Experimental poisoning by Baccharis megapotamica var. weirii in buffalo

Oliveira-Filho, José C.; Carmo, Priscila M.S.; Iversen, Anita; Nielsen, Kristian Fog; Barros, Claudio S. L.

Published in: Pesquisa Veterinária Brasileira

Publication date: 2012

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Experimental poisoning by *Baccharis megapotamica* var. *weirii* in buffalo

José C. Oliveira-Filho², Priscila M.S. Carmo², Anita Iversen³, Kristian F. Nielsen³ and Claudio S.L. Barros⁴


Five male 6-8 month-old Murrah buffalo calves were orally dosed with the fresh aerial parts of *Baccharis megapotamica* var. *weirii* at doses of 1, 3, 4, 5 and 10g/kg body weight (bw) (~1-10mg macrocyclic trichothecenes/kg/bw). The *B. megapotamica* used for the experiment was harvested on a farm where a recent spontaneous outbreak of poisoning caused by such plant had occurred. Clinical signs appeared 4-20 hours and 4 buffaloes died 18-49 hours after the ingestion of the plant. Clinical signs were apathy, anorexia, and watery diarrhea, fever, colic, drooling, muscle tremors, restlessness, laborious breathing and ruminal atony, and dehydration. The most consistent gross findings were restricted to the gastrointestinal (GI) tract consisted of varying degrees of edema and reddening of the mucosa of the forestomach. Histopathological findings consisted of varying degrees of necrosis of the epithelial lining of the forestomach and of lymphocytes within lymphoid organs and aggregates. Fibrin thrombi were consistently found in sub-mucosal vessels of the forestomach and in the lumen of hepatic sinusoids. It is suggested that dehydration, septicemia and disseminated intravascular coagulation participate in the pathogenesis of the intoxication and play a role as a cause of death. A subsample of *B. megapotamica* var. *weirii* was frozen-dried and ground and analyzed using UHPLC (Ultra High Performance Liquid Chromatography) with high resolution Time of Flight mass spectrometry and tandem mass spectrometry, it was shown that the plant material contained at least 51 different macrocyclic trichothecenes at a total level of 1.1-1.2mg/g. About 15-20% of the total trichothecenes contents was found to be monosaccharide conjugates, with two thirds of these being glucose conjugates and one third constituted by six aldopentose conjugates (probably xylene), which has never been reported in the literature.

INDEX TERMS: Poisonous plants, *Baccharis megapotamica*, buffalo, experimental plant poisoning, necrosis in forestomach, lymphoid tissue necrosis, macrocyclic trichothecenes, chemical analysis.

---

¹ Received on December 12, 2011.
² Accepted for publication on December 29, 2011.
³ Part of the Doctoral Thesis of the senior author. Part of this study was carried out in the Centro de Ciências e Tecnologia Rural, of the Federal University of Campina Grande, Patos, Paraíba. During a 12-month fellowship granted to the first author by the Program on Academic collaboration (PROCAD-NF) from CAPES. And the costs of the present publication were covered by the same source.
⁴ Post-Graduate Program in Veterinary Medicine, Major in Veterinary Pathology, Centro de Ciências Rurais (CCR), Universidade Federal de Santa Maria (UFSM), Camobi, Santa Maria, RS 97105-900, Brasil.
⁵ Center for Microbial Biotechnology, Institute for Systems Biology, Technical University of Denmark, Søltofts Plads, Building 221, DK-2800 Kgs. Lyngby, Denmark.
⁶ Laboratório de Patologia Veterinária, Departamento de Patologia, Centro de Ciências da Saúde, UFSM, Santa Maria, RS 97105-900. Pesquisador 1A do CNPq. *Corresponding author: claudioslbarros@uol.com.br
INTRODUCTION

The Baccharis genus (Asteraceae: tribe Asteraceae) includes nearly 500 species. All are found in the New World with the exception of B. halimifolia, which was introduced into Australia from the United States (Jarvis et al. 1991). This species is suspected of poisoning cattle in both countries (Everist 1981) and proved toxic when administered experimentally to chicks (Duncan et al. 1957). B. glomerulifolia, another North American species, was experimentally toxic to mice and chicks (Duncan et al. 1957), and B. pteronioioides has been associated with cattle poisoning in the southwestern United States (Marsh et al. 1920, Stegelmeier et al. 2009). B. pteronioioides toxicity was produced in hams dosed with 100-200mg of the plant (Steggemeier et al. 1957). B. artemioides causes disease in cattle in a restricted zone of Argentina, northwest of Buenos Aires and southeast of Cordoba (Rizzo et al. 1997).

