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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

**ABSTRACT.** Experimental poisoning by *Baccharis megapotamica* var. *weirii* in buffalo. Pesquisa Veterinária Brasileira 32(5):383-390. Departamento de Patologia, Universidade Federal de Santa Maria, Camobi, Santa Maria, RS 97105-900, Brazil. E-mail: claudioslbarros@uol.com.br

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 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.

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**RESUMO.** [Intoxicação experimental por *Baccharis megapotamica* var. *weirii* em búfalos.] As partes aéreas verdes de *Baccharis megapotamica* var. *weirii* foram administradas oralmente a cinco búfalos da raça Murrah de 6-8 meses de idade nas doses de 1, 3, 4, 5 e 10g/kg de peso corporal (pc) (~1-10mg de tritocetenos macrocíclicos/kg/pc). A planta usada no experimento foi colhida numá fazenda onde um surto recente de intoxicação espontânea por essa planta havia ocorrido. Nos búfalos deste experimento, os sinais clínicos apareceram 4-20 horas e 4 búfalos morreram 18-49 horas após a ingestão da planta. Os sinais clínicos consistiram de apatia, anorexia, diarreia aquosa, febre, cólica, salivação, tremores musculares, inquietação, respiração laboriosa, atonia ruminal e desidratação. Os achados
macrophages more consistent were restricted to the
lungs, gastrointestinal (GI) and consisted of muscle mass and
atherosclerotic plaques of the arterial vessels. Effects of
various drugs on the GIs are known, including changes in
growth and development of the gastrointestinal tract and
necrosis of lymphocytes in lymph nodes, spleen, tonsils, and
Soides (Rizzo et al. 1997) contain a series of potent cyto
toxic 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. megapo
	amica, the macrocyclic trichothecenes accumulate in the
plant as baccharinoids (B1, B2, B3, B4 etc.), roridins includ
ing their glycosides, and miotoxins (Jarvis et al. 1996). To
date, no macrocyclic trichothecenes have been detected in
B. halimifolia, B. pteronioides, or B. glomerulifolia.

Spontaneous poisoning by B. coridifolia occurs fre
quently 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. me
gapotamica var. weirii have been reported in cattle (Drie
meier 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 suscep
tibility increases considerably if the animals are subjec
ted to such stress factors as fatigue, hunger, or thirst (Bar
ros 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 poison
ning in buffalo diagnosed at our laboratory (Oliveira-Filho et al. 2011) prompted the undertaking of the current expe
rimetal study to determine the clinical and pathological
aspects of the B. megapotamica var. weirii poisoning in bu
falo, the pathogenesis of the toxicosis and the toxic prin
ciples involved in this plant.

INTRODUCTION

The Baccharis genus (Asteraceae: tribe Asteraceae) inclu
des 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 expe
rimentially 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. pteronioidi
des has been associated with cattle poisoning in the south
western United States (Marsh et al. 1920, Stegelmeyer et al.
2009). B. pteronioides toxicosis was produced in hams
ters dosed with 100-200mg of the plant (Stegelmeyer et al.
2009). B. artemioides causes disease in cattle in a restric
ted zone of Argentina, northwest of Buenos Aires and sou
theast of Cordoba (Rizzo et al. 1997).

Nearl 120 species of Baccharis have been recorded in
Brazil; of those, only B. coridifolia (Tokarnia & Dobeneiner
1975, Barros 1998) and B. Megapotamica (Tokarnia et al.
1992, Driemeier et al. 2000, Pedroso et al. 2010) have been
proved 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. mega
apotamica with essentially the same distribution and toxic
effects on livestock are known, namely B. megapotamica var. megapotamica and B. megapotamica var. weirii (Tokar

B. coridifolia and the two varieties of B. megapotamica cau
se a severe acute poisoning in livestock characterized by
degeneration and necrosis of the epithelial lining of gas
trointestinal tract and necrosis of lymphocytes in lymph
nodes, spleen, tonsils, and several lymphoid aggregates
(Tokarnia & Dobeneiner 1975, Tokarnia et al. 1992, Barros

megapotamica (Kupchan et al. 1977) B. coridifolia, (Busam
& Habermehl 1982, Habermehl et al.1985) and B. artemi
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falo, the pathogenesis of the toxicosis and the toxic prin
ciples 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, rumininal 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-
manto de Aguiar, state of Rio Grande do Sul, Brazil, from where a spontaneous outbreak of *B. megapotamica* var. *weirii* was recently reported (Oliveira-Filho et al. 2011). The harvest plant was kept at 4°C until fed to the experimental buffalo; the time elapsed from the harvesting and the feeding was never longer than 48 hours.

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 re-dissolved 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 0.37mg/kg, as well as 3 un-spiked matrix samples.

