Broom Snakeweed Extracts Dosed to Late-Term Pregnant Cattle Do Not Cause Premature Parturition

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**Recommended Citation**

DOI: [https://doi.org/10.26077/15eb-503b](https://doi.org/10.26077/15eb-503b)  
Available at: [https://digitalcommons.usu.edu/poisonousplantresearch/vol5/iss1/2](https://digitalcommons.usu.edu/poisonousplantresearch/vol5/iss1/2)

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Broom Snakeweed Extracts Dosed to Late-Term Pregnant Cattle Do Not Cause Premature Parturition

Abstract
Broom snakeweed [Gutierrezia sarothrae (Pursh) Britton and Rusby] and threadleaf snakeweed [G. microcephala (DC.) A. Gray] are found on many rangelands in western North America. Snakeweeds are generally unpalatable; however, animals will graze them when other forage is not available and there are field reports that pregnant cows that graze snakeweeds may abort calves. Subsequent feeding studies using fresh cut snakeweed, ground and gavaged into pregnant cattle have failed to reproduce abortions, though it was evident that at high doses snakeweed quickly damaged the rumen microflora resulting in severe rumen atony, bloating and acidosis. We report here an attempt to solvent extract the snakeweeds, mix the extracts with ground alfalfa hay, and test the extracts for abortifacient activity in late-term pregnant cattle. The dosed extracts again appear to be unable to reproduce snakeweed induced abortions in cattle. Extracts from snakeweed chemotype 1 plant appear to be quite toxic to the rumen and caused complete rumen stasis after 2-3 days. In contrast, cattle receiving chemotype 2 extracts were able to tolerate over twice the dosage as that given for chemotype 1 extracts. We conclude that broom snakeweed plants are unlikely to be directly responsible for cattle abortions observed in cattle grazing snakeweed infested rangelands. It is more likely that cattle may be affected by rumen toxicity and/or might suffer from poor nutritional factors given the lack of quality forage that might be available on rangelands with high snakeweed infestation.

Keywords
Snakeweed, cattle abortions, Gutierrezia sarothrae, Gutierrezia microcephala

This article is available in Poisonous Plant Research (PPR): https://digitalcommons.usu.edu/poisonousplantresearch/vol5/iss1/2
INTRODUCTION

Broom snakeweed [Gutierrezia sarothrae (Pursh) Britton and Rusby] and threadleaf snakeweed [G. microcephala (DC.) A. Gray] are found on many rangelands in western North America. Both species are commonly referred to as snakeweed. Snakeweeds are generally unpalatable; however, in many harsh rangelands where snakeweeds thrive and displace other nutritious plants, animals will graze them when other forage is not available (Ralphs, 2011). The major toxicity associated with snakeweeds is late gestation abortion (Kingsbury, 1964; McDaniel and Ross, 2002). However, others suggested it caused more a gastrointestinal toxicity resulting in loss of condition and wasting (Norris and Valentine, 1954). In early studies by Dollahite and Anthony (1956) abortion, premature weak calves and retained placentas were described in cows fed perennial snakeweed. However, subsequent feeding studies have not been able to reproduce that work. If an abortifacient toxin is present in snakeweeds the lack of a consistent biological response makes it difficult to identify. Snakeweeds are reported to contain a number of different secondary compounds, recently we have focused on their diterpene acid content as a result of similar compounds from Ponderosa pine needles being abortifacient in late-term pregnant cattle (Gardner et al., 1994). Recent phytochemical studies in our laboratory found the diterpene acid profiles of the snakeweeds differ significantly among collection sites and we reported finding the snakeweeds to be composed of at least eight different chemotypes (Gardner et al., 2020).

