Pathological aspects of experimental poisoning of goats by *Stryphnodendron fissuratum* pods (Fabaceae)

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Abstract

Stryphnodendron fissuratum Mart. (Fabaceae) is an importantly toxic plant for livestock in Central-Western Brazil. Despite the recently published clinical descriptions of the poisoning, the gross and histopathological lesions of the disease needed to be better characterized. For this reason the pods of *S. fissuratum* were given orally to 10 young goats. The goats that received single doses of 10 g/kg, 20 g/kg and 40 g/kg and 4 fractioned doses of 5 g/kg, died poisoned. One goat that received a single dose of 10 g/kg recovered. Only those animals that received one dose of 5 g/kg did not become ill. At *post mortem* examination, the main lesions were associated with the digestive system and consisted of broken up epithelium and congestion of the mucosa of forestomachs and abomasum. Widespread areas of erosion and ulcerations were observed at the reticula and abomasal mucosa. Int these areas, the main lesions were neutrophil infiltration, vacuolization of the epithelial lining, swelling and dissociation of cells with cytoplasmic eosinophilia, pyknosis, karyorrhexis and nuclear karyolysis. In the central nervous system, the main lesions were identified in the brain of goats that received 20 g/kg and 40 g/kg of pods and consisted of spongiosis of the brainstem white matter. Based on these results, it was proved that the pods of *S. fissuratum* are acutely toxic for goats under the conditions of this experiment. The poisoning was characterized by damage to the organs of the digestive system and the central nervous system.

Poisonous plants, rumen necrosis, spongiosis

The *Stryphnodendron* gender reunites 36 taxa of which 90% are found in Brazil and approximately 50% are found exclusively on the Brazilian territory in various biomes, but mostly in the type of savanna called "cerrado" areas and in the Amazon rainforest (Scalon 2007). Some species of this gender are poisonous for ruminants, such as *Stryphnodendron obovatum* Benth. (Camargo 1965; Brito et al. 2001a), S*tryphnodendron coriaceum* Benth. (Tokarnia et al. 2000) and *Stryphnodendron barbatimao* Mart. (Pereira et al. 1989).

Stryphnodendron fissuratum Mart., which belongs to the Fabaceae family, Mimosoideae sub family, commonly known as "doughnuts", has been pointed out as the cause of poisoning in bovines in the "cerrado" areas of Central Western of Brazil, in the States of Goiás, Mato-Grosso and Mato Grosso do Sul (Rodrigues et al. 2005a; Ferreira et al. 2009). *S. fissuratum* poisoning was experimentally recreated in bovines, sheep and goats (Rodrigues et al. 2005a; Mendonça et al. 2010). The clinical profiles in these species presented an acute evolution followed by death, especially due to circulation alterations in the gastrointestinal tract.

Triterpenoid saponins were isolated from *S. fissuratum* (Haraguchi et al. 2006; Yokosuka et al. 2008) and they seem to be the main toxin responsible for the pathogenesis of poisonings in ruminants (Tokarnia et al. 2000; Brito et al. 2001a). The mechanism of action of saponins is unknown, and it is believed that it may be related to the lytic action on

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the cell membranes of the affected tissues of all eukaryotic organisms that have sterols in their cell membranes (Molyneux et al. 1980; Yokosuka et al. 2008).

Aiming to better understand the poisoning, this study reports the necropsy and histopathological findings in goats poisoned by *Stryphnodendron fissuratum*.

Materials and Methods

Stryphnodendron fissuratum mature pods were collected in the Municipality of Rondonópolis, Mato Grosso, Brazil, directly from the trees and the soil. After collection, the pods were kept refrigerated for further use in the experiment. The botanic material classification was carried out at the Central Herbarium of the Federal University of Mato Grosso.

