

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/271709420>

# Toxicity of White Snakeroot ( *Ageratina altissima* ) and Chemical Extracts of White Snakeroot in Goats

ARTICLE in JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JANUARY 2015

Impact Factor: 2.91 · DOI: 10.1021/jf505614z · Source: PubMed

READS

75

7 AUTHORS, INCLUDING:



**Mark Grey Collett**

Massey University

57 PUBLICATIONS 487 CITATIONS

SEE PROFILE



**Bryan Stegelmeier**

United States Department of Agriculture

142 PUBLICATIONS 1,670 CITATIONS

SEE PROFILE



**Benedict T Green**

United States Department of Agriculture

82 PUBLICATIONS 891 CITATIONS

SEE PROFILE



**James A. Pfister**

United States Department of Agriculture

223 PUBLICATIONS 2,471 CITATIONS

SEE PROFILE

# Toxicity of White Snakeroot (*Ageratina altissima*) and Chemical Extracts of White Snakeroot in Goats

T. Zane Davis,<sup>\*,†</sup> Stephen T. Lee,<sup>†</sup> Mark G. Collett,<sup>‡</sup> Bryan L. Stegelmeier,<sup>†</sup> Benedict T. Green,<sup>†</sup> Steven R. Buck,<sup>§</sup> and James A. Pfister<sup>†</sup>

<sup>†</sup>Poisonous Plant Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 1150 East 1400 North, Logan, Utah 84341, United States

<sup>‡</sup>Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North, Manawatu 4442, New Zealand

<sup>§</sup>University of Illinois at Urbana–Champaign, 1816 South Oak Street, Champaign, Illinois 61820, United States

## S Supporting Information

**ABSTRACT:** White snakeroot (*Ageratina altissima*) is a sporadically toxic plant that causes trembles in livestock and milk sickness in humans that drink tainted milk. The putative toxin in white snakeroot is tremetone and possibly other benzofuran ketones, even though it has not been demonstrated *in vivo*. Toxic white snakeroot was dosed to goats, and they developed clinical signs of poisoning, exercise intolerance, significant increases in serum enzyme activities, and histological changes. Tremetone and the other benzofuran ketones were extracted with hexane; the extracts and residues were analyzed for tremetone and dosed to goats at tremetone and benzofuran ketone concentrations similar to the original plant material. However, none of the dosed goats developed the disease. The results demonstrate for the first time that white snakeroot is a potent myotoxin in goats and that other compound(s), which may be lost or modified during the extraction process, could be involved in causing trembles and milk sickness.

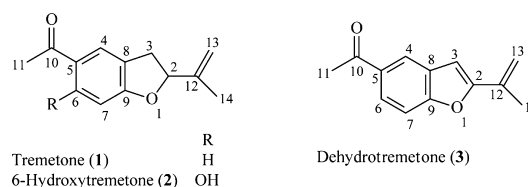
**KEYWORDS:** white snakeroot, tremetone, tremetol, *Ageratina altissima*, *Eupatorium rugosum*, trembles, milk sickness, benzofuran ketones, goats

## INTRODUCTION

White snakeroot (*Ageratina altissima* or previously *Eupatorium rugosum* (Asteraceae)) is a plant commonly found in the midwestern and eastern United States and is responsible for causing trembles in livestock and milk sickness in humans. Early reports suggest that livestock are poisoned after eating 0.5–1.5% of their body weight over a period of one to three weeks.<sup>1</sup> Initial signs of poisoning in most livestock include depression, reluctance to eat, and inactivity followed by tremors of the nose, flanks, and limbs, especially after exercise or activity. With prolonged and severe poisoning, animals develop tachypnea, tachycardia, a stiff gait, and altered posture (animals are reluctant to move and stand “hunched up” with a flexed back). Severely affected animals become debilitated, acidotic, and die. Poisoning is common in nursing neonates, suggesting the reported toxins are lipid soluble facilitating transmammary poisoning.

