

Pathological findings in fetuses of goats and cattle poisoned by *Sida carpinifolia* (Malvaceae)¹

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ABSTRACT- Pedroso P.M.O., Colodel E.M., Seitz A.L., Correa G.L.F., Soares M.P. & Driemeier D. 2012. [Pathological findings in fetuses of goats and cattle poisoned by *Sida carpinifolia*.] *Pesquisa Veterinária Brasileira* 32(3):227-230. Setor de Patologia Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9090, Porto Alegre, RS 91540-000, Brazil. E-mail: davetpat@ufrgs.br

In Brazil, the consumption of *Sida carpinifolia* by livestock has been associated with neurological diseases linked to lysosomal storage disorders. This paper describes the pathological findings in two caprine fetuses from dams that were experimentally poisoned with *S. carpinifolia*. The goats were orally dosed with 10 and 13g/kg of a paste of green chopped *S. carpinifolia* for 30 days and were observed for an additional 15 days period after the last dosage with the plant; thereafter they were euthanized and necropsied. The dams showed only slight clinical signs. The study also includes the findings in one bovine fetus from a naturally *S. carpinifolia* poisoned cow which showed mild incoordination, generalized tremors, staggering, and frequent falls. The cow was euthanized and necropsied. While there were no significant histopathological changes in the goats, in the cow vacuolation of Purkinje neurons of the cerebellum, pancreatic acinar cells, and thyroid follicular cells were observed. The main microscopic changes observed in the caprine and bovine fetuses were vacuolation in the epithelium of renal tubules, thyroid follicular cells, and Purkinje neurons of the cerebellum. Transmission electron microscopy of sections from CNS of the cow and its fetus revealed vacuoles containing fine granular material surrounded by membrane. Lectin-histochemistry of CNS sections from goat fetuses marked lightly to sWGA lectins, WGA, and Con-A.

INDEX TERMS: Poisonous plants, Swainsonine, *Sida carpinifolia*, Malvaceae, plant poisoning, lysosomal storage disease, pathological findings, lectin-histochemistry, transmission electron microscopy, caprine and bovine fetuses.

RESUMO.- [Achados patológicos em fetos de caprinos e bovinos intoxicados por *Sida carpinifolia* (Malvaceae).] No Brasil, o consumo de *Sida carpinifolia* por animais de produção tem sido associada a doenças neurológicas

relacionadas com doença de depósito lisossômico. Este trabalho descreve os achados patológicos observados em dois fetos caprinos de mães que foram experimentalmente intoxicadas por *S. carpinifolia*. As cabras foram intoxicadas experimentalmente com *S. carpinifolia* nas doses de 10 e 13g/kg durante 30 dias e foram acompanhadas durante 15 dias após o consumo da planta. Após este período foram eutanasiadas e necropsiadas. O estudo também inclui os achados patológicos encontrados em um feto de uma fêmea bovina intoxicada naturalmente pela planta, que mostrou leve incoordenação, tremores generalizados, andar desequilibrado e quedas frequentes. A vaca foi eutanasiada e necropsiada. Embora não houvesse alterações histológicas significativas nas cabras, vacuolização dos neurônios de Purkinje do cerebelo, das células acinares do pâncreas e nas células foliculares da tireoide foram observadas na

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vaca. As principais alterações histológicas observadas nos fetos caprinos e no feto bovino foram vacuolização no epitélio dos túbulos renais, nas células foliculares da tireoide e nos neurônios de Purkinje do cerebelo. Na microscopia eletrônica de transmissão do sistema nervoso central da vaca e de seu feto revelaram-se vacúolos contendo material finamente granuloso e delimitado por membrana. Na técnica de lectina-histoquímica dos fetos caprinos houve marcação leve no SNC para as lectinas sWGA, WGA e para Con-A.

TERMOS DE INDEXAÇÃO: Swainsonina, *Sida carpinifolia*, Malvaceae, doença de depósito lisossomal, achados patológicos, intoxicação por plantas, lectina-histoquímica, microscopia eletrônica de transmissão, fetos caprinos e bovinos.

INTRODUCTION

In Brazil, the consumption by livestock of *Sida carpinifolia*, also named *Sida acuta* var. *carpinifolia* (L.f.) K. Schum. (Malvaceae family), has been associated with lysosomal storage disorders (Driemeier et al. 2000, Colodel et al. 2002a). Although native to tropical America, *S. carpinifolia* has spread throughout the tropics and subtropics, where it is particularly abundant in humid and reduced sunlight areas (Kismann & Groth 2000, Lorenzi 2008). The disease is caused by the indolizidine alkaloid swainsonina (Colodel et al. 2002b) that inhibits the lysosomal enzyme α -mannosidase and induces the storage of mannose containing oligosaccharides in lysosomes of several cells, particularly neurons, hepatocytes, and acinar pancreatic cells (Stegelmeier et al. 1995, Jolly & Walker 1997).

