Poisoning by *Astragalus garbancillo* var*. garbancillo* in Sheep in Northwestern Argentina

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Abstract

Several toxic plants produce lysosomal storage of glycoproteins and mainly nervous clinical disorders. A disease caused by the consumption of *Astragalus garbancillo* var. *garbancillo* in sheep from northwestern Argentina is described here. The affected animals presented weight loss, listlessness, staggering gait, and ataxia. Histopathological studies revealed cellular vacuolation in several organs, mainly in the central nervous system (CNS). The material accumulated in the cells was positive for lectins LCA (*Lens culinaris* agglutinin), sWGA (succinyl–*Triticum vulgaris* agglutinin), and Con A (*Concanavalin* A). Finally, the presence of toxic levels of swainsonine was detected in composite samples of the plant. Poisoning by *Astragalus garbancillo* var. *garbancillo* was characterized as α-mannosidosis of plant origin.

Keywords: Northwestern Argentina, sheep, swainsonine, *Astragalus garbancillo* var. *garbancillo*

Introduction

Glycogenosis caused by the consumption of plants of the *Swainsona* genera in Australia and *Oxytropis* and *Astragalus* in many other parts of the world has been known for years (Huang et al. 2003). However, several species in these taxonomic groups are not toxic and even have potential as good forage resources (Cook et al. 2014).

The genus *Astragalus* is widely distributed and comprises between 2,500 and 3,000 species (Niknam and Lisar 2004), with 110 endemic species being present in South America from Ecuador, Peru, and Bolivia to Chile and Argentina. In Argentina, 70 species occur (Zuloaga et al. 2008) from Jujuy to Tierra del Fuego, mainly in the Andean-Patagonian region (Gómez-Sosa 1979, 1994; Daviña and Gómez-Sosa 1993). According to Gómez Sosa (2004), *Astragalus garbancillo* grows from northern Peru through Bolivia to central-western Argentina at elevations of 1,600-4,500 m. The author identifies two varieties: *A. garbancillo* var. *garbancillo* and *A. garbancillo* var. *mandoni,* which differ in the tip of the flower keel. Only the variety *garbancillo* grows in Argentina (Gómez Sosa 2004). The toxicity in the genus *Astragalus* is due to the presence of

swainsonine, an alkaloid that can inhibit lysosomal α-mannosidase and Golgi α-mannosidase II (Cook et al. 2014).

Williams and Gomez Sosa (1986) determined nitro compounds in 30 species of *Astragalus* in Argentina. Swainsonine has been measured only in *A. pehuenches* (Molyneux & Gómez-Sosa 1991, Robles et al. 2000) and now in *A. garbancillo*. Endophytic fungi have been shown to be responsible for swainsonine production in *Astragalus* and other plants (Cook et. al. 2014). The possible involvement of fungi in both *A. pehuenches* and *A. garbancillo* remains to be studied.

The present work describes locoweed cases in sheep associated with the consumption of *Astragalus garbancillo* var. *garbancillo* (*A. garbancillo*) in northwestern Argentina.

Materials and Methods

The study consisted of four visits to the locality of Pampa Llana, 26°61'S, 66°28'W, 3655 m, San Carlos department, Salta province, Argentina, where nervousness and death in several sheep flocks was described. The area corresponds to the Puna biogeographic region and is characterized by a dry and cold climate with large daily temperature variations reaching absolute minimum values down to –15 ºC (Reboratti 2005). The evaluation of the situation involved visiting five farmers and asking them about the disease. During the interviews, two criollo sheep affected by the condition mentioned above were identified. One of the animals was a 4-year-old ewe with obvious signs of nervousness (animal I), and the other was a 6-year-old ewe showing progressive weight loss and depression (animal II). Both animals were euthanized by administration of an overdose of xylazine or ketamine, and complete post-mortem examination was performed. Tissue samples from CNS, pancreas, heart, liver, and kidney were taken, fixed in 10% buffered formalin, and processed following the common procedure for histological examination. Selected sections of the CNS were subjected to lectin histochemistry using previously described procedures (Driemeier et al. 2000). Finally, suspected plants were collected from different areas of the grazing sites, pressed, and dried for identification at the MCNS Herbarium of the National University of Salta (Holmgren et al. 1990). Part of the plant material was dried to obtain a composite sample. This sample was used to determine swainsonine (SW) using the methods described by Gardner et al. (2001). Results are expressed in % SW (Dry Matter, DM).

