Plant Tannins Inhibit the Induction of Aberrant Crypt Foci and Colonic Tumors by 1,2-Dimethylhydrazine in Mice

Hala U. Gali-Muhtasib, Ihab H. Younes, Joseph J. Karchesy, and Marwan E. El-Sabban

Abstract: We have shown that naturally occurring tannins possess antitumor promotion activity in mouse skin. In the present investigation, we studied the ability of a hydrolyzable tannin, gallotannin (GT), and a condensed tannin extracted from red alder (RA) bark to inhibit 1,2-dimethylhydrazine (DMH)-induced colonic aberrant crypt foci (ACF) and tumors in Balb/c mice. In addition, we determined the ability of GT to inhibit the proliferation and to induce apoptosis in a human colon cancer cell line (T-84). Mice were given tannins by intraperitoneal injections, by gavage, or in drinking water before treatment with DMH for 24 weeks. Alternatively, mice were given tannins by intraperitoneal injection or gavage for only 2 weeks before DMH administration, then tannin administration was discontinued and mice were treated with DMH for 24 weeks. The multiplicity, size, and distribution of ACF and tumors were significantly inhibited by GT and RA in the above treatment regimens. The most effective treatments included GT by gavage, RA bark extract by intraperitoneal injection, and either tannin dissolved in drinking water. Extent of inhibition of ACF and tumors was gender independent. In cell culture experiments, GT treatment for three days inhibited the growth of T-84 cells, with a concentration resulting in half-maximal inhibition estimated to be 20 \( \mu \text{g/ml} \). The treatment was not cytotoxic to cells at 1–40 \( \mu \text{g/ml} \). Interestingly, at 10 \( \frac{\mu \text{g}}{\text{ml}} \), GT induced apoptosis in T-84 cells as determined by the Hoechst DNA staining technique. Collectively, these findings support a potential role for tannins as chemopreventive agents against colon cancer.

Introduction

Colon cancer is one of the most common visceral malignancies in the Western countries, with a lifetime risk of occurrence approaching 5% in some regions (1). This cancer originates from the epithelial lining of the mucosal layer of the large intestine, whereby loss of control occurs in the process of cell division, leading to thickened epithelial cells known as polyps or adenomas. Adenomatous polyps from the colon appear as a protruding mass of tissue into the lumen and are precursors of the malignant colon tumors. Adenocarcinomas almost always arise from the secretory cells in the epithelium (2).

Recently, a large number of natural food components have been screened for their protective or anticancer activities, among which plant polyphenols have proved to be a potent class. Tannins are types of plant polyphenols widely distributed in the plant kingdom that have been recently found to possess remarkable antitumor-promoting effects in various animal models and tumor systems (3–6). Classically, tannins are divided into two chemically and biologically distinct groups: the condensed tannins (CTs), also referred to as proanthocyanidins, and the hydrolyzable tannins (HTs) (7). There is interest in proanthocyanidins because of their potential health benefits. Recent interest in these food polyphenols has emerged because of their antioxidant, free radical-scavenging, and metal-chelating activities, which may lead to their possible role in the treatment and prevention of cancer and other pathologies (6). Several HTs and CTs have been found to block the activities of many mutagens and to have anticarcinogenic effects. When applied topically, injected, or added to the diet or drinking water, tannins were found to inhibit tumor initiation and carcinogenesis in the skin and the mammary gland (8–11). Plant tannins extracted from various sources have been also shown to possess antitumor-promoting effects in the skin of hairless mice by inhibiting several biochemical markers of tumor promotion induced by exposure to ultraviolet-B light (7,12). The protective effects of tannins against many types of cancers lead us to postulate that these polyphenols are universal antitumor agents (13). Other effects include their ability to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, and modulate immunologic responses depending on the tannin doses and types used (14). The aim of this project is to study the ability of tannins to protect against colon cancer in an animal model and to modulate the proliferation of a human colon cancer