The animal care and experimental design were approved by the Animal Ethics Committee of the University of Cuiabá, Brazil (Register number 175 CEP/UNIC). Twelve clinically healthy male goats of an age of 4–7 months, weighing 16–21 kg were used. Thirty days before the beginning of the experiments, the goats received antiparasitic treatment (ivermectina 1% Chemitec®, Chemitech Agro-Veterinária, São Paulo, Brazil) and were kept in a collective corral for adaptation and clinical observation. Early in the morning the animals were fed a commercial diet (Caprinotech crescimento®, Purina, São Paulo, Brazil), mineral supplement (Purinafós 70 caprinos®, Purina), tifton hay (*Cynodon dactylon*) and water *ad libitum*. Ten goats received the pods of this tree. Two goats were used as control (goats No. 1 and 2); these animals were kept under the same management only without receiving the pods of *S. fissuratum*. The experimental design is presented in Table 1.

Table 1. Design of experimental poisoning of goats by *Stryphnodendron fissuratum* pods.

Goat No.	Weight (kg)	Daily dose (g/kg)	Daily dose (g)	Number of ingestion (days)	Total dose (g/kg)	Total ingested (g)
1	20.7	Control	-	-	-	-
2	20.3	Control	-	-	-	-
3	19.3	40	732	1	40	732
4	16.8	40	672	1	40	672
5	18.8	20	376	1	20	376
6	18.3	20	366	1	20	366
7	21.0	10	210	1	10	210
8	19.3	10	193	1	10	193
9	18.9	5	94.5	1	5	94.5
10	19.7	5	98.5	1	5	98.5
11	19.2	5	96	4	20	384
12	20.5	5	102	4	20	410

The goats that died after administration of the pods were necropsied. During necropsy, the brain and lymph nodes, thong, trachea, thyroid, lung, heart, liver, spleen, adrenal glands, kidney, bladder, esophagus, rumen, reticulum, omasum, abomasum, pancreas, duodenum, jejunum, ileum, caecum, colon, rectum and skeletal muscle tissues were collected. Samples were processed by usual methods, inserted in paraffin, cut 5 µm in thickness and dyed by the haematoxylin-eosin (HE) technique for histological analysis.

Results

The goats that received single doses of 10 g/kg and 4 fractioned doses of 5 g/kg/day became ill and died. No clinical signs were observed in goats that received doses of 5 g/kg. Detailed results of the experimental poisoning by *S. fissuratum* in the goats that died are presented in Table 2.

Necropsy findings

Loss of fur at the dorsal region of the integument was observed in goats that received single doses of 20 g/kg and 40 g/kg. Major gross lesions were found mainly in the digestive

Table 2. Experimental design and results obtained in goats that died after poisoning by *Stryphodendron fissuratum*.

Goat No.	Weight (kg)	Daily dose (g/kg)	Daily dose (g)	Number of ingestion (days)	Total dose (g/kg)	Total ingested (g)	Symptoms the onset after intoxication	Days until death
3	19.3	40	732	1	40	732	first day	6
4	16.8	40	672	1	40	672	first day	4
5	18.8	20	376	1	20	376	first day	6
6	18.3	20	366	1	20	366	first day	25
7	21.0	10	210	1	10	210	first day	24
11	19.2	5	96	4	20	384	third day	16
12	20.5	5	102	4	20	410	fourth day	11

tract. The rumen was distended by the contents. The contents of the rumen, reticulum and omasum were slightly dried; the content of the abomasum was pasty, with a slightly blackened brown colour. A significant quantity of partially digested *S. fissuratum* seeds were identified predominantly in the rumen. An odor similar to this plant was found in the rumen content of goats in which the disease evolved acutely.

In the proximities of the reticulum-rumen fold, a light oedema in the walls was observed. Highly reddish papillae were identified close to the rumen-reticulum groove and the ventral ruminal sac. In the ruminal mucosa there were diffuse haemorrhages shaped as petechiae and epithelium detachment with evidences of highly reddish submucosa (Plate V, Fig. 2A). The omasum and reticulum had the same lesional pattern of those described in the rumen, with different intensity. These lesions were not observed in goats that received 20 g/kg in fractioned doses. In the goats that received single doses exceeding 10 g/kg and in the ones that received 20 g/kg in fractioned doses, the abomasum was diffusely reddish with blood clots in its content. The abomasal folds were swollen with multiple eroded or ulcerated areas (Plate V, Fig. 2B). The intestinal loops were distended, intensively reddish with mucus. The caecum was dilated with a darkened pasty content, sometimes with blood streaks. The colon mucosa presented petechiae and diffuse areas of congestion. Most of the mesenteric lymph nodes were increased and oedematous to the cut. The liver was congested, increased in volume, with evidences of the lobular pattern and with distended biliary vesicle.

