Antineoplastic and Antioxidant Properties of Some Fruits and Vegetables Using Experimental Models of Mammary Cancer

P. García-Solís  
Facultad de Ciencias Naturales  
Universidad Autónoma de Querétaro  
Querétaro and Facultad de Medicina  
Universidad Autónoma de Querétaro  
Querétaro  
Mexico

E.M. Yahia  
Facultad de Ciencias Naturales  
Universidad Autónoma de Querétaro  
Querétaro  
Mexico

V. Morales-Tlalpan, M. Díaz-Muñoz and C. Aceves  
Instituto de Neurobiología  
Universidad Nacional Autónoma de México  
Querétaro  
Mexico

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Abstract

Consumption of fruits and vegetables has been associated with reduced risk of chronic diseases. They are rich in several antioxidant phytochemicals such as carotenoids and phenolic compounds that could be responsible for the health benefits. We studied the antineoplastic properties of some fruits and vegetables using in vivo and in vitro models. First, we studied the effect of ‘Ataulfo’ mango consumption on chemically-induced mammary carcinogenesis and plasma antioxidant capacity (AC) in rats treated with the carcinogen N-methyl-N-nitrosourea (MNU). Mango was administered in the drinking water (0.02-0.06 g/ml) during both short-term and long-term periods to rats, and plasma antioxidant capacity was measured by ferric reducing/antioxidant power (FRAP) and total oxyradical scavenging capacity assays. Mango consumption did not have an effect on mammary carcinogenesis (incidence, latency and number of tumors), nor on plasma AC of rats treated with MNU. Second, we screened using methylthiazolydiphenyl-tetrazolium bromide assay the antiproliferative activity of aqueous extracts of avocado, black sapote, guava, mango, cactus cladodes (cooked and raw), papaya, pineapple, four different prickly pear fruit, grapes and tomato, on the breast cancer cell line MCF-7. β-carotene, gallic acid, total phenolic contents and AC were analyzed in each aqueous extract. In vitro study showed that only the papaya extract had a significant antiproliferative effect and we did not notice a relationship between total phenolic content and AC with antiproliferative effect. These results suggested that each plant food has a unique combination in quantity and quality of phytochemicals which could determine its biological activity.

INTRODUCTION

Consumption of fruits and vegetables (F&V) has been associated with reduced risk of some types of cancer. F&V are rich in several antioxidant phytochemicals such as carotenoids and phenolic compounds that could prevent the oxidative damage produced by free radicals (Liu, 2004). Free radicals oxidize lipids, proteins and DNA, and they are involved in the initiation and promotion/progression of carcinogenesis (Brown and Bicknell, 2001). Besides their antioxidant capacity (AC), phytochemical compounds have diverse biological effects such as antimutagenic activity, inhibition of cell proliferation, induction of apoptosis, etc. (Liu, 2004). It was proposed that the health benefits of F&V might result from multiple combined effects of their phytochemicals rather than from the action of a single active ingredient (Liu, 2004). In this work we studied the antineoplastic activities of whole extracts of F&V consumed in Mexico using in vivo and in vitro methods.
models of mammary cancer. Mango fruit is rich in phytochemicals such as carotenoids and phenolics, and has been reported to have in vitro antioxidant and antineoplastic effects suggesting important anticancer activity in vivo. We studied the effect of ‘Ataulfo’ mango consumption on chemically-induced mammary carcinogenesis and plasma AC in rats treated with the carcinogen N-methyl-N-nitrosourea (MNU). We also screened the antiproliferative activity of 14 aqueous extracts of F&V on the breast cancer cell line MCF-7 using methylthiazolydiphenyl-tetrazolium bromide (MTT) assay.