Results

Clinical and Epidemiological Findings

All of the farmers stated that between 0.5 and 2% of the flock is affected every year and that the problem is more severe in dry years, with cases usually occurring between August and December. The two affected animals belonged to different flocks that grazed in different areas of the same region. Animal I exhibited weight loss, listlessness, staggering gait, neck and head tremors, ataxia, and difficulty in standing. When animal I attempted to raise its head, the signs increased, and the animal showed the inability to stand, remaining instead in a dog's sitting position. Animal II exhibited gradual weight loss, pale mucous membranes, listlessness, and overall poor condition. Finally, all producers associated the observed disease with the consumption of *A. garbancillo*, indicating that, once affected, the animals leave the flock, walk erratically, and tend to consume the plant compulsively.

Macroscopic Findings

The observed macroscopic changes include lack of subcutaneous and abdominal fat. The animal with nervous signs exhibited mild bruises resulting from the difficulty standing (figure 1).

Figure 1. Adult sheep affected by the disease as shown in the sheep's "dog-sitting" position.

Botanical Identification and Analysis of Swainsonine

All the samples were identified as *A. garbancillo* var. *garbancillo* and recorded as specimen MCNS 12880 at the MCNS Herbarium of the National University of Salta. Swainsonine concentration in the composite samples of leaves and fine stems ground at early vegetative stage was 0.030% (in March 2014) and 0.034% (in December 2014) (figure 2).

Figure 2. *Astragalus garbancillo* var. *garbancillo*.

Histopathological Findings

Histopathological analyses in both animals revealed vacuolation of the neuronal cytoplasm, especially in cerebellum and basal nuclei. In animal II, vacuolation was less evident, and a loss of a large number of Purkinje cells in cerebellar folia was observed. Many of the remaining cells exhibited necrosis with pyknotic and condensed cytoplasm (figure 3). All these results are detailed in table 1. Vacuolation also extended to other tissues, such as the myocardium, liver, kidney, and pancreas. In the pancreas, vacuolation affected the exocrine cells, and islets of Langerhans exhibited atrophy (table 2).

Lectin Histochemistry

Results of lectin histochemistry are shown in table 2. Different labeling patterns were observed between animals. In animal I, the cells exhibited a “foamy” pattern, with multiple coalescent vacuoles in the cytoplasm. In animal II, the affected neurons exhibited a solid and homogenous, condensed labeling pattern. Some of these cells also exhibited a densely granulated cytoplasm (figure 4).

