Natural and Experimental Poisoning of Cattle by *Enterolobium contortisiliquum* Pods (Fabaceae Mimosoideae) in Central-Western Brazil

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Abstract

Enterolobium contortisiliquum pods are commonly identified as being the cause of abortions and photosensitivity in cattle. This paper describes the clinical and pathological aspects of a natural outbreak of hepatogenous photosensitization by the pods of *E. contortisiliquum* in Brazil and the results of experimental poisoning in three bovines. The history of natural poisoning was obtained at the site of the outbreak. Clinical examinations were carried out and hepatic enzymes were analyzed. A *post-mortem* examination was carried out at the outbreak location on a cow that had died. Several samples were collected from the animal's internal organs to carry out histopathological examinations. *E. contortisiliquum* pods were given to cows in order to reproduce the disease. The natural poisonings were characterized by photosensitization and aborted young. *Enterolobium* photosensitization was not experimentally reproduced, but the animals fell sick and recovered after 52 days.

Photosensitization, abortion, earpod tree

Enterolobium contortisiliquum (Vell.) Morong, a member of the tree family Fabaceae, sub-family Mimosoideae, commonly known in English as the earpod tree and in Portuguese as tamboril and timbauva, is widely distributed throughout Brazil, and is found in all of its geographic regions (Lorenzi 1992; Carvalho 1994; Tokarnia et al. 2000).

Recently it was demonstrated that the pods of *E. contortisiquum* have bisdesmosidic triterpene saponins which were given the trivial names contortisiliosides A-G (1-7). Two of these compounds (contortisiliosides A_1 (3β-hydroxy-21β-[(3-phenylprop-2enoyl)oxy] olean-12-en-28-oic acid) and contortisiliosides C_3 (β-D-glucopyranosyl-(1→3)-[α-L-arabinofuranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosy l21β-((E)-3phenylpro-2-enoyl)oxy]3β-[β-D-xylopyranosyl-(1→2)-β-D-galactopyranosyl-1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl)oxy]olean-12-en-28-oate) demonstrated *in vitro* cytotoxic activity (Mimaki et al. 2004). It was also shown that the pods contain enterolobin, a haemolytic protein that induces a potent pleural exudation, cellular infiltration and paw oedema in rats (Castro-Faria-Neto et al. 1991).

Earpod tree pods have often been blamed for abortions and outbreaks of photosensitization in cattle. Experiments have shown that pods cause hepatocellular necrosis but have not proved that they cause abortions (Tokarnia et al. 2000). Apart from the types of lesion arising from photodermatitis and abortions, it has been reported that natural poisoning by the plant has resulted in animals displaying symptoms of anorexia and depression. Recovery took 30-40 days following the first sign of symptoms (Grecco et al. 2002).

Although reports exist in the literature showing that *E. contortisiliquum* pods, when ingested in quantity, cause photosensitization and aborted foetuses, in experiments in

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which the maximum tolerable dose was administered, neither photosensitization nor abortions occurred (Tokarnia et al. 1999). The present paper aims to describe the clinical and pathological aspects of a natural outbreak of hepatogenous photosensitization by the pods of *E. contortisiliquum* in cattle in the state of Mato Grosso, Central West Brazil and the results of experimental poisoning in three bovines.

Materials and Methods

The outbreak occurred at the municipality of Jangada, in the Southern Central Microregion of the State of Mato Grosso – Brazil, during the dry season. The photodermatosis and abortion case histories were obtained from this region. Clinical examinations were carried out and blood samples collected to obtain the hepatic biochemical profile for three ill mixed breed animals (Animal B17, B186 and B467). One animal (B247), which had been found recumbent two days earlier, was sent to the University of Cuiabá Veterinary Hospital for treatment, but died immediately after being unloaded into the corral and was *post-mortem* examined in the sector for Veterinary Pathology at the same university.

Fragments of various organs were collected and preserved in 10% formalin. The material destined for histopathological examination was dealt with according to standard procedures, including immersion in paraffin, cut into 5 μ slices and stained with haematoxylin-eosin.