Historically, cases of white snakeroot poisoning are sporadic and unpredictable, making it difficult to associate plant toxicity with a specific plant compound. Recently, white snakeroot populations have been identified that have unique and different chemical profiles with varying benzofuran ketone (=benzofuran) concentrations or chemotypes.<sup>2</sup> In 1918, Wolf et al. demonstrated that an ether soluble resin of white snakeroot caused trembles.<sup>3</sup> A toxic, yellow, straw-colored oil was isolated from white snakeroot in the 1920s and identified as tremetol.<sup>4</sup> Tremetol was later shown to be a mixture of many lipophilic compounds including several benzofuran compounds (trem-

etone, 1, dehydrotremetone, 3, 6-hydroxytremetone, 2, and structurally related compounds, see Figure 1).<sup>2,5,6</sup> *In vitro* cell



**Figure 1.** Chemical structures of tremetone, 1, 6-hydroxytremetone, 2, and dehydrotremetone, 3.

culture studies using murine melanoma (B16F<sub>1</sub>) and five other mammalian cell lines suggested that microsomally activated product(s) of tremetone, 1, were the likely toxin(s).<sup>7,8</sup> Dehydrotremetone, 3, was not toxic to the same cell lines even when incubated with microsomes.<sup>8</sup> However, when animals were dosed with synthetic tremetone, 1, the animals did not become poisoned.<sup>9</sup> After nearly 100 years since the initial studies were conducted, tremetone, 1, although commonly accepted as the toxin, has not been demonstrated to be toxic *in vivo*. In the literature, there is a single report in

**Received:** November 20, 2014

**Revised:** January 29, 2015

**Accepted:** January 30, 2015

**Published:** January 31, 2015

**Table 1. Serum Enzyme Activities and Exercise Tolerance from Goats Dosed with White Snakeroot and White Snakeroot Extracts and Residues<sup>a</sup>**

	group (=material dosed)	day 1 (mean ± SD)	day 7 (mean ± SD)	day 9 (mean ± SD)	day 10 (mean ± SD)
alanine aminotransferase, ALT (U/L)	control	16 ± 3	36 ± 7	31 ± 7	13 ± 3
	ground white snakeroot	16 ± 3 <sup>a</sup>	128 ± 171 <sup>b*</sup>	199 ± 228 <sup>b*</sup>	108 ± 119 <sup>b*</sup>
	extracted plant residue	14 ± 3	36 ± 3	32 ± 7	15 ± 5
	hexane extract	18 ± 4	14 ± 7	14 ± 3	36 ± 5
	recombined extracts and residues	16 ± 2	14 ± 7	13 ± 1	36 ± 2
aspartate aminotransferase, AST (U/L)	control	60 ± 15	79 ± 19	76 ± 19	55 ± 13
	ground white snakeroot	65 ± 14 <sup>a</sup>	145 ± 53 <sup>b*</sup>	2611 ± 3867 <sup>bc*</sup>	965 ± 1143 <sup>bc*</sup>
	extracted plant residue	55 ± 5	90 ± 5	101 ± 10	73 ± 6
	hexane extract	55 ± 5	59 ± 30	59 ± 7	85 ± 10
	recombined extracts and residues	50 ± 8	58 ± 29	54 ± 5	96 ± 10
creatinine kinase, CK (U/L)	control	152 ± 62	79 ± 20	68 ± 19	121 ± 31
	ground white snakeroot	160 ± 66 <sup>a</sup>	3414 ± 6199 <sup>b*</sup>	1536 ± 1892 <sup>b*</sup>	2649 ± 2654 <sup>b*</sup>
	extracted plant residue	128 ± 13	95 ± 14	112 ± 26	190 ± 62
	hexane extract	122 ± 21	115 ± 25	115 ± 16	178 ± 78
	recombined extracts and residues	105 ± 11	126 ± 48	121 ± 49	160 ± 54
goats that were exercise intolerant (unable to run 5 min)	control	0/4	0/4	0/4	0/4
	ground white snakeroot	0/4	2/4	3/4	2/3
	extracted plant residue	0/4	0/4	0/4	0/4
	hexane extract	0/3	0/3	0/3	0/3
	recombined extracts and residues	0/3	0/3	0/3	0/3

<sup>a</sup>Note:  $n = 3$  on day 10 for the white snakeroot group because one goat was necropsied on day 9. Means that are significantly different ( $p < 0.05$ ) from others within the same group are indicated with superscript letters. Means that are significantly different from other groups on the same day are indicated with an asterisk (\*).

which Angora goats were exposed to white snakeroot and 53 of the goats died in Central Texas.<sup>10</sup> Dosing studies with the plant collected from that site in Texas resulted in acute death from hepatic necrosis with no myotoxic lesions and established an LD<sub>50</sub> of 5 mg/kg of fresh white snakeroot.<sup>10</sup> Spanish goats have been shown to be a good model to study the toxicity of the benzofuran-containing plant, rayless goldenrod (*Isocoma pluriflora*),<sup>11,12</sup> which is thought to contain the same toxin as white snakeroot. Recent research in our laboratory has focused on identifying the toxic component of white snakeroot and demonstrating its toxicity in a live animal model.