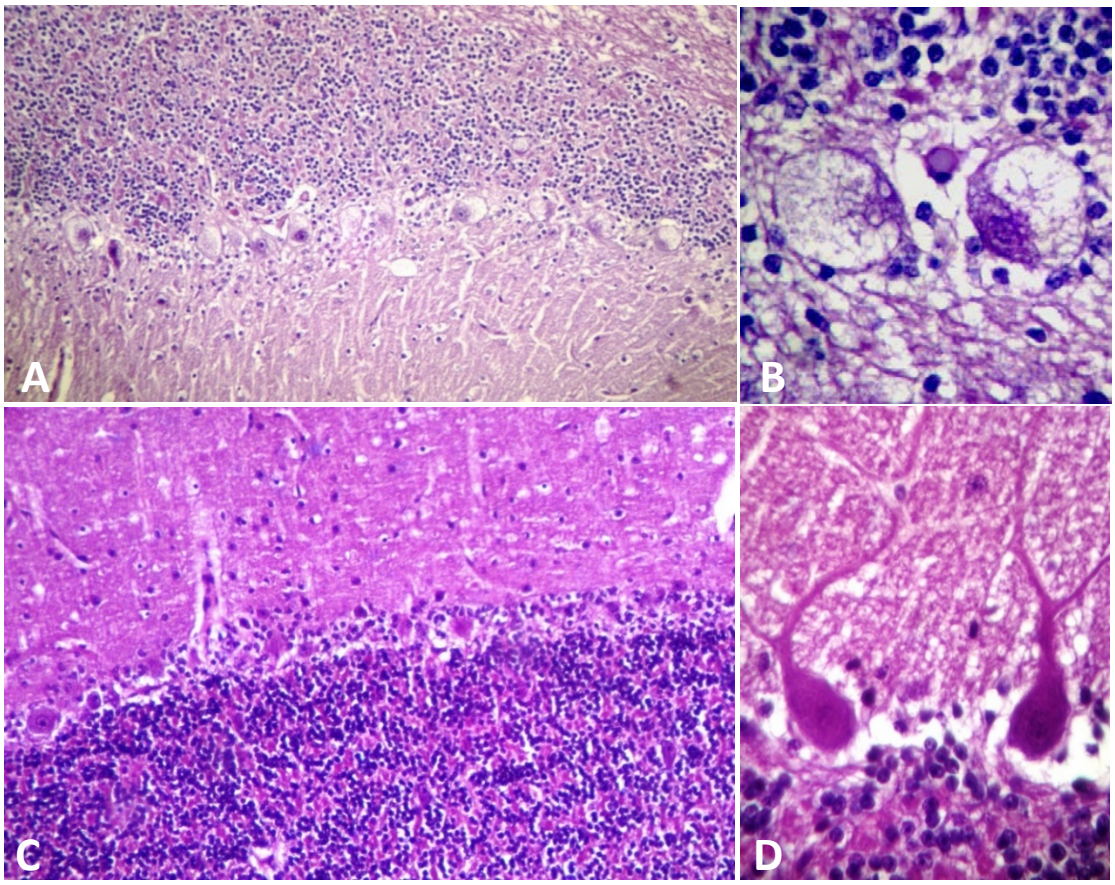

Figure 3. Animal I: (A) Severe vacuolation of the Purkinje cells (H&E 40X). (B) Detail of affected Purkinje cells (H&E 400X). Animal II: (C) Remarkable loss of Purkinje cells in the cerebellar folia. Retraction of the scarce remaining cells (H&E, 40X). (D) Detail of cellular lesions showing cytoplasmic retraction (H&E 400X).

Table 1. Histopathological findings in nervous system

Animal	Samples	Neuronal loss	Vacuolar changes	Gliosis
I	Cerebral cortex	-	++	+
	Basal ganglia	-	++++	+
	Cerebellum	+	++++	-
II	Cerebral cortex	-	+	++
	Basal ganglia	++	+	+++
	Cerebellum	+++	++	+++

- = no / + = minimal + + = mild ++ + = moderate ++++ = severe

Table 2. Lectinhistochemical analysis in nervous system

Animal	Samples	LCA	BS1	WGA	sWGA	Con A
I	Cerebellum	+	-	+	+	+
	Basal ganglia	+	-	+	+	+
II	Cerebellum	+	-	-	-	+
	Basal ganglia	+	-	+	+	+

+ = positive - = negative

Discussion

The clinical and pathological findings, along with the regional history and the concentration of swainsonine found in *A. garbancillo* var. *garbancillo* in the two sampling periods, allow us to attribute the severe clinical cases observed to the consumption of this plant. Swainsonine concentrations were about 0.030% of DM in both sampling periods; levels above 0.001% are considered harmful for livestock (Molyneux et al. 1994). Stegelmeier et al. (1999) indicated that doses of swainsonine equal to or above 0.2 mg/kg/day for at least 21 days can produce irreversible neurological disease.

