Toxicon 126 (2017) 90–95

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Enterolobium contortisiliquum is a cause of acute ruminal acidosis in sheep

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ARTICLE INFO

Article history:
Received 11 August 2016
Received in revised form
13 October 2016
Accepted 31 December 2016
Available online 3 January 2017

Keywords:
Poisonous plants
Enterolobium contortisiliquum
Lactic acidosis
Hepatotoxicity

ABSTRACT

The ingestion of pods of *Enterolobium contortisiliquum* is associated with digestive disturbances, photosensitivity and abortion in domestic ruminants. This experiment was designed to test the hypothesis that digestive disturbances in this toxicosis are really caused by acute ruminal acidosis. Three sheep fed large doses (10–15 g/kg/body weight [bw]) of *E. contortisiliquum* pods developed ruminal acidosis and were treated with sodium bicarbonate to try to control this metabolic disturbance, thus providing additional evidence of the involvement of ruminal acidosis in the pathogenesis of toxicosis. Two of the sheep died, and one recovered after treatment. In the two sheep that developed severe signs of ruminal acidosis, the values of blood lactate were 18 mg/dL and 196.88 mg/dL, indicating metabolic acidosis as the cause of death. Additionally, four sheep developed elevated serum levels of aspartate aminotransferase and gamma glutamyl transferase, indicating that the pods had hepatotoxic effects. Necropsy findings included the accentuation of the hepatic lobular pattern and multiple focally extensive red areas in the rumen mucosa and on the surface of the liver. Repeated ingestion of small doses induced tolerance but did not induce cumulative effects. Histopathologically, the epithelial mucosa of the rumen and reticulum exhibited swollen and vacuolated epithelia with intraepithelial pustules. Focal ulceration of the mucosa was also observed. Multifocal vacuolar degeneration of hepatocytes and scattered individual hepatocellular necrosis were evident in the liver. We concluded that the main clinical manifestation of intoxication by *E. contortisiliquum* pods in sheep was acute ruminal lactic acidosis and metabolic acidosis. Ingestion of repeated sublethal doses could stimulate proliferation of the ruminal fauna that degrades the sugar present in the pods, and thereby prevent the occurrence of ruminal acidosis. The plant is also hepatotoxic, and no abortions were observed.

1. Introduction

Enterolobium contortisiliquum is a toxic leguminous tree (Fig. 1) of the family Fabaceae and the subfamily Mimosoideae (Riet-Correa et al., 2011). The consumption of the pods of *E. contortisiliquum* has been associated with digestive disturbances, photosensitivity and abortion in cattle (Silva et al., 2006; Assis et al., 2009; Mello et al., 2010; Schons, 2011; Bezerra et al., 2012; Tokarnia et al., 2012; Sant'ana et al., 2014, Souza et al., 2015), as well as diarrhea and abortion in goats (Benício et al., 2005; Assis et al., 2009) and photosensitivity in sheep (Bezerra et al., 2012). In birds, *E. contortisiliquum* causes diffuse hepatocellular necrosis (Gadelha et al., 2015).

Several triterpenoid saponins have been identified in the seeds and fruits of *E. contortisiliquum*, mainly enterolosaponin A and contortisilioside B, which are toxic to macrophages, and contortisilioside A and C, which are toxic to both macrophages and

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http://dx.doi.org/10.1016/j.toxicon.2016.12.015
0041-0101/

Fig. 1. *Enterolobium contortisiliquum*: (A) the tree and (B) pods.

murine lymphoma cells (Mimaki et al., 2003, 2004). Other saponins that have been identified in *E. contortisiliquum* include enterolosaponin B, contortisilioides D – G and enterolobin (Castro-Faria-Neto et al., 1991; Mimaki et al., 2003, 2004). The latter is a hemolytic, cytolytic and pro-inflammatory protein (Castro-Faria-Neto et al., 1991). Saponins found in plants of the *Enterolobium* genus have been associated with short-term ruminal antiprotozoal activity in wethers (Koenig et al., 2007).

