Toxicological investigations on Toano, Wasatch, and stinking milkvetches

M. COBURN WILLIAMS

Abstract

Toano milkvetch (Astragalus toanus Jones) synthesizes the $\beta$-D-glucoside of 3-nitro-1-propanol (miserotoxin), a highly toxic aliphatic nitro compound, and also accumulates toxic levels of selenium. The toxicity of Toano milkvetch to 1-week-old chicks was compared with Wasatch milkvetch [Astragalus miser var. oblongifolius (Rydib.) Cronq.], which contains only miserotoxin but does not accumulate selenium; stinking milkvetch (Astragalus praelongus Sheld.), which accumulates selenium but does not contain miserotoxin; and a combination of Wasatch milkvetch and stinking milkvetch. The LD$_{90}$ for chicks fed Toano milkvetch was 67.8 mg NO$_2$/kg plus 2.7 mg Se/kg of body weight. The LD$_{90}$ for Wasatch milkvetch was 105 mg NO$_2$/kg and for stinking milkvetch 5.9 mg Se/kg. The LD$_{90}$ of a combination of Wasatch milkvetch and stinking milkvetch was 66.1 mg NO$_2$/kg and 2.7 mg Se/kg. When miserotoxin and selenium were fed together, either in Toano milkvetch or the Wasatch-stinking milkvetch combination, the LD$_{90}$ for each compound was significantly lower than when they were fed separately. If seleniferous and nitro-bearing species grow sympatrically, livestock might be poisoned at lower concentrations of the individual toxic compounds if they grazed both species.

Key Words: Astragalus toanus, Astragalus miser var. oblongifolius, Astragalus praelongus, selenium, nitro compound, miserotoxin

Toxic compounds in the genus Astragalus include aliphatic nitro compounds (Williams and Barney 1977), selenium (Rosenfeld and Beath 1964), and swainsonine (Molyneux and James 1982). The latter compound is an indolizidine alkaloid that causes locoism. Generally these poisonous compounds do not occur in the same species of Astragalus. Although swainsonine has been detected in nitro-containing and selenium-accumulating species, the species in which the concentration of swainsonine is high enough to cause the loco syndrome neither contain nitro compounds nor accumulate selenium.

Toano milkvetch (Astragalus toanus Jones) is the only species in the genus known to absorb toxic levels of selenium (Barney 1964) and to synthesize aliphatic nitro compounds (Williams and Barney 1977). The nitro compound was subsequently identified as miserotoxin, the $\beta$-D-glucoside of 3-nitro-1-propanol (Stemitz and Yost 1978). Miserotoxin hydrolyzes to 3-nitro-1-propanol (3-NPOH) in the rumens of cattle and sheep (Williams et al. 1970).

This nitro compound is rapidly absorbed into the circulatory system and subsequently converted to 3-nitropropionic acid (3-NPA) (Murier et al. 1984, Pass et al. 1984). The effects of 3-NPA are two-fold: the nitrite complexes with ferrous hemoglobin to produce methemoglobin, and the 3-NPA affects the brain, central nervous system, and vital organs (James et al. 1980). Selenium adversely affects most vital organs.

Toano milkvetch is a member of the Astragalus section Pectinati (Barney 1964). The plant is a robust, sparsely leafy perennial adapted to desert and semidesert conditions. Toano milkvetch is found in western Box Elder County, Utah, westward to the upper reaches of the Humboldt River in Nevada and along the Snake River in western Idaho. The showy pink-purple flowers appear in early May to early June. Toano milkvetch is not known to cause livestock losses, probably because it is not abundant, and it completes its life cycle and becomes dormant quickly.

The presence of dual poisons in plants has been noted in galenia (Galenia pubescens [Eckl. and Zeyh.] Druce) (Williams 1979), red spinach (Triatoma iriqueira Rottb. ex Willd.) (Everist 1981), and kikuyugrass (Pennisetum clandestinum Hochst. ex Chiov.) (Everist 1981), all of which may accumulate toxic levels of soluble oxalates and nitrates if ample water and nitrogen are available.