In the first description of the disease in goats (Driemeier et al. 2000), high rates of abortions and stillbirths were reported. The clinical and pathological features seen in herbivores poisoned by *S. carpinifolia* are indistinguishable from those described in livestock poisoned by plants of the genera *Swainsona* in Australia (Colegate et al. 1979), *Astragalus* and *Oxytropis* in the United States (Stegelmeier et al. 1995, Pfister et al. 2003), *Ipomoea* spp. (Barbosa et al. 2006, Barbosa et al. 2007, Armien et al. 2007, Antoniassi et al. 2007), and *Turbina cordata* (Dantas et al. 2007) in Brazil. This paper describes the pathological findings observed in fetuses from livestock poisoned by *Sida carpinifolia*.

MATERIALS AND METHODS

Experimental animals

Two 2-year-old Saanen goats (Goats 1 and 2), at about six months of pregnancy, were dosed with 10 and 13g/kg of green *Sida carpinifolia* for 30 days. Animals were observed for an additional period of 15 days after the consumption of the plant. The goats originated from farms free from *S. carpinifolia* infestation. The plant was chopped and moistened to form a paste, which was orally administered to the goats using an adapted 60ml syringe. The goats were kept in individual pens, where they also received water *ad libitum* and 200g of commercial concentrate and alfalfa hay.

A for 6-month-pregnant control goat received alfalfa hay, 200g of commercial concentrate and water *ad libitum*. After 15 days of the last dosage, the goats were euthanized and necropsied (Colodel et al. 2002a). The experiment was approved by the Ethic Committee of *Universidade Federal do Rio Grande do Sul* (UFRGS), and the animal protocol was approved by the Research and Post-

graduate Committee of *Hospital de Clínicas de Porto Alegre*, Brazil (Bedin et al. 2010).

Necropsy and histopathology

The study also included a 5-month-old bovine fetus, whose dam had been spontaneously poisoned after consumption of *S. carpinifolia*. The cow and the two goats were necropsied, and fragments of organs were collected and fixed in 10% formalin, processed routinely for histology and stained with hematoxylin and eosin. The same procedures were conducted with the caprine fetuses (Fetus 1 and 2) and the bovine fetus (Fetus 3) and their controls respectively.

Transmission electron microscopy

Fragments (measuring approximately 3mm) from CNS from the cow and its fetus were fixed in 2% glutaraldehyde in phosphate buffer. Later, they were post-fixed in 1% osmium tetroxide, dehydrated in increasing concentrations of ethanol and embedded in Epon. The ultra-thin sections were contrasted with uranyl acetate and lead citrate and examined under a transmission electron microscope.

Lectin-histochemical technique

Fragments of central nervous system (CNS) from the two caprine fetuses were prepared and studied under lectin-histochemistry. Lectins used are shown in Table 1. The slides were deparaffinized in xylene and hydrated in decreasing concentrations of alcohol. The inactivation of endogenous peroxidase was performed with 3% hydrogen peroxide for 30 minutes and with heat recovery using a solution of 10mM citrate buffer (pH 6.0) in a water bath (100°C) for 15 minutes. For the reduction of specific binding (background), sections were treated with skim milk (Molico®) 5% for 30 minutes. Subsequently, the lectins were applied at a dilution of 5µg/ml in PBS (phosphate buffered saline) pH 7.4, except for the lectin Con-A, which was diluted at 1 µg/ml. Then the slides were incubated in a moist chamber at 40°C for 12-14 hours "overnight".

Table 1. Lectins used in histochemical study of poisoning by *Sida carpinifolia*

Lectin	Abbreviation	Carbohydrate specificity ^a
<i>Concanavalia ensiformis</i>	Con-A	α -D-Man; α -D-Glc
<i>Glycine max</i>	SBA	α -D-GalNac; β -D-GalNac; α e β -Gal
<i>Dolichos biflorus</i>	DBA	α -D-GalNac
<i>Ulex europaeus-1</i>	UEA-1	α -L-Fuc
<i>Triticum vulgare</i>	WGA	β -D-GlcNac, NeuNac
<i>Succinyl-WGA</i>	sWGA	(β -(1-4)-D-GlcNac) ²
<i>Arachis hypogaea</i>	PNA	β -D-Gal(1-3) GalNac
<i>Ricinus communis-1</i>	RCA-1	β -D-Gal > α -D-Gal

^a Goldstein I.J. & Hayes C.E. (1978). Fuc = fucose, Gal = galactose, GalNac = N-acetil-galactosamina, Glc = glicose, GlcNac = N-acetil-glicosamina, Man = manose, NeuNac = N-acetil-neuraminic acid.

