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A Simple and Accurate Method for Determining Antioxidative Activity

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Antioxidative activity is an important factor in inhibiting oxidative stress. The usual methods for determining antioxidative activity are time-consuming and cumbersome. They are also indirect processes that use biological material such as brain or liver microsome. This study therefore proposed a new method. Redoxpotential was determined using galvanic cell with or without the addition of various antioxidants or herbal extracts in zinc sulfate solution. The result was compared with the results from the TBA method and the peroxide value from sodium thiosulfate titration. All methods showed significant and dose-dependent enhancement of antioxidative activity by adding ascorbic acid, quercetin, ginseng, or gingko biloba extract. The result of redox potential using galvanic cell showed the smallest standard deviation and took the shortest time among the three methods. Therefore, the antioxidative potential of chemical substances and herbal extracts can be determined simply, directly and accurately in a short period of time using galvanic cell.

Key Words: Antioxidative activity, Redoxpotential, Galvanic cell, Thiobarbituric acid, Sodium thiosulfate

INTRODUCTION

Antioxidative activity is an important protective factor against oxidative stress (Ahlemeyer et al, 2003; Imai et al, 2003; Latchoumycandane et al, 2003; Loguercio et al, 2003; MacDonald-Wicks et al, 2003; Onodera et al, 2003). Many herbal medicines have such function (Lee et al, 2000; Yun et al, 2000; Lin et al, 2001) and there are also several methods to determine it. Most of the methods are time-consuming and cumbersome; they are also indirect processes that use biological materials such as brain or liver microsome (Slater, 1984; Moore et al, 1998). Therefore, a simple, accurate, and direct method was introduced using electrochemical principle.

METHODS

Determining antioxidative activity using galvanic cell

Zinc and copper plates (20×15×1 mm) were soaked in zinc sulfate solution and copper sulfate solution (0.01 M in water), as illustrated in Fig. 1. After connecting the two plates to a voltage meter (Fluke 27/FM Multimeter, PSM-45) through an electric cable, various substances were added in the zinc sulfate solution. The solution was left to stand for 5 minutes to stabilize the potential. The potentials were compared by adding various antioxidants (Vasiljeva et al, 2000; Sanders et al, 2001) or putative antioxidative herbal extracts in the experimental groups and by not

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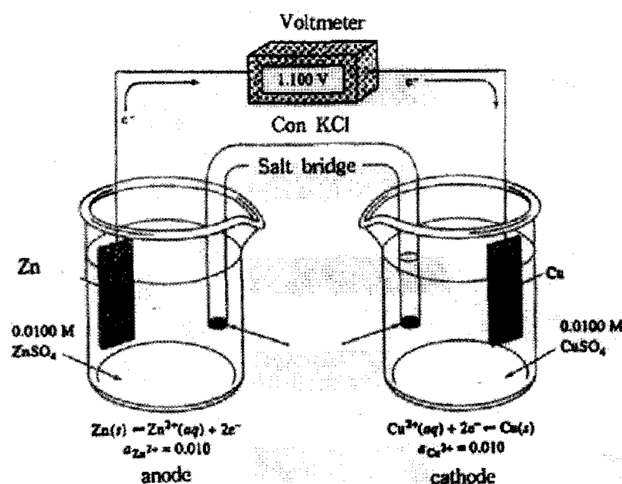

Fig. 1. Illustration of galvanic cell. The zinc plate in the zinc sulfate solution and the copper plate in the copper sulfate solution were soaked. The two metal plates were connected to the voltage meter through an electric cable and the two kinds of solution linked by a salt bridge of concentrated KCl. Change in voltage was observed by adding various antioxidants and herbal extracts.

ABBREVIATIONS: TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substance.

adding anything in the control group. This study used ascorbic acid (0.5 M, 0.05 M) and glutathione (10 mM, 1 mM) for the antioxidants. Red ginseng extract (5 g/dl, 0.5 g/dl, Korean Ginseng Corp, Korea) (Jeon et al, 1996; Choi et al, 1997; Kim et al, 2000; Sung et al, 2000) and ginkgo biloba extract (2 g/dl. 0.2 g/dl, donated by Sunkeong Chemicals, Suwoon, Korea) (Bridi et al, 2001) were also used as putative antioxidative herbs.

