Toxicity of Nitro-containing *Astragalus* to Sheep and Chicks

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Highlight: *Several species of* Astragalus *that contain organic nitro compounds were tested for toxicity to sheep and 1-week-old chicks. Methemoglobin analyses in sheep indicated that nitro compounds in* A. diversifolius, A. convallarius, *and* A. pterocarpus *resembled 3-nitro-1-propanol in toxicity and rate of absorption from the digestive tract. Nitro compounds in* A. cibarius *and* A. canadensis *were more closely related to 3-nitropropanoic acid in toxicity and rate of absorption.* A. pterocarpus, A. convallarius, *and* A. diversifolius *have been categorized as "Class I" species because they produce acute oral toxicity in sheep at less than 100 mg NO_2/kg of body weight. "Class II"* Astragalus *(*A. canadensis *and* A. cibarius*) produce acute toxicity in sheep only if oral dosage exceeds 100 mg NO_2/kg. Class I species are more likely to cause livestock losses on the range.*

Domestic livestock grazing western desert and mountain range are often poisoned by species of the genus *Astragalus.* The poisonous species have been broadly divided into three categories: those that accumulate selenium; those that produce the loco syndrome; and those that cause chronic or acute poisoning, but for which the toxic principle is unknown (Kingsbury, 1964).

Organic nitro compounds are poisonous materials reported in *Astragalus* (Williams and Parker, 1974) that fall within the last category.

Inorganic nitrites are rarely found in higher plants, because the absorbed nitrates are rapidly metabolized to ammonia and then incorporated into amino acids, proteins, and other nitrogenous compounds. Nearly all nitrite poisoning in domestic animals results from reduction of nitrate to nitrite in the digestive tract and not from ingestion of nitrite *per se.* Nitrate is reduced often in ruminants and less often in monogastric animals.

A few plant families, particularly the Leguminosae, synthesize aliphatic nitro compounds. Norfeldt and Younge (1949) reported that dairy heifers were poisoned by eating creeping indigo (*Indigofera endecaphylla* Jacq.), a legume that was being studied in pasture trials in Hawaii. Cooke (1955) identified the toxic principle as 3-nitropropanoic acid (3-NPA).

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Thirty-nine cattle died overnight after grazing Wasatch milkvetch (*Astragalus miser* var. *oblongifolius* (Rydb.) Cron.), on a mountain meadow near Logan, Utah. Workers at Logan identified the poison as the β-glucoside of 3-nitro-1-propanol, which they named miserotoxin (Stermitz et al., 1969). The compound is rapidly converted to 3-nitro-1-propanol (3-NPOH) in the rumen (Williams et al., 1970). The poison is rapidly absorbed from the rumen and results in loss of motor control and automatic responses. Two other varieties of timber milkvetch also synthesize miserotoxin. Yellowstone milkvetch (*A. miser* var. *hylophilus* (Rydb.) Barneby) and Columbia milkvetch (*A. miser* var. *serotinus* (Gray) Barneby) were found to be as toxic as Wasatch milkvetch (Williams and Norris, 1969). No losses from Yellowstone milkvetch have been reported, but Columbia milkvetch is a troublesome poisonous plant of British Columbia.

Stermitz et al. (1972) qualitatively examined 115 species and varieties of *Astragalus* in the Colorado State University herbarium for aliphatic nitro compounds. Fifteen were positive. Williams and Parker (1974) conducted a similar study on *Astragalus* at the Intermountain Herbarium at Utah State University. They examined 251 species of *Astragalus,* representing about two-thirds of the 368 North American species. Fifty-six (22%) were positive for organic nitro compounds. Two positive species, *A. emoryanus* (Rydb.) Cory and *A. tetrapterus* Gray, were known to cause livestock losses. An introduced species, *A. falcatus* Lam., was also found high in nitro compounds. These findings indicated that such compounds were widespread in *Astragalus* and might occur in 20% or more of the species. Many times, the organic nitro compounds might be the poisonous principle or one of the poisonous principles in those species known to be poisonous, but in which the toxic agent has not been identified.

These *Astragalus* species were collected during 1973 for biochemical analyses and feeding trials: *A. tetrapterus; A. pterocarpus* Wats.; *A. canadensis* var. *brevidens* (Gand.) Barneby; *A. convallarius* Greene; *A. diversifolius* Gray; *A. cibarius* Sheld.; *A. toanus* Jones; and *A. falcatus. A. emoryanus* was collected in 1972 in New Mexico.