Toano milkvetch is of interest because it can be used to compare the synergistic effects of 2 poisons, both within the same plant, with other poisonous milkvetches that contain either miserotoxin or selenium but not both. The study compares the toxicity to chicks of Toano milkvetch (selenium and miserotoxin) with Wasatch milkvetch [Astragalus miser var. oblongifolius (Rydib.) Cronq.] (miserotoxin), and stinking milkvetch (Astragalus praelongus Sheld.) (selenium), and a combination of Wasatch milkvetch and stinking milkvetch.

Materials and Methods

Toano milkvetch was collected 4 May 1987 (full flower) and 12 May 1987 (late flower and pod), along Utah Highway 30, 10 miles east of the Nevada-Utah line. Wasatch milkvetch was collected during vegetative growth in the mountains near Logan, Utah, and vegetative stinking milkvetch was collected in the Henry Mountains of southern Utah. Wasatch and Toano milkvetch were oven-dried at 50°C for 24 hr. Stinking milkvetch was air-dried. All material was ground to pass a 40-mesh screen and stored in sealed containers until used.

The 3 milkvetches were analyzed for aliphatic nitro compounds using the method described by Williams and Norris (1969). Selenium was analyzed fluorometrically (Olson 1969) by H.F. Mayland, USDA-ARS, Kimberly, Idaho.

One-week-old Leghorn cockerels (50 ± 4 g) were used to bioassay the test material for toxicity. The chicks were removed from food and water overnight (15 hr) to empty the crop. Twenty chicks were used per dose and the experiment was repeated once. The plant material was encapsulated in No. 4 gelatin capsules and administered by hand each morning.

Wasatch milkvetch was fed at 114.0, 106.5, 99.6, and 93.1 mg NO$_2$/kg. Toano milkvetch was fed at 79.4, 71.1, 63.9, and 57.5 mg NO$_2$/kg. Since Toano milkvetch contained 350 ppm selenium, the above dosage also contained 3.2, 2.8, 2.6, and 2.3 mg Se/kg. Stinking milkvetch was fed at 6.6, 6.1, 5.6, and 5.3 mg Se/kg. Wasatch milkvetch was fed at 77.9, 68.8, 60.7, and 53.6 mg NO$_2$/kg plus stinking milkvetch at 3.1, 2.8, 2.4, and 2.1 mg Se/kg, or the same amount of selenium found in Toano milkvetch with the equivalent concentration of NO$_2$. Chicks were dosed between 8 and 9 a.m. on 2 consecutive days, observed for toxic signs, and the LD$_{90}$ was determined 24 hr after the second dosage.

Author is plant physiologist, Poisonous Plant Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Logan, Utah 84321, in cooperation with the Utah Agricultural Experiment Station, Logan.

Utah Agricultural Experiment Station Journal Contribution No. 3529.

Manuscript accepted 28 February 1989.
Dosages within the range of $L_{D0}$ and $L_{D100}$ were determined from preliminary experiments. Geometrical progression for doses within these parameters were determined in accordance with the method of Weil (1952) for determining $L_{D0}$. $L_{D0}$ were determined by probit transformation with a confidence level of 95%.

### Results and Discussion

Miserotoxin analyses (expressed as mg NO$_2$/g of plant) for the 3 milkvetches were: Wasatch milkvetch, 11.6 mg/g; Toano milkvetch, 8.8 mg/g; and stinking milkvetch, 0.0 mg/g. Toano milkvetches were: Wasatch milkvetch, 11.6 mg/g; Toano milkvetch, 8.8 mg/g; and stinking milkvetch, 6.6 mg/g. Toano milkvetch contained 1,040 ppm selenium. (Table 1). The mortality among chicks fed Toano milkvetch was 100% at 77.9 mg NO$_2$/kg but no deaths occurred at the lowest dose. Stinking milkvetch contained 1,040 ppm selenium.