Enterolobium contortisiliquum pods were collected from the site of the outbreak and given in a single dose to three animals at 5 g/kg (bovine P098), 9 g/kg (bovine P107) and 12 g/kg (bovine P471).

The *E. contortisiliquum* pods were force-fed at the large animal clinic of the Veterinary Hospital. The animals were left unshaded in a corral for 40 days and were evaluated clinically for the appearance of lesions arising from photodermatitis. Additionally, the animals were given water *ad libitum*, alfalfa (*Medicago sativa*), and commercially available salt licks for cattle.

The study was approved by the Ethics Committee of the University of Cuiabá.

Results

In the herd, the clinical symptoms were observed from September to November 2007. Of 98 animals, 15 presented, apart from digestive ailments, lesions arising from photodermatitis (15.3%). In total, 7 cows aborted (7.14%), 3 died (3%) and the rest recovered. According to information obtained from the farm, outbreaks of this disease occur in the herd on an annual basis, and always in the dry season, and include diarrhoea and photodermatitis, whilst aborted foetuses are not always recorded. The history obtained lead to the conclusion that, during this period, the cattle eat earpod tree pods without restriction, due to the scarcity of pasture (Plate V, Fig. 1).

The first signs of natural poisoning observed in the cattle included green-coloured diarrhoea followed by dehydration, anorexia and progressive weight loss. Jaundice was another condition commonly observed among the natural poisonings. The most consistent lesions were those arising from photodermatitis on patches of skin with little pigment, mainly on the udders (Plate V, Fig. 2) and nostrils. Weight-loss, goose pimples, hair loss and scarring were also observed.

Table 1. Clinical alterations, evolution and outcome following experimental intoxication by the pods of *E. contortisiliquum* in cattle

Clinical symptoms	P098	P107	P471
Appetite reduction	+[a]	++	++
Anorexia	+	++	+
Jaundice	-	++	+
Pungent diarrhea	+	+++	+++
Dehydration	-	+	+
Paleness of conjunctival mucosa	-	+	+
Hypotonia of rumen movement	-	++	++
Prolonged sternal recumbency	-	++	++
Outcome	Recovered	Recovered	Recovered

[a] Clinical signs: +++ accentuated, ++ moderate, + slight, - absent.

The main clinical alterations observed in the cattle experimented upon are summarized in Table 1. Animal P098 displayed mild apathy and green diarrhoea appeared about 14 days after the administration of the pods. These alterations became less intensive and 24 days later, the animal was showing signs of improvement, and during evaluation it showed no signs of disease. Animals P107 and P471 showed similar symptoms. Six days after the *E. contortisiliquum* pods were administered, marked lethargy and anorexia were observed. The symptoms developed and after 8 days included pale conjunctival mucosa, hypotonia of ruminal muscle movements, green diarrhoea containing a large amount of seeds of *E. contortisitiquum*, dehydration, lassitude, and prolonged sternal recumbency (Plate VI, Fig. 3). These changes were observed for a month. After 37 days, the animals were showing signs of improvement and, 52 days after the administration of the plant pods, the cattle had recovered completely and showed no signs of photodermatitis.

The blood samples collected from the cattle in the natural cases of poisoning demonstrated that the serum activities of gamma-glutamyl transferase (GGT) and aspartate amino transferase (AST) were markedly higher and in the experimental cases these levels were slightly higher.

Post mortem examination of animal B247 revealed a low body condition score, photodermatitic scarring and generalized jaundice. The skin of the udders, mainly that of the teats, had dry gangrene associated with purulent exudation, and there was hypertrophy of the lymph nodes which, like the pre-scapular and inguinal lymph nodes, had a green colour. Examination of the viscera of the thoracic cavity revealed pulmonary interstitial emphysema and haemopericardium. Vascular alterations characterized by haemorrhages were seen in the pre-gastric diverticuli and abomasum (Plate VI, Fig. 4), which, incidentally, contained a large quantity of *E. contortisiliquum* seeds. The rectal ampulla was distended due to a large quantity of separated hard lump faeces that were covered in mucus. Distention of the biliary vesiculae, marked oedema of the kidney wall and yellowed parenchyma were recorded in the liver capsule.