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cell line (T-84) in vitro. The representative HT used was gallotannin (GT); the representative CT was an extract of red alder (RA) bark, *Alnus rubra* Bong, a tree found abundantly in the Pacific Northwest coastal forests of North America. The bark extract of this tree is known to be rich in procyanidins used by the indigenous people in the form of purgatives, general tonics, and teas to treat several ailments such as cold, digestive problems, respiratory problems, and heart pain (15–17). We used the established 1,2-dimethyldihydrazine (DMH) model of colon cancer in BALB/c mice to test the efficacy of different routes of administration of tannins as chemopreventive agents against colon cancer. GT or RA was given to mice by intraperitoneal injection or gavage two weeks before DMH (pretreatment), then tannin treatment was discontinued and mice were injected with DMH for 24 weeks. Alternatively, tannins were delivered by intraperitoneal injection, by gavage, or in drinking water 15 minutes before each DMH injection (cotreatment) for the length of the experiment (24 wk). In this study, we present evidence for a role of tannins in the prevention of colon cancer induced by DMH in mice and report that GT inhibited cell growth and induced apoptosis in a human colon cancer cell line.

**Materials and Methods**

**Reagents**

Dulbecco’s modified Eagle’s medium (with L-glutamine, 1,000 mg/ml D-glucose, and sodium pyruvate), Ham’s F-12, trypsin-EDTA, Dulbecco’s phosphate-buffered saline (PBS), fetal bovine serum, sodium bicarbonate, and penicillin-streptomycin were obtained from GIBCO BRL Life Technologies. Prolong Antifade and Hoechst-33342 stain were purchased from Molecular Probes (Eugene, OR). Trypan blue, DMH, and ribonuclease A were purchased from Sigma (Fluka) Chemical (St. Louis, MO). [3H]thymidine was obtained from Amersham Life Sciences. Biodegradable scintillation cocktail was obtained from Research Products International. Basic fuchsin and sodium metabisulfite were purchased from Fisher Scientific. GT (FW 1701) was obtained from J. T. Baker Chemical. RA extract (70% aqueous acetone) was obtained from freshly collected bark from a tree cut in McDonald State Forest near Corvallis, OR, and freeze-dried to give the tannin-rich powder (16). The oligomeric CT content of the RA extract is 16% by weight as determined by chromatography (16).

**Determination of the Dose of Tannins at Which 50% of the Mice Died**

To determine the dose of tannin that could be administered intraperitoneally without being lethal to mice, the dose at which 50% of the mice died (LD₅₀) was estimated. For this experiment, mice were divided into five groups of four mice each. Tannin extracts were dissolved in isotonic saline (0.9%) and injected intraperitoneally at 1, 5, 10, 20, and 30 mg/kg body wt. A control group of mice received intraperitoneal injections of 0.9% saline. Each mouse was injected with 0.2 ml of solution on a daily basis for two weeks.

**Tumor Induction and Treatment Regimen**

The protective effects of tannins were tested in adult BALB/c mice bred in the animal care facility at the American University of Beirut. Mice were housed under optimum conditions of temperature (22 ± 2°C) and light (12:12-hour light-dark cycle). Mice (6–8 wk old) were kept in plastic cages covered with sawdust and had unrestricted access to a commercial mouse diet (24% protein, 4.5% fat, 4% fiber) and water (except the groups receiving tannins in drinking water). Mice were randomly distributed to the control or treatment groups; each group consisted of 14 mice (7 males and 7 females placed in separate cages). Details of the various treatments are shown in Table 1. The carcinogen DMH was freshly prepared in isotonic saline at a dose of 20 mg/kg body wt and injected subcutaneously (0.2 ml) at the dorsal side of each mouse once per week for 24 weeks. Mice were weighed weekly and routinely inspected for any symptoms of colon cancer, such as diarrhea, bloody stools, and loss of body weight. Tannins were administered to mice by three different methods: 1) intraperitoneal injection of a 0.2-ml solution prepared in 0.9% saline and delivered at a dose of 10 mg/kg body wt, 2) force feeding (gavage) of a 0.3-ml tannin solution prepared in 0.9% saline and delivered at a dose of 1% (wt/vol) into the esophagus region, and 3) drinking water containing 0.25% (wt/vol) tannins, a dose that did not affect the drinking behavior of mice. Mice consumed an average of 6 ml of water daily, which is equivalent to an intake of 15 mg of tannins per mouse per day. For drinking water studies, solutions were offered to mice on Days 1–4 of every week. On Day 5 (day of DMH injection), the remaining solution was diluted with distilled water to a volume of 200 ml and offered to mice for the rest of the week. Fresh tannin solutions were prepared on a weekly basis. One week after the last DMH injection, mice were sacrificed by diethyl ether inhalation.