Nearly 120 species of Baccharis have been recorded in Brazil; of those, only B. coridifolia (Tokarnia & Döbereiner 1975, Barros 1998) and B. megapotamica (Tokarnia et al. 1992, Driemeier et al. 2000, Pedroso et al. 2010) have been proven to be toxic to livestock. Both B. megapotamica and B. coridifolia are found in southern Brazil, but they occupy different habitats; B. megapotamica is found in marshy areas (Tokarnia et al. 1992) whereas B. coridifolia grows in pastureland (Barros 1998). Two varieties of B. megapotamica with essentially the same distribution and toxic effects on livestock are known, namely B. megapotamica var. megapotamica and B. megapotamica var. weirii (Tokarnia et al. 1992).

B. coridifolia and the two varieties of B. megapotamica cause a severe acute poisoning in livestock characterized by degeneration and necrosis of the epithelial lining of gastrointestinal tract and necrosis of lymphocytes in lymph nodes, spleen, tonsils, and several lymphoid aggregates (Tokarnia & Döbereiner 1975, Tokarnia et al. 1992, Barros 1998, Varaschin et al. 1998, Varaschin & Alessi 2003). B. megapotamica (Kupchan et al. 1977) B. coridifolia, (Busam & Habermehl 1982, Habermehl et al.1985) and B. artemioides (Rizzo et al. 1997) contain a series of potent cytotoxic agents belonging to the highly cytotoxic macrocyclic trichothecone complex previously believed to be produced only by fungi (Jarvis et al. 1996). In the case of B. megapotamica, the macrocyclic trichothecones accumulate in the plant as baccharinoids (B1, B2, B3, B4 etc.), roridins including their glycosides, and miotoxins (Jarvis et al. 1996). To date, no macrocyclic trichothecones have been detected in B. halimifolia, B. pteronioioides, or B. glomerulifolia.

Spontaneous poisoning by B. coridifolia occurs frequently in cattle (Rissi et al. 2005) occasionally in sheep (Rozza et al. 2006) and rarely in horses (Alda et al. 2009). Isolated reports of spontaneous outbreaks involving B. megapotamica var. weirii have been reported in cattle (Driemeier et al. 2000) sheep (Pedroso et al. 2010) and buffaloes (Oliveira-Filho et al. 2011). There are also some anecdotal accounts of spontaneous toxicosis by B. megapotamica var. weirii in cattle. Typically, the toxicosis in livestock occurs when naive animals raised in areas free of Baccharis spp. are transferred to pastures infested by the plant. The susceptibility increases considerably if the animals are subjected to such stress factors as fatigue, hunger, or thirst (Barros 1998). Interestingly, cattle that are raised in pastures where Baccharis spp. exist will graze it very rarely if ever, although in the case of B. megapotamica var. weirii there are anecdotal accounts that particularly hungry cattle familiar with the plant, may, on occasion, ingest it and get poisoned.

A recent outbreak of B. megapotamica var. weirii poisoning in buffalo diagnosed at our laboratory (Oliveira-Filho et al. 2011) prompted the undertaking of the current experimental study to determine the clinical and pathological aspects of the B. megapotamica var. weirii poisoning in buffaloes, the pathogenesis of the toxicosis and the toxic principles involved in this plant.

MATERIALS AND METHODS

Five male 6-8-month-old, 122-143 kg Murrah buffaloes identified by numerals 1-5 were used in the experiment. Each buffalo was force-fed orally with a single dose of fresh Baccharis megapotamica var. weirii respectively at doses of 1, 3, 4, 5 and 10g/body weight (bw) (Table 1). Only the top 10cm of the aerial parts of the plant were fed to the buffaloes. Just before the administration of the plant and every four hour after the dosing, the buffaloes were clinically evaluated for the following parameters: respiratory and cardiac rates, rectal temperature, time of capillary filling, ruminal movements, posture, ambulation and behavioral changes. During the whole duration of the experiment the buffalo were kept in a fenced paddock and were offered Tifton hay and water ad libitum.