All species synthesize organic nitro compounds (Williams and Parker, 1974). *A. tetrapterus* was listed by Marsh and Clawson (1920) as a poisonous plant of southwestern Utah and eastern Nevada, but the toxic principle was unknown. *A. pterocarpus,* found principally at the confluence of the Reese and Humboldt Rivers in Nevada, is closely related to *A. tetrapterus* both genetically and morphologically. They comprise two of three species in the section Pterocarpi (Barneby, 1964). Although not previously reported as a

Table 1. Toxicity of *Astragalus* to 1-week-old chicks.

Species	Mg NO_2/g of plant	Mg NO_2/kg of body weight[1]		Number of grams of plant required for	
		Toxic signs	LD_{50}[2]	Toxic signs	LD_{50}
A. diversifolius	7	109	218	1	2
A. pterocarpus	11	169	339	1	2
A. tetrapterus	12	185	370	1	2
A. convallarius	12	185	370	1	2
A. canadensis	60	924	1,848	1	2
A. cibarius	14	647	1,077	3	5
A. toanus	7	540	755	5	7
A. falcatus	14	862	1,294	4	6
A. emoryanus	8	493	862[3]	4	7[3]

[1] Chick weight averaged 65 g.
[2] LD_{50} = dose required to kill 50% of the test chicks.
[3] Lethal dose for *A. emoryanus* not established, but greater than 862 mg NO_2/kg.

poisonous plant, genetic similarity suggested that *A. pterocarpus* would most likely produce the same type of poisoning caused by *A. tetrapterus.*

A. convallarius and *A. diversifolius,* together with *A. miser,* comprise the three species of section Genistoidei. *A. miser,* already extensively studied, contained 3-NPOH. Genetic relationships suggested that *A. convallarius* and *A. diversifolius* might be equally as toxic, although neither was known to be poisonous.

A. canadensis was sent to our laboratory from Oregon. Ranchers reported that the plant fatally poisoned cattle, and that range on which it grew abundantly could not be safely used until the plant disappeared. Peavine is a well-known poisonous plant of the Rio Grande valley of New Mexico and Texas (Mathews, 1940; Sperry et al., 1964). *A. falcatus* is an introduced species currently being evaluated for agronomic properties at several state experiment stations. *A. cibarius,* a common vetch of foothills in Utah and Idaho, contains 3-NPA glucosides (Stermitz et al., 1972). *A. toanus* was the only selenium accumulator found to synthesize organic nitro compounds.

These studies were begun to determine the aliphatic nitro content in nine species of *Astragalus* and to study the toxicology of all species on 1-week-old chicks and of five species on sheep.

Methods

The plants were collected in flower or early pod, dried at 60°C in a forced-air drier, ground to 40 and 20 mesh for chemical analysis and feeding, respectively, and stored in sealed containers.

Plant samples were analyzed for organic nitro compounds by the method of Cooke (1955) as modified by Williams and Norris (1969). All calculations are reported as nitrite, as computed from a standard curve for 3-NPA.

Chick Bioassay

All species were extracted on a Soxhlet extractor with ethanol as previously described (Williams and Norris, 1969). The final aqueous solution was concentrated, so that 1 ml of extract was equal to 1 g of plant. The chicks, 1-week-old and weighing an average of 65 g, were taken off feed and water the previous night so that the crop would be void of food and liquid. The crop at this age holds about 7 ml of liquid. Extracts were administered via a rubber catheter into the crop. Dosages, administered in duplicate, were progressively increased by 1-ml increments until the mean lethal dose (LD_{50}) was achieved. The chick bioassay was used to determine the relative toxicity and thereby indicate probable doses of plant required to kill cattle and sheep.

Sheep Investigations

A. diversifolius, A. convallarius, A. pterocarpus, A. canadensis, and *A. cibarius* were fed to sheep. We also orally administered 3-NPA, 3-NPOH, and sodium nitrite. One sheep was used per treatment. *A. emoryanus, A. toanus, A. falcatus,* and *A. tetrapterus* were not fed, because of not enough material. All doses, administered orally via a stomach pump as an aqueous slurry (plants) or in water (3-NPOH, 3-NPA, and sodium nitrite), were calculated as mg NO_2/kg of body weight. In species found highly poisonous to chicks, dosages began at 25 mg NO_2/kg and were raised in 12.5-mg NO_2/kg increments, until a fatal dose was reached. Dosages of the less toxic *A. canadensis* and *A. cibarius* were begun at 50 mg NO_2/kg and raised in 50-mg NO_2/kg increments, until a fatal dose was reached. The fatal dose was then reduced by 25 mg NO_2/kg and fed to one sheep to determine whether half or all of the final 50-mg increment was required for acute poisoning. Sodium nitrite, 3-NPA, and 3-NPOH were fed first at 50, 50, and 25 mg NO_2/kg, respectively, with dosages progressively increased in 25, 25 and 12.5 mg NO_2/kg increments, until a fatal dose was reached. Sheep fed more than one level of nitro compounds were rested and given normal rations for 1 week between treatments. Sheep were dosed between 8 and 9 am. Blood samples from the jugular vein were drawn hourly for 5 hours for methemoglobin determinations.