**Tissue Processing**

Directly after the mice were sacrificed, the colon was excised, flushed with PBS, and kept in ethanol-acetic acid (3:1) at 4°C until later staining and analysis, as described previously (18). At the time of analysis, colons were placed in 50% aqueous ethanol for 15 minutes at room temperature and transferred to distilled water for 10 minutes, to 1 N HCl for 7 minutes at 60°C, and finally to Schiff’s reagent at room temperature for 10 minutes. At this final stage, the colons were dark pink in color. They were rinsed in distilled water, cut longitudinally, pinned flat on a cork board, and stored...
overnight in 45% aqueous acetic acid at 4°C. On the following day, the length of all colons was measured, and they were cut into three parts (proximal, middle, and distal colon) and placed on microscopic slides. Gross tumors were identified, their size and location were recorded, and some were preserved in 4% buffered formaldehyde for histopathological examination as previously described (19). The yield or average number of tumors in each group and the diameter and location of tumors were recorded. Tumors were classified as small (0.1–1.0 mm diameter), medium (1.1–2.0 mm diameter), or large (2.1–4.0 mm diameter). Aberrant crypts were identified as being larger in diameter and having enlarged luminal openings and thickened crypt walls compared with neighboring normal crypts. Aberrant crypt foci (ACF) were classified as small (1–3 crypts/focus), medium (4–6 crypts/focus), and large (>7 crypts/focus). For statistical analysis, data were first analyzed by analysis of variance to test for differences between groups and then by Tukey’s test to determine which particular groups were significantly different. Student’s t-test was used to check for differences between males and females in each treatment.

Cell Culture

Human colon cancer cells (T-84) were kindly provided by Dr. Fadia Homeidan (American University of Beirut) and cultured in Dulbecco’s modified Eagle’s medium-Ham’s F-12 (1:1) supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (50 µg/ml) in a humidified incubator (37°C) in 95% air-5% CO2. A plating density of 4 x 10^4 cells/ml was used for all experiments, except as otherwise mentioned. In cell culture experiments, only the effect of GT was studied because of its higher solubility in ethanol (the vehicle used for tannin dissolution) than that of RA bark extract.

Cell Proliferation Assays

Cell growth was assessed by counting cells at predetermined intervals using trypan blue exclusion assays. Cells were treated with tannins 24 hours after they were plated (Day 0) and harvested at Days 1, 3, and 5 after treatment; fresh medium containing tannins was replenished on Days 2 and 4. Tannins were dissolved in ethanol; the ethanol concentration did not exceed 0.9%/well. Control plates were treated with ethanol (<0.9%) only. At harvest time, cells were trypsinized, stained with trypan blue dye, and counted using a hemocytometer. For thymidine uptake studies, cells were plated in 96-well plates and incubated overnight to allow the cells to attach. Fresh medium (1 ml/well) containing different concentrations of GT was added to the cells 24 hours after they were plated, and the culture was continued for 24 hours. [3H]thymidine was then added to each well for a final dose of 1 µCi/well, and the cells were further incubated for 4 hours. Cells were harvested on a PHD cell harvester (Brandel) and dried on filters overnight. Biodegradable liquid scintillation cocktail was then added, and radioactivity was detected and expressed as counts per minute using a scintillation counter. Cell proliferation was expressed as the percentage of the control. Each experiment was performed in nine replicates and repeated twice.