In goats that received single doses of 20 g/kg and 40 g/kg, the kidneys were congested and the urinary bladder was filled with congested serous vessels and with petechial haemorrhages in the mucosa. Other organs did not present important lesions.

Histopathological findings

Degenerative and necrotic alterations of mucosa of different segments in the digestive tract were the main histological findings (Plate V, Fig. 2C). In goats that received single doses of 20 g/kg and 40 g/kg, degeneration and diffuse necrosis of the ruminal, reticular and omasal mucosa were observed. Spongiosis of the epithelium of the rumen, reticulum and omasum was also observed in degrees varying from light to mild. In the ruminal, reticular and omasal epithelium, there were several degrees of hydropic degeneration, swelling and dissociation of cells, acantholytic keratinocytes and necrosis. Close to the necrotic epithelium areas, serofibrinous effusion, and mononuclear and polymorphonuclear infiltrates were observed. In the lamina propria of forestomachs (goats that received single doses of 20 g/kg and 40 g/kg), congestion areas of the abomasum and duodenum (goats that received single doses of 20 g/kg, 40 g/kg and 4 fractioned doses of 5 g/kg), were found, ranging from light to severe, and in other areas, oedemas, haemorrhages and necrosis of the muscular tunic were found. Serofibrinous effusion and inflammatory reaction occurred

especially in the ulcerated areas, from the rumen to the duodenum mucosa. In the large intestine, alterations of the lamina propria were discrete and consisted of oedema, fibrinous secretion, or haemorrhages. Alterations in the liver consisted of diffuse hepatocellular vacuolization and, occasionally, mild centrilobular necrosis. Alterations in the kidneys were mild and consisted of tubular epithelium necrosis, renal glomeruli atrophy, sometimes with a deposit of eosinophilic material in the Bowman's space and in the light of renal tubules.

Lesions in the central nervous systems were identified in the brainstem (Plate V, Fig. 2D) of goats that received 20 g/kg and 40 g/kg of the plant. The main alterations consisted of spongiosis of the medulla oblongata white substance and cerebellar peduncles. In the brain and cerebellum, the alterations consisted mainly of congestion and presence of focal areas of haemorrhage.

Discussion

In this study, *Stryphnodendron fissuratum* pods were poisonous to goats at single doses starting from 10 g/kg onwards and at 4 fractioned doses of 5 g/kg/day. The poisonings evolved acutely or subacutely and caused degenerative and necrotic alterations in the digestive tract and spongiosis in the central nervous system.

In Brazil, three major toxic plants affecting livestock with pathologic alterations in the digestive tract are *Baccharis coridifolia* in sheep and bovines (Rozza et al. 2006), *B. megapotamica* var. *megapotamica* and var. *weirii* in bovines (Tokarnia et al. 1992), and *B. megapotamica* var. *weirii* in goats (Barbosa et al. 1994). In these poisonings, the necropsy and histopathological findings are similar to the ones described in the *S. fissuratum* poisoning in goats. However, in *Baccharis* spp. poisonings in bovines, necrosis of the lympho-haematopoietic system can happen beside digestive tract disturbances. Moreover, hepatocyte necrosis is more acute than in the *S. fissuratum* poisoning in goats.

The digestive lesions of goats in this study were similar to those described in spontaneous and experimental *S. fissuratum* poisoning in bovines. Naturally, in *S. fissuratum* intoxication in bovines, hepatic photosensitization has been reported (Ferreira et al. 2009), as well as hepatocellular swelling and individual hepatocyte necrosis. Similar lesions were observed in the goats of this study, but at lower intensity. Deaths in goats are probably related to the irritating action of the toxins of *S. fissuratum* pods on the digestive tract, with consequent necrosis of the mucosa and circulatory alterations, as occurs in bovines poisoned by *S. obovatum* (Brito et al. 2001b), *S. barbatimao* (Tokarnia et al. 1991), *S. coriaceum* (Tokarnia et al. 2000) and *Enterolobium contortisiliquum* (Mendonça et al. 2009).