The purpose of this study was 4-fold: First, to determine if white snakeroot that contained known concentrations of tremetone, 1, 6-hydroxytremetone, 2, and dehydrotremetone, 3, was myotoxic in a goat model; second, to determine if a hexane extract of white snakeroot containing 1, 2, and 3, adsorbed onto alfalfa at the same concentration as the white snakeroot plant material was toxic; third, to determine if the plant residue, following the hexane extraction of white snakeroot, that did not contain 1, 2, and 3, was toxic; and fourth, to determine if the hexane extract recombined with the white snakeroot plant residue was toxic.

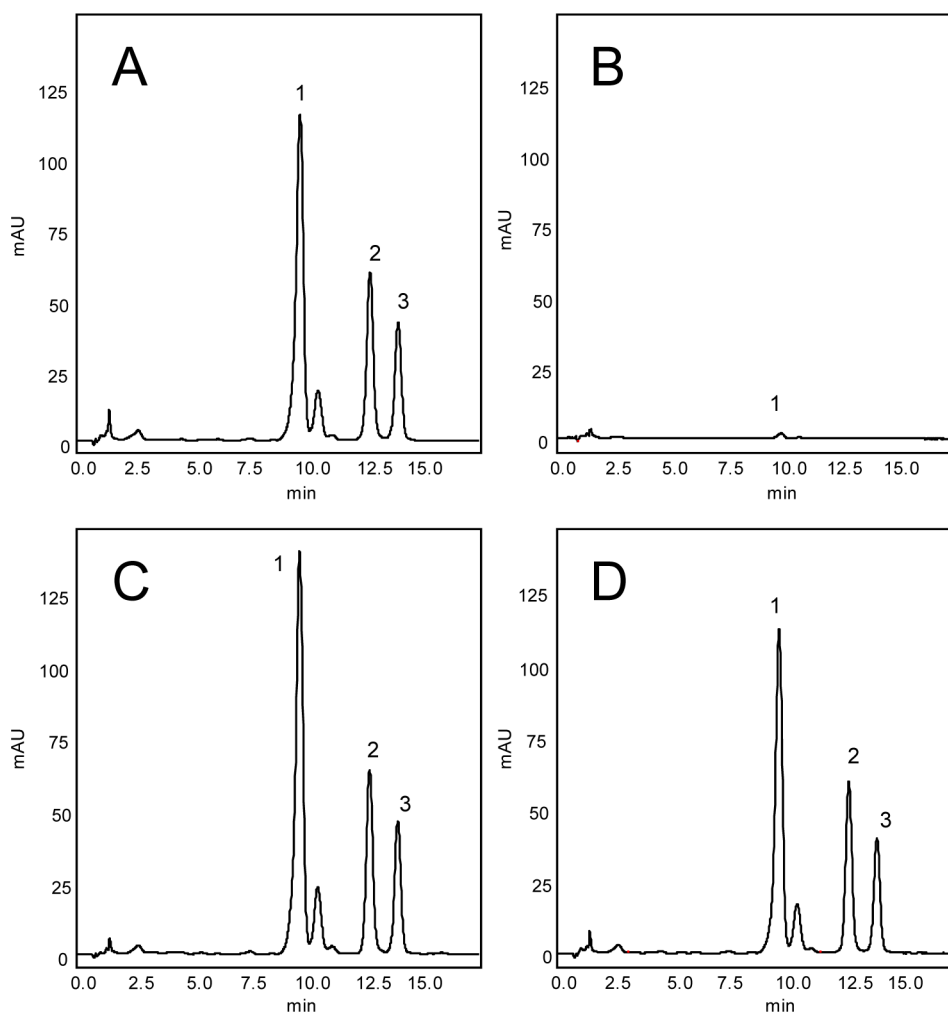
## MATERIALS AND METHODS

**Collection of Plant Material.** The aerial parts of white snakeroot plants were collected from two locations near each other in Vermilion County, Illinois. The collection locations and GPS coordinates were: Interstate, 40°06.483' N/87°40.866' W, and Salt Fork, 40°05.536' N/87°49.683' W. The plants were collected in September while in early to full flower stages of growth. White snakeroot plants were identified by Dr. Stanley L. Welsh curator at the Stanley L. Welsh Herbarium at Brigham Young University, Provo, UT, and Dr. David S. Seigler, Professor, Department of Plant Biology,

University of Illinois, Urbana–Champaign, Illinois. The current taxonomic classification for this plant is *Ageratina altissima* (L.) King & H. Rob. var. *altissima* as per the USDA, National Resources Conservation Service, Plant Database. Voucher specimens of white snakeroot, accession nos. 3555 and 3562 for the Salt Fork and the Interstate collections, respectively, were deposited in the Poisonous Plant Research Laboratory Herbarium, Logan, UT.