Past efforts at our laboratory to reproduce the abortifacient effects using fresh cut snakeweed, ground and gavaged into pregnant cattle have failed. Because of the resinous nature of snakeweeds, we have found it is physically difficult to grind and administer the plant material in animal trials. Working past these obstacles, both pregnant goats and cows were repeatedly dosed. This produced no convincing abortions, though it was evident that at high doses snakeweed quickly damaged the rumen microflora resulting in severe rumen atony, bloating and acidosis. These changes were severe and without extensive rumenotomies, transfaunations, and aggressive correction of acidosis and dehydration poisoning could be fatal. As such gastrointestinal damage is not reported in range animals because it is likely they never ingest such toxic doses (unpublished data).

As ponderosa pine needle induced abortion in late term pregnant cattle is similar to that described in snakeweed associated toxicity, it was hypothesized that the toxins might be similar. In past work on ponderosa pine needles we were able to successfully administer crude solvent extracts that had been mixed with ground alfalfa hay and consistently reproduced the abortifacient effects in a bovine model (Gardner et al., 1994). Thus, we report here an attempt to solvent extract two of the most populous snakeweed chemotypes, mix the extracts with ground alfalfa hay, and test the extracts for abortifacient activity in late-term pregnant cattle.
MATERIALS AND METHODS

Plant Material. Broom snakeweed plant material (*Gutierrezia sarothrae*) representative of chemotype 1 (see Gardner *et al.*, 2020 for chemotype descriptions) was collected from areas near Sublett, Idaho (42° 20’ 20” N, 113° 12’ 54” W) and Bothwell Utah (42° 43’ 28” N, 112° 13’ 24” W) during October 2020. *Gutierrezia microcephala*, representative of chemotype 2, was collected east of Carlsbad, NM (32° 33’ 17” N; 103° 43’ 38” W) and north of Roswell, NM (33° 56’ 11” N; 104° 35’ 40” W) during September 2018. The above ground plant material was cut, collected, and allowed to dry at ambient temperature and stored in covered areas at ambient temperature. Chemotype classification as either type 1 or type 2 was verified by GC-MS analysis (Gardner *et al.*, 2020).

Solvent Extraction and Dose Preparations. A single dose of snakeweed was set at 2.27 kg (5 lbs). Late-term pregnant cattle were scheduled to receive two doses/day for 10 days. Each dose was prepared as follows: the above ground plant material (2.27 kg) was extracted by steeping with methylene chloride (40 L) for 16 hrs. The solvent was drained and filtered through several layers of cheese cloth and then evaporated under reduced pressure at 50° C by use of a roto-evaporator. The extract was rinsed with methylene chloride, transferred to a glass container, and the volume adjusted to approximately 800 mL by addition of methylene chloride. This procedure was repeated 60 times for each chemotype to provide enough extract for the treatment of three cows dosed twice daily for ten days. The average mass of extract obtained from chemotype 1 extractions was 218 g/dose and was 264 g/dose for chemotype 2 plant material. One dose equivalent of extract in 800 mL of methylene chloride was mixed with 0.9 kg (2 lbs) of ground alfalfa hay. Any residual solvent was removed by air-drying in a fume hood at ambient temperature for 16 hrs. The extract/alfalfa hay mixtures were then ground through a 2 mm screen (Wiley mill) one additional time before treatment.

Animal Trials. The protocol for animal use in this research was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC #11404), Utah State University. The experimental procedure for testing abortifacient diterpene acids has been previously documented (Gardner *et al.*, 1994) and repeated here with the snakeweed extracts. Six pregnant Hereford cows (525 kg ± 42 kg; first calf heifers condition 9 - good) with known breeding dates and with no previous exposure to snakeweed plants, were divided into two groups of three cows and treated with either chemotype 1 or chemotype 2 extracts. The extract/alfalfa mixture was administered by oral gavage (hand pumped with stomach tube) to pregnant cows starting on day 250-252 of pregnancy, in two doses per day (morning and afternoon) for up to 10 days in the form of a water slurry as previously described (James *et al.*, 1994; Gardner *et al.*, 1994). During the experiment, the cattle were maintained on alfalfa hay with half a daily ration (a
daily ration consisted of approximately 2% of body weight) given to them after each oral dosing and free access to water. The parturition was considered to be premature (an abortion) if it occurred before 270 days of gestation and fetal membranes were retained for more than 12 hrs. Blood was collected via jugular venipuncture every morning prior to the first dosage of the day. Serum was separated from red blood cells by centrifugation and stored frozen at −20°C.