The most important alterations in the *S. obovatum* poisoning in bovines consisted of rumen papilla redness and adherence, detachment of epithelium and congestion of lamina propria, especially in the rumen and with lower intensity in the reticulum and omasum, beside congestion and ulcerating areas in the abomasum (Brito et al. 2001b). In *S. coriaceum* and *S. barbatimao* poisoning in bovines, necropsy findings consisted of circulatory alterations, with inflammation and necrosis of the forestomach and abomasal epithelium. Moreover, renal and hepatic regressive alterations, hepatic photosensitization and jaundice are also mentioned in these poisonings (Camargo 1965; Pereira et al. 1989; Tokarnia et al. 1991; Tokarnia et al. 2000). The fur loss described in goats 3–5 can be considered as a light hepatic photosensitization, given that the livers of these animals presented diffuse hepatocellular vacuolation and centrilobular necrosis. However, additional studies need to be conducted to prove the photosensitizing effects of *S. fissuratum*, since the goats of this experiment remained in a shaded place.

Renal alterations were equally identified in the histological exam and proven by the increase of creatine and urea serum concentrations (Mendonça et al. 2010). These same alterations were also described in the spontaneous and experimental poisoning by *S. fissuratum* pods in bovines (Ferreira et al. 2009).

Neurological disturbances such as motor incoordination, tottering, abnormal postures, depression and muscular trembling also occurred in poisonings by species of the *Stryphnodendron* gender in bovines and goats (Pereira et al. 1989; Brito et al. 1995; Tokarnia et al. 2000; Mendonça et al. 2010), being associated with hepatic encephalopathy. The hepatic alterations in the goats of this study were mild. The brainstem spongiosis described in this work can be ascribed to the direct action of *S. fissuratum* toxic principles (Haraguchi et al. 2006; Yokosuka et al. 2008), given that triterpene saponins can open calcium and potassium dependent channels in neuron cell membranes (McManus et al. 1993), with a consequent cytotoxic oedema (McGavin and Zachary 2007). Central nervous system spongiosis also occurs in poisonings by plants such as *Tetrapterys multiglandulosa* in bovines and goats (Tokarnia et al. 2000; Riet-Correa et al. 2005), *Ateleia glazioviana* in bovines and sheep (Gava et al. 2001; Raffi et al. 2006), *Helichrysum argyrosphaerum* in sheep and goats (Van der Lugt et al. 1996), beside *Ornithogalum saundersiae* and *O. prasinum* in bovines (Van der Lugt 2002). In these intoxications, electronic microscopic studies have shown that the lesion is a consequence of intramyelinic oedema of the white substance (Van der Lugt 2002; Riet-Correa et al. 2005; Raffi et al. 2006).

Based on these results, it was proved that the pods of *S. fissuratum* are acutely toxic for goats under the conditions of this experiment. The poisoning was characterized by damage to organs of the digestive system, kidney and central nervous system.