**Preparation of Plant Material and Extracts.** The white snakeroot from the Salt Fork and Interstate collections were air-dried at ambient temperature, mixed together in a ratio of approximately 0.40:0.60, and ground to pass through a 2.38 mm screen and mixed using a model 55 Mix-All, (Gehl Company, West Bend, WI).

Ground plant material (30 kg) was extensively extracted in 15 Soxhlet extractions of approximately 2 kg portions with *n*-hexane (50 h). The hexane extract from 8 of the extractions were concentrated to dryness by rotary evaporation, combined, and reconstituted to 4.0 L with dichloromethane and referred to as the hexane extract. The hexane extract was quantitatively adsorbed onto alfalfa (ratio of 1 mL hexane extract per 4 g alfalfa, which is equal to the hexane to plant ratio from which it was extracted) and allowed to dry overnight in a fume hood. The ground plant material extracted with *n*-hexane was recovered from the Soxhlet thimble, transferred to a paper bag, and dried in a fume hood for 24 h at ambient temperature to allow residual hexane to evaporate and is referred to as the extracted plant residue (=plant residue). The hexane extract and plant residue from seven Soxhlet extractions were quantitatively recombined and are referred to as the combined extract and residue (=extract+residue). The ground white snakeroot (=snakeroot), hexane extract, plant residue, and extract+residue, which were dosed to the snakeroot group, hexane extract group, plant residue group, and extract+residue



**Figure 2.** HPLC chromatograms from (A) white snakeroot plant material (white snakeroot); (B) white snakeroot plant material after extraction by hexane, (extracted plant residue); (C) white snakeroot hexane extract adsorbed onto alfalfa (hexane extract); (D) white snakeroot hexane extract adsorbed back onto extracted white snakeroot residue (combine extract and residue). Peak numbers refer to tremetone, 1, 6-hydroxytremetone, 2, and dehydrotremetone, 3, whose chemical structures are shown in Figure 1.

group, respectively, were analyzed for the benzofurans. It was confirmed that the snakeroot, hexane extract, and extract +residue contained the same benzofuran compounds and at nearly equal concentrations. The plant residue contained only trace amounts of the benzofurans in the same dosed mass.

**HPLC Analysis.** The analysis of tremetone, 1, 6-acetyl-7-methoxy-2,2-dimethylchromene, 6-hydroxytremetone, 2, and dehydrotremetone, 3, in snakeroot, hexane extract, plant residue, and extract+residue treatments was performed by HPLC as described previously.<sup>2,13</sup>

**Dosing of Goats.** Eighteen yearling Spanish goats in good body condition that weighed  $29.1 \pm 5.4$  kg (mean  $\pm$  SD) were selected from the same herd at the Poisonous Plant Research Laboratory. The goats were randomly divided into five groups with three or four animals per group (Table 1). The animals were trained to lead and to run for 5 min on a treadmill moving at 12 km/h on a 10% incline for 15 d before the start of the study. The day before the initial dosing, all animals were weighed, bled by jugular venipuncture, and exercised on a treadmill. The snakeroot, plant residue, hexane extract, and extract+residue groups were dosed with 1.67% body weight intraruminally via oral gavage, for 9 d. A negative control group was also dosed ground alfalfa hay via oral gavage at 1.67% of

body weight. The dose was split and given twice per day at 9:00 AM and 9:00 PM. Throughout the duration of the study, the goats had access to water and long stem alfalfa hay *ad libitum*. One goat from the snakeroot group developed severe exercise intolerance, reluctance to move, and anorexia. The goat was euthanized on day 9. The rest of the goats were euthanized and necropsied during the morning of day 10.