During dosing, cows were closely monitored for signs of potential abortion (vulvar enlargement) and rumen health [rumen contractions (frequency and strength)], rumen pH and smell. Normal cows have between two and four contractions/minute. Normal rumen material has a unique fermenting smell with a pH of about 7.0. Treatment of cows that develop rumen atony and acidosis characterized by markedly decreased contractions and force, decreased rumen pH, and a fetid rumen smell was discontinued and cows were treated to restore rumen flora and function. Rumen treatment included normalization of acidosis with sodium bicarbonate, electrolytes and fluid replacement, transfaunations and mild nicotinic stimulation with finely ground tobacco.

**Serum Analysis.** Sera samples were analyzed for detection of possible diterpene acid metabolites such as tetrahydroagathic acid which is a known metabolite of isocupressic acid and related diterpenes known to cause abortions in cattle (Gardner et al., 1999) with the following modifications. Sera (1.0 mL) was placed into an 8 mL screw cap vial and 1.0 ml of saturated KH₂PO₄ added and the samples extracted twice with 2 mL CHCl₃. The combined extracts were filtered through anhydrous sodium sulfate, the solvent removed by evaporation under nitrogen at 60°C. The samples were then dissolved in 0.200 mL of pyridine and 0.050 mL of BSTFA silylation reagent and heated at 60°C for 30 min. Samples were then transferred to an autosample vial with a low volume (0.300 mL) glass insert. The samples were then analyzed by GC-MS for the presence of diterpene acid metabolites. For comparison, the dosed plant material (alfalfa + snakeweed extract) (100 mg aliquot) was extracted with 4 mL of dichloromethane. A portion

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1 Bovine rumen stimulation/acidosis treatment: This therapy should be modified according to animal condition. If the rumen or urine pH indicates there is significant acidosis, the sodium bicarbonate can be increased. If the rumen is impacted the animal may not tolerate the entire dose and it should be treated with less water and mineral oil as indicated. The treatment may need to be divided and given twice or three times a day. Severe impaction lasting several days may require rumenotomy with complete evacuation of all ingesta, transfaunations and electrolyte replacement. Treatment by rumen gavage was done using a Frick oral speculum, a Springer Magrath pump, and a 2.5 M long 2 cm Tygon plastic tubing. When initially placing the tube, relieve as much rumen gas as possible. Mix the indicated treatments well in the bucket and slowly pump the indicated treatments into the rumen. Monitor the cow closely and discontinue treatment if the animal is distressed or regurgitates. The treatment cocktail included 2-3 kg finely ground alfalfa, 800 gm NaCl, 800 gm NaHCO₃, 100 gm water soluble vitamins, 12-16 L warm water. Optional reagents: 500 mL fresh rumen juice (if transfaunations is indicated), 1-2 quarts mineral oil, 500-600 ml propylene glycol, 200 gm finely ground loose shag tobacco.
of the sample extract was filtered, and a 0.200 mL aliquot was placed into a 7 mL vial and the solvent evaporated. Samples were then dissolved in 0.200 mL of pyridine and 0.050 mL of BSTFA silylation reagent and heated at 60°C for 30 min. Chloroform (0.800 mL) was added, and the samples were then transferred to an autosample vial for GC-MS analysis using the previously described GC-MS parameters (Gardner et al., 2020).

**Figure 1.** The major diterpene acids from the two chemotypes.

RESULTS AND DISCUSSION

The two most populous snakeweed chemotypes were selected for testing for abortifacient activity based on representing the chemistry for a large percentage of the snakeweeds and their geographic occurrence from the eastern New Mexico and western Texas rangelands from which most of the reports of proposed snakeweed induced abortions have been reported. The major diterpene acids from these two chemotypes are shown in Figure 1, and although they look similar, they differ in the configuration of the acid group at C-4 and the attached side chains.