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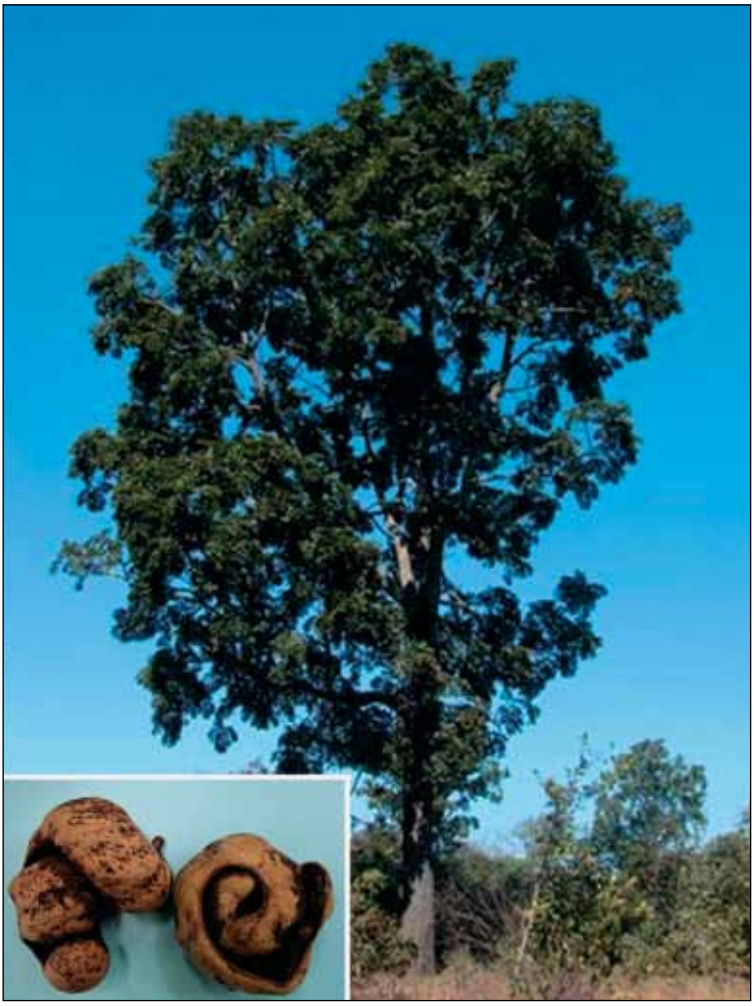

Fig. 1. *Stryphnodendron fissuratum*, Municipality of Rondonópolis, State of Mato Grosso, Brazil. Inset: *S. fissuratum* pods, "doughnuts".

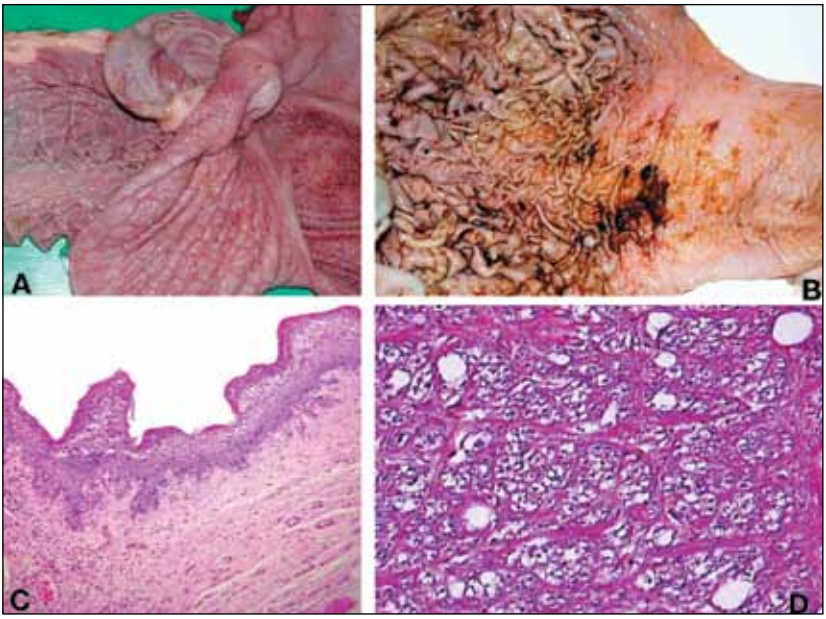

Fig. 2. A. Mucosa of the rumen, omasum and abomasum with hyperemia, petechial hemorrhages and necrosis (goat 6). B. Mucosa of the abomasum with edema of the folds, ulcers and hemorrhagic content (goat 11). C. Necrosis of the rumen mucosa and formation of vesicles with polymorphonuclear cells (goat 6). Obj. 10 ×. HE. D. Brainstem spongiosis (goat 3). Obj. 10 ×. HE.