation of aloe-emodin with human skin fibroblasts, and then irradiation with visible light, resulted in prooxidant damage to cellular DNA and RNA. These discrepant results may be due to the different light intensities used in experimental systems, or some intermediates formed between cellular constituents and aloe-emodin under the treatment with light. Moreover, there is no intense light existing in cells where aloin and aloe-emodin react with biomolecules in vivo.

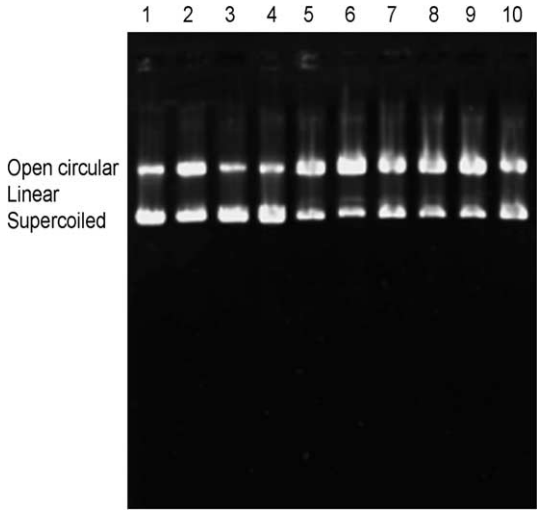

Fig. 6. Agarose gel electrophoretic patterns of plasmid DNA breaks by ·OH generated from Fenton reaction in the presence of aloe-emodin. 0.5 μg of pBR322 DNA was incubated at 37 °C for 1 h with 2 mM $FeSO_4$, 30% H_2O_2 and the following additives: Lane 1, no addition (plasmid DNA); Lane 2, $FeSO_4$ and H_2O_2 (DNA damage control); Lane 3, H_2O_2 treatment alone; Lane 4, Fe^{2+} treatment alone; Lanes 5–10, $FeSO_4$ and H_2O_2 in the presence of aloe-emodin with concentrations of 2.5 mM, 1.25 mM, 0.3 mM, 70 μM, 16 μM, and 8 μM, respectively.

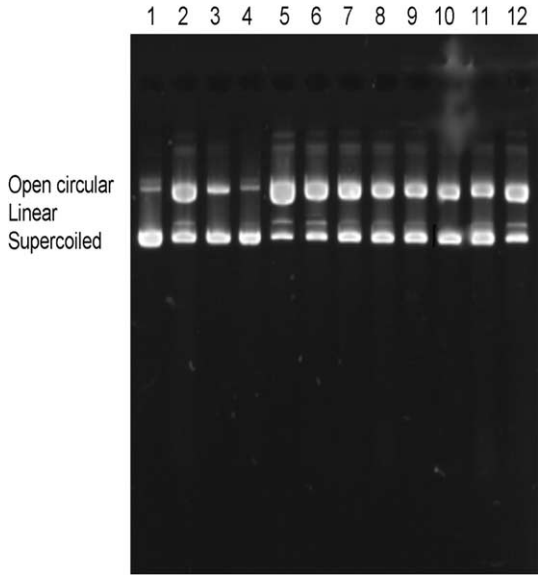

Fig. 7. Agarose gel electrophoretic patterns of plasmid DNA breaks by ·OH generated from Fenton reaction in the presence of EGCG. 0.5 μg of pBR322 DNA was incubated at 37 °C for 1 h with 2 mM $FeSO_4$, 30% H_2O_2 and the following additives: Lane 1, no addition (plasmid DNA); Lane 2, $FeSO_4$ and H_2O_2 (DNA damage control); Lane 3, H_2O_2 treatment alone; Lane 4, Fe^{2+} treatment alone; Lanes 5–12, $FeSO_4$ and H_2O_2 in the presence of EGCG with concentrations of 1.25 mM, 0.3 mM, 70 μM, 16 μM, and 8 μM, 1.7 μM, 0.5 μM, and 0.27 μM, respectively.

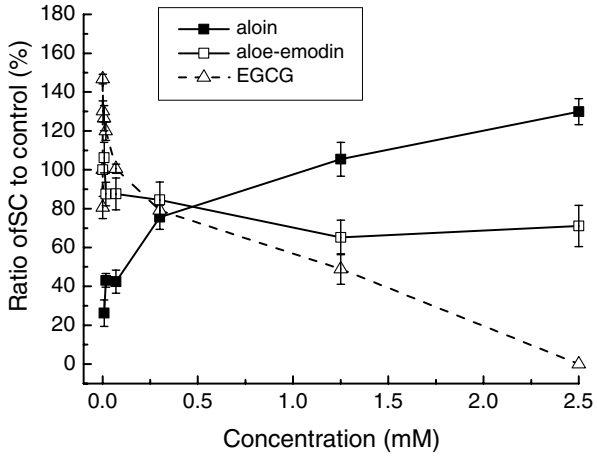

Fig. 8. Concentration-dependent antioxidant or prooxidant effects of aloin, aloe-emodin and EGCG on DNA. Antioxidant or prooxidant effect on DNA was represented by the ratio of percentage of supercoiled form of tested sample to that of the control. ■, aloin; □, aloe-emodin; △, EGCG. Average values of three experiments are shown.

Results in this paper show that aloin and aloe-emodin had different actions on DNA at different concentrations, indicating that they had different operating mechanisms on biomolecules. The difference may be explained by their different molecular structures and their concentration-dependent activities. Relatively higher reducing power of aloin at low concentrations, reducing Fe^{3+} to Fe^{2+}, which can enhance the generation of $^{\cdot}OH$ from the Fenton reaction, may play a key role in its prooxidant effect on DNA. With the increase of concentration, however, free radical-scavenging activity of aloin increased due to the ease of abstraction of the anomeric hydrogen from the C-10 glucoside. This scavenging ability on free radicals may gradually predominate over its reducing power on Fe^{3+}, resulting in antioxidant effect on DNA. For aloe-emodin at high concentrations, predomination of reducing power over free radical-scavenging activity resulted in its prooxidant effect. It has been reported that several chemopreventive agents that are antioxidants at some concentrations became prooxidants at other concentrations (Lee & Park, 2003). Therefore, dose selection may be very important in the application of antioxidants. Aloin and aloe-emodin have been considered to have antibacterial (Tian, Hua, Ma, & Wang, 2003) and antiviral activities (Sydiskis, Owen, Lohr, Rosler, & Blomster, 1991), and the Fenton reaction was also proved to be possible in vivo (Meneghini, 1997). Therefore, prooxidant effects of aloin and aloe-emodin on biomolecules might be helpful for explaining their antibacterial and antiviral activities. Hydroxyl radicals produced in the Fenton reaction can kill bacteria or damage the envelopes of viruses. Moreover, cytotoxic effects of aloin and aloe-emodin on human cells can also be explained by their prooxidant effects.

In summary, aloin and aloe-emodin, the main active components in *aloe*, were investigated for their antioxidant and prooxidant effects on DNA in vitro. Aloin and aloe-emodin showed antioxidant or prooxidant on plasmid DNA, depending on their nature (structure) and their concentrations. Their prooxidant or antioxidant effect on DNA may be due to the balance of two activities, free radical-scavenging activity and reducing power on iron ions, which may drive the Fenton reaction via reduction of iron ions. The predomination of reducing power (on iron ions) over free radical-scavenging activity results in the prooxidant effect on DNA. Further research is necessary to investigate the antioxidant and prooxidant mechanisms of aloin and aloe-emdin at physiological levels in vivo.

Acknowledgements

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