Microscopically, the hepatic lesions were characterized by centrilobular coagulation necrosis and hepatocyte degeneration (Plate VII, Fig. 5), as well as cholestasis, pericholangitis, and oedema of the spaces of Disse. Oedema was present in the submucosa of the omasum; in the abomasum there was ulceration of the mucosa and extensive haemorrhaging.

Discussion

In the semiarid region of Brazil poisoning by *Enterolobium contortisiliquum* is sporadic. In the mentioned cases, the disease was also characterized by diarrhoea, abortions and photodermatitis (Silva et al. 2006). However, in the State of Mato Grosso (Central-Western of Brazil) the disease occurs often (Grecco et al. 2002). The diagnosis of natural cattle poisoning by the pods of *E. contortisiliquum* was based upon clinical findings, *post mortem* examination results (including the finding of plant seeds in the rumen and abomasum) and histopathological analysis of the digestive tract, which revealed lesions in the fore-stomachs, abomasum and liver, where centrilobular hepatic necrosis with bilial retention was particularly obvious. According to the literature, the clinical cycle of intoxication by *E. contortisiliquum* manifests itself just a few hours following ingestion of the pods, with its evolution characterized clinically by jaundice, reduced appetite and putrid diarrhoea (Afonso and Pott 2001).

It is known that saponins have a lytic action on cell membranes. Some bisdesmosidic triterpene saponins can interfere with fluidity of cell membrane and affect the role in ion transport. Apparently, the ability of saponins to affect this indicator may explain their effects on hepatocyte functions (Francis et al. 2002). Therefore, the haemorrhages seen in

the pericardium, pre-gastric diverticuli and abomasum of animal B247 could be linked to hepatic dysfunction, due to inhibition of the formation of fibrinogen, the synthesis of which depends fundamentally on hepatic parenchyma (Jones et al. 2000).

Apart from that, it is suggested that the hepatic necrosis described in the bovine might be related to the retention of phylloerythrin and consequent photodermatitis. However, lesions similar to those described have been seen in other hepatotoxicoses which occur alongside photodermatitis in cattle (Kellerman et al. 1990). Therefore, the differential diagnosis of hepatogenous photosensitization by *Brachiaria* spp. was taken into consideration, in which, apart from hepatic necrosis and cholangiopathy, crystalloid structures in ducts, miniature canals and hepatocytes as well as macrophage aggregates of foamy cytoplasm are observed (Driemeier et al. 1999). These alterations were not seen in this study.

According to Tokarnia et al. (1999), cattle poisoned by the pods of *E. contortisiliquum* manifest restlessness, swish vigorously the tail, shake the head and try to escape the sun and seek shade due to the lesions caused by the photodermatitis, similar to those described in the present work. In experiments with *E. contortisiliquum* pods in cattle, the evolution of the disease was acute, with symptoms appearing just a few hours after ingestion, as was seen in animals P098, P107 and P471, in which, six hours after administration of the *E. contortisiliquum* pods, the animals displayed lethargy, anorexia and prolonged recumbency. However, in the experiment described here and in the experiments of other authors, the photodermatitic lesions as those seen in the natural poisonings were not reproduced.

In experiments in which the pods of *E. contortisiliquum* were given to cattle, fatal cases were reported, with acute evolution of the disease between 18 and 27 h after pod ingestion. It was also reported that those animals that did not die recovered quickly and displayed tolerance to *E. contortisiliquum* seeds (Tokarnia et al. 2000). In the experiment carried out on animals P107 and P471, recovery from digestive disturbances took place 52 days after the administration of the pods. There was no further display of photodermatitic lesions by the fortieth day of observation. The values of gamma-glutamyl transferase and aspartate transferase of these animals were slightly increased. Based on this, it is suggested that whilst the animals displayed hepatic lesions, these were insufficient to cause photosensitization, because it is known that ruminants with hepatogenous photosensitization have markedly elevated serum levels of GGT and AST (Bonel-Raposo et al. 2004) as observed in the natural cases of poisoning of this study.