Results and Discussion

Chick Bioassay

Extracts of *A. pterocarpus, A. convallarius, A. diversifolius, A. canadensis,* and *A. tetrapterus* were highly toxic to 1-week-old chicks per gram of plant fed (Table 1). *A. cibarius, A. toanus, A. emoryanus,* and *A. falcatus* required three to five times more extract than the highly toxic species to produce toxic signs and two to three times more extract for LD_{50}. *A. canadensis,* despite its high nitro content (Table 1), was not more toxic per gram of plant than the four highly toxic plants that were relatively low in nitro compounds. *A. canadensis* appeared to contain a less toxic form of nitro compound, more closely related to that found in *A. cibarius* and other less toxic species. The potency of *A. canadensis* was caused by its very high nitro compound concentration. The nitro compounds in *A. pterocarpus, A. tetrapterus, A. diversifolius,* and *A. convallarius* were more toxic to chicks per mg of NO_2/kg of body weight than nitro compounds in *A. cibarius, A. canadensis, A. falcatus, A. emoryanus,* and *A. toanus.*

The toxic signs observed in chicks after the test species

Table 2. Toxicity of 3-nitro-1-propanol (3-NPOH), 3-nitropropanoic acid (3-NPA), sodium nitrite, and five *Astragalus* species to sheep.

Treatment	Mg NO_2/g of plant	Mg NO_2/kg of body weight	
		Nonlethal dose	Lethal dose
Sodium nitrite	–	100.0	125.0
3-NPA	–	100.0	125.0
3-NPOH	–	37.5	50.0
A. pterocarpus	7.8	25.0	37.5
A. convallarius	6.2	50.0	62.5
A. diversifolius	6.4	50.0	62.5
A. cibarius	16.3	175.0	200.0
A. canadensis	36.0	250.0	275.0

were fed were identical to those caused by Wasatch milkvetch (Williams et al., 1969). The birds became depressed and stood with eyes closed and heads lowered. Motor control was affected, causing the chicks to be unsteady and often fall backwards or sideways. Collapse and paralysis preceded death.

Sheep Investigations

The toxicity of species fed to sheep corresponded to that found for chicks (Table 2). *A. pterocarpus, A. convallarius,* and *A. diversifolius* were highly toxic per milligram of nitrite. *A. canadensis,* because of its high nitro compound content, was highly toxic per gram of plant. *A. canadensis* contained 5.6 times more NO_2/g of plant than *A. diversifolius,* but was 4.4 times less toxic. Therefore, fewer grams of *A. canadensis* were fed per kilogram of body weight to produce acute poisoning.

By these sharp differences in toxicity per milligram of nitrite, we have divided the *Astragalus* tested into two groups:

1. Class I plants: those highly toxic per milligram of contained NO_2. These plants (*A. pterocarpus, A. convallarius,* and *A. diversifolius*) may cause acute poisoning in sheep when fed at 99 mg NO_2/kg of body weight or below.

2. Class II plants: those low in toxicity per milligram of contained NO_2. These plants (*A. canadensis* and *A. cibarius*) cause acute poisoning in sheep only if fed at 100 mg NO_2/kg of body weight or above. Classification of *A. toanus, A. tetrapterus, A. emoryanus,* and *A. falcatus* requires further study. However, the data (Table 1) suggests that *A. tetrapterus* and *A. falcatus* would be Class I and Class II species respectively. In the plants studied, the breaking point between Class I and II plants for sheep was 62.5 mg NO_2 and 200.0 mg NO_2. Cattle appear to be more susceptible to nitro compounds; therefore, levels of these materials required for acute poisoning may be proportionately lower than levels listed for sheep.

One major reason for the difference in toxicity between Class I and Class II plants was the rate of absorption of the nitro compound from the digestive tract, as determined by the formation of blood methemoglobin. As nitro compounds enter the circulatory system, they complex with ferrous hemoglobin to form methemoglobin. Thus, the rate of absorption and the relative concentration of the nitro compound in the blood can be determined by assay for percentage of methemoglobin.