Thereafter, each slide with their respective lectins was individually washed with distilled water and PBS. Streptavidin was then applied (DAKO LSAB 2 kit, DAKO Corp., Carpinteria, CA) for 20 minutes in a moist chamber. The revelation of the sections was performed with the chromogen diaminobenzidine (DAB) (DAKO) for 10 minutes and then counterstained with Harris hematoxylin for 30 seconds and mounted with Entellan (Merck, Darmstadt, Germany Sigma Chemical Co., Saint Louis, USA) and observed under optical microscope.

RESULTS

During the experimental period, goats showed only mild clinical signs such as slow moving and dullness, but no

change in feces consistency was noticed. After the end of daily dosages, goats started to increase body weight, at a rate of 119-110g/day. The sole significant gross findings at *post mortem* examination of the goats were edema and increased size of lymph nodes, but without significant histopathological changes. The cow presented a clinical picture of incoordination, generalized tremors, staggering and frequent falls when it was stimulated to move. While the cow had no gross changes at necropsy, histopathological changes included vacuolation of Purkinje neurons of cerebellum, pancreatic acinar cells, and thyroid follicular cells. Transmission electron microscopy of sections from thalamus of the cow revealed vacuoles containing fine granular material surrounded by a membrane.

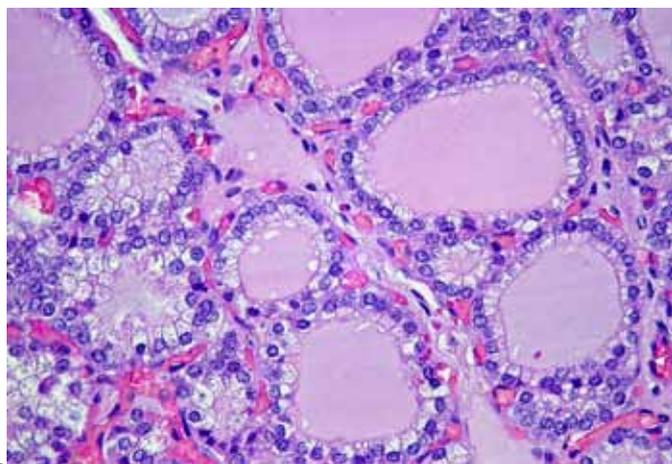
The main histopathological changes observed in the two caprine fetuses were moderate distention and vacuolation of the epithelium of renal tubules and slight disappearance of Nissl substance in neurons of the CNS. There were no pathological changes in the control goat and her fetuses. The main histological findings in the bovine fetus were marked vacuolation of the epithelial cells of renal tubules and moderate vacuolation of the follicular cells of the thyroid (Fig.1). There were no pathological changes in the control

of the bovine fetus (Fig.2). The cerebellum of the bovine fetus had mild vacuolation of the Purkinje neurons, which under transmission electron microscopy revealed small vacuoles containing finely granular material membrane-delimited (Fig.3). Lectin-histochemistry of CNS sections from caprine fetuses showed mild staining with the sWGA (Fig.4), WGA and Con-A.

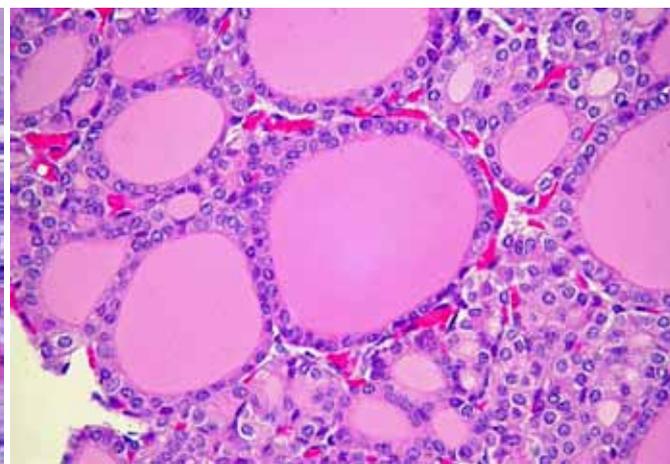
DISCUSSION AND CONCLUSION

Poisoning by *Sida carpinifolia* in these animals was consistent with clinical, histopathological, ultrastructural, and lectin-histochemical findings from previous reports. It has been demonstrated that livestock poisoned by plants containing swainsonine such as *Oxytropis* spp. and *Astragalus* spp. (locoweeds) have also been affected by congenital defects, birth of weak offspring, and abortions (James 1972, Hartley & James 1975), which have been associated with transplacental toxicosis (James 1971) interfering with embryonic and fetal development (James et al. 1992).

