

Cerebellar Cortical Degeneration in Cattle Poisoned With *Solanum* spp. in South America: An Epidemiological, Clinicopathological, Pathological, and Toxicological Review

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Abstract

Cattle that consume *Solanum bonariense* L (= *Solanum fastigiatum* Willd.) or *Solanum paniculatum* L. develop a typical cerebellar cortical degeneration characterized by periodic episodes of ataxia, hypermetria, hyperesthesia, head and thoracic limb extension, opisthotonus, nystagmus, and falling to the side or backward. Histological lesions include vacuolation, degeneration, and loss of Purkinje cells. Axonal spheroids, microcavitations, and other changes of Wallerian degeneration in cerebellar granular layer and white matter are also observed. Neurotoxic compounds in *Solanum* spp. causing neurologic dysfunction in ruminants were not definitively elucidated. The same Solanaceae species are extensively used with culinary purposes or for the treatment of liver and gastrointestinal disorders as hangovers in humans. In the present paper, we review the epidemiology, clinical signs, and pathological hallmarks of poisoning by *Solanum* —*S. bonariense* L. (= *S. fastigiatum* Willd.) and *S. paniculatum*—with emphasis in histopathology, ultrastructural, and lectin- and immuno-histochemical changes in spontaneous and experimentally poisoned cattle in South America. The current knowledge of the pathogenesis of these bovine cerebellar cortical degenerations is discussed, and some advances in botanical and toxicological aspects of these Solanaceae species are presented, taking into account the potential risk of human poisoning.

Keywords: cerebellar degeneration, diseases of cattle, poisonous plants, Solanaceae

Introduction

From the beginning of the 16th century when cattle were first introduced into South America, the use of autochthonous plants as forage contributed to livestock losses from toxic plants that were novel to Spanish and Portuguese settlers. To overcome this drawback, the settlers had to recognize and consider these potential risks to minimize animal deaths, and in some cases, learn about the aboriginal practices or

develop new strategies to avoid plant poisoning. A good example of these practices is the description of *Baccharis coridifolia* (mio-mio) poisoning and control by the Spanish Jesuit chronicler Bernabé Cobo (Cobo 1653).

At present, poisonous plants still constitute a very important problem causing direct and indirect losses to farmers in South America. An estimation of

total cattle losses obtained from different regional veterinary diagnostic laboratories from Brazil and Uruguay indicates an annual mortality rate of 5 percent, of which 10 to 14 percent is due to poisonous plants (Riet-Correa and Medeiros 2001). A recent survey of cattle necropsies examined at the Laboratory of Veterinary Pathology of Federal University of Santa Maria from 1990 to 2005 (2,912 cases) indicates that in 15.83 percent of the cases, the cause of death was attributed to the ingestion of poisonous plants (Rissi et al. 2007). *Solanum fastigiatum* Willd. var. *fastigiatum* (“Jurubeba”) causes serious intoxication problems in cattle in Rio Grande do Sul, southern Brazil (Rissi et al. 2007, Sant’Ana et al. 2011), causing an irreversible cerebellar disorder (Riet-Correa et al. 1983, Zambrano et al. 1985, Barros et al. 1987, Paulovich et al. 2002, Rech et al. 2006, Sant’Ana et al. 2011). Similar cerebellar syndromes were described for *Solanum bonariense* L. (“Naranjillo”) in Uruguay and Argentina (Podestá et al. 1971, Riet-Correa et al. 1983, Verdes 2006, Verdes et al. 2006, Verdes et al. 2007, Verdes et al. 2010, Odriozola et al. 2012) and for *Solanum paniculatum* L. (“Jurubeba”) in Brazil (Barros et al. 1987; Medeiros et al. 2004; Guaraná et al. 2011a,b). These Solanaceae species belong to a genus distributed worldwide that encompasses other well-recognized toxic species that cause multiple disease conditions and death by different mechanisms, and that are especially abundant in tropical and subtropical regions of Central and South America (Baker et al. 1989). The intoxication has been experimentally reproduced in cattle (Riet-Correa et al. 1983, Barros et al. 1987, Medeiros et al. 2004, Verdes et al. 2006).