Apoptosis Studies

Apoptosis was studied by nuclear staining techniques. Cells were plated on autoclaved glass coverslips in six-well culture plates. Various doses of tannins (1, 10, and 20 µg/ml) were applied to cells 24 hours later, and the culture was continued for 24 hours. The medium was then aspirated, and cells were washed twice with warm PBS. Cellular DNA was stained by 1 ml of Hoechst-33342 stain (1 µg/ml) for 20 minutes in the dark and at room temperature. Cells were then washed with PBS and mounted on slides using Prolong

Table 1. Body Weights of Mice at the Beginning (Week 1), the Middle (Week 10), and the End (Week 20) of the Experiment

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>No. of Mice</th>
<th>Week 1</th>
<th>Week 10</th>
<th>Week 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (saline) ip</td>
<td>15</td>
<td>31</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>DMH only sc</td>
<td>16</td>
<td>32</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>3b</td>
<td>GT → DMH ip</td>
<td>15</td>
<td>27</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>4b</td>
<td>GT → DMH gavage</td>
<td>15</td>
<td>30</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>GT only ip</td>
<td>15</td>
<td>29</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>RA → DMH ip</td>
<td>15</td>
<td>30</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>RA only</td>
<td>15</td>
<td>31</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>8</td>
<td>GT → DMH gavage</td>
<td>15</td>
<td>30</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>9</td>
<td>GT only gavage</td>
<td>15</td>
<td>29</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td>10</td>
<td>RA → DMH gavage</td>
<td>15</td>
<td>31</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>11</td>
<td>RA only gavage</td>
<td>15</td>
<td>28</td>
<td>30</td>
<td>33</td>
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<td>12</td>
<td>GT → DMH drinking</td>
<td>15</td>
<td>27</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>13</td>
<td>RA → DMH drinking water</td>
<td>15</td>
<td>26</td>
<td>31</td>
<td>31</td>
</tr>
</tbody>
</table>

*a:* Abbreviations are as follows: DMH, 1,2-dimethylhydrazine; GT, gallotannin; RA, red alder bark.

*b:* Groups 3 and 4 received GT daily for only 2 wk (14 treatments), then tannin treatment was discontinued and mice were injected with DMH for the rest of the experiment (24 wk). In all other groups, mice were treated with tannins 15 min before DMH injection.
Antifade and subsequently stored at 4°C until analysis. Nuclear condensation was observed under a fluorescence microscope (model LSM 410, Zeiss).

**Results**

Mice in all groups treated with DMH, GT, and/or RA bark extracts gained weight progressively with no noticeable adverse effects (Table 1). The highest safe dose of tannins that could be administered intraperitoneally was 10 mg/kg body wt, the highest safe dose that could be force-fed was 1% (wt/vol), and the highest safe dose that could be consumed in drinking water was 0.25% (wt/vol). Thus, throughout the study, these tannin doses were used to assess the effects on DMH-induced colon cancer in mice.

**Tannins Decrease ACF and Tumor Multiplicity**

Tannins administered by various means (except RA bark by gavage) were found to significantly decrease the number of ACF or tumors in the colon of mice compared with the DMH-treated mice (Figure 1). The most effective treatments at reducing the number of ACF were intraperitoneal injection of RA bark (62% inhibition, \( p < 0.05 \)) and force feeding of GT (55% inhibition, \( p < 0.05 \); Figure 1, A and B). Interestingly, treatment with GT by gavage for two weeks before a 24-week DMH treatment inhibited ACF numbers by 54% \( (p < 0.05) \). The inhibition of gross tumors by tannins was different from that of ACF numbers (Figure 1, C and D). Treatments that were effective at reducing ACF numbers (GT or RA in drinking water) did not reduce the number of gross tumors in the colon of mice. In some cases, however, the same treatments that reduced ACF numbers (GT by gavage, RA by intraperitoneal injection, GT pretreatment) were also effective at reducing the number of gross tumors. Treatment of mice with RA by gavage did not reduce the number of ACF and/or gross tumors.