**Histological Analysis.** At necropsy, samples of left lateral retroocular, tongue, masseter, superficial pectoral, triceps, intercostals, longissimus dorsi, semitendinosus, diaphragm, biceps femoris, biceps brachii, quadriceps femoris, gluteus medius, psoas major, adductor, and semimembranosus skeletal muscles were collected, attached to wooden tongue depressors, and fixed in 10% neutral buffered formalin. The heart was opened, cleaned with water, closely examined, fixed intact, and sectioned to examine portions of the right atrium, the right papillary muscle, the right ventricular free wall, the septum, the left atrium, the left papillary muscle, and the left ventricular free wall. Other tissues including brain, spinal cord, lung, liver, right and left kidneys, adrenal gland, urinary bladder, thyroid gland, mesenteric lymph node from the small intestine, esophagus, rumen omasum, abomasums, duodenum, pancreas, jejunum ileum, cecum, and colon were collected fixed, and prepared for

examination. Tissues were processed, sectioned, and stained using standard histologic techniques. Special stains of specific skeletal muscle, myocardium, and liver lesions included diastase-positive and diastase-resistant periodic acid-Schiff (PAS), Masson trichrome stain for collagen, and Congo red. Lesions were scored by distribution (percentage of tissue affected) and graded by the severity of lesion, (0 = none, 1 = minimal [loss of striation and hypereosinophilia], 2 = mild [sarcomeric clumping with myocyte swelling], 3 = moderate [sarcomere disruption with focal monocytic inflammation and mild nuclear proliferation], 4 = severe [extensive inflammation, regeneration with fibrosis]), by two pathologists who were “blinded” to the treatment groups. All animal work was done under veterinary supervision with the approval and supervision of the Utah State University Institutional Animal Care and Use Committee.

**Exercise Tolerance.** Goats were run on a treadmill (Horse Gym 2000 GmbH, Großsorheim, Germany) for 5 min to determine exercise tolerance. The treadmill was moving at 12 km/h on a 10% incline. If movement became labored and the goat was unable to keep pace before completion of the 5 min exercise period, as determined by an observer “blinded” to the treatment groups, then the treadmill was stopped and the animal was removed and considered to be exercise intolerant.

**Serum Analyses.** Serum biochemistries were analyzed as previously described.<sup>12</sup> Significant analytes that are reported herein include the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatine kinase (CK).

**Statistical Analyses.** Serum chemistry variables were analyzed using a mixed linear model approach (Proc Mixed in SAS 9.3). Serum variables were evaluated for normality, and all distributions were non-normal. Thus, a log transformation was performed before analysis; however, all means presented herein are untransformed values. The repeated measures model contained treatments, days and the day x treatment interaction, with animals as a random factor, and animals were nested within treatment groups. The appropriate covariance structure was determined using the lowest value of Akaike’s information criteria (AIC) compared across models. Least square means were separated after a significant F test using the PDIF option in SAS.

Subjective pathologic scores for each group were compared using an analysis of variance. Means that were significantly different were separated using Tukey’s analysis ( $p < 0.05$ , SAS 9.3 Proc GLM).

## ■ RESULTS

The combined white snakeroot plant material from the Salt Fork and Interstate collections was analyzed by HPLC and found to contain 2.1  $\mu\text{g}/\text{mg}$ , 0.72  $\mu\text{g}/\text{mg}$ , 0.96  $\mu\text{g}/\text{mg}$ , and 0.53  $\mu\text{g}/\text{mg}$  of tremetone, 1, 6-acetyl-7-methoxy-2,2-dimethylchromene, 6-hydroxytremetone, 2, and dehydrotremetone, 3, respectively. These compounds were quantitatively extracted into hexane when the white snakeroot plant material was Soxhlet extracted with hexane, evaporated and resolubilized in dichloromethane and quantitatively reabsorbed on the same amount of ground alfalfa or plant residue and dosed as hexane extract and extract+residue, respectively. Dosed extracts were analyzed for benzofuran content after applying the extracts resuspended in dichloromethane to the alfalfa or plant residue and dried overnight. The chromatograms showing the benzofurans in each treatment are shown in Figure 2. The

amounts of the hexane extract and the dosed extract+residue material contained tremetone, 1, and total benzofurans at the same dosages (35.1 and 60 mg/kg body weight, respectively) as the dosed snakeroot. The dosed plant residue contained only trace amounts of tremetone and the other benzofurans.

On day 5, one goat from the snakeroot group developed clinical signs of poisoning and was unable to run on the treadmill for 5 min. The condition of the goat became more severe and it was euthanized on day 9. A second and third goat from the snakeroot group developed the same clinical signs and became exercise intolerant on days 6 and 9. None of the goats from the plant residue, hexane extract, or extract+residue groups exhibited clinical signs of poisoning or became exercise intolerant.