As in other cases of *Solanum* intoxication that cause cerebellar degeneration in South Africa, the United States, and Australia (Piennar et al. 1976, Menzies et al. 1979, Bourke 1997, Porter et al. 2003), the main pathophysiological features of South American species are the vacuolation of the perikaria in Purkinje cells followed by loss of these neurons and the presence of axonal spheroids, as well as microcavitations in the cerebellar granular layer and/or white matter (Riet-Correa et al. 1983; Barros et al. 1987; Medeiros et al. 2004; Verdes et al. 2006; Guaraná et al. 2011a,b).

On the other hand, *S. paniculatum* and *S. fastigiatum* are extensively used for culinary purposes, in alcoholic beverages (figure 1), or as infusions in Brazilian folk medicine for the treatment of liver and gastrointestinal disorders from excess alcohol consumption in humans (Vieira et al. 2010). They are potent inhibitors of gastric acid

secretion (Mesia-Vela et al. 2002), and their aqueous extract has antioxidant and hepatoprotective activity in mice (Sabir and Rocha 2008).



Figure 1. *Solanum* sp. (“Jurubeba”) is used often for culinary purposes or in alcoholic beverages. Left, “Jurubeba” fruit sauce offered as a condiment in a traditional self-service restaurant in Goiás near Brasília, Brazil. Right, a bottle of “Jurubeba” wine purchased in the interior of the State of Pernambuco, Brazil.

Until recently the neurotoxic compounds in *Solanum* spp. causing neurologic dysfunction in ruminants were not definitively elucidated (Baker et al. 1989, Verdes et al. 2007, Guaraná et al. 2011b). However, different substances or fractions were characterized from some of these South American species, stimulating bioassay-guided studies in animal models (Mesia-Vela et al. 2002, Ruiz-Diaz et al. 2004, Higa et al. 2006, Sabir and Rocha 2008).

Although rodents seem to be adequate biomodels to test some pharmacological effects of *Solanum* spp., at the present time, no susceptible laboratory animals have been found to reproduce cerebellar dysfunction similar to that reported in cattle (Riet-Correa et al. 1983, Zambrano et al. 1985, Ruiz-Diaz et al. 2004). Nevertheless, sheep (Zambrano et al. 1985) and goats (Verdes et al. unpublished results) seem to be alternative biomodels to study this neurodegeneration.

The identification and characterization of neuroactive principles present in aerial parts of *Solanum* spp., as well as its ruminal by-products, could become a key step to definitively clarify its specific toxicological pathways towards its target, the cerebellar Purkinje cells. This would contribute to an understanding of the pathogenesis of these acquired cerebellar degenerations in ruminants and also to the safe use of these plants as natural or folk medicine for humans.

The objective of the present paper is to review the epidemiology, clinical signs, and pathological hallmarks of poisoning by *Solanum*–*S. bonariense* L. (= *S. fastigiatum* Willd.) and *S. paniculatum*—with emphasis in histopathology, ultrastructural, lectin- and immuno-histochemical changes in spontaneous and experimental poisoned cattle in South America. The current knowledge of the pathogenesis of these bovine cerebellar cortical degenerations is discussed, and some advances in botanical and toxicological aspects of these Solanaceae species are presented.

Epidemiology and Clinicopathology of the Poisoning in Cattle and Animal Models

The spontaneous disease caused by *Solanum* spp. affects cattle older than 8 months of age of various breeds, but dairy cattle and crossbred animals are most affected. Morbidity varies from 1 to 25 percent. Deaths are uncommon and are associated with progressive weakening, drowning accidents, or traumatic injuries; in some specific cases, mortality can reach 20 percent (Guaraná et al. 2011b). Farmers usually sell affected animals for slaughter when clinical signs are observed. No seasonal differences were detected in the incidence of the disease (Riet-Correa et al. 1983).