For the experiment, specimens of B. megapotamica var. weirii were harvest in a farm in the municipality of Diler-
For chemical analysis plant material was frozen-dried and ground to a fine powder using a domestic coffee mill; then 0.50±0.01g material was distributed into 15ml Falcon tubes. 6.5ml 55% MeOH was added and the tubes placed on a shaking table for 4 hr. Tubes were then centrifuged at 7000 g for 3 minutes and 4.5ml of the supernatant transferred to new tubes and evaporated to dryness with N2. Samples were then redissolved in 50% methanol and filtered through a 0.45µm teflon syringe filter into an autosampler vial.

Quantification was done in spiked matrix, by spiking ground freeze dried material with 75 µl methanol solution containing roridin A (leaving to dry for 2 hr) to a final concentration of 375, 188, 94, 47, 23, 12, 5.9, 2.9, 1.5, 0.73, and 3.0 mg/kg, as well as 3 un-spiked matrix samples.

Table 1. Experimental poisoning by Baccharis megapotamica var. weirii in buffalo

<table>
<thead>
<tr>
<th>Buffalo</th>
<th>Weight (kg)</th>
<th>Dose of plant administered (g/kg)a</th>
<th>Time spent in the administration of the plant</th>
<th>Severity of clinical signs</th>
<th>Time between the termination of the ingestion of the plant and death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>143</td>
<td>10g</td>
<td>2h15min</td>
<td>+++b</td>
<td>18h30min</td>
</tr>
<tr>
<td>2</td>
<td>132</td>
<td>5g</td>
<td>55 min</td>
<td>+++</td>
<td>19h45min</td>
</tr>
<tr>
<td>3</td>
<td>135</td>
<td>4g</td>
<td>1h05min</td>
<td>+++</td>
<td>49h5min</td>
</tr>
<tr>
<td>4</td>
<td>122</td>
<td>3g</td>
<td>1h33min</td>
<td>+++</td>
<td>21h40min</td>
</tr>
<tr>
<td>5</td>
<td>146</td>
<td>1g</td>
<td>30 min</td>
<td>+c</td>
<td>Survived</td>
</tr>
</tbody>
</table>

*a All administrations of the plant were in single doses, (+) (+++) marked, (+) mild.

RESULTS

Chemical analysis of the plant revealed 51 one different major macrocyclic trichothecenes; the major peaks are...
shown in Figure 1. All five treated buffalo showed clinical signs and four of them died. Data regarding the time elapsed from the ingestion of the plant, the severity of clinical signs showed by each buffalo and the time elapsed between

Figure 1. Chemical analysis of the plant *Baccharis megapotamica* var. *weirii* used in the experiment. Base peak chromatogram (black m/z 400-900) overlaid with extracted ion chromatograms of the [M+H]^+ ion the macrocyclic trichothecenes and the [M+NH_4]^+ of the glycosylated/xylanated derivatives, showing that in this crude extract most the major peaks in this time frame are macrocyclic trichothecenes.

ingestion of the plant and death of the animal are on Table 1. The onset of clinical signs varied from 4 hours (Buffalo 1) up to 20 hours (Buffalo 5) after the ingestion of the plant. The time elapsed from the ingestion of the plant to the death of the animal varied from 18 hours and 30 minutes (Buffalo 1) to 49 hours and 5 minutes (Buffalo 3).

The first observed clinical signs were apathy, anorexia, and watery profuse diarrhea (Fig.2). Fever was observed in all experimental buffalo, except Buffalo 5, and reached 40.9°C in Buffalo 1. The clinical signs evolved rapidly to colic (tenesmus), drooling (Fig.3), muscle tremors, restlessness, and loss of strength of ruminal movements eventually terminating in complete ruminal atony, laborious breathing, and dehydration, seen as sunken eyes in the orbit pockets and loss of normal cutaneous turgidity. Tachycardia and increased time of capillary filling were also observed. With the exception of Buffalo 5, the capillary filling time was up to 5 minutes. Of the 5 buffaloes fed *B. megapotamica* var. *weirii*, only Buffalo 5 which was fed 1g/kg/bw of the plant, survived after running a short clinical course consisting of moderate liquid diarrhea and apathy. After 48 hours of the onset of the clinical signs this buffalo recovered and was euthanized in the following day for necropsy.