MATERIALS AND METHODS

In Vivo Experiment

At 5 weeks of age, rats received different quantities of ‘Ataulfo’ mango in the drinking water: a) Control (1.2% of sucrose), b) Mango-1 (0.02 g/ml); c) Mango-2 (0.04 g/ml), and d) Mango-3 (0.06 g/ml). Two weeks later, rats were i.p. injected with MNU (50 mg/kg bw) or vehicle (0.9% saline). At 8 weeks of age mango treatments were discontinued (blue arrow) for half of the rats (STI: short-term intake), and they were supplied with control beverage. The other half of the rats (LTI: long-term intake) continued with mango intake until the end of the experiment (Green arrow). Plasma AC was measured by ferric reducing/antioxidant power (FRAP) (Benzie and Strain, 1999) and total oxyradical scavenging capacity (TOSC) assays (Winston et al., 1998). In both assays a standard curve with ascorbic acid was used to estimate the total capacity of the samples. All animal procedures followed the Official Mexican Norm NOM-062-ZOO-1999.

Fruit and Vegetables

‘Hass’ avocado, black sapote, ‘Media China’ guava, ‘Ataulfo’ mango, ‘Milpa Alta’ nopal (prickly pear cactus cladodes: cooked and raw), ‘Maradol’ papaya, ‘MD2’ pineapple, four different prickly pear (PP) fruit, ‘Flames seedless’ red grapes and ‘Clermon’ tomato. Edible portions of F&V were frozen with liquid nitrogen and stored at -70°C.

Aqueous Extract Preparation

Aqueous extracts were prepared according to Percival et al. (2006). Frozen F&V were mixed with 1:1 HPLC grade water, homogenized and sonicated for 5 min. After centrifugation at 9000 × g for 20 min at 4°C, the supernatant was set aside. The pellet was resuspended with water, shaken and centrifuged and the procedure was repeated a third time. Supernatants were then pooled and one aliquot of each extract was sterilized by filtration.

Chemical Analysis and AC Assays in Aqueous Extract

β-carotene and gallic acid contents were analyzed by HPLC (García-Solís et al., 2009), total phenolic content was analyzed by Folin-Ciocalteu assay (Singleton et al., 1999). Total AC was measured by FRAP and the 2, 2-diphenyl-1-1-picrylhydrazyl (DPPH) radical scavenging assays (Brand-Williams et al., 1995).

Cell Culture

Breast cancer cells MCF-7 were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 5% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin. Cells were incubated in a humidified 90% air, 5% CO₂ atmosphere at 37°C (García-Solís and Aceves, 2003). The antiproliferative effect was assessed by MMT assay (Arroyo-Helguera et al., 2006).

RESULTS AND DISCUSSION

The present study is, to our knowledge, the first to test in vivo antineoplastic effect of mango juice on mammary gland. Previous studies showed that mangoes have in vitro

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antioxidant, antimutagenic, and antineoplastic activities (Botting et al., 1999; Percival et al., 2006). However, we have shown here that short- and long-term mango consumption at physiological levels did not prevent mammary carcinogenesis in rats treated with the carcinogen MNU. This null effect of short-term and long-term intake of mango indicated that the kind and quantity of ‘Ataulfo’ mango phytochemicals do not inhibit the MNU-induced mammary cancer, neither at the initiation nor at the promotion/progression steps of carcinogenesis. Table 1 summarizes mammary tumorigenesis data in terms of incidence, latency, and both number and size of tumors for both control and mango-fed groups. Mango juice treatments did not modify TO SC values in the plasma of rats fed with different levels of mango (Table 2). The FRAP assay indicated that healthy rats (not treated with MNU) with long-term intake of mango, the FRAP values tended to increase in a dose-dependent manner (Table 2). The mango-3 group had a 3.1-fold increase in FRAP value with respect to the control, whereas Mango-1 and Mango-2 groups had 1.9- and 2.2-fold increases in FRAP values, respectively.

