

⟨Original Article⟩

Effect of cigarette extract on vitamin C contents of broccoli sprouts

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Summary Quitting smoking is very difficult, and it is important to educate young children before they run into cigarette. By measuring the length of sprouts, we previously reported that model of practical examination learning about why children must not smoke although adults were allowed to smoke. In this study, vitamin C (ascorbic acid) contents of broccoli sprouts in different growth stages exposed cigarette extract were measured. Relationship ascorbic acid contents and growth stage cigarette extract was not observed.

Key words: Sprouts, Education experiment, Help a smoker quit, Non-smoking education, Vitamin C, High-performance liquid chromatography

Introduction

It is well known that the hazards of smoking to the health^{1,2}. Smoking have more powerful negative effect on children than on adults. It is very difficult to give children a short description of necessity of non-smoking although adults smoke with relish.

To accomplish this purpose, student practice that the effect of cigarette extract on plants was examined, with a special focus on its effect upon plant growth. In this study, broccoli under four different conditions were used; broccoli seeds, newly germinated broccoli, broccoli sprouts of about 1 cm long and the control group broccoli which isn't given exposure to the cigarette extract. This study

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showed that the growth of broccoli that is exposed from the earliest stage of its growth was most affected by the cigarette extract and showed the least growth³. This result might be helpful in convincing small children of the harm of smoking habit. Our model experiment can simply explain difficult these scientific issues. In this study, we determined how much effect the cigarette extract has on vitamin C content of broccoli leaf.

Materials and methods

Materials

The samples of broccoli were prepared from seeds [“Atariya” company (<http://www.atariya.net/>)] in this study. We purchased ascorbic acid (vitamin C) standard from Sigma-Aldrich Japan.

Methods

Broccoli under four different conditions at the point of cigarette extra exposure were used for this study; broccoli seeds (group 1), newly germinated broccoli (group 2), broccoli sprouts of about 0.5-1 cm long (group 3) and the control group broccoli which isn’t given exposure to the cigarette extract (group 4) . Then we picked green leaves and measured ascorbic acid after freezing at -80°C for 60 days (Table 1).

Tissue samples (1.0 g) were mashed and essence from leaves extracted by 10 mL

meta-phosphoric acid (1->50) and we filter out impurities with PEFE membrane filter (pore size = 0.45 μm).

The high-performance liquid chromatography (HPLC) system comprised a Shimadzu LC-20 AD pump (Shimadzu, Kyoto, Japan) and Rheodyne 7125 sample injector with a 20 μL sample loop (Rheodyne, Cotati, CA, USA). An inertsil-4 column (5.0 × 150 mm; GL Sciences, Tokyo, Japan) was used, and the flow rate of the mobile phase was 0.5 mL/min. The mobile phase components were methanol and 20 mmol/L phosphate buffer (pH 2.2). The solvent ratio of methanol and phosphate buffer was 3:7. Detection was carried out at a wavelength of 242 nm (4.0 absorbance/ 1000 mV).

We performed statistical analysis using a one way analysis of variance (ANOVA by JMP 8.0). We set the significance level was set at $p < 0.05$.

Results

Figure 1 shows the standard curve of ascorbic acid (vitamin C). The area of peak was proportional to the amount of ascorbic acid administrated. A strong relationship was observed between peak area and the amount of ascorbic acid (vitamin C) standard. These data show the amount of vitamin C we measure is reliable quite.

Ascorbic acid content in broccoli sprouts was raised under 4 different conditions at the point of

Table 1 Treatment condition of broccoli sprout with cigarette extra.

	group 1	group 2	group 3	group 4
the day before planting				
soak seeds in ...	cigarette extra	tap water	tap water	tap water
sow seeds	cigarette extra	tap water	tap water	tap water
1 day after sow	cigarette extra	cigarette extra	tap water	tap water
3 days after sow	cigarette extra	cigarette extra	cigarette extra	tap water
7 days after sow	cigarette extra	cigarette extra	cigarette extra	tap water

The seventh day after soak seeds, we picked green leaves of broccoli sprout and measured ascorbic acid (vitamin C). Cigarette extract ; 3 cigarettes in 200 mL tap water and leave a day. The method for treating sprout is as shown us previously³.