A few extracts (from 2-3g material) were further extracted and purified by redissolving the extract in dichloromethane and subsequently passing the extract through a polyethylene imine silica column (Jarvis 1992, Jarvis et al. 1996). The column was eluted with dichloromethane (fraction 1) and methanol (fraction 2). These were both evaporated to dryness with N₂, and redissolved as above.

Samples were analysed by Ultra High Performance Liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS) on a maxis G3 quadrupole time of flight mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an electrospray (ESI) source. The MS was connected to an Ultimate 3000 UHPLC system (Dionex, Sunnyvale, CA). Separation of 1µl samples were performed at 40 °C on a 100mm × 2.1mm ID, 2.6µm Kinexet C₁₈ column (Phenomenex, Torrance, CA) using a linear water-acetonitrile gradient (both buffered with 20mM formic acid) at a flow of 0.5mL/min starting from 20% acetonitrile and increased to 65% in 10 minutes, then 1 min to 100%, keeping this 2 min, keeping this for 3 minutes. HRMS was performed in ESI⁻ with a data acquisition range of 10 scans per sec at m/z 100-1000. The MS was calibrated using sodium formate automatically infused prior to each analytical run, this provided a mass accuracy of less than 1.5ppm in MS mode. Trichothecenes were detected as extracted ion chromatograms (±0.002 Da) of the [M+H]⁺ ion with [M+NH₄]⁺ and [M+Na]⁺ used as qualifiers. The monosaccharide conjugated trichothecenes were detected as [M+NH₄]⁺ and [M+Na]⁺ ions. Tandem experiments were conducted on the [M+H]⁺ and [M+NH₄]⁺ ions with a collision energy of 20-30 eV and nitrogen as collision gas. Reference standards of trichodermin, roridins E, -H, and L-2, verrucarins J, -A and -H, satratoxins G, -H, and iso-F, trichoverrols A and B, as well as trichoverrins A and B were all co-analyzed with the samples. Extracted ion chromatograms were constructed for all trichothecenes known from *Baccharis* and *Myrothecium* species as noted in Antibase2010 (Nielsen et al. 2011), as well as for baccharinoids, miophytocens, miotoxins and trichothece (Jarvis et al. 1988, 1991, 1996, Jarvis 1992) including several glucose conjugated trichothecenes.

Blood Samples were collected from the jugular vein of the experimental buffalo for complete blood count, proteinogram, and determination of seric activity of aspartate transaminase (AST), and determination of fibrin degradation products (FDPs). Hemoculture were performed in blood samples drawn aseptically from Buffalo 1 and 3 after they presented overt clinical signs of intoxication. All buffalo were necropsied and several tissue samples were collected during the necropsies and fixed in 10% buffered formalin, processed routinely for histopathology and stained by hematoxilin and eosin. Selected samples of liver and kidney were stained by Fraser-Lendurm method for the demonstration of fibrin; in this method fibrin, keratin, and some cytoplasmic granules appears red, erythrocytes appear orange, and collagen appears green.

For histopathological evaluation of the epithelial changes in the gastrointestinal tract fragments of the following organs were sampled: rumenoreticulum (dorsal sac, ventral sac, reticular fold, cranial pillar, caudoventral blind sac, and caudodorsal blind sac), omasum, abomasum, duodenum, jejunum, ileum, proximal colon, spiral colon, cecum, and rectum. For histopathological evaluation of the lymphoid system fragments of following organ/tissues were sampled: tonsils, spleen, gut associated lymphoid tissue, bronchial associated lymphoid tissue; and 10 lymph nodes (prescapular, axilar, mandibular, popliteal, gastric, jejunal, hepatic, bronchial, internal iliac, and renal).

### RESULTS

Chemical analysis of the plant revealed 51 one different major macrocyclic trichothecenes; the major peaks are

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**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)*</th>
<th>Time spent in the administration of the plant</th>
<th>Severity of clinical signs</th>
<th>Time between the terminus 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>+++⁺⁺⁺</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></td>
</tr>
</tbody>
</table>

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

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.

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.

Fig.3. Drooling in Buffalo 3 experimentally poisoned by Baccharis megapotamica var. weirii.
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 caudoventral blind sac, ruminal cranial pillar, reticulum, caudodorsal blind sac, dorsal sac, and omasum. In the 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.

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 Baccharis megapotamica var. weirii. Dehydration was clearly a participant 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 of 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 buffalos 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 poisoning by Baccharis 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 macroyclic trichothe cenes (Jarvis et al 1996, Varaschin et al. 1998, Varaschin & Alessi 2003).

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 Baccharis 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).

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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/B14, Baccharinoid B17/B17, iso-baccharin/baccharin and the conjugate xylose-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 hydroxylated 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 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).

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REFERENCES


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