**Effect of Gender or Locale on Tannin Inhibition of Colon Cancer**

There was no statistical significance between males and females in the multiplicity of ACF or tumors (Figure 2). ACF and tumor numbers were remarkably higher in the distal colon than in the middle or proximal colon (Figure 3), which is in accordance with published literature (20). Interestingly, all tannin treatments (except RA by gavage) resulted in significantly fewer ACF in all parts of the colon (proximal, middle, and distal; Figure 3, A and B). However, tumors were fewer in number only in the distal part of the colon (Figure 3, C and D). The size of ACF (number of crypts per focus) or tumors (diameter) is an indicator of their degree of aggressiveness and likelihood of progression to

![Figure 1. Inhibition of 1,2-dimethylhydrazine (DMH)-induced aberrant crypt foci (ACF) and tumors by gallotannin (GT) and red alder (RA) bark extract administered by gavage (G), in drinking water (D), or intraperitoneally (IP) for length of experiment or for only 2 wk (P, pretreatment). Bars denoted by same letter (a, b) are not significantly different (Tukey’s test, \( p < 0.05) \).](image-url)
Table 2 summarizes results of the average weighted sizes of tumors and the effects of various tannin treatments on tumor size. Small (1–3 crypts/focus) ACF numbers were significantly less in most tannin treatment groups. The most effective treatments at attenuating ACF numbers were GT given by gavage, GT in drinking water, or GT injected intraperitoneally (pretreatment) (Figure 1). Similarly, the number of small, medium, and large tumors was reduced on treatment with tannins (Table 2). When the inhibition of tumor numbers was analyzed on the basis of size, we observed that pretreatment with GT (intraperitoneal injection) resulted in a significant reduction (72%) in the number of large tumors. However, the administration of GT by gavage inhibited the total tumor number by 75%, while small size tumors were reduced by 85% (Table 2).

Effects of Tannins on Colon Cancer Cells In Vitro

When T-84 cells were treated with 1–40 μg/ml of GT for 5 days, cell growth was significantly inhibited at ≥20 μg/ml (p < 0.001; Figure 4A). At Day 1, GT marginally reduced T-84 cell growth; at Days 3 and 5, however, a dose-dependent inhibition of growth was evident. At Day 5, GT reduced growth by 58% and 81% at 20 and 40 μg/ml, respectively (p < 0.001). This pattern of T-84 cell growth was confirmed by the [3H]thymidine incorporation assay. DNA synthesis was significantly reduced on treatment with 1–50 μg/ml of GT (p < 0.001; Figure 4B). To rule out the possibility that the observed inhibition in cell growth was due to cytotoxicity, the effect of tannins on cell viability was determined. At up to 30 μg/ml, GT had no significant effect on T-84 cell viability on either day (data not shown). Interestingly, nuclear DNA staining by Hoechst-33342 indicated that the increase in the apoptotic index due to GT was apparent at doses as low as 1 μg/ml (2.5-fold increase) and became pronounced at 10 μg/ml (~4-fold increase). No further increase in the apoptotic index was noticed at higher concentrations (20 μg/ml; Figure 5).

Discussion

Plant tannins have been previously shown to possess antimutagenic activities in various animal models and organ systems. In the skin, tannins have been found to inhibit ultraviolet-B- and chemical-induced carcinogenesis (5,13). Tannins have been also found to be effective treatments against mammary gland (11), hepatic (22), stomach (23), and lung (24) cancers. The main dietary sources of these compounds include fruits and beverages (fruit juice, wine, beer, tea, coffee, and chocolate) and, to a lesser extent, vegetables, dry legumes, and cereals. Possible mechanisms for the protective effects of tannins include their ability to inhibit some biochemical markers of tumor promotion, including the stimula-
Figure 3. Distribution of ACF and tumors in proximal, middle, and distal colon. *, Significantly different from DMH treatment groups (Tukey’s test, $p < 0.05$). Comparisons were only made with DMH group, and not between treatments.