Sheep appear to be particularly sensitive to the effects of swainsonine, which has been attributed to differences in the toxicokinetics of the alkaloid (Stegelmeier et al. 1995). The observed vacuolation in tissues is a typical characteristic of poisoning by plants containing swainsonine (Jolly and Walkley 1997), and the histological differences found between the two cases studied can be attributed to different stages of disease development. Lesions similar to those found in animal II were mentioned for goats naturally intoxicated with *Ipomoea carnea* (De Balogh et al. 1999), which indicated more chronic stages of the disease as the possible causes for these findings. James and Van Kampen (1971) also mentioned the presence of residual lesions in experimentally poisoned horses and cattle that were euthanized 1 month after the end of plant consumption. Accordingly, the accumulation of sugars in cytoplasm may lead to cellular death (Dorling et al. 1989, Elbein 1989, Takeda et al. 2014), which is in agreement with the high loss of Purkinje cells in the cerebellum observed in animal II.

In agreement with other cases of α-mannosidosis, lectin histochemistry showed positive reactivity with Con-A, WGA, sWGA, and LCA in cytoplasmic vacuoles of Purkinje cells (Stegelmeier et al. 2005). As with histological differences, the different labeling patterns observed may be due to the different development stages of the disease. Lectin labeling with Con A and LCA suggests accumulation of mannose, whereas WGA and sWGA indicate accumulation of N-acetyl-D-glucosamine and sialic acid (Goldstein and Hayes 1978, Goldstein 1980). Thus, the type of sugar accumulated by the cells would vary with stage of disease progress both in the cerebellum and in the basal nuclei.

Other normally affected tissues are the reproductive organs, the nervous system, and the endocrine and immunological system (Elbein et al. 1981, Driemeier et al. 2000, McLain et al. 2004, Stegelmeier et al. 2005, Dantas & Riet-Correa 2007, Ríos et al. 2008, 2015). In this case, pancreatic, hepatic, and renal lesions described agree with those produced by other plant species that induce lysosomal accumulation due to the presence of swainsonine (Van Kampen and James 1972, Driemeier et al. 2000, Dantas and Riet-Correa 2007, Ríos et al. 2008). Cardiac lesions observed seem to

be much less frequent, although they were described in experimentally poisoned rats (Stegelmeier et al.1995). Therefore, the myocardium seems to be more resistant to the action of swainsonine than other tissues. Accordingly, sheep experimentally intoxicated with *Astragalus lentiginosus* did not reveal cardiac lesions 32 days after administration of the plant (Van Kampen and James 1972).

Molecular phylogenetic studies of South American species of *Astragalus* grouped the species in two different clades (Wojciechowski et al. 1999). Scherson et al. (2008) included *A. garbancillo* in a subclade with several species including *Astragalus amatus, A. arnotianus, A. berteroanus*, *A. cruckshanksii, A. cryptobotrys, A. nivicola,* and *A. uniflorus*. However, none of these species have been reported as being toxic due to the presence of swainsonine. Swainsonine levels found in *A. garbancillo* var. *garbancillo* were similar to those reported in other species (Ralphs et al. 2008). However, the concentration of swainsonine may vary according to the presence of endophytes, the rate of growth, and environmental stress conditions (Ralphs et al. 2008).