The most consistent gross findings were restricted to the gastrointestinal (GI) tract and consisted of varying degrees of edema and reddening of the mucosa of the forestomachs especially of the rumen (Fig.4) and reticulum (Fig.5). Mucosal reddening of the rumen was more intense in the cranial pillar (3 out of 5 buffalo examined), ruminal antrum (3/5), coronary pillar (1/5) and dorsal sac (1/5). Marked edema was observed in the reticulo-ruminal fold (3/5) and omasum (1/5). Petechiae and paint-brush hemorrhages were observed, mainly in the dorsal sac (3/4 buffalo).

Varying sized recent ulcers were observed in the abomasum of Buffalo 1 ad 3. In all the four buffalo that died spontaneously diffuse reddening was observed in the mucosa of the duodenum, jejunum, ileum, and cecum. In this latter viscus dark-red fetid content was found. Additional, in Buffalo 2 and 4, similar reddening was observed in the mucosa of spiral colon. Linear ulcers were observed in the distal third of the esophagus in Buffalo 4. Buffalo 2-4 had enlarged gastric and jejunal lymph nodes which were red and juice to the cut surface.

Histopathological findings in the forestomach consisted of varying degrees of necrosis of the epithelial lining. This variation occurred from animal to animal and even within the same animal. In some instances only the basal layer was affected (Fig.6), in others both the basal and squamous layer were affect and still in other the whole thickness of the ruminal squamous stratified epithelium was affected (Fig.7). These changes were associated with hyperemia, edema, and inflammatory infiltrate predominantly neutrophilic, bacterial aggregates in the submucosa. Bacterial ag-
Experimental poisoning by *Baccharis megapotamica* var. *weirii* in buffalo

gregates were found over the forestomach epithelial deprived submucosa and in one case surrounding blood vessels of the submucosa (Fig.8). Fibrin thrombi were consistently found in submucosal vessels of the forestomach and in the lumen of hepatic sinusoids. These thrombi were positive for fibrin by the Fraser-Lendrum method (Fig.9).
The necrosis in the epithelial lining of the forestomach were more intense in the following order of decreasing intensity: reticular fold, ruminal ventral sac, ruminal caudal ventral blind sac, ruminal cranial pillar, reticulum, caudal dorsal blind sac, dorsal sac, and omasum. In reticular fold of one animal (Buffalo 1) there was also necrosis of the smooth muscle layer beneath the areas of epithelial necrosis. Mild necrosis (Buffalo 2 and 3) were observed in the parietal cells of the abomasal mucosa.

Hepatic necrosis was observed in the four buffalos that died due to the intoxication. It consisted of multifocal individual foci of necrosis or individual hepatocellular necrosis. In on animal (Buffalo 3) the necrosis was more prominent in the hepatocytes adjacent to the portal triads. In two cases (Buffalo 1 and 3) diffuse moderate cytoplasmic vacuolization was observed.

Necrosis of lymphocytes (Fig.10) was observed in all four buffalos dying from the intoxication and in all lymphoid organ/tissues sampled. The intensity of lymphocyte necrosis was dose dependent and varied depending of the type of lymphoid tissue examined, being more prominent in the gut associated lymphoid aggregates and in the jejunal and mesenteric lymph nodes.

**DISCUSSION**

The clinical signs, clinical course, gross findings and histopathology observed in the buffalo of the current study are similar to those described in the naturally occurring *Baccharis megapotamica* var. *weirii* poisoning by in buffalo (Oliveira-Filho et al. 2011) in cattle (Driemeier et al. 2000) and sheep (Pedroso et al. 2010) and the experimental intoxication in cattle with this plant species (Tokarnia et al. 1992). The lesions are also similar to those produced by *B. cordifolia* in cattle (Tokarnia & Döbereiner 1975, Barros 1998, Rissi et al. 2005) and the experimental intoxication was dose dependent and varied depending of the type of lymphoid tissue examined, being more prominent in the gut associated lymphoid aggregates and in the jejunal and mesenteric lymph nodes.