Table 2. Weighted Average Size of Tumors and Fraction of Tumors With Different Sizes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total No. of Tumors</th>
<th>Weighted Size$^a$</th>
<th>Average Number of Tumors of Different Sizes$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Small</td>
</tr>
<tr>
<td>DMH</td>
<td>378</td>
<td>1.18</td>
<td>10.88 ± 5.71 (185)</td>
</tr>
<tr>
<td>GT → DMH ip (pre)$^c$</td>
<td>142</td>
<td>1.19</td>
<td>4.21 ± 3.20 (59)</td>
</tr>
<tr>
<td>GT → DMH gavage (pre)$^c$</td>
<td>151</td>
<td>1.20</td>
<td>4.86 ± 4.40 (68)</td>
</tr>
<tr>
<td>GT → DMH gavage</td>
<td>95</td>
<td>1.49</td>
<td>2.55 ± 3.05 (28)</td>
</tr>
<tr>
<td>GT → DMH drinking water</td>
<td>194</td>
<td>1.30</td>
<td>5.50 ± 4.82 (77)</td>
</tr>
<tr>
<td>RA → DMH drinking water</td>
<td>198</td>
<td>1.18</td>
<td>7.69 ± 5.38 (100)</td>
</tr>
<tr>
<td>RA → DMH ip</td>
<td>103</td>
<td>1.19</td>
<td>3.08 ± 1.50 (40)</td>
</tr>
<tr>
<td>RA → DMH gavage</td>
<td>200</td>
<td>1.24</td>
<td>7.45 ± 4.87 (82)</td>
</tr>
</tbody>
</table>

$^a$: Weighted sizes were calculated by dividing the sum of all tumor sizes (assigning 0.5, 1.5, and 3.0 mm for small, medium, and large tumors as a size indicator) by the total number of tumors.

$^b$: Values are means ± SE. Values in parentheses represent total number; values in brackets represent percentage of DMH.

$^c$: Groups received GT daily for only 2 wk (14 treatments), then tannin treatment was discontinued and mice were injected with DMH for the rest of the experiment (24 wk). In all other groups, mice were treated with tannins 15 min before DMH injection.
Figure 4. Effect of GT on cell growth (A) and incorporation of $[^3H]$thymidine (B) in T-84 cells. Cells were plated at a density of $4 \times 10^5$ cells/ml in 6-well (cell count) or 96-well (thymidine uptake) plates in absence or presence of GT. Tannins were dissolved in ethanol (<0.9%). Control plates were treated with vehicle only. All comparisons were done with control (ethanol). A: on Day 1, 1, 20, 30, and 40 g/ml GT significantly reduced growth ($p < 0.01$); on Day 3, 30 and 40 g/ml GT reduced growth ($p < 0.05$); on Day 5, 1 g/ml GT significantly reduced growth ($p < 0.05$), while 20, 30, and 40 g/ml reduced growth more significantly ($p < 0.001$). B: 1, 10, 20, 30, and 50 µg/ml significantly reduced $[^3H]$thymidine incorporation into cells ($p < 0.001$).

Figure 5. Effect of GT on induction of apoptosis. Apoptotic cells were labeled with Hoechst-33342 stain (1 µg/ml) for 20 min in dark at room temperature. Nuclear condensation was observed under a fluorescent confocal microscope. There was a 2.5-fold increase in apoptotic index due to GT (1 µg/ml). This was increased to ~4-fold at 10 µg/ml.
tion of the enzyme ornithine decarboxylase (5,12), and DNA synthesis (7). Other mechanisms include their ability to enhance the immune system (25), inhibit polycyclic aromatic hydrocarbon-DNA adduct formation (24), scavenge free radicals (6), induce DNA fragmentation (26), and inhibit invasion of metastatic cells through the extracellular matrix (27).

An established rodent model of DMH-induced colorectal carcinogenesis was used in this study to evaluate the chemopreventive activity of two types of tannins administered through different routes. We also investigated the ability of these polyphenols to inhibit growth and/or induce apoptosis in a human colon cancer cell line (T-84). It is estimated that the total intake of tannins is 1 g/day (31,32). This dose is equivalent to 16 mg/kg body wt of an average person and, thus, compares very well with the doses of tannins chosen for this study.