**Chemotype 1 trial.** Three late-term pregnant cattle were treated with extracts from snakeweed chemotype 1 plant material (Table 1). These plants account for the largest percentage (47%) of any of the eight chemotypes. The first cow was dosed with 1.4 kg of hay/extract mixture or 218 g extract/dose. On day 3
(six doses), the rumen of this cow developed a foul odor, and had reduced/weak rumen contractions (< 1/min), so the trial was terminated. The animal was treated as directed by the attending veterinarian and though there was no impaction the rumen acidosis had damaged the rumen to the extent that its recovery was impossible, and the cow succumbed. At necropsy the rumen had massive mucosal necrosis with submucosal inflammation and fibrosis. Other tissues were diffusely congested with focal paragranulomas in the liver and kidneys suggestive of septicemia and toxemia. For the next two animals on chemotype 1, the dosage was cut in half (109 g extract/dose) and rumen function was closely monitored allowing termination of treatment at the initial signs of decreased function. Both animals received treatments for eight days after which the animals were taken off the treatments due to observed reduced rumen activity, decreased rumen pH, and the fetid smell of rumen material. Animals were treated as described with the acidosis and ruminate stimulus. Both recovered after several days of treatment and gave birth to normal calves after full gestation period (Table 1).

Table 1. In Vivo Treatment of Late-Term Pregnant Cattle with Snakeweed (Gutierrezia sarothrae and G. microcephala) Diterpenoid Extracts.

<table>
<thead>
<tr>
<th>Animal I.D.</th>
<th>Extract Dosed* (g/dose)</th>
<th>Weight (Kg)</th>
<th>Total Doses</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>18861</td>
<td>Type-1 (109 g)</td>
<td>496</td>
<td>6</td>
<td>Rumen complications</td>
</tr>
<tr>
<td>18874</td>
<td>Type-1 (109 g)</td>
<td>462</td>
<td>16</td>
<td>Rumen complications</td>
</tr>
<tr>
<td>18867</td>
<td>Type-1 (109 g)</td>
<td>526</td>
<td>15</td>
<td>Rumen complications</td>
</tr>
<tr>
<td>18875</td>
<td>Type-2 (264 g)</td>
<td>550</td>
<td>7</td>
<td>Rumen complications from straw hay impaction.</td>
</tr>
<tr>
<td>18870</td>
<td>Type-2 (264 g)</td>
<td>536</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>18871</td>
<td>Type-2 (264 g)</td>
<td>582</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

* Extracts from snakeweed plant material (Gutierrezia sarothrae) representative of chemotype 1 and G. microcephala, representative of chemotype 2 (see Gardner et al., 2020 for chemotype descriptions).

**Animal died before parturition. Any animal with rumen complications was treated as outlined in the methods and materials.
Chemotype 2 trial. Three late-term pregnant cattle were treated with extracts from snakeweed chemotype 2. These plants account for the second largest percentage (13%) of the plants among the chemotypes found. All cows were treated with a dose of 264 g/dose twice daily. The initial cow dosed received only 7 doses. During dosing she also developed rumen and gastrointestinal impairment that was seen as decreased rumen contraction frequency and force (less that one weak contraction per minute). She also developed pica eating large quantities of straw bedding material. This resulted in rumen impaction and subsequent acidosis. The bedding material in the pens was removed, and exchanged for wood shavings. With extensive acidosis and rumen stimulant therapy this animal recovered. Wood shavings were used as bedding for all other animals. The other two pregnant cows on the trial received all 20 doses. No abortions were observed, and all animals gave birth to normal calves after the full gestational period (Table 1).

