Comparative Potencies of Nutraceuticals in Chemically Induced Skin Tumor Prevention

Irene M. Villaseñor, Ma Karenina B. Simon, and Ainstein M. A. Villanueva

Abstract: Four nutraceuticals, sugar beet roots, cucumber fruits, New Zealand spinach leaves, and turmeric rhizomes, were evaluated for their comparative effectiveness against dimethylbenz[a]anthracene (DMBA)-initiated and croton oil-promoted skin tumors. Three different protocols were used. The most effective protocol (Protocol 2) is the topical application of the nutraceuticals 1 h before croton oil. There was a decrease in the percent skin tumor incidence, a decrease in multiplicity of skin tumors, and a later onset of skin tumors compared with the positive control for all the nutraceuticals tested, with turmeric being the most potent, as evidenced by 30% skin tumor incidence, 87.2% decrease in skin tumors, and a 5-wk delay in skin tumor formation compared with the positive control. Topical application of the nutraceuticals daily for 5 days before DMBA and 1 h before croton oil (Protocol 1) and immediately after croton oil (Protocol 3) did not have an additional protective effect against skin tumors compared with Protocol 2. Kruskal-Wallis analysis of variance by ranks showed that Protocol 2 is the most effective, with the treatment groups belonging to different populations at the 0.05 level of significance compared with $\alpha = 0.20$ for Protocols 1 and 3. Turmeric is the most potent nutraceutical, because the average number of tumors formed after application of tumeric is statistically different from the positive control at $\alpha = 0.01$.

Introduction

Nutraceutical, from the words nutrition and pharmaceutical, is any substance that may be considered a food or part of a food and provides medical and health benefits. It is also called functional foods, designer foods, medical foods, pharmafoods, and FoSHU (food for specified health use).

According to the World Health Organization, $\geq 35\%$ of cancer causes are diet related. Proper nutrition could prevent 50–90% of all cancers. An internet search lists some examples of nutraceuticals used in cancer prevention (1). Those with high anticancer activity are soybeans, garlic, ginger, cabbage, carrots, celery, parsley, parsnips, and licorice. Those with moderate levels are citrus fruits, onions, flax, broccoli, brussels sprouts, cauliflower, tomatoes, peppers, turmeric, brown rice, and whole wheat, whereas oats, barley, rosemary, mint, thyme, oregano, sage, basil, cucumber, cantaloupe, and berries have measurable levels of anticancer activity.

Antioxidants have been implicated in the prevention of skin photocarcinogenesis (2–9). This research aims to establish the comparative effectiveness of some nutraceuticals that exhibited antioxidant activity in preventing chemically induced skin tumors. These nutraceuticals are sugar beet roots (Beta vulgaris L.), New Zealand spinach leaves (Tetragonia expansa Murr.), and turmeric rhizomes (Curcuma longa L.). Cucumber fruits (Cucumis sativus L.) were also investigated because of their popularity in skin care.

Materials and Methods

Preparation of Nutraceuticals

Expressions of cucumber fruits and sugar beet roots and decoctions of spinach leaves and turmeric rhizomes were prepared. Cucumber fruits were homogenized, and the juices were expressed. An expression of the roots of sugar beets was prepared by homogenizing 500 g of beets in 200 ml of water. Decoctions of turmeric and spinach were made by processing 500 g of the samples in 250 ml of water and then boiling for 15 min. These extracts were then separately filtered through suction and freeze-dried (Vertis freeze dryer). The lyophilized extracts of spinach and cucumber were light green flakes, beet was a sticky red-violet solid, and turmeric was yellow and sticky.

Animals

Swiss Webster albino mice were purchased from the Bureau of Animal Industry, Department of Agriculture. They were acclimatized for $\geq 1$ wk before the start of the experiments. They were fed regular chow and randomly di-
vided into groups of five per cage. They were given water ad libitum.

**Mouse Skin Tumor Assay**

Skin tumors were assayed as described by Mehta et al. (10). The back of the albino mouse was shaved. On the 6th day, 0.2 ml of 410 µg of dimethylbenz[a]anthracene (DMBA; Sigma Chemical, St. Louis, MO) in acetone was applied to the shaved portion. Beginning on the 4th day, 0.2 ml of 0.03% croton oil (Sigma Chemical) in acetone was applied three times a week for 20 wk. An initial concentration of 5.0 mg extract/0.2 ml acetone was applied 5 days before DMBA application and 1 h before croton oil (Protocol 1), 1 h before croton oil (Protocol 2), and immediately after croton oil was dried (Protocol 3). Ten mice were used per test sample and per test protocol. The number of skin tumors was counted weekly. The diameters of the skin tumors were measured on the 20th wk.

**Results**

An initial concentration of 5.0 mg nutraceutical/0.2 ml acetone, when splashed on the dorsal back of albino mice, led to 60–80% mortality before tumors developed. This concentration was then decreased to 3.2 mg/0.2 ml of acetone for the sugar beet roots and spinach leaves. A concentration of 2.5 mg/0.2 ml of acetone was used for cucumber fruits and turmeric rhizomes.