The mean  $\pm$  SD of serum enzyme activities of AST, ALT, and CK for each of the dosed groups are shown in Table 1. There were significant ( $p < 0.05$ ) treatment by day interactions for AST, ALT, and CK. There were significant increases in the serum activities of AST, ALT, and CK on day 7 in the snakeroot dosed goats. Serum CK activities of the snakeroot dosed goats that were poisoned increased to between 2285 and 17704 U/L within 1 day of becoming exercise intolerant. There were no significant changes in CK, AST, and ALT activities of control, hexane extract, plant residue, and extract+residue dosed groups during the 10 days of the study.

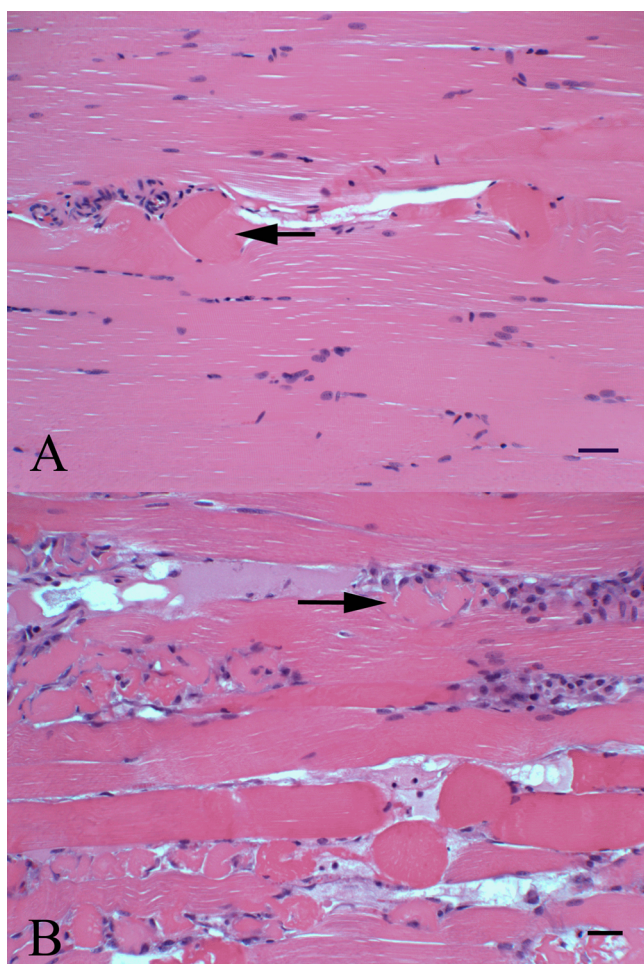
Occasionally, skeletal muscle from goats in the control, hexane extract, plant residue, and extract+residue groups had rare myofiber swelling and edema. These lesions were also seen histologically as focal myocyte degeneration and necrosis (Figure 3A). The degeneration was characterized by myocyte swelling with loss of striation and rarely individual myocyte necrosis with focal inflammation. These lesions were small and rare involving small clusters of myocytes suggesting they were background changes. All but one of the snakeroot dosed goats had severe skeletal muscle myodegeneration and necrosis. This was evident grossly as many of the muscles of both the appendicular and axial skeleton were swollen with pale streaking. Histologically, these lesions were characterized as extensive Zenker’s necrosis with loss of striation, swelling, coagulation and clumping of sarcomere proteins, focally extensive inflammation and phagocytosis of myocyte debris and focal regeneration (Figure 3B). The lesions were most severe in many of the large appendicular muscles including the biceps brachii, biceps femoris, semitendinosus, semimembranosus, quadriceps femoris, and gluteus medius. Fewer skeletal muscles of the axial skeleton were also affected with smaller, less severe lesions in the psoas major, longissimus dorsi, and intercostal skeletal muscles.

The livers from all dosed animals, including the control goats, had mild centrilobular hepatocyte swelling with occasional vacuolation. The vacuoles were clear with prominent margins suggestive of lipid accumulation. No additional liver lesions were identified in any of the groups including the snakeroot dosed goats. No significant histologic lesions were identified in the other sampled tissues from goats in either the treated or control groups.