It is suggested that, in natural cases, degenerative lesions occur associated with lesions of the biliary system with retention of phylloerythrin and consequent photodermatitis. It is probable that the retention of phylloerythrin occurs as a consequence of interruption to the draining of bile by the swelling of the hepatocytes. Apparently, this could be the main mechanism of retention of phylloerythrin in the cases of photosensitization described in this work. Perhaps the rapid recovery of the cattle poisoned by *E. contortisiliquum* described in this work might be explained by the presence of a damaged area of the hepatic parenchyma. In such cases photosensitization is unusual, because a sufficient number of hepatocytes commonly escape such damage, removing the phylloerythrin of circulation (Yager and Scott 1993). However, further experiments with the pods of *E. contortisiliquum* should be carried out on cattle so that the plant's importance as a photosensitizer can be proved, because it is known that the contortisiliosides found in the plant's pods, display moderate cytotoxic activity *in vitro* (Mimaki et al. 2004).

Otrava lusky *Enterolobium contortisiliquum* (Fabaceae, Mimosoideae) u skotu v přirozených a experimentálních podmínkách středozápadní Brazílie

Lusky *Enterolobium contortisiliquum* jsou jednou z běžných příčin abortů a fotosenzitivity u skotu. Tato práce popisuje klinické příznaky a patologicko-morfologický nález

při hepatogenní fotosenzibilizaci vyvolané lusky *E. contortisiliquum* po hromadné otravě v přirozených podmínkách a po experimentálním podání třem jedincům skotu v Brazílii. Anamnestické údaje o otravách způsobených touto rostlinou v přírodě byly získány přímo na místě výskytu nemoci. Nemocná zvířata byla klinicky vyšetřena, včetně analýzy jaterních enzymů. V místě otravy bylo tělo uhynulé krávy podrobeno pato-anatomickému vyšetření. Během pitvy byly odebrány vzorky vnitřních orgánů k histopatologickému vyšetření. K potvrzení reprodukovatelnosti zjištěných údajů byly vybraným kravám podány lusky *E. contortisiliquum.* Náhodná přirozená otrava se vyznačovala fotosenzibilizací a aborty plodu. Při experimentálně reprodukované otravě však k fotosenzibilizaci nedocházelo, u zvířat byla pozorována nevolnost a zdravotní stav se upravil po 52 dnech.

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Fig. 1. Pod collection site where a specimen of *E. contortisiquum* and a herd of cattle with open access to the area can be seen.

Fig. 2. Lesions of photodermatitis on the teats of a heifer from the site of the *E. contortisiliquum* pod poisoning outbreak.

Fig. 3. Experimental poisoning by *E. contortisiquum* pods. Animal P107 displaying prostration and prolonged sternal recumbency.

Fig. 4. Natural poisoning by the pods of *E. contortisiquum*. Animal B247. Abomasum mucosa with haemorrhages. Insert: seeds removed from the stomach.

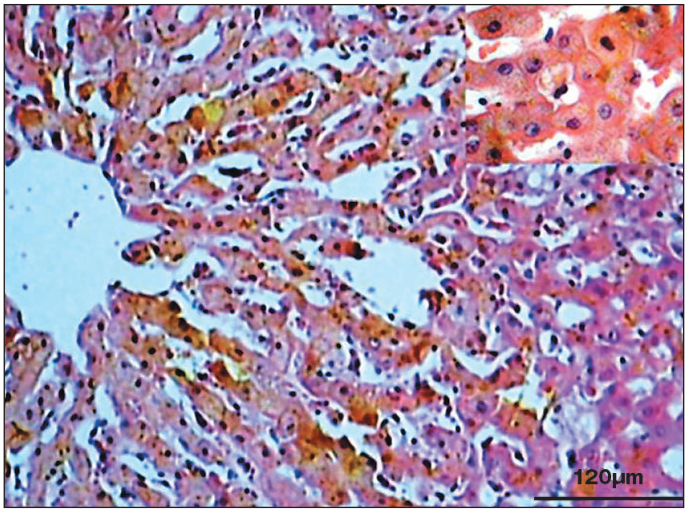

Fig. 5. Natural poisoning by the pods of *E. contortisiquum*. Animal B247. Liver centrilobular coagulation necrosis and cholestasis. Insert: hepatocyte necrosis and the standard of degeneration more in detail.