Toxicological investigations on Toano, Wasatch, and stinking milkvetches

M. COBURN WILLIAMS

Abstract

Toano milkvetch (*Astragalus toanus* Jones) synthesizes the β-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* (Rydb.) 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_{50} for chicks fed Toano milkvetch was 67.8 mg NO_2/kg plus 2.7 mg Se/kg of body weight. The LD_{50} for Wasatch milkvetch was 105 mg NO_2/kg and for stinking milkvetch 5.9 mg Se/kg. The LD_{50}s of a combination of Wasatch milkvetch and stinking milkvetch were 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_{50} 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 Barneby 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 (Barneby 1964) and to synthesize aliphatic nitro compounds (Williams and Barneby 1977). The nitro compound was subsequently identified as miserotoxin, the β-D-glucoside of 3-nitro-1-propanol (Stermitz 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) (Muir 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 (Barneby 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 (*Trianthema triquetra* 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* (Rydb.) 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_{50} 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.
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Table 1. Toxicity to chicks of miserotoxin and selenium from Wasatch, Toano, and stinking milkvetches.

Species	Dose Mg NO_2/kg	Dose Mg Se/kg	No. chicks died[1]	LD_{50} Mg NO_2/kg	LD_{50} Mg Se/kg	LD_{50} 95% C.I. (mg/kg)
Toano milkvetch	79.4	3.2	20	67.8	2.7	NO_2 = 66.22 – 69.34
	71.1	2.8	17			Se = 2.70 – 2.72
	63.9	2.6	2			
	57.5	2.3	0			
Wasatch milkvetch + stinking milkvetch	77.9	3.1	20	66.1	2.7	NO_2 = 64.86 – 67.30
	68.8	2.8	14			Se = 2.58 – 2.72
	60.7	2.4	2			
	53.6	2.1	0			
Wasatch milkvetch	114.0	—	20	105.0	—	NO_2 = 103.28 – 106.66
	106.5	—	12			
	99.6	—	2			
	93.1	—	0			
Stinking milkvetch	—	6.6	20	—	5.9	Se = 5.77 – 5.98
	—	6.1	15			
	—	5.6	3			
	—	5.3	0			

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

Dosages within the range of LD_0 and LD_{100} were determined from preliminary experiments. Geometrical progression for doses within these parameters were determined in accordance with the method of Weil (1952) for determining LD_{50}s. LD_{50}s 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 milkvetch contained 350 ppm selenium when collected 4 May and 150 ppm selenium on 12 May. Only the 4 May material was used. 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 (Table 1). The mortality among chicks fed Toano milkvetch was 100% at 79.4 mg NO_2/kg but no deaths occurred at the lowest dose. The toxicity of a combination of Wasatch and stinking milkvetch was similar to Toano milkvetch with 100% mortality at 77.9 mg NO_2/kg and 3.1 mg Se/kg. Compared to the selenium in Toano milkvetch, or in the Wasatch and Toano milkvetch combination, twice as much selenium from stinking milkvetch was required to produce 100% mortality.

The LD_{50} for chicks fed 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}s 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}s 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}s 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 fequently 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.

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