Toxicologic Response in Mice Fed Cucurbita Fruit

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ABSTRACT

Reports from Australia of illness in consumers eating bitter squash prompted a 10-wk mouse-feeding study containing increased levels of the fruit of two cultivars of Cucurbita pepo, L., 'Blackjack' and 'Straightneck', and an accession of the bitter species, Cucurbita texana, Gray, was conducted. The latter produced poor growth, severe diarrhea, anemia and 40% mortality in mice fed diets containing 1% freeze-dried fruit. Diets containing 10 or 20% C. texana caused 100% mortality within a few days. The cultivar contained 3.56 and 3.79 mg per g of fresh fruit cucurbitacins E glycoside and I, respectively. The cultivars of C. pepo, 'Blackjack' and 'Straightneck', contained no detectable cucurbitacins; animals fed up to 20% freeze-dried squash in their diets showed no toxicity, with normal growth and hematology.

The presence of purgative bitter principals in various Cucurbitaceae has been known since ancient times. These compounds, isolated and identified as a number of tetracyclic triterpenes, are known as cucurbitacins (Cts) (4-5). Some of the seventeen Cts that have been isolated are reported to possess antineoplastic, purgative, emetic and narcotic properties (8). The very high toxicity of these compounds has resulted in a present general abandonment for medical uses (10). Acute oral toxicity (LD50) in mice of these various Cts range between 5 to 650 mg/kg body weight (9).

The present study was concerned with the acceptability and response of mice fed diets containing various levels of two cultivars of C. pepo compared to mice fed a bitter species (Cucurbita texana, Gray) with known amounts of Cts.

MATERIALS AND METHODS

Weanling, male, Swiss-Webster mice were fed one of the following dietary treatments: (a) modified AIN-76 purified, basal (control) (2), (b) 1% 'Straightneck' squash (SN), (c) 1% 'Blackjack' squash (BJ), (d) 1% C. texana (Tex), (e) 10% SN, (f) 10% BJ, (g) 10% Tex, (h) 20% SN, (i) 20% BJ and (j) 20% Tex. The squash was added as dried material replacing sucrose in the basal diet. They were grown and harvested at the New York State Agricultural Experiment Station, Geneva, NY, and then peeled, deseeded, freeze-dried and ground to a fine powder. Eight mice were fed each of the ten diets for 10 wk ad libitum individually in wire mesh cages in a room set at 23°C with a 12-h light/dark cycle. Water was also supplied ad libitum. Animals were weighed, and their mean estimated food intake recorded weekly.

Analysis of Cts in the three cucurbita fruits was done by HPLC (7) after a chloroform extraction and TLC development on Baker Si 500 F plates with methanol:water (45:55, vol/vol). After drying, the Cts were located by UV light fluorescence, scraped off and filtered through a 0.45-μm 9-mm diam. cellulose acetate membrane filter via centrifugation. The Cts were determined by a Waters UV detector at 257 nm after separation on a Waters C-18 Radial-Pak column with methanol:water (70:30, vol/vol) eluted at 1.0 ml per min. Standards were prepared by classical isolation techniques for qualitative and quantitative comparisons (10).

At the end of 10 wk, mice were anesthetized with CO2 and blood was obtained by heart puncture in a syringe rinsed with 0.1% heparin. Blood was analyzed for hemoglobin (Sigma Chemical Co., St. Louis) and red and white blood cell counts (12).

RESULTS

All of the mice died between 3 to 6 d when fed either the 10 or 20% Tex diets. About 40% of the mice fed 1% Tex died during the 10-wk study (Table 1). The survivors showed severely depressed weight gains together with severe diarrhea as compared to the basal or other squash fed groups. Significantly depressed levels of hemoglobin, hematocrit and red blood cell counts also occurred in the mice fed 1% Tex (Table 1). Normal growth, food intake and hematology were observed in mice fed all levels of the BJ and SN summer squash.

1Department of Food Science and Technology.
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sent in the SN or BJ cultivars, but 3.56 and 1.39 mg/g dC.

Blackjack.

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Significantly lower (P<0.05) values than all other groups. All values are means ± SEM.

mg/kg in mice. Ct I has a mouse oral LD 50 of 5 mg/kg and a high laxative effect at a dose level of 5

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80% lethality in 10 wk (9). Calculation of the lethal dietary concentration of the Cts in mice fed the 1% Tex diet (~40% lethality in 10

wk) was ca. 50 ppm. No Cts were observed in SN and 

10% BJ

1% BJ

1% SN

10% SN

10% Tex

All died within first week.