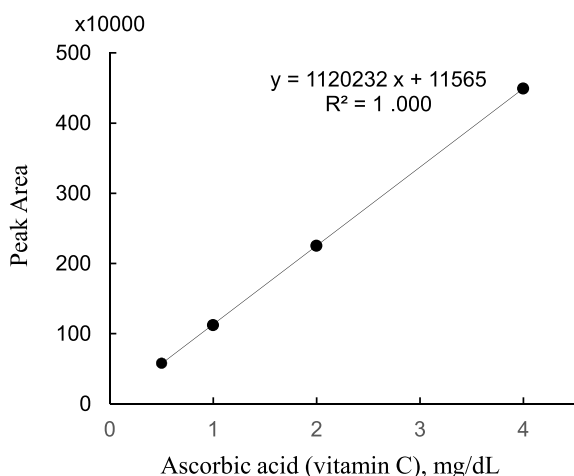


Fig. 1 Standard curve of ascorbic acid (vitamin C). We found a correlation between the leaves' peak areas and ascorbic acid contents, and correlation coefficient (R^2) was 1.000.

cigarette exposure (Table 2).

When one-way analysis of variance analysis was conducted, there was a significant difference between the plural combinations. Growth was affected by cigarette extracts according to the growth stage, but vitamin C content was not affected similarly. The results of comparison for each pair by student's t-test are shown in Table 3. There is a

significant difference when the numerical value between groups is positive.

Discussion

Because it is very difficult to quit smoking once children start it, it is important to conduct smoking cessation education before meeting tobacco. Although everyone knows tobacco is harmful to health, it is very difficult to explain why adults are smoking but children are prohibited. When doing smoking education for young children, group 4 shows a person who does not smoke at all (Table 1). Group 3 can be thought of as a model that started smoking when becoming a youth. Group 2 can be thought as a model when young children ("It's about you right now") and group 1 as a model; it is smoking from that you are in the belly of the mother. Using our education experiment, we can learn that harm becomes bigger as we start smoking early³.

Vitamin C in humans must be ingested for survival. Vitamin C is reported to be good for beauty and health to have antioxidant effect on lipid, protein and DNA⁴. Vitamin C is an electron donor, and this property accounts for all its known functions. As an

Table 2 Ascorbic acid (vitamin C) content in broccoli sprout grown by 4 different conditions at the point of cigarette exposure.

group	1	2	3	4
ascorbic acid (mg/100 g)	31.8	13.3	12.9	3.26
SD	20.5	4.94	4.43	1.49

Table 3 Comparison of average values.

	group 1	group 2	group 3	group 4
group 1	-16.6299	1.8026	2.2251	11.8701
group 2	1.8026	-16.6299	-16.2074	-6.5624
group 3	2.2251	-16.2074	-16.6299	-6.9849
group 4	11.8701	-6.5624	-6.9849	-16.6299

There is a significant difference when the numerical value between groups is positive. Statistical analysis was performed using one way ANOVA (JMP 8.0). The significance level was set at $p < 0.05$.

electron donor, vitamin C is a potent water-soluble antioxidant in humans. Antioxidant effects of vitamin C have been demonstrated in many experiments *in vitro* and *in vivo*⁴. It is known that the vitamin C concentration in the serum of smokers is lower than that of nonsmokers, which affects the high morbidity rate of smokers⁵. Although smokers are pleased by the idea that vitamin C is to suppress health damage caused by smoking^{6,7}, it was reported vitamin C isn't smoker's salvation^{4,8}.

Using the model experiment proposed here, we measured the vitamin C contents in plants that cultivated different ways (Table 1). We investigated whether the time of exposure to cigarette extract influences the amount of vitamin C in broccoli sprouts. There was no correlation between the amount of vitamin C and the time of treatment (Table 2). Disadvantage is that it was measured after freezing the measurement sample for 60 days. It will be considered to have some influence on the vitamin C measurement value by the long-term freezing. In future study, we must measure vitamin C immediately after harvesting sprouts. In this study, we showed teaching materials to learn difficult concepts through simple experiments⁹⁻¹¹. The next research must be a model for a more intuitive smoking cessation education.

Conflicting Interests

The authors have declared that no conflict of interest exists.

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