## ■ DISCUSSION

White snakeroot is a potent myotoxin in most livestock species. It has caused sporadic cases of poisoning in livestock and humans since the early 1800s. The results from this study demonstrate for the first time that white snakeroot is a potent myotoxin in goats that affects both appendicular and axial





**Figure 3.** Photomicrograph of the skeletal muscle, quadriceps femoris from a goat from the (A) control group dosed with ground alfalfa; (B) white snakeroot dosed group that was treated with ground snakeroot, at 1.67% body weight for 9 d. Notice the rare myonecrosis and clumping of myocyte proteins (arrow) in part A. Such lesions never affected more than 1% of the myocytes in control animals. Also, notice the extensive myonecrosis, clumping of myocyte proteins (arrow) and focally extensive inflammation in Figure 3B. Such lesions were often severe and in some goats affected more than 60% of the myocytes. H&E, bar = 50  $\mu$ m.

skeletal muscles in goats. The goats had severe skeletal muscle myodegeneration and necrosis and significant increases in serum activities (AST, ALT, and CK) of enzymes that can be used as indicators of muscle damage. The myotoxic lesions observed in this study are in contrast to the only reports of white snakeroot poisoning of goats in the literature,<sup>10</sup> in which a single fresh dose of white snakeroot from Texas was lethal to Angora goats within 24 to 48 h of dosing and was characterized by extensive para-acinar hepatic necrosis, and increases in serum LDH and SGOT (=AST) activities. The histological hepatic changes we observed appeared related mainly to increased lipid mobilization and metabolism. Similar hepatic changes in the control group suggest that it is not likely a toxic response but more likely associated with altered metabolism relating to the treatment (oral gavage and subsequent physiologic exercise). It may be that the chemical constituents of the white snakeroot from Texas are different from the white snakeroot from Illinois that was used in this study. The

differences in the clinical disease produced by the two different populations of white snakeroot should be further investigated.

Previous dosing of goats with rayless goldenrod, which is thought to contain the same toxin as white snakeroot, caused lesions to develop mostly in the large appendicular muscles when orally gavaged at 2 and 3% body weight.<sup>11</sup> The rayless goldenrod dosed in that study contained tremetone, 1, (0.53  $\mu$ g/mg), dehydrotremetone, 3, (1.34  $\mu$ g/mg), and 3-oxyangeloyl-tremetone (2.39  $\mu$ g/mg). The white snakeroot dosed in this study contained 2.1  $\mu$ g/mg, 0.96  $\mu$ g/mg, and 0.53  $\mu$ g/mg of tremetone, 1, 6-hydroxytremetone, 2, and dehydrotremetone, 3, respectively. The goats dosed with rayless goldenrod at 3% body weight received a total dose of 60 mg total benzofurans/kg body weight, which was the same dosage of total benzofurans administered to the snakeroot, hexane extract, and extract+residue dosed goats in this study even though they were orally gavaged with plant material at 1.67% body weight. The goats in the snakeroot, hexane extract, and extract+residue dosed groups in this study received nearly 3.3 times more tremetone than the goats dosed with rayless goldenrod at 2% of BW. If tremetone, 1, is the sole toxin in white snakeroot and rayless goldenrod then the goats dosed with hexane extract and extract+residue should have poisoned similar to the goats dosed dried snakeroot.

Tremetol has been commonly accepted as the toxin in white snakeroot. In fact, as recently as 1985 it was referred to as the toxin in white snakeroot.<sup>14</sup> When tremetone, 1, was dosed orally or by injection into breast muscle of cockerels for 7 days, no signs of poisoning or distress were observed.<sup>9</sup> Bowen and co-workers<sup>9</sup> concluded that tremetone, 1, was not the toxin in white snakeroot. Results of cell culture cytotoxicity studies led to the proposal that microsomally activated tremetone, 1, was responsible for the toxicity of the plant;<sup>8</sup> however, it still has not been demonstrated in any animal model. The hexane extract and the extract+residue groups in this study were gavaged with the same dosages of tremetone, 1, 6-hydroxytremetone, 2, and dehydrotremetone, 3, as the snakeroot group. The hexane extract and extract+residue dosed groups did not develop clinical signs, exercise intolerance, serum enzyme changes, or histological lesions observed in the snakeroot dosed group. These results suggest that tremetone, 1, and/or 6-hydroxytremetone, 2, and dehydrotremetone, 3, are not the singular toxin/toxins in white snakeroot. Consequently, there may be another compound or group of compounds that act either independently or synergistically with tremetone, 1, or other benzofurans. The lack of toxicity observed in the extract+residue group further suggests that if another agent that acts singularly or synergistically with tremetone, 1, and the other benzofurans is present in white snakeroot, this agent is modified and inactivated during the extraction-recombination process. However, since white snakeroot and rayless goldenrod both cause the same disease in livestock and both plants contain tremetone, 1, and dehydrotremetone, 3, it is possible that tremetone, 1, (or a structurally related compound) is a marker for the active agent or that there is another compound that acts synergistically with one of the benzofurans.

