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):
Experimental poisoning by Baccharis megapotamica var. weirii in buffalo

José C. Oliveira-Filho, Priscila M.S. Carmo, Anita Iversen, Kristian F. Nielsen and Claudio S.L. Barros*


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, labious 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 xylose), 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.
macroscópicos mais consistentes estavam restritos ao trato gastrointestinal (GI) e consistiram de graus variados de edema e a vermelhamento da mucosa dos pré-estômagos. Os achados histopatológicos consistiam de vários graus de necrose do epitélio de revestimento dos pré-estômagos e de linfócitos em agregados e órgãos linfoides. Trombos de fibrina foram consistentemente encontrados nos vasos da submucosa dos pré-estômagos e na luz dos sinusoides hepáticos. É sugerido que desidratação, septicemia e coagulação intravascular disseminada participem da patogênese da intoxicação e sejam fatores responsáveis pela morte dos animais afetados pela intoxicação. Uma subamostra de B. megapotamica var. weirii foi congelada a seco, moída e analisada usando UHPLC (Cromatografia Líquida de Ultra Alta Performance) com espectrometria de tempo-de-vôo de alta resolução e espectrometria de massa em tandem. Foi demonstrado que o material de planta analisado continha pelo menos 51 tricotopecos macrocíclicos diferentes num nível total de 1,1-1,2mg/g. Cerca de 15-20% do conteúdo total de tricotopecos eram conjugados de monossacarídeos, sendo dois terços desses, conjugados de glicose e um terço constituídos por seis conjugados de aldopentose (provavelmente xilose), o que nunca tinha sido antes relatado na literatura.

TERMOS DE INDEXAÇÃO: Plantas tóxicas, Baccharis megapotamica, búfalo, intoxicação experimental por planta, necrose dos pré-estômagos, necrose do tecido linfóide, tricotopecos macrocíclicos, análise química.

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. pteroniioides has been associated with cattle poisoning in the southwestern United States (Marsh et al. 1920, Stegelmeier et al. 2009). B. pteroniioides toxicity was produced in hams ters dosed with 100-200mg of the plant (Stegelmeier et al. 2009). B. artemisioides causes disease in cattle in a restricted zone of Argentina, northwest of Buenos Aires and southeast of Córdoba (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. artemisioides (Rizzo et al. 1997) contain a series of potent cytotoxic agents belonging to the highly cytotoxic macrocyclic trichothecene complex previously believed to be produced only by fungi (Jarvis et al. 1996). In the case of B. megapotamica, the macrocyclic trichothecenes 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 trichothecenes have been detected in B. halimifolia, B. pteroniioides, 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-
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)*</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>+++<em>h</em></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>+*</td>
<td>Survived</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Dose of plant administered (g/kg)*</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>10g</td>
<td>2h15min</td>
<td>+++<em>h</em></td>
<td>18h30min</td>
</tr>
<tr>
<td>5g</td>
<td>55 min</td>
<td>+++</td>
<td>19h45min</td>
</tr>
<tr>
<td>4g</td>
<td>1h05min</td>
<td>+++</td>
<td>49h5min</td>
</tr>
<tr>
<td>3g</td>
<td>1h33min</td>
<td>+++</td>
<td>21h40min</td>
</tr>
<tr>
<td>1g</td>
<td>30 min</td>
<td>+*</td>
<td>Survived</td>
</tr>
</tbody>
</table>

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

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-

![Fig.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+NH4]+ of the glycosylated/xylanated derivatives, showing that in this crude extract most the major peaks in this time frame are macrocyclic trichothecenes.](image1)

![Fig.2. Buffalo 2, affected in the experimental poisoning by Baccharis megapotamica var. weirii. Watery profuse diarrhea like the one illustrated here was one of the first clinical signs observed in the affected buffalo.](image2)

![Fig.3. Drooling in Buffalo 3 experimentally poisoned by Baccharis megapotamica var. weirii.](image3)
Experimental poisoning by *Baccharis megapotamica* var. *weirii* in buffalo

Gregarious 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 blind sac, ruminal cranial pillar, reticulum, caudodorsal 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 buffaloes 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 buffaloes 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. coridifolia* in cattle (Tokarnia & Döbereiner 1975, Barros 1998, Rissi et al. 2005) and sheep (Rozza et al. 2006). Mild intoxication was induced by a dose as small as 1g/kg/bw; however the smallest lethal dose of tested dose was 3g/kg/bw. This indicates that buffalo are somewhat more resistant to poisoning by *B. megapotamica* var. *weirii* than cattle (*Bos taurus*) since an oral dose of 1g/kg/bw of this plant was lethal to bovine (Tokarnia et al. 1992).

The primary target organs in this poisoning are those of GI, especially the forestomach and the lesions are grossly characterized by reddening of the mucosal surface of these gastric compartments and submucosal edema. Characteristic histopathology includes necroses of the epithelial lining of the forestomach and to a lesser extent abomasum and intestines and necrosis of lymphocytes in lymphoid organs and lymphoid aggregates. These lesions are in accordance with the described fort the action of macrocyclic trichotheecenes (Jarvis et al. 1996, Varaschin et al. 1998, Varaschin & Alessi 2003).

The pathogenesis and cause of death in *Baccharis* spp. in livestock was never completely resolved (Barros 1998, Rissi et al. 2005). It was suggested that dehydration, electrolyte imbalance and septicemia stemming from bacterial invasion through breakdowns in necrotic ruminal epithelium (Rissi et al. 2005) and disseminated vascular coagulation (Stegelmeier et al. 2009) could play a part. Data from the current experiment suggest that several of these mechanisms of disease production could act concomitantly or in sequence in the pathogenesis and cause of death of buffalo experimentally poisoned by *B. megapotamica* var. *weirii*. Dehydration was clearly a participante as judged by the hemoconcentration as demonstrated in Table 2 by radi and marked elevation in the packed cell volumes and total protein concentration in the serum. These are classical signs of dehydration (Smith and & Magdesian 2002, Fettman 2006) and were not observed only in the one buffalo (Buffalo 5) which developed just a mild form o intoxication and survived.

Evidences of disseminated intravascular coagulation (DIC), a serious manifestation of abnormal coagulation (Morris 2002) was observed in all experimental animals excepting Buffalo 5. This was reflected in the sharp increase in fibrin degradation products which were detected in the serum of the four buffaloes that died. DIC is a severe break down in the hemodynamic homeostasis caused by the generation of excess thrombin. There are many causes, inducing diffuse vascular damage which results in exposure of blood to tissue factor (Mosier 2011). In the case of the four buffalo that died it was possible to detect thrombi formation in the liver by the method of Fraser-Lendrum and those are associated with foci of liver necrosis; thus generation of tissue factor in response to

![Image](image-url)

**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 a</th>
<th>Total plasma protein</th>
<th>AST b</th>
<th>FDP c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE</td>
<td>AE</td>
<td>PE</td>
<td>AE</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>

a Packed cell volume, b aspartate transaminase, c fibrin degradation products, d pre-experiment sample, e sample taken after the administration of the plant a just hours before death of the buffalo.

---

José C. Oliveira-Filho et al.

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 threees 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 septicemia 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 septicemia, 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 septicemia 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-soridin 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 soridin L-2 were detected. Expressed as soridin 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 loses 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., Pierzian F. & Barros C.S.L.