The absorption of the nitro compounds from *A. convallarius, A. diversifolius,* and *A. pterocarpus* was very rapid and closely followed the curve for 3-NPOH through the first 3 hours after dosing (Fig. 1). When lethal doses of these species or 3-NPOH were administered, methemoglobin concentration surpassed 20% in about 3 hours. A concentration of 21 to 26% methemoglobin was not a fatal

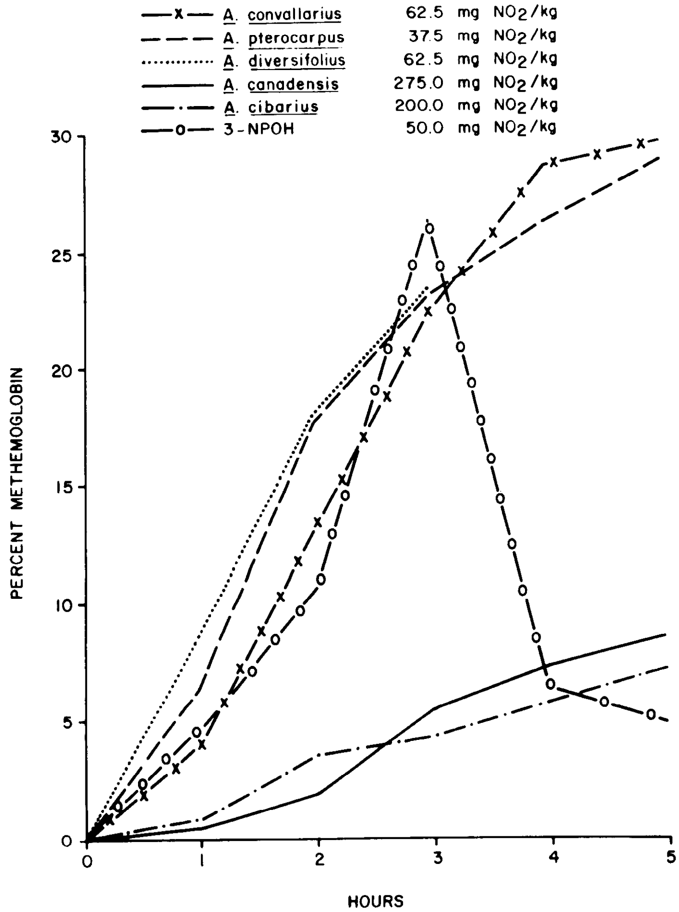

Fig. 1. *Percent methemoglobin in blood of sheep fed on minimum lethal dose (mg NO_2/kg of body weight) of five* Astragalus *species and 3-NPOH. The animal fed* A. diversifolius *died between 3 and 4 hours after treatment.*

level of methemoglobin *per se* but rather indicated that enough of the nitro compound for acute poisoning had entered the circulatory system. The animals usually died 4 to 6 hours after treatment, but no animal survived more than 12 to 14 hours. When *A. diversifolius* and *A. convallarius* were fed to sheep at 50 mg NO_2/kg, methemoglobin reached 21 and 25%, respectively. Although the animals survived, they were severely affected. Thus, the lethal dose for these species was probably closer to 50 than 62.5 mg NO_2/kg.

The methemoglobin curves of *A. diversifolius, A. pterocarpus,* and *A. convallarius* are similar to that of 3-NPOH, the metabolite found in Wasatch milkvetch. Because two of these species are closely related to Wasatch milkvetch, 3-NPOH may be the toxic compound in them, also. Genetic and morphological relationships between *A. pterocarpus* and *A. tetrapterus* indicate that both may contain the same poison, probably 3-NPOH.

The nitro compounds from *A. cibarius* and *A. canadensis* were slowly absorbed, as indicated by methemoglobin concentration (Fig. 1). They were more slowly absorbed than a lower concentration of 3-NPOH. Thus, the nitro compound from these species appears to be both slowly released from the plant to the digestive tract and slowly absorbed into the circulatory system. Therefore, nitro compounds from these species must be ingested in large amounts before intoxication occurs. The animals fed *A. cibarius* and *A. canadensis* died during the night, more than 12 hours after dosing. Because