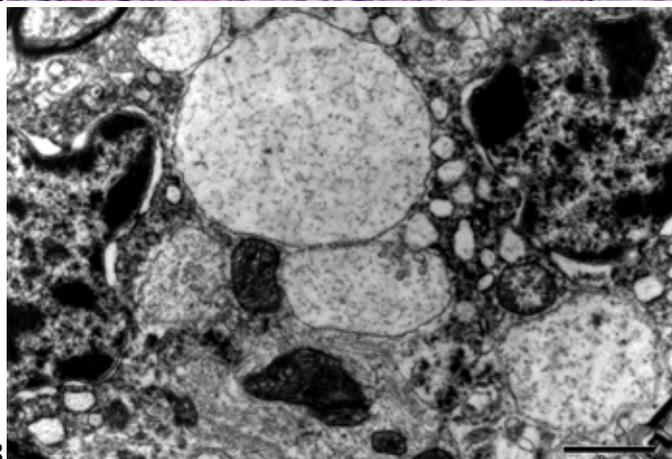
The consumption by pregnant goats of *Sida carpinifolia* has been associated with abortions and stillbirths; however, after stopping consumption of *S. carpinifolia*, goats conceive and due normal kids (Driemeier et al. 2000, Colodel et al.



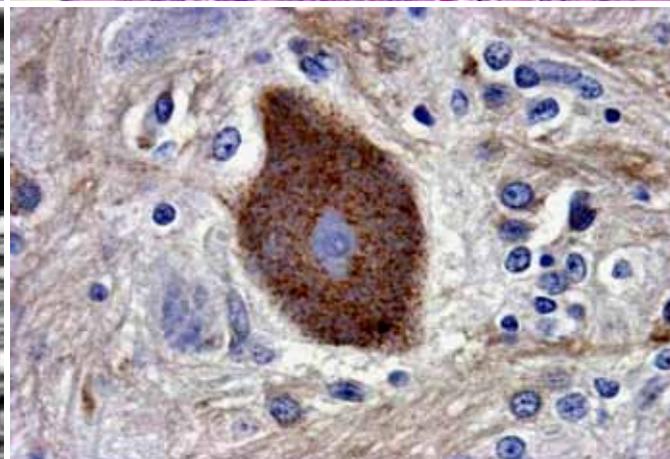
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2



3



4

Fig.1. Bovine fetus. Thyroid. Moderate vacuolation of follicular cells. Hematoxylin and Eosin, obj.40x.

Fig.3. Bovine fetus. Cerebellum. Small vacuoles containing finely granular material membrane delimited. Transmission electron microscopy. Bar: 1 μ m.

Fig.2. Control bovine fetus. Thyroid. Hematoxylin and Eosin, obj.40x.

Fig.4. Caprine fetus 1. CNS. Marking for *Succinyl* WGA (sWGA). Lectin-histochemical, obj.40x.

2002a). The aforementioned factors suggest that *S. carpinifolia* consumption may affect fertility in open goats, as well as may cause pregnancy losses. In addition, sheep experimentally poisoned with *S. carpinifolia* experienced complete clinical recovery after ceasing consumption for a few days (Seitz et al. 2005a). Similar findings were observed here.

The caprine and bovine fetuses from this study had not their gestation interrupted; however, if animals had consumed the plant for longer periods, it may be said that abortion or other disorder could have affected them. These fetuses exhibited histopathological lesions mainly located in epithelial cells of proximal convoluted renal tubules and follicular cells of thyroid; therefore, similar findings as observed in sheep experimentally poisoned by *Astragalus lentiginosus* (James 1971, James 1972, Hartley & James 1975). If such lesions would be capable of producing abortion, stillbirth, or other reproductive disorder in these fetuses is unknown and remain to be proved.

Poisoning by *S. carpinifolia* has been characterized as an α -mannosidase (Driemeier et al. 2000), in which accumulation of oligosaccharides containing α -mannose and β -N-acetyl-glucosamine (GlcNAc) has been demonstrated (Beaudet 1983). Sections from fetuses of this showed affinity by the lectins sWGA, WGA, and Con-A. Lectins WGA and sWGA characteristically mark GlcNAc residues (Monsigny et al. 1980), and lectin Con-A binds specifically to α -mannose (Goldstein & Hayes 1978). Pattern seen on lectin-histochemistry here is similar to that observed previously in *S. carpinifolia* poisoned sheep, deer and cattle (Seitz et al. 2005b, Pedroso et al. 2009, Pedroso et al. 2010, 2011).

Findings reported here confirm that *Sida carpinifolia* must be included in the list of poisonous plants linked to reproduction disorders in Brazilian livestock such as *Aspidosperma pyriforme*, *Ateleia glazioviana*, *Tetrapteryx* spp., *Stryphnodendron* spp., and *Mimosa tenuiflora* (Riet-Correa 2007, Lima & Soto-Blanco 2010).

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