Poisoning by the pods of *E. contortisiliquum* induces clinical signs of gastrointestinal disturbances, such as diarrhea, anorexia, ruminal atony, apathy, dehydration and tachypnea (Tokarnia et al., 1999; Grecco et al., 2002; Mendonça et al., 2009; Dias et al., 2011; Schons, 2011; Bacha, 2012; Tokarnia et al., 2012; Bacha et al., 2016). A few documented studies have reported histopathological findings in the fore stomachs that included hydropic degeneration and pustules in the ruminal stratified epithelium (Grecco et al., 2002; Dias et al., 2011; Bacha, 2012; Bacha et al., 2016). In one of these studies involving sheep from our laboratory (Bacha, 2012), a ruminal pH of <5.0 was observed, but the authors did not associate this finding with intoxication. In a subsequent experiment, high sugar content was identified in the pods of *E. contortisiliquum* (Bacha et al., 2016).

Therefore, we hypothesized that ruminants ingesting large doses of the pods of *E. contortisiliquum* develop acute ruminal lactic acidosis due to the fermentable carbohydrates present in the plant, and this may be the cause of the reported digestive disturbances associated with poisoning by *E. contortisiliquum*.

2. Materials and methods

Mature pods of *E. contortisiliquum* were harvested in October 2015 from a farm in Midwestern Brazil (latitude 20°14′26″ S, longitude 56°22′42″ W, altitude 125 m) where abortions and photosensitization outbreaks in cattle had been reported by the owner and were tentatively associated with the ingestion *E. contortisiliquum* pods. The plant was identified as *Enterolobium contortisiliquum* (Vell.) Morong. at the Botany Laboratory, and a voucher was kept in the Herbarium CGMS (no. 44247) of the Federal University of Mato Grosso do Sul (UFMS), Brazil.

The pods were dried for a week in a well ventilated and illuminated environment and then ground in a Trapp® grinding mill TRF70. The powdered pods were kept in burlap bags at room temperature for two days until the start of the experiment, when they were administered intra-ruminally to sheep through a rumen cannulated fistula. Seven adult, mixed breed ewes between 30 and 40 kg were used in the experiment. Five of them were 3–4 months pregnant, since abortions are reportedly associated with toxicosis. Before the start of the experiment, all of the sheep were subjected to parasitological examinations through the quantification of eggs per gram of feces.

The sheep were kept in metal cages placed on grass (*Paspalum notatum*), and the cages were moved daily and exposed to sunlight. During the hotter hours of the day (10 a.m.–2 p.m.) the cages were covered with canvas.

The daily diet consisted of commercial rations for sheep (250 g/sheep), commercial mineral salts for sheep (30 g/sheep), water *ad libitum*, corn silage and green grass (*Paspalum notatum*).

To evaluate whether sheep develop tolerance to the toxic effects of *E. contortisiliquum,* daily doses of 10 or 15 g/kg/bw were administered to 5 sheep for 1–10 days (Table 1). Daily clinical exams were performed on each sheep to evaluate cardiac and respiratory function, rectal temperature and ruminal pH (using an electronic pH meter). When clinical signs of acute ruminal acidosis, such as anorexia, diarrhea, drooling, dehydration, prolonged recumbence, and foamy and/or yellowish content, were observed, the pH level was measured every 3 h. If the pH reached a level of 5.5 or less, the sheep was treated intra-ruminally with 1 g/kg/bw of sodium bicarbonate based on a previously published protocol (Oliveira et al., 2009).

The experimental design including the doses of *E. contortisiliquum* received and the outcome for each sheep is provided in Table 1.

Considering that hepatotoxic action has been reported due to intoxication by the pods of *E. contortisiliquum* (Bezerra et al., 2012), blood samples were collected from each sheep immediately prior to the start of the experiment, which was considered day 0 (D0), and then daily throughout most of the experiment to determine the serum activities of AST and GGT. Due to the large range of reported reference values for AST (60–280 U/L) and GGT (20–52 U/L) (Kaneko et al., 2008), the reference value for the sheep in this experiment was considered to be the one measured at D0, immediately prior to the start of the experiment. Blood was collected from the jugular veins of sheep that developed signs of severe ruminal and metabolic acidosis into tubes containing fluoride to determine blood lactate levels.

The sheep that died were necropsied, and fragments of several organs were collected in 10% formalin and routinely processed for histopathological examination.