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The Effects of Cigarette Extract on the Vitamin C Contents of Broccoli Sprout: Second Report

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Summary：We know that quitting smoking is very difficult, and it is important to educate young children before they run into cigarette smoking. We already published the model of practical examination by learning about why children must not smoke, although adults were allowed to smoke. This study is aimed at explaining why children should avoid smoking through an illustration using broccoli sprouts. In this study, the vitamin C (ascorbic acid) content of broccoli sprout in different growth stages exposed to cigarette extract was measured immediately after extract treatment. The ascorbic acid content per milligram wet weight was higher in group 1 (exposed from the earliest growth stage) than that in the other groups both in the stems and the leaves. At the same time, we recalculated the value of ascorbic acid content per milligram of sowed seed, and the ascorbic acid content in group 1 was significantly lower than that in the other groups. In other words, when broccoli was fed on cigarette water at the early stage, it hardly grows up, but ascorbic acid content per wet weight was higher than non-cigarette treatment. However, when the vitamin C content is converted to sowed seed weight rather than wet weight, different elements are visible. Group 1 (grown from seeds with cigarette extract) and group 4 (grown with tap water until the end of the experiment) had the lowest and highest amount of vitamin C taken from 1 g of the sown seeds, respectively. Thus, the longer the tobacco exposure period, the lower the sown seed content is due to the consumption of vitamin C. We believe that these results are meaningful in educating people about smoking avoidance and cessation.

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1. Introduction

It is well known that smoking is hazardous to our health[1, 2]. Smoking has more powerful negative effects on children than on adults. It is very difficult to give children a short description of the need to avoid smoking because adults smoke with relish. Nicotinic sensitivity varies from individual to individual, and these differences in sensitivity affect smoking habits and smoking cessation treatments[3, 4].

The effects of cigarette extracts on plants were examined in this research, with a special focus on its effect upon plant growth. Broccoli, under four different growth conditions (broccoli seeds, newly germinated broccoli, broccoli sprouts approximately 1 cm long, and broccoli without exposure to cigarette extract (control group)), was used for this study. We showed that the growth of broccoli that is exposed to cigarette extract from the earliest growth stage was most affected by the cigarette extract and showed the least growth[5, 6]. This result might be helpful in convincing small children of the harmful effects of smoking. Our previous model experiment can simply explain these complex scientific issues. In this study, we determined how much effect cigarette extract has on the vitamin C content of broccoli stem and leaf under four different growth conditions. The content was measured separately for stems and leaves.

2. Materials and Methods

Materials

Broccoli seeds were purchased from Atariya company, Chiba, Japan. We bought standard ascorbic acid (vitamin C) at Sigma-Aldrich, Tokyo, Japan.

Methods

Four different growth stages of broccoli were used in this study, specifically broccoli seeds, newly germinated broccoli, broccoli sprouts of about 0.5–1 cm long, and the control group, which did not have any exposure to cigarette extract[5]. Then, we harvested the sprouts and measured their ascorbic acid contents immediately (Table 1).

The broccoli seeds were germinated and grown under the conditions described in the previous text (samples 1–4). Broccoli sprouts with a height of approximately 3–5 cm were harvested, and only the leaves and stems were used. Each sample was weighed precisely (50–90 mg), ground in a mortar, and then mixed well with 10 mL of 10% metaphosphoric acid. The mixture was filtered through a 0.45-μm membrane filter, and 20 μL of the sample was subjected to high-performance liquid chromatography (HPLC) analysis.

The HPLC system comprised a Shimadzu LC-20 AD pump (Shimadzu, Kyoto, Japan) and Rheodyne7125 sample injector with a 20-μL sample loop (Rheodyne, Cotati, CA, USA). An

Table 1 Treatment conditions of broccoli sprouts exposed to cigarette extract. The broccoli sprouts were harvested 7 days after sowing, and the vitamin C content was measured immediately. Cigarette extract (CE) preparation: three cigarettes were soaked in 200 mL tap water (TW) for 24 h. The method for treating the sprouts was as previously described[5].

	Group 1	Group 2	Group 3	Group 4
day before planting	CE	TW	TW	TW
day of sowing	CE	TW	TW	TW
1 day after sowing	CE	CE	TW	TW
3 days after sowing	CE	CE	CE	TW
7 days after sowing	CE	CE	CE	TW

inertsil-4 column (5.0 mm × 150 mm ; GL Sciences, Tokyo, Japan) was used, and the flow rate of the mobile phase was 1.0 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 nm absorbance/1,000 mV).