Three protocols were used for the skin tumor assay. Protocol 1 involved the application of the nutraceuticals daily for 5 days before the application of DMBA. They were also applied 1 h before croton oil. There was a 100% skin tumor incidence for the positive control, DMBA + croton oil, with the formation of 47 tumors and the appearance of the first tumor in the 9th wk (Fig. 1). In Protocol 1, the most effective extract is turmeric, with five of eight surviving mice developing skin tumors, for a 62.5% skin tumor incidence (Table 1). The other three nutraceuticals showed a 70% skin tumor incidence. The first tumor for the turmeric-treated mice appeared in the 14th wk (Fig. 1), a 5-wk delay compared with the positive control group. In cucumber- and spinach-treated mice, the first tumor appeared in the 12th wk. The first tumor developed earliest, in the 10th wk, in sugar beet-treated mice. In terms of tumor multiplicity, Kruskal-Wallis analysis of variance by ranks showed that the average number of tumors for spinach, cucumber, and turmeric are statistically different from that of the positive control at α = 0.20, whereas the average number of tumors for sugar beet differs from the positive control at α = 0.50.

Table 2 summarizes the results of the assay when the nutraceuticals were applied 1 h before croton oil. The most effective nutraceutical is turmeric, with a 30% skin tumor incidence. Turmeric-treated mice also developed the fewest skin tumors, with a total of only six skin tumors for the three mice with skin tumors, which is statistically different from that of the positive control at α = 0.01. The average number of tumors for spinach- and beet-treated mice differs from that for the positive control at α = 0.10 and α = 0.30, respectively. The least effective nutraceutical is cucumber, because the average number of tumors differs from that of the positive control at α = 0.70. There were no significant differences in the delay of skin tumor formation, because the first tumor appeared on the 13th wk for turmeric, 12th wk for cucumber, and 11th wk for sugar beet and spinach.

In Protocol 3, the most potent nutraceutical is again turmeric (Table 3), with 62.5% skin tumor incidence, followed

![Figure 1. Number of skin tumors counted per week for nutraceuticals applied 5 days before dimethylbenz[a]anthracene (DMBA) and 1 h before croton oil (CO).](image)

<table>
<thead>
<tr>
<th>Test Sample</th>
<th>No. of Mice With Tumors</th>
<th>%Tumor Incidence&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Avg No. of Tumors/Mouse&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Avg Tumor Diam, mm</th>
<th>%Mortality&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>DMBA + croton oil</td>
<td>10</td>
<td>100.0</td>
<td>4.7 ± 3.3</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Beets</td>
<td>7</td>
<td>70.0</td>
<td>2.9 ± 1.3</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Cucumber</td>
<td>7</td>
<td>70.0</td>
<td>2.3 ± 1.1</td>
<td>1.8</td>
<td>0</td>
</tr>
<tr>
<td>Spinach</td>
<td>7</td>
<td>70.0</td>
<td>2.3 ± 0.8</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td>Turmeric</td>
<td>5</td>
<td>62.5</td>
<td>2.4 ± 1.3</td>
<td>1.5</td>
<td>20</td>
</tr>
</tbody>
</table>

<sup>a</sup> DMBA, dimethylbenz[a]anthracene.  
<sup>b</sup> %Tumor Incidence = (no. of mice with skin tumors/no. of surviving mice) × 100.  
<sup>c</sup> Values are means ± SD; avg no. of tumors/mouse = total no. of skin tumors/no. of mice with skin tumors.  
<sup>d</sup> Mortality = (no. of mice dead before developing tumors/total no. of mice) × 100.
by cucumber with six of nine mice (66.7%) developing tumors. The average number of tumors per mouse is lowest for beets, which differs from the positive control at \( p = 0.10 \). The average number of tumors for spinach-, turmeric-, and cucumber-treated mice differs from the positive control at \( p = 0.20, 0.50, \) and \( 0.90 \), respectively.

### Discussion

In the two-step carcinogenesis, the mice were treated with a low concentration of DMBA, which does not lead to carcinogenesis but irreversibly activates normal cells. In Protocol 1, the nutraceuticals were applied daily for 5 days before DMBA to determine whether they can negate the genetic alterations induced by DMBA.

Croton oil, the seed oil of *Croton tiglium* L., the active ingredient of which is 12-O-tetradecanoylphorbol-13-acetate, was used as the tumor promoter. It was applied continuously for 20 wk, three times a week. The nutraceuticals were applied 1 h before croton oil in Protocol 2 to determine whether they can retard the tumor promotion stage. Protocol 3 involved application of the nutraceuticals at the same time as croton oil to observe whether they can potentiate its tumor-promoting activity.