In the second part of this work, we studied the effect of 14 aqueous extracts obtained from F&V consumed in Mexico on cell proliferation of breast cancer cell line MCF-7. The aim of our study was to test complete and not fractioned extracts. Initially, the total content of β-carotene and phenolics, including gallic acid, in the aqueous extracts were analyzed. Among all the F&V analyzed we only detected β-carotene in papaya extract (21.3 µg/ml) (data not shown), and we were unable to detect β-carotene in other aqueous extracts from fruit and vegetables rich in this compound, such as mango (Ornelas-Paz et al., 2007), presumably because of the very low dilutions used. This could also be explained because the papaya matrix is softer than other F&V used in this study. In papaya and other yellow fruits, carotenoids are dissolved in oil droplets in chromoplasts and can be readily extracted whereas in other foods, such as nopal, carotenoids could be entrapped and form a complex with proteins in diverse cellular structures (Castenmiller and West, 1998). This result is very important because carotenoids are a family of nearly 600 fat-soluble plant pigments that have received substantial attention because some act as provitamin A, and many have antioxidant activity (Liu 2004). Figure 1 (upper panel) shows the values of phenolic contents, expressed as µg of GAE/ml, of the 14 fruits and vegetable aqueous extracts tested in this study. Among all the aqueous extracts analyzed, guava had the highest phenolic content, followed by black sapote, nopal (cooked and raw), mango, pineapple, papaya, PP ‘Rojo pelota’, red grapes, PP ‘2-651’, PP ‘Reina’, and avocado. Tomato extract had the lowest phenolic content. It is convenient to indicate that Folin-Ciocalteu is not specific for analysis of individual compounds; however, it gives a global quantity of phenolics present in each sample (Singleton et al., 1999). Among all the aqueous extract analyzed, grape had the highest gallic acid content, followed by mango, pineapple, papaya, PP ‘Reina’, PP ‘Naranjona’, guava, black sapote, PP ‘2-651’, PP ‘Rojo pelota’, and tomato (Fig. 1, bottom panel). FINALLY, in avocado and nopal (cooked and raw) extracts, we were unable to detect gallic acid (Fig. 1, bottom panel). Phenolic compounds are important constituents in fruits and vegetables, and their presence relates to the AC, food quality, and potential health benefits (Mahattanatawee et al., 2006).

AC by FRAP and DPPH assays in aqueous extracts of F&V (Fig. 2) showed a very similar pattern. The five aqueous extracts with highest AC were guava, mango, black sapote, papaya and pineapple. It is interesting to note that the aqueous extract of black sapote, a fruit poorly characterized, had high phenolic content and high AC. Further research is warranted to explore the correlation between the phenolic content, AC and potential health benefits of this fruit. Extracts with lower AC were avocado, tomato and nopal (cooked and raw). Both nopal extracts, cooked and raw, had high phenolic content (Fig. 1, upper panel) but very low AC (Fig. 2).

After testing the 14 aqueous extracts at 0.01, 0.1, 0.5 and 1% for 24, 48 and 72h, we found that only papaya extract had a significant but modest inhibitory effect on proliferation of MCF-7 cells after 72h of treatment (Fig. 3, bottom panel). Therefore, we increased the doses to 2 and 4% for black sapote, guava, mango, papaya and raw nopal
extracts, which were those with the highest phenolic content and AC in the case of the four first extracts, and with high phenolic content but poor AC in the case of nopal (Figs. 1 and 2). With the higher doses, we have noticed that only mango and papaya extracts had a significant inhibitory effect on cell proliferation (Fig. 3). The effect of the mango extract was significant only at 4% and after 72h of treatment (Fig. 3, bottom panel). However, papaya extract was more effective, showing an inhibitory effect on cell proliferation at 4% after 24h (Fig. 3, upper panel). At longer treatment duration (48 and 72h), the papaya’s antiproliferative action was more evident (Fig. 3, middle and bottom panel). The maximum effect of papaya extract was shown after 72h of treatment at 2 and 4%, causing 30 and 53% inhibition of MCF-7 cells proliferation, respectively (Fig. 3, bottom panel).

CONCLUSIONS

‘Ataulfo’ mango intake did not prevent mammary carcinogenesis nor did it increase AC in plasma of MNU-treated rats. Only papaya extract has a consistent antiproliferative activity on MCF-7 cells. This antineoplastic effect seems to be related with carotenoid content. Antiproliferative effects have not been related with phenolic content and AC. These results suggested that each plant food has a unique combination in quantity and quality of phytochemicals which could determine its biological activity, and papaya represents a very interesting fruit to explore its antineoplastic properties.