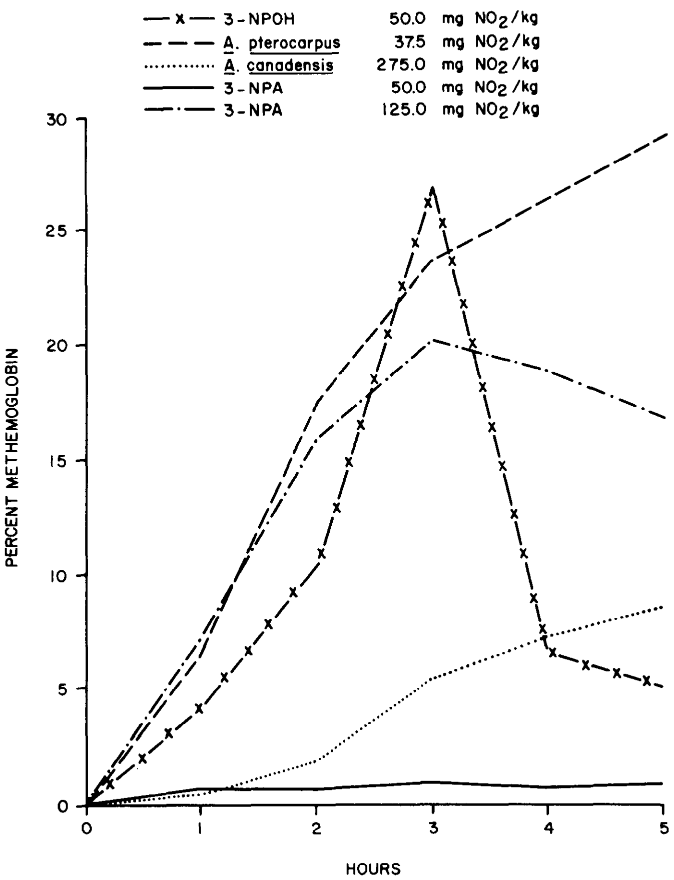

Fig. 2. *Percent methemoglobin in blood of sheep fed* Astragalus, *3-NPOH, and 3-NPA. All except 3-NPA at 50 mg NO_2/kg are minimum lethal doses.*

blood samples were drawn only for 5 hours, the methemoglobin levels near the time of death were not determined.

The absorption rate of 3-NPA and 3-NPOH differed markedly (Fig. 2). When 3-NPA was fed at 50 mg NO_2/kg, methemoglobin level did not exceed 1.5% in 5 hours, and animals showed no signs of poisoning. A sheep fed 3-NPOH at 50 mg NO_2/kg had 26.7% methemoglobin at 3 hours. Although the methemoglobin level fell rapidly thereafter, the animal died 7 hours after treatment. Sheep were killed only when 3-NPA was fed at 125 mg NO_2/kg, 2½ times the lethal dose of 3-NPOH. Even at this rate, methemoglobin concentration was less than that found with the lower rate of 3-NPOH.

A. cibarius synthesizes 3-NPA (Stermitz et al., 1972). Although the form of organic nitro compound in *A. canadensis* var. *brevidens* is unknown,[1] the quantity required to poison sheep and the slow absorption of the nitro compound suggest that the toxic principle may be 3-NPA.

Methemoglobin level reached 46.8% in a sheep fed 100 mg NO_2/kg as sodium nitrite. The animal survived. The fatal level of sodium nitrite for cattle is estimated at 100 mg NO_2/kg (Garner, 1957). Organic nitro compounds are thus more toxic per milligram of nitrite than inorganic nitrite. Although the lethal dose of 3-NPA and sodium nitrite (as nitrite) to sheep was the same, methemoglobin levels indicate that far less nitrite from 3-NPA was actually absorbed. Therefore, 3-NPA was more toxic than sodium nitrite per milligram of nitrite absorbed into the circulatory system.

Cattle are poisoned more often by nitro-bearing *Astragalus* than sheep, because they tend to be more susceptible to nitro compounds. A steer died 4.5 hours after oral dosing with 3-NPOH at 25 mg NO_2/kg (Williams et al., 1969), whereas sheep required 3-NPOH at 50 mg NO_2/kg.

Although none of the species studied are listed by Kingsbury (1964) as causing the loco syndrome, the term loco is broadly applied to any chronic condition caused by plants, particularly *Astragalus,* in which loss of motor control, muscular incoordination, and staggering are evident. Many *Astragalus* species have the word "loco" as part of their common name. The toxic signs associated with organic nitro poisoning by Wasatch milkvetch (Williams et al., 1969) and other species described here are identical to those described by Marsh and Clawson (1920) for *A. tetrapterus* and by Sperry et al. (1964) for *A. emoryanus.* Chronic poisoning would most likely occur if any of these speecies were consumed in sublethal amounts over several days or weeks.

[1]*A. canadensis* var. *mortonii* (Nutt.) Wats. has been found to contain 3-NPA glucosides. F. R. Stermitz and M. E. Martin, Colorado State University personal communication.

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