Statistical analysis was performed using one-way ANOVA (by JMP8.0). The significance level was set at $p < 0.05$.

3. Results

Fig. 1 shows the standard curve of ascorbic acid (vitamin C). The HPLC system comprised a Shimadzu LC-20 AD pump and Rheodyne7125 sample injector with a 20-μL sample loop. An inertsil-4 column (5.0 mm × 150 mm) was used, and the flow rate of the mobile phase was 1.0 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. The peak area was proportional to the amount of ascorbic acid. A strong relationship was observed between the peak area and the amount of ascorbic acid (vitamin C). These data showed that the amount of vitamin C that we measured is quite reliable. Vitamin C content in broccoli sprouts and the detailed values were shown in Table 2. Fig. 2 and Table 2 show two phenomena : 1) the vitamin C content of stems was higher than that in the leaves, and 2) the vitamin C content of group 1 was higher than that in the other groups cultivated under their respective growth conditions. Fig. 3 shows Vitamin C content in broccoli sprouts and the amount of vitamin C harvested from sown seeds and the detailed values were shown in Table 4. The vitamin C content measured, as shown in Fig. 2, was converted to the amount of vitamin C harvested from the sown seeds. Group 1 had the lowest amount of vitamin C (exposure to CE at the earliest growth stage).

Broccoli growth was affected by exposure to CE, considering the different growth stages as we described already[5], but the vitamin C content was higher in group 1,

whose growth was also affected by CE. The results of comparison for each pair using Student's t-test are shown in Table 3 and Table 5. There is a significant difference when the numerical value between groups is positive.

Table 3 shows a comparison of the mean value shown in Table 2. There is a significant difference when the numerical value between groups is positive. For example, in the case of stems, the vitamin C level of group 1 is significantly higher than that of other groups (Table 3). Since the value is negative there is no significant difference between group 3 and group 4. Statistical analysis was performed using one-way ANOVA (JMP8.0). The significance level was set at $p < 0.05$.

Table 5 shows a comparison of the mean value shown in Table 4. There was a significant difference between the plural combinations. In the case of stems, since the values was positive, there was a significant difference was found in all group combinations.

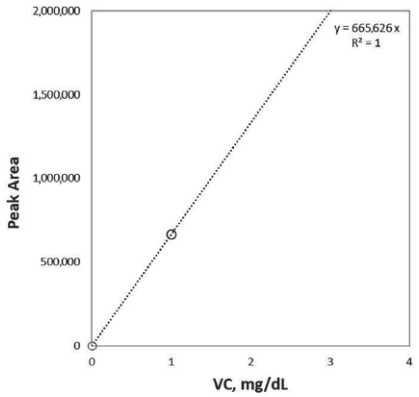

Fig. 1 Standard curve of ascorbic acid (vitamin C) content
We found a correlation between the leaves' peak areas and ascorbic acid contents. Correlation coefficient (R^2) = 1.000.

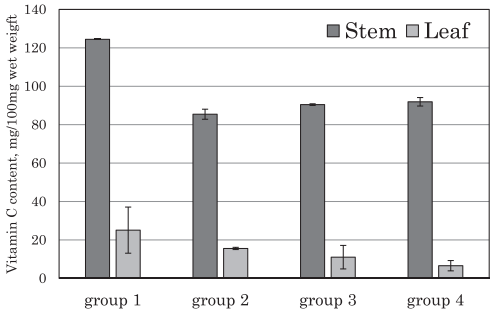

Fig. 2 Vitamin C content in broccoli sprouts
Broccoli seeds were germinated and grown under several conditions described in the text (samples 1–4). Broccoli sprouts were grown to 3–5 cm, and the leaves and stems were harvested. Each sample was weighed precisely (50–90 mg) and ground in a mortar, and then mixed well with 10 mL of 10% metaphosphoric acid. The mixture was filtered through a 0.45-μm membrane filter, and 20 μL of the sample was subjected to HPLC analysis. Error bars indicate measured values ± SD.