Analysis of Sera Samples for Abortifacient Metabolites. Sera samples collected before an animal was dosed (blank sample) and then for each day of dosing and were analyzed by GC-MS for the presence diterpene acids and possible metabolites. A sample of the extract/hay mixture from both chemotypes was also analyzed for comparison to the sera samples. Diterpene acids were detected in sera samples within 24 h after the first dose (Figure 2). Compounds 2, 3 and 4 appear not be metabolized and are found unchanged in the sera. Compound 1 is absent in the sera and presumably is metabolized to an unknown metabolite (Met-A). However, the detected metabolite is not tetrahydroagathic acid (THAA) as it possesses a different GC retention time and different mass spectrum. The similarities of Met-A with THAA might suggest it has abortifacient activity; however, no abortions were observed in the dosing of chemotype 1. Sera obtained from animals dosed chemotype 2 plant material also contained diterpene acids. No metabolism of the major diterpene acid (compound 6) was detected (Figure 2B) and no abortions occurred.

Broom snakeweed has historically been linked to poisoning and abortion in livestock. Initial feeding trials reported gastrointestinal toxicity seen as loss of appetite, colic, diarrhea and hematuria (Matthews, 1936). Latter Dollahite and coworkers repeated these trials in various livestock reporting additional gastrointestinal changes including constipation, vulvar swelling, udder development, and abortion (1956, 1957 and 1959). These studies lead to the inclusion of snakeweed as a poisonous plant that has been associated with abortion in livestock in several different texts (Kingsbury, 1964 and Burrows and Tyrl, 2013). Anecdotal evidence of snakeweed supported these conclusions as producer surveys suggested that heavy snakeweed infested ranges were reported to have 16.6% abortions compared to abortion rates of 0.6% in snakeweed free ranges (Carpenter et al., 1990). As infestation in many ranges is extensive, it is estimated that snakeweed costs millions of dollars in animal losses each year (Torell et al.,
Figure 2. Analyses of Snakeweed Extracts and Corresponding Sera from dosed animals. Peak numbers correspond to the identified diterpene acids (Figure 1).

A. Chemotype – 1 Extract and Sera

B. Chemotype – 2 Extract and Sera
1984). In spite of this evidence, further identification of broom snakeweed abortifacient compounds has been elusive. In multiple studies using sheep, goats, cattle, various snakeweed populations, and chemotypes, abortions have not been reproduced and a dependable model to study toxicity has not been developed (unpublished data from various laboratories). As the described late term snakeweed associated abortions were similar to those described in pine needle induced abortion, it has been hypothesized that the toxins are similar. The snakeweed chemotypes were selected as they contained the diterpene acids similar to pine needles. Extracts of the plants were used as previous studies using whole ground plant material were difficult to dose to cattle and all caused severe damage to rumen microflora and function (unpublished data).

Conclusion. Experimental trials appear to be unable to reproduce snakeweed induced abortions in cattle. Snakeweed chemotype 1 plants appear to be quite toxic to the rumen and may cause complete rumen stasis after 2-3 days of heavy snakeweed consumption. In contrast, cattle receiving chemotype 2 plants were able to tolerate over twice the dosage as that given for chemotype 1 plants. We conclude that broom snakeweed plants are unlikely to be directly responsible for cattle abortions observed in cattle grazing snakeweed invested rangelands. It is more likely that cattle may be affected by rumen toxicity and/or might suffer from poor nutritional factors given the lack of quality forage that might be available on rangelands with high snakeweed infestation. Body condition may be a factor in toxicity-abortion for those animals grazing snakeweeds. It is not uncommon that cattle grazing snakeweed infested rangelands may not be in the best condition due to the lack of high-quality forage. Pfister et al., (2008) did show that cattle with lower body scores would graze more pine needles than cattle with higher body scores. However, a direct correlation between body score, diterpene acid dose and abortion has not been demonstrated. We have clearly demonstrated previously the abortifacient activity of isocupressic acid in ponderosa pine needles in cattle that were scored good or better (Gardner et al., 1994), and thus would have expected if the snakeweed diterpene acids were abortifacient they would have induced abortions under the same experimental conditions.

REFERENCES