All chicks died when dosed with Wasatch milkvetch at 114 mg NO$_2$/kg, but there were no fatalities in the group fed at 93.1 mg/ kg. The LD$_{50}$ for Toano milkvetch was 67.8 mg NO$_2$/kg and 2.7 mg Se/kg. When Wasatch milkvetch and stinking milkvetch were fed together, the LD$_{50}$ were 66.1 mg NO$_2$/kg and 2.7 mg Se/kg. When miserotoxin and selenium were fed in Toano milkvetch, or in the Wasatch and Toano milkvetch combination, twice as much selenium from stinking milkvetch was required to produce 10% mortality. The LD$_{50}$ for Wasatch milkvetch was 105.0 mg NO$_2$/kg and the LD$_{50}$ for stinking milkvetch 5.9 mg Se/kg (Table 1). The LD$_{50}$ for Toano milkvetch was 67.8 mg NO$_2$/kg and 2.7 mg Se/kg. When Wasatch milkvetch and stinking milkvetch were fed together, the LD$_{50}$ were 66.1 mg NO$_2$/kg and 2.7 mg Se/kg. When miserotoxin and selenium were fed in Toano milkvetch, or from a combination of Wasatch and stinking milkvetches, the LD$_{50}$ for mg NO$_2$/kg and mg Se/kg were not significantly different. The LD$_{50}$ for both mg NO$_2$/kg and mg Se/kg in Toano milkvetch and the Wasatch-stinking milkvetch combination was significantly different when compared with the LD$_{50}$ of NO$_2$/kg in Wasatch milkvetch and selenium in stinking milkvetch fed alone.

Chicks affected by miserotoxin or a combination of miserotoxin and selenium became depressed and stood with heads lowered and feathers ruffled. They frequently lost their balance and fell on their sides. In fatal cases the birds became paralyzed, comatose, and died. Chicks fed only selenium became depressed, stood with head lowered and ruffled feathers, but did not exhibit the loss of muscular coordination noted in chicks that received miserotoxin. In fatal cases, these chicks gradually became weaker, then comatose before death.

These data indicated that significantly larger amounts of selenium and miserotoxin must occur in separate plants to equal the toxicity of both compounds in one plant. If seleniferous and nitro-bearing species grew sympatrically, animals might be poisoned at lower concentrations of the individual compounds if they grazed both species.

The effects of dual poisoning in chicks by selenium and miserotoxin cannot be used to predict responses in domestic livestock that might consume the same compounds. Livestock are generally poisoned at lower doses (mg/kg of body weight) of these poisons than are chicks. The minimum lethal doses of selenium (administered orally as selenite) are 3.3 mg/kg of the body weight for horses, 3 mg for cattle, and 15 mg for swine (Rosenfeld and Beath 1964). A steer and a sheep died after they were gavaged with miserotoxin-containing plants at 25 and 37.5 mg NO$_2$/kg, respectively (Williams and James 1975, Williams and James 1978).

The additive or synergistic effect of two or more compounds involving selenium has practical application not only on the range but also where selenium, nitrates, and other compounds accumulate from runoff from seleniferous soils and cropped (thus fertilized) lands. Problems from the accumulation of selenium and other toxic compounds are particularly likely to occur when runoff terminates in basins that have no natural outlets. Deformities in waterfowl and embryos in their eggs have been observed in wildlife refuges fed by water from seleniferous soils and crop lands (Ohlendorf et al. 1986). The ingestion of selenium in combination with other poisonous elements or compounds may cause toxic syndromes in humans, wildlife, and livestock not observed with toxicosis from selenium alone.

### Literature Cited


---

**Table 1. Toxicity to chicks of miserotoxin and selenium from Wasatch, Toano, and stinking milkvetches.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>LD$_{50}$</th>
<th>LD$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg NO$_2$/kg</td>
<td>Mg Se/kg</td>
<td>No. chicks died</td>
</tr>
<tr>
<td>Toano milkvetch</td>
<td>79.4</td>
<td>3.2</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>71.1</td>
<td>2.8</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>63.9</td>
<td>2.6</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>57.5</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td>Wasatch milkvetch +</td>
<td>77.9</td>
<td>3.1</td>
<td>20</td>
</tr>
<tr>
<td>stinking milkvetch</td>
<td>68.8</td>
<td>2.8</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>60.7</td>
<td>2.4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>53.6</td>
<td>2.1</td>
<td>0</td>
</tr>
<tr>
<td>Wasatch milkvetch</td>
<td>114.0</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>106.5</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>99.6</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>93.1</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Stinking milkvetch</td>
<td>-</td>
<td>6.6</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>6.1</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>5.6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>5.3</td>
<td>0</td>
</tr>
</tbody>
</table>

*Twenty chicks were used per dose. Mean of 2 experiments. Number of deaths 24 hr after second feeding.*


Weil, C.S. 1952. Tables for convenient calculation of median-effective dose (LD$_{50}$ or ED$_{50}$) and instructions in their use. Biometrics 8:249-263.