A major clinical feature of affected cattle is the occurrence of periodic attacks in which CNS dysfunction leads to falls and an inability to rise, without loss of consciousness, lasting up to 1 minute; most animals appear normal between episodes. Other clinical signs include ataxia, hypermetria, hyperesthesia, staggering gait, muscle tremors, head and thoracic limb extension, opisthotonus, nystagmus, and in those animals most severely affected, falling to the side or backward. Nervous signs occur spontaneously or are induced when affected animals became excited or intentionally stressed, for instance with the head-raising test (Pienaar et al. 1976, Riet-Correa et al. 1983, Verdes et al. 2006, Guaraná et al. 2011a). A few animals show permanent neurological signs, including dysmetria and a “star gazing” attitude. In affected animals, serum levels of AP, AST, and GGT are usually normal (Riet-Correa et al. 1983, Verdes et al. 2006).

The poisoning has been experimentally reproduced in cattle, inducing clinical signs similar to those observed in the spontaneous intoxication (Riet-Correa et al. 1983, Zambrano et al. 1985, Barros et al. 1987, Medeiros et al. 2004, Verdes et al. 2006, Guaraná et al. 2011a) (table 1). All

experimental studies clearly demonstrate that cattle had to consume considerable quantities of the plant, over 76 to 260 days, in order to show clinical signs (table 1). Thus, the occurrence of clinical signs is more likely when pastures invaded by *Solanum* spp. are overgrazed (Riet-Correa et al. 1983, Zambrano et al. 1985, Barros et al. 1987, Medeiros et al. 2004, Guaraná et al. 2011a) or when the grass in the affected paddocks was previously mowed before introduction of the animals. This situation promotes the sprouting of *Solanum* spp. in the pastures, which likely facilitates their intake by cattle (Guaraná et al. 2011b).

In sheep, similar cerebellar lesions as those described for cattle were observed after administration of a commercial ration containing 20 percent of dry plant for 202 to 370 days (total dose 0.43 kg of dried leaves/kg BW). However, none of the guinea pigs, rabbits, or rats were intoxicated after being given an oral dose via commercial ration containing 10 percent of dry plant for 120 days (Zambrano et al. 1985). Recently, similar cerebellar lesions to those in cattle and sheep were observed in goats after oral administration of dry leaves (Verdes et al. unpublished results).

Finally, it is important to note the existence of several diseases of cattle characterized by signs of cerebellar insufficiency, some of which also produce degeneration and vacuolation of Purkinje cells and axonal spheroids as observed in *Solanum* spp. poisoning (table 2). A very intriguing characteristic of cerebellar cortical degeneration induced by *Solanum* spp. is the highly selective damage done to Purkinje cells. The basis for this selectivity remains unknown; the identification of the stored material within these neurons or determination of the neuroactive substances present in these shrubs would help to clarify this aspect (Riet-Correa et al. 1983, Verdes 2006).

Pathology, Histopathology, Transmission Electronic Microscopy (TEM), and Lectin Histochemistry

Natural cases of *Solanum* poisoning usually do not present significant macroscopic lesions (Riet-Correa et al. 1983, Verdes et al. 2006). However, Rech et al. (2006) observed a case of encephalic traumatic subdural hemorrhage and another case with gross atrophy of the cerebellum out of 19 cases reported. Similarly, Guaraná et al. (2011b) found gross atrophy of the cerebellar grey matter in one of two cases of *S. paniculatum* poisoning, and Sant’Ana et

Table 1. Summary of different strategies used to induce experimental cerebellar cortical degeneration in cattle with *Solanum* spp.

Species	Dosage schedule and method used	Cumulative dose	Days before first clinical signs	Reference
<i>S. fastigiatum</i> Willd. (1809) var. <i>fastigiatum</i> *	0.4 kg/day of dried leaves via rumen cannula	0.18 kg of dried leaves/kg BW	155 days	Riet-Correa et al. 1983
<i>S. fastigiatum</i> Willd. (1809) var. <i>fastigiatum</i> *	Oral administration of commercial ration containing 10% of dry plant	0.14 kg of dried leaves/ kg BW	76 days	Riet-Correa et al. 1983
<i>Solanum</i> sp.**	Green leaves <i>ad libitum</i> for 639 days	0.94 kg of green leaves/ kg BW	260 days	Barros et al. 1987
<i>Solanum</i> sp.**	5 g/kg BW of dried leaves, via rumen cannula (5 days/week)	0.17 kg of dried leaves/kg BW	106 days	Barros et al. 1987
<i>Solanum paniculatum</i> L. (1762)	5 g/kg BW of dried leaves, via rumen cannula (5 days/week)	Not showed	93 to 102 days	Medeiros et al. 2004
<i>Solanum bonariense</i> L. (1753)*	Green leaves at 1% BW, via rumen cannula, daily	1.024 kg of green leaves/kg BW	128 to 136 days	Verdes et al. 2006
<i>Solanum paniculatum</i> L. (1762)	Oral administration of commercial ration containing 3g of dry plant/kg BW during 3 months and after that, 4g of dry plant/kg BW during 1 month more	Not showed	120 days	Guaraná et al. 2011a