**Table 2. Laboratory data from blood of five buffalo poisoned by Baccharis megapotamica var. weirii in buffalo**

<table>
<thead>
<tr>
<th>Buffalo</th>
<th>PCV</th>
<th>Total plasma protein</th>
<th>AST</th>
<th>FDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>AE</td>
<td>PE</td>
<td>AE</td>
<td>PE</td>
</tr>
<tr>
<td>1</td>
<td>39</td>
<td>48</td>
<td>6.5</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>42</td>
<td>7.2</td>
<td>8.9</td>
</tr>
<tr>
<td>3</td>
<td>39.5</td>
<td>54.5</td>
<td>7.1</td>
<td>7.8</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>54</td>
<td>7.3</td>
<td>9.4</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>37</td>
<td>7.5</td>
<td>7.1</td>
</tr>
</tbody>
</table>

*Packed cell volume, †aspartate transaminase, ‡fibrin degradation products, °pre-experiment sample, † sample taken after the administration of the plant a just hours before death of the buffalo.

Fig.10. Histopathology showing necrosis of lymphoid lymphocytes in follicles of the lymphoid tissue in experimentally poisoning by *Baccharis megapotamica* var. *weirii* in buffalo. (A) Internal iliac lymph node of Buffalo 3. HE, obj.40x. (B) Tonsil of Buffalo 2. HE, obj.20x.
necrosis endothelial cells and hepatocytes is a distinct possibility, resulting in tissue factor-induced activation of extrinsic coagulation to produce thrombin. Thrombin causes platelet aggregation and activation of coagulation factors V, VIII, and I to form fibrin, could result in the widespread microvascular clots observed in threes cases. Concurrently, the high levels of thrombin stimulate clot dissolution by binding to thrombomodulin to activate protein C, by converting plasminogen into plasmin, and by binding to anti-thrombin III to become inactivated. The widespread nature of the coagulation response results in the consumption of these and other factors, resulting in widespread hemorrhages. Although hemorrhages were not seen in the buffalo of this study, this could be explained by the extremely short course of the disease.

Evidences of septicaemia could be observed in Buffalo 1 from which bacterial culture of the blood yield non-enterococci Streptococcus Group; Bacteria of this group D can cause septicaemia, among other clinical dysfunctions (Greene & Prescott 2006). Some findings in the experimental buffalo of this study as fever and bacterial aggregates surrounding blood vessels or associated with b reached ruminal epithelium are consistent with septicaemia plain a role in this intoxication.

UHPLC with high resolution Time of Flight mass spectrometry proved to be a powerful technique for the analysis of the macrocyclic trichothecenes in plant extracts detecting 51 different major macrocyclic trichothecenes. The major findings were Baccharin B2/B1/4, Baccharinoid B12/B17, iso-baccharin/baccharin and the conjugate xylo-side-roridin L-2. Out of 14 available trichothecene standards only 2 (roridins E and A) could be detected in the samples. Trichothecenes found in the sample with no possibility of standard matching were identified tentatively on the basis of the MS/MS with spectra showing a fragmentation pattern (accurate mass) consistent with macrocyclic trichothecenes (Nielsen et al. 2011). These identification points included several water loss ions as well as the m/z 231 and 249 ions seen from macrocyclic trichothecenes, or m/z 229 and 247 seen in case of hydroxylation of the trichothecene skeleton. Interestingly neither verrucarins nor roridin L-2 were detected. Expressed as roridin A equivalents the 51 macrocyclic trichothecenes summed up to a total content of 1.1-1.2mg/g which is in the same range found by Jarvis et al. (1996) where 0.04-0.7mg/g was detected. Using MS/HRMS and in-source fragmentation, 15-20% of the total trichothecenes contents was found to be conjugated to a glucose (seen by the losses of a glucose moiety) also found by Jarvis et al. (1996) and one third constituted by 6 aldopentose conjugates, probably xylose conjugates, which was also partly confirmed by better retention of these compared to their glucose analogues. These aldopentose derivatives have to our knowledge has never been reported in the literature. All these monosaccharide derivatives could easily be identified as they only produced [M+NH₄⁺] and [M+Na⁺] pseudomolecular ions, whereas the normal trichothecenes produced these as minor ions and [M+H⁺] as the major ion. The monosaccharide conjugate fraction of 15-20% fits well with the finding of Jarvis et al. (1996), and toxicology wise it is not known if these are toxic in vivo as the case for deoxynivalenol-3-glucoside (Berthiller et al. 2011).

Acknowledgements.- To Dra. Raquel Rubia Rech for technical assistance with photography.

REFERENCES


Olivera-Filho J.C., Carmo P.M.S., Lucena R.B., Pierezan F. & Barros C.S.L.