Determining antioxidative activity using the TBA method

The thiobarbituric acid reacting substances (TBARS) were measured using the thiobarbituric acid (TBA) method applied by Ohkawa (Ohkawa et al, 1979) and modified by Jeon (Jeon et al, 2000) and Sung (Sung et al, 2000). Briefly, 0.1 ml of linoleic acid (60 per cent) and 1.9 ml of mixed solution {methanol : phosphate buffer (0.1 M, pH 7.0)=1 : 4, v/v} were transferred to two glass tubes. And then 1.9 ml of the phosphate buffer and 0.1 ml of the various antioxidants or herb extracts were added in one of the tubes for the experimental group and only phosphate buffer in the other tube for the control group. The tubes were incubated for 24 hours at 40°C and stirred at 100 rpm. The ginseng extract (5 g/dl, 0.5 g/dl), ginkgo biloba extract (2 g/dl, 0.2 g/dl), and quercetin (10 mM, 1 mM) were dissolved in absolute methanol, while the ascorbic acid (0.5 M, 0.05 M) was dissolved in water. After incubation, 1 ml of thiobarbituric acid (0.67%), which was dissolved in 0.1 M phosphate buffer, was added, and heated for half an hour at 80°C in bath water. After immediate cooling in the water bath, 1 ml of chloroform was added; the solution was shaken gently and allowed to stand for 30 minutes to separate the chloroform layer. The optical density of the upper layer was determined at 532 nm on a spectrophotometer (Kontron 850, Swiss).

Determining the peroxide value through titration with sodium thiosulfate (Cramer et al, 1991)

The inhibitory effect of various substances on the peroxidation of linoleic acid was measured through titration with sodium thiosulfate. Briefly, 0.1 ml of linoleic acid (60 per cent) with 0.1 ml of antioxidants or herb extracts in a glass tube was incubated for 24 hours at 50°C. After incubation, 3.5 ml of mixed solution of chloroform with acetic acid (2 : 3, v/v) and 0.1 ml of saturated potassium iodide was added. The solution was stirred and allowed to stand for 5 minutes in a dark room, and then 1 ml of starch solution (5 g/dl in water) was added, which turned the solutions color to purple. The solution was titrated with sodium thiosulfate (0.01 N) to blend the purple color. In this study, ginseng extract (5 g/dl, 0.05 g/dl), ginkgo biloba extract (2 g/dl, 0.2 g/dl), and quercetin (10 mM, 1 mM) were dissolved in absolute methanol, while ascorbic acid was dissolved in water.

Chemicals

All pure chemicals were supplied by Sigma (USA). Ginseng was extracted by water and ethanol and ginkgo biloba by alcohol and supplied in powder form. These are commercial products in Korea.

Statistical analysis

Each experiment was repeated seven times. Data are presented as mean±standard deviation. The statistical significance was evaluated using the Ryan-Elinot-Gabriel-Welsch multiple range test of SAS 8.2 that recognized probability lower than 0.05 as significantly different.

RESULTS

Determining antioxidative activity using galvanic cell

Fig. 2 presents the antioxidative activity of various substances as detected by the galvanic cell. The addition of quercetin, ascorbic acid, ginseng extract, or ginkgo biloba extract in the zinc sulfate solution decreased the potential of the control group (1.102±0.001 V) significantly and made it dose-dependent. Among them, highly concentrated ginkgo biloba extract (2 g/dl) showed the most significant decrease (1.021±0.002 V), followed by highly concentrated ascorbic acid (1.053±0.0009 V) and ginseng extract (1.055 ±0.0017 V).

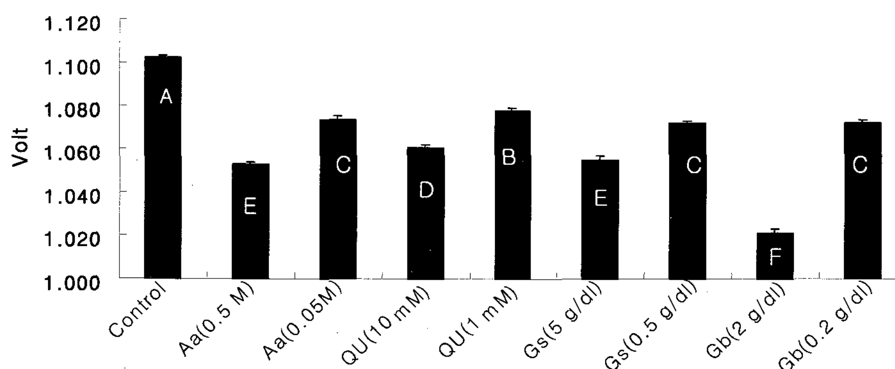

Fig. 2. Electric potential (in volts) of galvanic cell with various antioxidants and herbal extracts added. The potential was highest in the control group. However, it was significantly decreased by the addition of ascorbic acid (Aa), quercetin (Qu), ginseng extract (Gs), and ginkgo biloba extract (Gb), which was an indication of antioxidative activity.