Literature Cited


Percival, S.S., Talcott, S.T., Chin, S.T., Mallak, A.C., Lound-Singleton, A. and Pettit-


### Tables

Table 1. Effect of mango intake on mammary tumorigenesis after MNU administration.

LTI: Long-term Intake; STI: Short-term Intake. †Results are expressed as the mean ± SD.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats with tumor</th>
<th>Tumor incidence (%)</th>
<th>Latency of tumors (weeks)†</th>
<th>Tumors per rat†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9/12</td>
<td>75.0</td>
<td>16.8 ± 3.2</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>STI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mango-1</td>
<td>9/12</td>
<td>75.0</td>
<td>16.9 ± 3.8</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>Mango-2</td>
<td>9/12</td>
<td>75.0</td>
<td>14.2 ± 3.8</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>Mango-3</td>
<td>8/12</td>
<td>66.7</td>
<td>15.4 ± 4.1</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>LTI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mango-1</td>
<td>7/12</td>
<td>58.3</td>
<td>18.4 ± 3.5</td>
<td>1.9 ± 0.7</td>
</tr>
<tr>
<td>Mango-2</td>
<td>6/12</td>
<td>50.0</td>
<td>17.5 ± 3.6</td>
<td>1.5 ± 0.8</td>
</tr>
<tr>
<td>Mango-3</td>
<td>8/12</td>
<td>66.7</td>
<td>14.6 ± 5.1</td>
<td>1.8 ± 1.2</td>
</tr>
</tbody>
</table>
Table 2. Antioxidant capacity in plasma of rats with long-term intake of mango. AAE: Ascorbic acid equivalents; FRAP: Ferric reducing/antioxidant ability of plasma; TOSC: Total oxyradical scavenging capacity. Results are expressed as the mean ± SD (Range).

<table>
<thead>
<tr>
<th>Group</th>
<th>TOSC value (µmol of AAE /L)</th>
<th>FRAP value (µmol of AAE /L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=4)</td>
<td>405.8 ± 45.1 (346-455)</td>
<td>53.7 ± 20.7 (38-84)</td>
</tr>
<tr>
<td>Mango-1 (n=4)</td>
<td>366.1 ± 37.7 (323-636)</td>
<td>102.2 ± 73.8 (57-210)</td>
</tr>
<tr>
<td>Mango-2 (n=4)</td>
<td>430.3 ± 50.0 (370-478)</td>
<td>119.9 ± 68.9 (29-190)</td>
</tr>
<tr>
<td>Mango-3 (n=4)</td>
<td>443.6 ± 139.7 (325-415)</td>
<td>168.6 ± 76.0 (57-223)</td>
</tr>
<tr>
<td>MNU-treated rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>421.8 ± 94.8 (306-530)</td>
<td>97.1 ± 126.0 (21-229)</td>
</tr>
<tr>
<td>Mango-3 (n=6)</td>
<td>468.2 ± 55.2 (406-529)</td>
<td>106.0 ± 104.4 (16-245)</td>
</tr>
</tbody>
</table>

Figures

Fig. 1. Total phenolics and gallic acid in F&V aqueous extracts. Nopal: prickly pear cactus stems; PP: prickly pear fruit. Data are expressed as mean ± SD. The dashed lines represent the mean of all values of each graph.
Fig. 2. Total antioxidant capacity in F&V aqueous extracts. Nopal: prickly pear cactus stems; PP: prickly pear fruit. Data are expressed as mean ± SD. The dashed lines represent the mean of all values of each graph.
Fig. 3. Effect of mango and papaya extracts on proliferation of MCF-7 cells. Cells were incubated during 24, 48 and 72 h with different doses of mango and papaya extracts. Control= 0.00. Data represent mean ± SD, n = 6. Differences between experimental groups were analyzed using a one-way ANOVA and Tukey Test. (*) Asterisk indicates significant difference with respect to the control (p<0.05 %).