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White snakeroot poisoning in goats: Variations in toxicity with different plant chemotypes

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ABSTRACT

Tremetone and possibly other benzofuran ketones are believed to be the toxic compounds in white snakeroot. However, disease has not been reproduced with purified toxins and the concentrations of the benzofuran ketones in white snakeroot populations that cause toxicosis have not been documented. The objectives of this study were to compare the toxicity of seven plant populations, better characterize the clinical and pathologic changes of poisoning, and correlate intoxication with benzofuran ketone content. Four of the seven white snakeroot collections were toxic at the dose and duration used in the study. Affected goats became exercise intolerant, had significant serum enzyme changes and histological lesions in the large appendicular muscles. The incidence and severity of poisoning was not correlated with total doses of tremetone or total benzofuran ketone concentrations suggesting they may not be closely involved in producing toxicity and the possible involvement of an unidentified toxin. The results also demonstrate that white snakeroot populations vary chemically and toxicologically.

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1. Introduction

White snakeroot [*Ageratina altissima* var. *altissima* previously known as *Eupatorium rugosum* Houtt. also known as *Ageratina altissima* (L.) *R.M. King* & *H.Rob. or Eupatorium urticaefolium* Reichard] is an herbaceous perennial with stiff stems that grow to be 0.3 to 1.5 m tall. White snakeroot leaves are 7.5 to 15 cm long, have three distinct veins, are opposite and sharply serrated while forming a pointed tip. The plant is easily identified during early fall by its small, bright, white flowers that are in composite heads of 10 to 30 flowers. White snakeroot is most commonly found in the eastern half of North America in moist, shaded, uncultivated areas along stream beds, near tree lines or on terraces (Burrows and Tyrl, 2001). Historically it is of considerable significance because of the suffering it caused for many of the midwest settlers in the United States in the early 1800s. It is reported that in some parts of Indiana and Ohio up to 50% of the deaths of early settlers were a result of white snakeroot-induced "milk sickness" (Wolf et al., 1918). The disease was so named as it was caused by drinking tainted milk from a cow that had ingested the plant. Livestock are also poisoned at white snakeroot doses of 1 to 1.5% of their body weight (BW) ingested daily for 1 to 3 weeks with the disease being known as "trembles". It is so named as intoxicated animals develop violent trembling when they are forced to move or are agitated (Kingsbury, 1964). Poisoning of sheep, goats (Reagor et al., 1989), cattle (Meyerholtz et al., 2011), horses (Olson et al., 1984, Smetzer et al., 1983; White et al., 1985), and even humans (Hartman et al., 1963) has been reported.

Initial signs of poisoning in most livestock are depression, reluctance to eat, and inactivity followed by fine muscle tremors of the nose, flanks, and legs especially after exercise or activity. The affected animal will often have tachypnea, tachycardia, a stiff gait, and altered posture as poisoned animals are reluctant to move and stand hunched up with a flexed back.

In 1918 it was shown that extracts of white snakeroot caused milk sickness and trembles (Wolf et al., 1918). Later Couch (1929) identified a straw-colored oil from white snakeroot that he named tremetol. Initially tremetol was believed to be a pure compound with the chemical formula of $C_{16}H_{32}O_3$. However, later work demonstrated that it was a complex mixture of terpenes, sterol and ketones including the benzofuran ketones (BFKs) tremetone, dehydrotremetone, 6-hydroxytremetone and 3-hydroxytremetone (Bonner and DeGraw, 1962; Bonner et al., 1961; Bowen et al., 1963). Additional work demonstrated that rayless goldenrod (*Isocoma pluriflora*) also contains the same BFKs and produces similar diseases (Couch, 1930; Burke, 1966).

Cases of white snakeroot poisoning are sporadic and unpredictable. However, *in vitro* cell culture studies suggested that tremetone was the likely toxin due to its cytotoxicity in several different cell lines

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after its microsomal activation (Beier et al., 1987; Beier et al., 1993). Nevertheless, tremetone toxicity in animal models has not been proven. In fact, neither, sheep nor cockerels developed disease when treated with synthetic tremetone (Bowen et al., 1963). Correlating clinical poisoning with toxin presence and concentrations in the plant has been hindered by the inability to quantitatively detect the specific BFKs thought to be associated with these toxicoses. Recently Lee et al. (2009) developed a quantitative high performance liquid chromatography (HPLC) assay to measure the concentrations of the proposed toxins tremetone, dehydrotremetone, and 6-hydroxytremetone in white snakeroot. Analysis of white snakeroot from different plant populations identified plant chemotypes with distinct benzofuran ketone types and concentrations (Lee et al., 2010). The differences in chemotypes may explain the sporadic incidence of poisoning and suggests that simply comparing doses based on percent of BW of plant material ingested is unreliable. The objectives of this study were to compare the toxicity of various white snakeroot populations, characterize the clinical and pathologic changes of poisoning in goats, and correlate intoxication with BFK content and concentrations.

2. Materials and methods

2.1. Collection of plant material

Seven collections of white snakeroot were made in four states (Missouri, Illinois, Indiana, and Ohio). The plant material was taxonomically identified as white snakeroot (*Ageratina altissima*) by laboratory taxonomists and by Dr. Stanley L. Welsh, curator of the Stanley L. Welsh Herbarium at Brigham Young University, Provo, UT. The collection site coordinates, dates of collection, and accession numbers of voucher specimens are shown in Table 1. The white snakeroot plant was air-dried and stored in a dry cool room until used. The day before beginning the study, the plant material was ground to pass through a 2.38 mm screen and mixed using a Gehl Mix-All, model 55 (Gehl Company, West Bend, WI).