Table 2 Ascorbic acid content in broccoli sprouts grown under four different conditions of cigarette exposure. The chromatographic conditions are described under the Materials and methods section.

group	1	2	3	4
stems				
ascorbic acid ± SD (mg/100 g stems)	124.5 ± 0.04	85.4 ± 2.26	90.4 ± 0.44	91.9 ± 2.21
leaves				
ascorbic acid ± SD (mg/100 g leaves)	25.1 ± 12.1	15.6 ± 0.58	11.0 ± 6.13	6.59 ± 2.66

Table 3 Comparison of average values. There is a significant difference when the numerical value between groups is positive. For example, in the case of stems, the vitamin C level of group 1 is significantly higher than that of other groups. Statistical analysis was performed using one-way ANOVA (JMP8.0). The significance level was set at $p < 0.05$.

stems	group 1	group 2	group 3	group 4
group 1	−5.79766	33.9891	28.9816	27.5141
group 2	33.9891	−4.09956	0.907935	2.37544
group 3	28.9816	28.9816	−4.09956	−2.63206
group 4	27.5141	2.37544	−2.63206	−4.09956

leaves	group 1	group 2	group 3	group 4
group 1	−14.4418	−4.91183	−0.374330	4.06567
group 2	−4.91183	−14.4418	−9.99043	−5.46433
group 3	−0.374330	−9.99043	−14.4418	−10.0018
group 4	4.06567	−5.46433	−10.0018	−14.4418

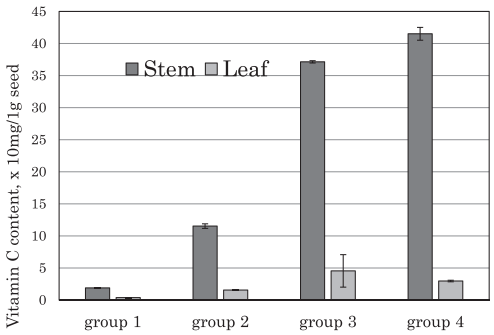

Fig. 3 Vitamin C content in broccoli sprouts and the amount of vitamin C harvested from sown seeds
The vitamin C content measured, as shown in Fig. 2, was converted to the amount of vitamin C harvested from the sown seeds. Group 1 had the lowest amount of vitamin C (exposure to CE at the earliest growth stage). Error bars indicate measured values ± SD.

Table 4 The amount of vitamin C harvested from sown seeds and the ascorbic acid content in broccoli sprouts grown under four different conditions of CE exposure. The chromatographic conditions are described under the Materials and methods section.

group	1	2	3	4
stems				
ascorbic acid ± SD (×10 mg/1 g seed)	1.90 ± 0.01	11.5 ± 0.35	37.1 ± 0.18	41.5 ± 0.45
leaves				
ascorbic acid ± SD (×10 mg/1 g seed)	0.40 ± 0.22	1.60 ± 0.06	4.50 ± 2.53	3.00 ± 0.12

Table 5 Comparison of average values. There is a significant difference when the numerical value between groups is positive. Statistical analysis was performed using one-way ANOVA (JMP8.0). The significance level was set at $p < 0.05$.

stems	group 1	group 2	group 3	group 4
group 1	−0.127560	0.808469	3.36857	3.80559
group 2	0.808469	−0.127560	2.43254	2.86956
group 3	3.36857	2.43254	−0.127560	0.309463
group 4	3.80559	2.86957	0.309463	−0.127560

leaves	group 1	group 2	group 3	group 4
group 1	−0.295570	−0.178020	0.120759	−0.037120
group 2	−0.178020	−0.295570	0.0032090	−0.154670
group 3	0.120759	0.0032090	−0.295570	−0.137690
group 4	−0.037120	−0.154670	−0.137690	−0.295570

4. Discussion

It is very difficult to quit smoking once you start it; hence, it is important to conduct smoking cessation education before initiating cigarette smoking. Everyone knows that tobacco is harmful to health, but it is very difficult to explain why adults are allowed to smoke but children are prohibited. When conducting smoking education for young children, Group 4 in this study portrays a person who does not smoke at all (Table 1). Group 3 can be thought of as a model that started smoking during one's youth. Group 2 can be thought as a model when young children ("It is about you right now"), and group 1 as a model portraying exposure to cigarette smoke while still in the belly of the mother. Using our educational experiment, we can learn that harm becomes bigger as we start smoking early.

Vitamin C in humans must be ingested for survival. Vitamin C is said to be good for

beauty and health, having antioxidant effects on lipid, protein, and DNA[7]. Vitamin C is an electron donor, and this property accounts for all its known functions. As an electron donor, vitamin C is a potent water-soluble antioxidant in humans. The antioxidant effects of vitamin C have been demonstrated in many *in vitro* and *in vivo* experiments[7]. It is said that the vitamin C concentration in the serum of smokers is lower than that of non-smokers, which affects the high morbidity rate of smokers[8]. Although smokers are pleased by the idea that vitamin C suppresses health damage caused by smoking[9, 10], it was reported that vitamin C does not save smokers from the harmful effects of smoking[7, 11].