In conclusion, the results from this study demonstrate for the first time that white snakeroot is a potent myotoxin in goats. The results also suggest that another compound besides tremetone, 1, may have a significant role in producing trembles in livestock and milk sickness in humans.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Histologic score of lesion severity and distribution in skeletal muscle of goats dosed with snakeroot, hexane extract, plant residue, and extract+residue. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*E-mail: [zane.davis@ars.usda.gov](mailto:zane.davis@ars.usda.gov). Tel: 435-752-2941. Fax: 435-753-5681.

### Funding

This research was supported by USDA/ARS.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The authors thank Katie Lott, Andrea Dolbear, and Ed Knoppel for their assistance with this research. We thank Dr. Stan Welsh of the Stan Welsh Herbarium and Dr. David Seigler of the University of Illinois at Urbana–Champaign for their assistance in identifying the plant material.

## ■ REFERENCES

- (1) Kingsbury, J. M. *Poisonous Plants of the United States and Canada*; Prentice Hall: Englewood Cliffs, NJ, 1964.
- (2) Lee, S. T.; Davis, T. Z.; Gardner, D. R.; Colgate, S. M.; Cook, D.; Green, B. T.; Meyerholtz, K. A.; Wilson, C. R.; Stegelmeier, B. L.; Evans, T. J. Tremetone and structurally related compounds in white snakeroot (*Ageratina altissima*): A plant associated with trembles and milk. *J. Agric. Food Chem.* **2010**, *58*, 8560–8565.
- (3) Wolf, F. A.; Curtis, R. S.; Kaupp, B. F. A monograph on trembles or sickness and white snakeroot. *North Carolina Agric. Exp. Stn. Technol. Bull.* **1918**, *15*.
- (4) Couch, J. F. The toxic constituent of richweed or white snakeroot (*Eupatorium urticaefolium*). *J. Agric. Res.* **1927**, *35*, 547–576.
- (5) Bonner, W. A.; DeGraw, J. I., Jr.; Bowen, D. M.; Shah, V. R. Toxic constituents of white snakeroot. *Tetrahedron Lett.* **1961**, *12*, 417–420.
- (6) Bonner, W. A.; DeGraw, J. I., Jr. Ketones from “white snakeroot. *Eupatorium urticaefolium*. *Tetrahedron Lett.* **1962**, *18*, 1295–1309.
- (7) Beier, R. C.; Norman, J. O.; Irvin, R. T.; Witzel, D. A. Microsomal activation of constituents of white snakeroot (*Eupatorium rugosum* Houtt) to form toxic products. *Am. J. Vet. Res.* **1987**, *48*, 583–585.
- (8) Beier, R. C.; Norman, J. O.; Reagor, J. C.; Rees, M. S.; Mundy, B. P. Isolation of the major component in white snakeroot that is toxic after microsomal activation: Possible explanation of sporadic toxicity of white snakeroot plants and extracts. *Nat. Toxins* **1993**, *1*, 286–293.
- (9) Bowen, D. M.; DeGraw, J. I., Jr.; Shah, V. R.; Bonner, W. A. The synthesis and pharmacological action of tremetone. *J. Med. Chem.* **1963**, *6*, 315–319.
- (10) Beier, R. C.; Norman, J. O. The toxic factor in white snakeroot: Identity, analysis, and prevention. *Vet. Hum. Toxicol.* **1990**, *32*, 81–88.
- (11) Stegelmeier, B. L.; Davis, T. Z.; Green, B. T.; Lee, S. T.; Hall, J. O. Experimental rayless goldenrod (*Isocoma pluriflora*) toxicosis in goats. *J. Vet. Diagn. Invest.* **2010**, *22*, 570–577.
- (12) Davis, T. Z.; Green, B. T.; Stegelmeier, B. L.; Lee, S. T.; Welch, K. D.; Pfister, J. A. Physiological and serum biochemical changes associated with rayless goldenrod (*Isocoma pluriflora*) poisoning in goats. *Toxicon* **2013**, *76*, 247–254.
- (13) Lee, S. T.; Davis, T. Z.; Gardner, D. R.; Stegelmeier, B. L.; Evans, T. J. A quantitative method for the measurement of three benzofuran ketones in rayless goldenrod (*Isocoma pluriflora*) and white snakeroot (*Ageratina altissima*) by HPLC. *J. Agric. Food Chem.* **2009**, *57*, 5639–5642.
- (14) Niederhofer, R. E. The milk sickness. *J. Am. Med. Assoc.* **1985**, *254*, 2123–2125.