The nutraceuticals exhibited antioxidant activity. The antioxidant in sugar beet is betanin, a betacyanin responsible for its red color. The leaves of New Zealand spinach contain lutein and zeaxanthin, both antioxidant carotenoids, whereas turmeric rhizomes contain the very potent antioxidant curcumin. Lutein and zeaxanthin showed inverse associations with ovarian cancer (11), prostate cancer (12), and breast cancer (13), but their dietary intake is not related to the risk of estrogen-related endometrial cancer (14).

Curcumin exhibits cytotoxic effects against human acute myelogenous leukemia HL-60 cells (15), human-derived Hep G2 cells (16), mouse embryonal PCC4 cells (17), human melanoma cell lines (18), human androgen-independent (DU145) and -dependent (LNCaP) prostate cancer cell lines (19,20), HT-29 human colon cancer cells (21), UMUC human and MBT-2 mouse bladder cell lines (22), and Colo 320 human colorectal cancer cells (23). It also exhibits antinitiation and antipromotion activities in radiation-induced rat mammary tumors (24) and inhibits the carcinogenesis of murine skin, stomach, intestine, and liver (25,26).

The results of the skin tumor assay show a decrease in the percent skin tumor incidence, 30–70%, when the nutraceuticals were applied using the three different protocols, compared with 100% skin tumor incidence for the positive control. Hence, the nutraceuticals did not exhibit cocarcinogenicity or co-tumor-promoting potentials. Rather, they showed evidence of being antitumor promoters and/or chemopreventers, as demonstrated in the decrease in percent skin tumor incidence, decrease in the average number of skin tumors per mouse, and delay in tumor formation compared with the positive control.

Compared with the positive control, there was a drastic decrease in the number of skin tumors when the nutraceuticals were applied 1 h before croton oil (Fig. 2). With this protocol, Kruskal-Wallis analysis of variance by ranks showed differences in the responses of mice to the different treatments at \( p = 0.05 \). Comparative analysis shows that tur-

### Table 2. Anti-Skin Tumor Potentials of Beets, Cucumber, Spinach, and Turmeric Extracts Applied 1 h Before Croton Oil

<table>
<thead>
<tr>
<th>Test Sample</th>
<th>No. of Mice With Tumors</th>
<th>%Tumor Incidence</th>
<th>Avg No. of Tumors/Mouse</th>
<th>Avg Tumor Diam, mm</th>
<th>%Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>DMBA + croton oil</td>
<td>10</td>
<td>100.0</td>
<td>4.7 ± 3.3</td>
<td>1.6</td>
<td>0</td>
</tr>
<tr>
<td>Beets</td>
<td>6</td>
<td>60.0</td>
<td>3.0 ± 1.1</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td>Cucumber</td>
<td>5</td>
<td>55.6</td>
<td>4.2 ± 0.8</td>
<td>1.6</td>
<td>10</td>
</tr>
<tr>
<td>Spinach</td>
<td>5</td>
<td>50.0</td>
<td>3.2 ± 1.3</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Turmeric</td>
<td>3</td>
<td>30.0</td>
<td>2.0 ± 1.0</td>
<td>1.0</td>
<td>0</td>
</tr>
</tbody>
</table>

a: Values are means ± SD.

### Table 3. Anti-Skin Tumor Potentials of Beets, Cucumber, Spinach, and Turmeric Extracts Applied Immediately After Croton Oil

<table>
<thead>
<tr>
<th>Test Sample</th>
<th>No. of Mice With Tumors</th>
<th>%Tumor Incidence</th>
<th>Avg No. of Tumors/Mouse</th>
<th>Avg Tumor Diam, mm</th>
<th>%Mortality</th>
</tr>
</thead>
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<tr>
<td>Spontaneous</td>
<td>0</td>
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<td>DMBA + croton oil</td>
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</tr>
<tr>
<td>Beets</td>
<td>7</td>
<td>70.0</td>
<td>2.1 ± 1.1</td>
<td>1.1</td>
<td>0</td>
</tr>
<tr>
<td>Cucumber</td>
<td>6</td>
<td>66.7</td>
<td>3.8 ± 1.3</td>
<td>1.9</td>
<td>10</td>
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<tr>
<td>Spinach</td>
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<td>70.0</td>
<td>2.3 ± 1.1</td>
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<td>0</td>
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<td>62.5</td>
<td>3.4 ± 1.5</td>
<td>1.3</td>
<td>20</td>
</tr>
</tbody>
</table>

a: Values are means ± SD.
This is the first report on the effectiveness of sugar beet roots, cucumber fruits, New Zealand spinach leaves, and turmeric rhizomes in the prevention of chemically induced skin tumor formation. Interestingly, even the known anticancer agent curcumin was ineffective in inhibiting the formation of dibenzo[a]pyrene-DNA adducts in the dibenzo[a]pyrene-induced human breast cell line MCF-7 (27). A lack of chemopreventive effect was also manifested by curcumin in 3,2′-dimethyl-4-aminobiphenol- and 2-amino-1-methylimidazo[4,5-b]pyridine-induced rat ventral prostate cancer models (28).

Acknowledgments and Notes

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References