2.2. Benzofuran ketone extraction and chemical analysis by analytical scale HPLC

Concentrations of the benzofuran ketones, chromenes, and phenols were determined by reversed phase HPLC on a Shimadzu LC-20AT equipped with an autosampler and photodiode array detector (PDA) from the same vendor and a 100 mm × 2 mm i.d., 5 μm, Betasil C_{18} column (Thermo Hypersil-Keysone, Bellefonte, PA) using the procedure reported in Lee et al. (2009). The compounds were quantified against a seven-point standard curve using previously isolated compounds. The standard curve was prepared over the range of 1.56 μg/mL–100.0 μg/mL by serial dilution.

2.3. Dosing of the goats

Thirty-two, yearling female Spanish goats in good body condition that weighed 22.6 ± 2.2 kg with a range of 17.4 to 29.0 kg were selected from the same herd. The goats were randomly divided into eight groups ($n = 4$). One goat from the Columbia group was removed from the study on the fourth day because of dosing difficulties. The animals were trained to lead and to run on a treadmill for two weeks before the start of the study. Four extra goats were trained for the study, two goats were eliminated because they would not run without stopping and two other goats were randomly eliminated so that there would be four animals per group. The day before the initial dosing, all animals were weighed, ran on treadmill for 5 min and bled by jugular venipuncture. All goats (except for 3 goats in each the Interstate 09 and Interstate 10 groups whose treatments were discontinued on days 8 or 9 to prevent further stress) were dosed intraruminally via oral gavage, for 9 days, with freshly ground dried white snakeroot at 2% of their body weight each day. The control group was dosed via oral gavage with similar amounts of ground alfalfa/grass hay. The dose was split and given twice per day at 1300 and 2200 h. The goats had access to water and long stem alfalfa hay *ad libitum* during the study. When animals became clinically affected (developed reluctance to stand, anorexia, or exercise intolerance) dosing was discontinued. All animals were humanely euthanized during the morning of day 10 after 9 days of dosing. At necropsy, samples of skeletal muscle approximately 0.5 × 1.0 × 4 cm in size were collected, attached to wood tongue depressors, and fixed in formalin. Skeletal muscles collected included: *retro*-ocular, tongue, masseter, cricothyroideus, pectoralis major, biceps brachii, triceps brachii, intercostals, diaphragm, longissimus dorsi, psoas major, biceps femoris, quadriceps femoris, semimembranous, semitendinosus, adductor, and gluteus medius. Other tissues collected, fixed, and examined included: brain, spinal cord, thyroid gland, heart, lung, liver, spleen, pancreas, rumen, abomasum, duodenum, jejunum, ileum, colon, mesenteric lymph node, adrenal gland, kidney, and urinary bladder. Lesions were subjectively scored by two pathologists (BLS, MGC) as: 0 = no change; 1 = minimal with myocyte degeneration characterized by rare myocyte swelling and hypereosinophilia; 2 = mild with occasional myocyte degeneration (myocyte swelling and hypereosinophilia) with rare myocyte necrosis; 3 = moderate with patchy myocyte degeneration and necrosis involving small portions (<35%) of the skeletal muscle fibers or sample; and 4 = severe with extensive myocyte degeneration and necrosis with or without subsequent inflammation and regeneration with diffuse distribution involving 40% or more myocytes. All animal work was done under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee.

2.4. Measure of muscle strength and endurance

The ability of the goat to run on a treadmill was used as a measure of muscle strength and endurance. Goats were run on a treadmill (Horse Gym 2000 GmbH, GroBsorheim, Germany) starting at 0700 h, at a 10% incline, moving at approximately 12 km per hour, for 5 min. If movement became labored and the animal was unable to keep pace before the 5 min running period was complete the animal was taken off of the treadmill and considered to be exercise intolerant.

Table 1
White snakeroot collection sites, dates, and USDA-ARS-Poisonous Plant Research Laboratory herbarium accession numbers.

Collection name	Collection location (county, state)	Coordinates	Collection date	Accession number
Zanesville	Muskingum, OH	N 39°52′35″, W 81°42′52″ "Spears" N 38°47′00″, W 92°15′01″; "University" N 38°56′24″, W 92°18′54″;	9/20/2010	4229, 4230 "Spears" 4222 "University" 4219, 4220
Columbia	Boone, MO	"East 163" N 38°52′07″, W 92°16′07″	9/18/2010	"East" 4217, 4218
Allerton	Piatt, IL	N 40°00′22″, W 88°39′11″	9/17/2009	3560
VRO	Vermillion, IL	N 40°03.582′, W 87°33.847′	9/15/2009	3550
Interstate 2009	Vermillion, IL	N 40°06′29″, W 87°40′52″	9/18/2009	3562
Interstate 2010	Vermillion, IL	N 40°06′29″, W 87°40′52″ "Wabash River" N 40°25′19″, W 86°53′53″	9/23/2010	4241 "Wabash River" 4235, 4236
Wabash	Tippecanoe, IN	"Happy Hollow" N 40°26′05″, W 86°53′54″	9/21/2010	"Happy Hollow" 4237