We have shown that tannins reduce the number of DMH-induced ACF (precursors of tumors) in mice, with GT being a more effective treatment than RA bark extract. Tannins also reduced the number of gross tumors, albeit to a lesser extent (Figure 1). With the exception of 1% RA bark extract administered by gavage, all tannin treatments reduced ACF or tumor multiplicity (Figure 1) and the number of tumors of each size (Table 2). Intraperitoneal injection and administration in drinking water were better routes than gavage, since ACF or tumor multiplicity was reduced to a greater extent by these routes (Figure 1). These results are in agreement with previous reports showing that polyphenols extracted from green tea possess potent inhibitory effects against the development of colonic tumors when given in drinking water (28,29). The latter route allows the continuous administration of small amounts over extended periods (4 days) and, thus, the steady absorption of tannins in amounts sufficient for effective inhibition. When injected intraperitoneally, tannins are directly delivered to the area surrounding the colon and, thus, exert their inhibitory effects directly on the target tissue. In addition, intraperitoneal injection could enhance the immune system of mice (25) because of its absorption into the circulation, a possibility that should be investigated. Remarkably, when mice were pretreated with GT for only two weeks before DMH treatment, ACF and tumor numbers were significantly lessened (Figure 1). This demonstrates the potent chemopreventive abilities of these compounds against DMH-induced colon cancer in mice. It is possible that GT treatment induced genetic changes in the colonic mucosa that, in turn, altered DMH metabolism by this layer. Interestingly, treatment of T-84 cells with 25 μg/ml GT for 12 or 24 hours did not allow cells to recover to control levels at 24 or 48 hours after withdrawal of GT (data not shown). Recovery of cell growth was observed only at Day 3 after withdrawal of GT, a time that is sufficient for the remaining cells to proliferate. Thus it is plausible that the in vivo GT pretreatment regimen over a short period of two weeks induced some yet to be determined effects that protected these cells from the effect of the carcinogen offered over a six-month period.

Although females were slightly more susceptible to DMH-induced cancer than males, tannins inhibited ACF and tumor multiplicity in both genders to the same extent (Figure 2). There was no indication that the inhibition in any one gender contributed to the total prevention of any treatment. Although in human populations the incidence of colon cancer among men and women depends on the geographical location, there are no large differences between the two genders.

Very few tumors were found to be located in the proximal or middle parts of the colon, whereas >60% of the tumors were found in the distal colon. This is due to the greater metabolism of DMH by the mucosa of the distal colon than by the middle or proximal parts (30). GT reduced ACF numbers in all parts of the colon except for the mice pretreated with GT by gavage, a treatment that reduced ACF numbers only in the proximal colon (Figure 3). Although exactly how dietary tannins exert their protective effect is not well understood, it has been shown that the conditions of the gut may fragment tannin polymers into a complex mixture of smaller units that may be readily absorbed through the digestive tract and recovered in the serum (32). The effect of tannin could be exerted directly through absorption by the colonocytes or through the blood concentration as shown by the results of intraperitoneal injection of tannins. To our knowledge, no studies have been performed to determine how much of intact tannins reaches the distal portion of the colon and where in the colon absorption occurs.

We have also used the human colonic carcinoma cell line T-84, an established model for many biological functions (33,34), as an in vitro model to test tannin’s ability to modify cell proliferation and, perhaps, the induction of apoptosis. Treatment of T-84 cells with GT inhibited their growth, with no apparent cytotoxic effect, in a dose-dependent manner at 72 hours, with maximal effect observed at 120 hours after treatment (Figure 4). When treated with 10 μg/ml of GT, T-84 cells exhibited numerous apoptotic bodies (fragmented nuclei), suggesting that programmed cell death (apoptosis) could be the major factor contributing to the inhibition of cancer cell growth (Figure 5).

In conclusion, the growth of colon cancer cells was inhibited by tannins, a finding that supports our in vivo results and further confirms a protective role for these compounds against colon cancer. The exact mechanism by which tannins exert their antitumor effect is currently under investigation in our laboratories.

Acknowledgments and Notes

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References