20% SN

34.6±1.5 436±17 16.2±0.1 49±1 8.9±0.2 6.4±1.0

20% BJ

40.4±2.4 440±22 16.7±0.5 48±1 9.2±0.3 6.0±1.0

20% Tex

All died within first week.

aPacked cell volume (Hematocrit).

bStraightneck.

cBlackjack.

dC. texana. 5 of 8 mice survived 10 wk but developed severe diarrhea.

eSignificantly lower (P<0.05) values than groups with RBC values of 9.3 and higher.

Analysis of the squash indicated no detectable Cts present in the SN or BJ cultivars, but 3.56 and 1.39 mg/g fresh weight of Cts E glycoside and I, respectively, in Tex. This species also contained trace amounts of D, B, and E Cts.

DISCUSSION

The high toxicity, severe diarrhea and anemia in mice fed Tex were undoubtedly due to the relatively high levels of Cts present. Ct E glycoside has an LD₅₀ of 40 mg/kg and a high laxative effect at a dose level of 5 mg/kg in mice. Ct I has a mouse oral LD₅₀ of 5 mg/kg (9). Calculation of the lethal dietary concentration of the Cts in mice fed the 1% Tex diet (~40% lethality in 10 wk) was ca. 50 ppm. No Cts were observed in SN and BJ. Indeed, mice fed 20% BJ had the highest weight gains (Table 1). Purgative properties of Cucurbetiae, later shown to be due to Cts, have been known for al­

most 80 years (1).

The cultivar BJ, did not have a bitter flavor and did not contain detectable Cts. The report of bitterness and illness associated with this variety of squash (6), as well as Ct E levels of 3.10 mg/g in straightneck squash grown in Alabama (11), is probably due to seed contamination. A small proportion of plants probably contained a gene for high Cts levels in the fruit. This effect could occur as a result of outcrossing in the seed production field. Indeed, only one plant in a home garden in Alabama had bitter fruit (11).

ACKNOWLEDGMENT

We appreciate the excellent animal care and hematology done for this study by Judy L. Anderson.

REFERENCES


TABLE 1. Mean weight gain, food intake and hematology of mice fed three cultivars of cucurbita for 10 wk.

<table>
<thead>
<tr>
<th>Dietary</th>
<th>Weight gain (g)</th>
<th>Food intake (g)</th>
<th>Hemoglobin (g%)</th>
<th>PCV(%)</th>
<th>RBC (×10⁶/mm³)</th>
<th>WBC (×10³/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>35.4±1.2</td>
<td>454±24</td>
<td>17.5±1.3</td>
<td>53±4</td>
<td>10.4±0.9</td>
<td>8.6±1.1</td>
</tr>
<tr>
<td>1% SN</td>
<td>39.0±4.6</td>
<td>415±27</td>
<td>16.4±0.6</td>
<td>47±1</td>
<td>9.4±0.4</td>
<td>7.2±0.5</td>
</tr>
<tr>
<td>1% BJ</td>
<td>39.1±2.0</td>
<td>432±16</td>
<td>15.6±0.5</td>
<td>46±2</td>
<td>9.3±0.4</td>
<td>5.6±1.5</td>
</tr>
<tr>
<td>1% Tex</td>
<td>11.9±1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>303±38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.9±1.0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>38±4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.6±1.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>7.7±0.2</td>
</tr>
<tr>
<td>10% SN</td>
<td>35.1±2.4</td>
<td>463±16</td>
<td>16.1±0.8</td>
<td>48±2</td>
<td>8.9±0.3</td>
<td>4.5±1.1</td>
</tr>
<tr>
<td>10% BJ</td>
<td>35.9±1.4</td>
<td>445±13</td>
<td>16.3±0.4</td>
<td>49±1</td>
<td>10.0±0.6</td>
<td>8.8±1.7</td>
</tr>
<tr>
<td>10% Tex</td>
<td>All died within first week.</td>
<td></td>
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<tr>
<td>20% SN</td>
<td>34.6±1.5</td>
<td>436±17</td>
<td>16.2±0.1</td>
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<td>8.9±0.2</td>
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<tr>
<td>20% BJ</td>
<td>40.4±2.4</td>
<td>440±22</td>
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<td>48±1</td>
<td>9.2±0.3</td>
<td>6.0±1.0</td>
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<sup>a</sup>Packed cell volume (Hematocrit).

<sup>b</sup>Straightneck.

<sup>c</sup>Blackjack.

<sup>d</sup>C. texana. 5 of 8 mice survived 10 wk but developed severe diarrhea.

<sup>e</sup>Significantly lower (P<0.05) values than groups with RBC values of 9.3 and higher.

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