A study on embryonic death in goats due to *Nicotiana glauca* ingestion

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ARTICLE INFO

ABSTRACT

Numerous plants are known to be teratogenic in livestock. In addition to causing malformations, several plants can also cause embryonic death. These losses decrease the reproductive efficiency of animals exposed to these plants. The aim of this study was to determine if teratogenic plants such as lupines or tobaccos cause embryonic losses. A goat model using the plant *Nicotiana glauca* was used in this study, as this model has been used to characterize the mechanism of *Lupinus*, *Conium*, and *Nicotiana*-induced terata. Four groups of goats were dosed from gestational day 1–10, 11–20, 21–30, and 31–40. Goats were evaluated via ultrasound imaging for pregnancy after completion of the dosing regimen and kids were evaluated for malformations at the time of parturition. Overall, there was no evidence from this study that *N. glauca* (anabasine) at this dose (2 g/kg/day) would cause embryonic losses in goats. However, the dose of *N. glauca* used in this study was at the lower threshold that would be expected to produce terata. Therefore it is possible that higher doses of anabasine could cause embryonic loss. Further work is also needed to characterize the kinetic profile of anabasine, and other teratogenic alkaloids, in the fetal compartments.

Published by Elsevier Ltd.

1. Introduction

Reproductive success is dependent on a large number of events that must occur in a specifically timed sequence (Hafez, 1993; Hood, 2006). Interference with one or more of these events may result in subtle reduction in reproductive potential, malformed offspring or total reproductive failure (Hood, 2006). Substantial research has demonstrated that numerous poisonous plants contain compounds that are teratogenic to livestock species (Burrows and Tyrl, 2013; Green et al., 2013a, 2013b; Kingsbury, 1964; Panter and Stegelmeier, 2000; Panter et al., 2000, 2011, 2013). In addition to teratogenesis, research and field observations suggest that ingestion of some of the "teratogenic plants" may result in early embryonic loss (Panter et al., 2011).

Previous research has demonstrated that when pregnant ewes grazed *Veratrum californicum* early in gestation, holoprosencephaly and related craniofacial deformities, called "monkey face lamb disease" were produced in lamb fetuses (Binns et al., 1963, 1962, 1965). Further studies demonstrated that maternal *Veratrum* ingestion produced a variety of congenital defects including tracheal stenosis (Keeler et al., 1985) as well as carpal and tarsal shortening (Keeler and Stuart, 1987). Maternal ingestion of *Veratrum* was also shown to induce early embryonic death and resorption (Keeler, 1990). Retrospectively, low reproductive rates in sheep in southeastern Idaho were linked to *V. californicum* induced early embryonic death and resorption (Binns et al., 1963; Keeler, 1990; Van Kampen et al., 1969).

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Locoweeds are plants from the *Oxytropis* and *Astragalus* genera that contain the toxin swainsonine. They have been shown to affect almost every aspect of female reproduction in several livestock species, including estrus behavior, estrous cycle length, ovarian function, conception, embryonic and fetal viability, and maternal/infant bonding (Panter et al., 1987, 1999b; Panter and Stegelmeier, 2000; Pfister et al., 2006a, 2006b). Research suggested that the negative effects locoweed had on early embryo viability and development are not from direct effects of swainsonine on the oocyte or the pre-implantation embryo (Wang et al., 1999), suggesting that another mechanism is responsible for embryonic losses.

Lupines are responsible for a condition in cattle referred to as "crooked calf syndrome" that occurs when pregnant cattle graze lupines containing the quinolizidine alkaloid anagyrine or the piperidine alkaloids ammodendrine, *N*-methyl-ammodendrine or *N*-acetyl hystrine (Panter et al., 1999a). Similar malformations are also seen in animals poisoned by *Conium*, containing the alkaloids coniine and *N*-methylconiine, and *Nicotiana* species, containing the alkaloid anabasine, with a similar mechanism of action (Panter et al., 1999a). A goat model using *N. glauca* has been established to study the mechanism of action of the cleft palate and crooked calf syndrome (Panter et al., 1990a, 1990b). In goats, the teratogenic insult period includes gestation days (GD) 30–60 (Keeler, 1984; Panter et al., 1990a, 1990b) with a narrow period for cleft palate induction that includes GD 35–41 (Panter and Keeler, 1992). The severity and type of the malformations are dependent on the amount of alkaloid(s) in the plants, the stage of pregnancy when the plants are eaten, and the length of time ingestion takes place (Panter et al., 2013). It has been suspected for many years by livestock producers, clinical veterinarians, extension agents, and scientists that early embryonic loss may be associated with lupine ingestion in the first trimester of pregnancy. However, it is not known if lupines, poison hemlock, or tobaccos (these plants induce terata by the same biological mechanism; Panter et al., 1999a) affect the viability of the embryo, causing embryonic losses. Therefore, the objective of this study was to compare the incidence of embryonic death in goats fed *N. glauca* (wild tree tobacco) at varying time points during the embryonic period of gestation.