Using the proposed model experiment, we measured the vitamin C contained in plants that were cultivated in different ways (Table 1). We investigated whether the time of exposure to CE influences the amount of vitamin C in broccoli sprouts. In our previous study, we measured the vitamin C content after freezing the samples for 60 days, although long-term freezing is considered to have some influence on vitamin C content[6]. In this study, we measured vitamin C immediately after harvesting the sprouts.

The vitamin C content per wet weight was higher in group 1 than that in the other groups. This seems inconsistent with the prevailing theory that the antioxidant effects of vitamin C on cigarette oxidation are mediated by the consumption of vitamin C. However, when the vitamin C content is converted to weight rather than per wet weight, different elements became visible. Group 1 and group 4 were the lowest and the highest, respectively, in terms of the amount of vitamin C taken from 1 g of sown seeds. Thus, the longer the exposure period, the lower the vitamin C content becomes, because of the consumption of vitamin C. We believe that these results are meaningful in educating people on smoking avoidance and cessation.

We have published teaching materials to learn difficult concepts through simple experiments[12–14]. The next research must be a model for a more intuitive smoking cessation education.

Conflicts of interest

The authors have no conflicts of interest.

References

1 WHO. World Health Organisation, Tabacco, fact sheet No339. http://www.who.int/mediacentre/factsheets/fs339/en/index.html
2 The Stationery Office. Smoking Kills—A White Paper on Tabacco. http://www.archive.official-documents.co.uk/document/cm41/4177/4177.htm
3 Nakajima M, Yamagishi S, Yamamoto H et al.: Deficient cotinine formation from nicotine is attributed to the whole delection of the CYP2A6 gene in humans. Clin Pharmacol Ther 67 : 57–69, 2000.
4 Dempsey DA, St Helen G, Jacob P et al.: Genetic and pharmacokinetic determinants of response to transdermal nicotine in white, black and Asian non-smokers. Clin Pharmacol Ther 94 : 687–694 2013.
5 Goroumaru-Shinkai M, Nishiguchi Y, Morishita M: The effect of cigarette extract on plants: special focus on its effect upon plant growth. J Res Sci Edu, 50 : 29–34, 2009.

6 Goroumaru-Shinkai M, Kuroda J, Nakazawa K et al.: Effect of cigarette extract on vitamin C contents of broccoli sprouts. Int J Anal Bio-Sci, 6 : 51–54, 2018.
7 Pachyatty SJ, Katz A, Wang Y et al.: Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Natr 22 : 18–35. 2003.
8 Schectam G, Byrd JC, Grunchow HW: The influence of smoking on vitamin C status in adult. Am J Public Health, 79 : 158–162, 1989.
9 McEvoy CT, Milner KF, Scherman AJ et al.: Vitamin C decrease the effect of smoking in pregnancy on infant lung function (VCSIP): rationale, design, and methods of a randomized, controlled trial of vitamin C supplementation in pregnancy for the primary prevention of effects of in utero tobacco smoke exposure on infant lung function and respiratory health. Contem Clin Trials, 58 : 66–77 2017
10 Panda K, Chattopadhyay R, Ghosh MK et al.: Vitamin C prevents cigarette smoke induced oxidative damage of proteins and increased proteolysis. Free Radic Bio Med, 27 : 1064–1079 1999
11 Raitakari OT, Adamus MR, Robyn F et al.: Oral vitamin C and endothelial function in smokers: short-term improvement, but no sustained beneficial. J Ame Coll Cardiology, 35 : 1616–1621 2000
12 Goroumaru-Shinkai M, Nishiguchi Y, Narusuye K et al.: Biological practice for students of Pharmaceuticals; Understanding of diuretic effect of mannitol using *Paramecium caudatum*. Medical and Biology, 153 : 407–415, 2009.
13 Goroumaru-Shinkai M, Nishiguchi Y, Narusuye K et al.: Development of method for measurement of visible absorption spectrum using pigments extracted from colored vegetable. Medical and Biology, 155(4) : 169–174, 2011.
14 Goromaru-Shinkai M, Nishiguchi Y, Nakazawa K et al.: Comparison of ultraviolet absorption by botanical extracts and development of educational material. J Analy Bio-Sci, 37 : 220–227, 2014.