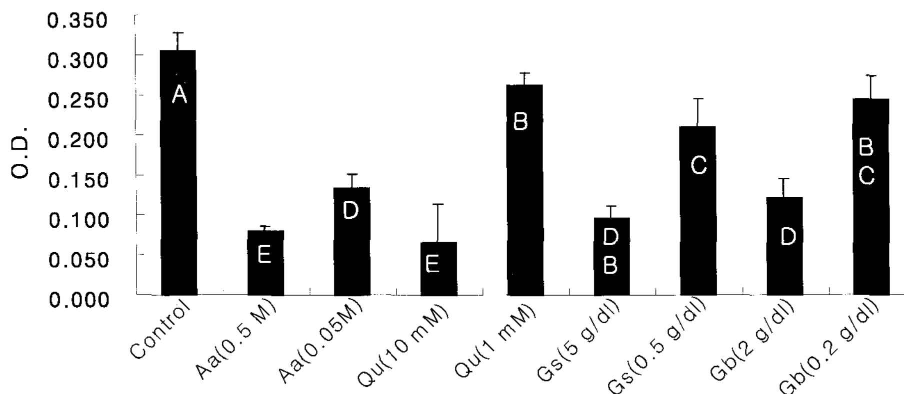

Fig. 3. Optical density (O.D.) in an arbitrary unit. After incubation of linoleic acid for 24 hours at 40°C, the optical density of upper layer that was determined at 532 nm using the TBA method. Optical density was highest in the control group. This was significantly decreased by the addition of ascorbic acid (Aa), quercetin (Qu), ginseng extract (Gs), and ginkgo biloba extract (Gb), which was an indication of antioxidative activity.

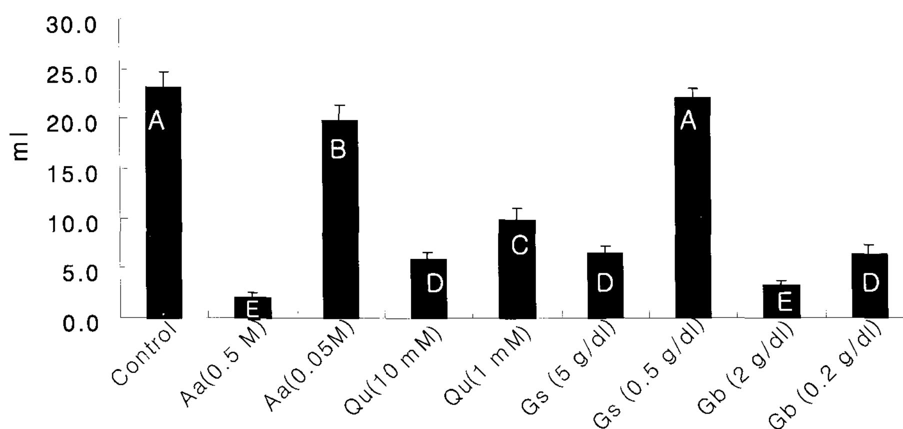

Fig. 4. Volume (in ml) of sodium thiosulfate to blend the purple color. Volume was greatest in the control group. This was significantly decreased by the addition of ascorbic acid (Aa), quercetin (Qu), ginseng extract (Gs), and ginkgo biloba extract (Gb), except weak ginseng extract.

Determining antioxidative activity using the TBA method

Fig. 3 presents the change in optical density at 532 nm by adding various substances in linoleic acid. The addition of quercetin, ascorbic acid, ginseng extract, or ginkgo biloba extract in linoleic acid significantly decreased the optical density of the control group (0.306±0.023) and made it dose-dependent. Adding highly concentrated ascorbic acid (0.08±0.006), quercetin (0.066±0.048), and ginseng (0.096 ±0.015) reduced optical density considerably.

Volume of thiosulfate for titration

Fig. 4 presents the volume of sodium thiosulfate to blend the purple color induced by the reaction of potassium iodide with the starch solution. The intensity of the color indicated the intensity of peroxidation. The volume of sodium thiosulfate in the control group was greatest and significantly different (23±1.5 ml) than in the other experimental groups, except low concentrated ginseng extract group (22 ±1.02 ml). The volume of sodium thiosulfate was smallest in highly concentrated ascorbic acid (2±0.57 ml) and ginkgo biloba extract (3.4±0.42 ml).

DISCUSSION

Principle of galvanic voltage has been well known for a long time. But there has'nt been any report to determine antioxidative activity using this principle. So, we made an attempt to assess usefulness of galvanic cell in determining antioxidative activity. The result obtained by using galvanic cell showed the smallest standard deviation of mean among these three methods (Fig. 2). Thus, determination of antioxidative activity using galvanic cell is considered to be very accurate and good reproducible. While linoleic acid requires 24 hours of oxidation process to determine antioxidative activity using the TBA method or titration with sodium thiosulfate, determination of antioxidative activity using galvanic cell can be done immediately without any pretreatment. Galvanic cell can

subsequently determine antioxidative activity through direct chemical reaction between zinc plate and antioxidants in zinc sulfate solution. Since the titration point for blending the purple color was not clear, we could safely assume the result from the galvanic cell showed more correlation with the result using the TBA method, than titration with sodium thiosulfate.

In conclusion, the antioxidative potential of chemical substances and herbal extracts can be determined simply and accurately using galvanic cell.

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