2. Materials and methods

2.1. Plant

N. glauca was collected in July 2004 near Kingman, Arizona. Green plant including leaves, stems, flowers, and woody parts were collected, chopped, and spread on tarps and allowed to air dry. The dry, coarsely chopped plant material was further ground to pass through a 2.4 mm mesh screen using a Gehl Mix-All model 55 (Gehl Company, West Bend, WI, USA). After processing, the ground plant material was stored in plastic bags away from direct light at ambient temperature in an enclosed shed until use. Percent anabasine was measured just prior to treatment using previously published methods (Keeler et al., 1981).

2.2. Animals

Twenty five female crossbred Spanish-type goats, weighing 46 ± 9 kg, were naturally bred to Spanish-type bucks. Gestation day (GD) 0 was considered to be the day of the last standing heat. The goats were randomly assigned into five groups of five goats each, one control group and four treated groups designated: GD 1–10, GD 11–20, GD 21–30, and GD 31–40. *N. glauca* was administered twice daily (7 am and 3 pm) at 1 g plant/kg BW (5.5 mg anabasine/kg BW) for the 10 day period. The control group was dosed with 1 g alfalfa/kg BW for 10 days from GD 16–25. The dried finely ground plant material was administered via oral gavage in approximately 1 L of tap water. All animals had free access to alfalfa hay and fresh water. Animals were observed throughout each day for symptoms of toxicity. All procedures were conducted under veterinary supervision and were approved by the Utah State University Institutional Animal Care and Use Committee.

2.3. Toxicokinetics

To monitor the daily serum concentration of anabasine, blood was collected via jugular venipuncture daily, immediately prior to and 1 h after the morning dose. Additionally, blood was collected from the goats in the GD 21–30 group at 0, 0.25, 0.50, 0.75, 1.0, 1.5, 2, 4, and 8 h after the first dose and at 0, 0.25, 0.50, 0.75, 1.0, 1.5, 2, 4, 8, 24 and 32 h after the last dose. The serum fraction of the blood samples was collected and stored at $-20\ ^\circ C$ until analysis.

2.4. Serum anabasine analysis

After thawing, the sera were thoroughly mixed and then centrifuged at $1360 \times g$ for 10 min. A 3 mL aliquot of sera was transferred to a clean 10 mL glass test tube. Fifty µL of 85% phosphoric acid was added to each sera sample and mixed well. Using a Supelco Visiprep 24 vacuum manifold (Supelco, Bellefonte, PA USA) the serum samples were each applied to a Strata X–C; 60 mg/3 mL SPE cartridge (Phenomenex, Torrance, CA USA) that had been conditioned with 2 mL MeOH followed by 2 mL deionized H_2O. Three milliliter of Sera was added to the SPE cartridge. Following the addition of the sera the cartridge was washed with 2 mL deionized H_2O and then with 2 mL MeOH. The cartridge was eluted 3 times with 1.5 mL 5% NH_4OH/MeOH solution (4.5 mL total volume) to remove the anabasine from the SPE cartridges. The NH_4OH/MeOH solution was evaporated to dryness under a gentle flow of nitrogen in a heating block at $60\ ^\circ C$. The residue from each sample was dissolved in 1 mL H_2O/methanol (50/50) and transferred to an autosample vial for HPLC-MS/MS analysis.

Analysis of anabasine in the samples was accomplished using a Surveyor HPLC and autosampler system coupled to a ThermoFinnigan LCQ Advantage Max mass spectrometer (Thermo Finnigan, San Jose, CA). Samples (10 µL) were injected with a Surveyor autosampler onto an Aquasil C 18 reversed phase column (100×2.1 mm i.d., 3 µm particle size) (Thermo Electron Corporation, Waltham, MA USA) protected by a guard column of the same adsorbent. Anabasine was eluted from the column with a gradient (0.2 mL/