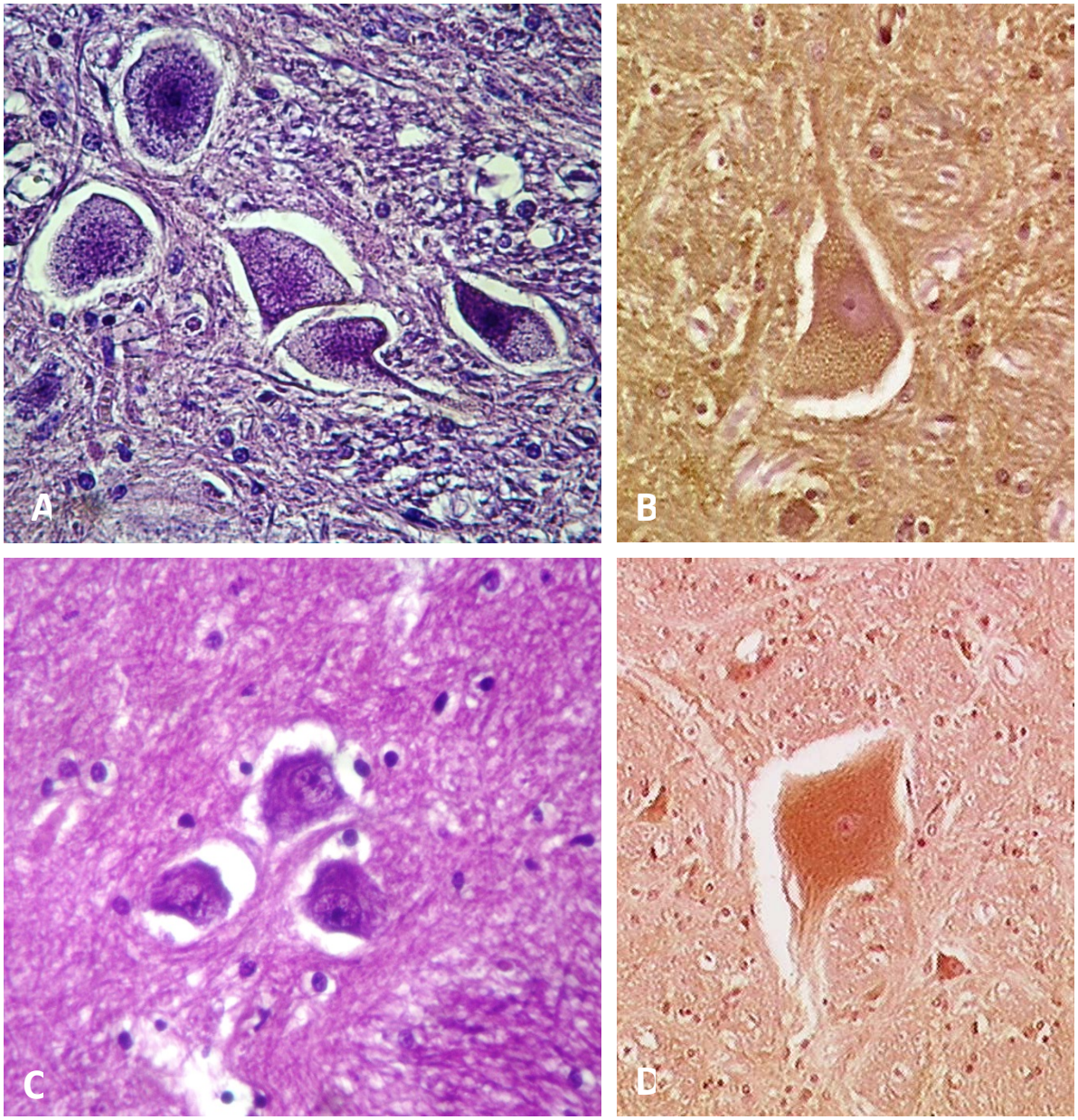

Figure 4. Animal I: (A) Severe neuronal vacuolation at the basal cerebellar nuclei (H&E 40X). (B) Vacuolated perikaryon positive for LCA (400X). Animal II: (C) neurons at the basal cerebellar nuclei showed condensed perikaryon (H&E 40X) and labeled positive with LCA (400X).

Overall, the observed clinical signs, the histopathological lesions of vacuolation, and neuronal loss associated with lectin labeling allow us to conclude that the animals were affected by α-mannosidosis. The abundance of *A. garbancillo* var. *garbancillo* in the grazing areas and the high swainsonine levels detected in the two sampling periods, along with the knowledge of the local farmers, suggest that this plant was the cause of the condition. The variations in the lesion patterns observed, as well as the diversity of the genus in South America, require further studies on this topic.

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