* *S. bonariense* L. (= *Solanum fastigiatum* Willd.) (Chiarini et al. 2007, Wagstaff 2008).

** Later identified as *S. paniculatum* L. (Franklin Riet-Correa, unpublished data).

Table 2. Diseases with clinical signs and pathology similar to *Solanum* spp. poisoning in cattle

In utero infection with BVD (at birth)

Cerebellar abiotrophia (hereditary, appears up to 8 months of age)

Cerebellar cortical atrophy (unknown cause, up to 8 months of age)

Familial convulsions in Angus cattle (inherited)

Mannosidosis or pseudolipidosis of Angus cattle (inherited)

GM1 gangliosidosis of Friesian cattle (inherited)

Others inherited Lysosomal Storage Diseases*

Lysosomal Storage Diseases acquired by plant poisonings (i. e.: *Swainsona* spp, *Astragalus* spp.,

Oxytropis spp., *Sida* spp., and *Ipomoea* spp.)*

*Cell vacuolation are present in visceral tissues and in other regions of CNS

al. (2011) reported a moderate atrophy of the cerebellum in 2 out of 33 spontaneously poisoned cattle. Other traumatic lesions like hip luxation are probably caused by traumatic injury during sporadic poisoning episodes (Riet-Correa et al. 1983).

The microscopic lesions are almost always localized only in the cerebellum. Vacuolation of the perikarya is present in Purkinje neurons (figure 2). Eosinophilic, round, homogeneous, occasionally fine granular axonal spheroids and microcavitations are found in the granular layer and cerebellar white matter (Riet-Correa et al. 1983, Verdes et al. 2006, Guaraná et al. 2011b) (figure 3). The quantification of Purkinje cells in spontaneous cases shows a decrease in number (Verdes et al. 2006, Rech et al. 2006) and in association with a consequent decrease

in the thickness of the molecular layer (Rech et al. 2006).

In the experimental poisoning of cattle, the decrease in numbers of Purkinje cells is associated with an increment of axonal spheroids and microcavitations in the cerebellar granular layer and white matter (Verdes et al. 2006). The severity of these alterations are associated with the quantity of plant consumed (Zambrano et al. 1985, Verdes et al. 2006).

Other interesting features of this plant poisoning are the proliferation of Bergmann glia in the cerebellar cortex or gliosis in the cerebral cortex and pons (Riet-Correa et al. 1983, Verdes et al. 2006, Guaraná et al. 2011b). Axonal spheroids and some vacuolated neurons are observed in fastigial,

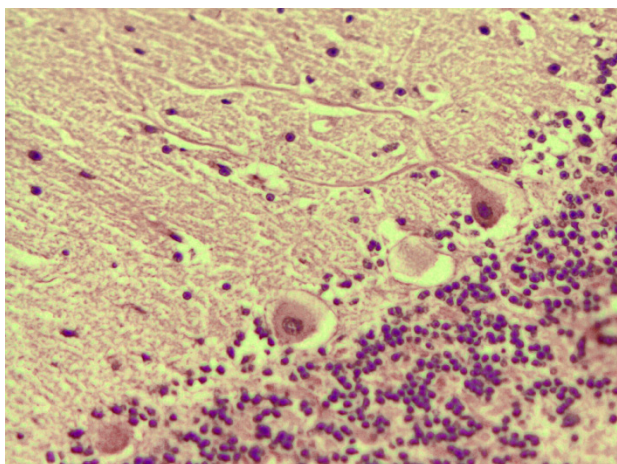


Figure 2. Cerebellar cortex of a steer experimentally poisoned with *Solanum bonariense* L. Note Purkinje neurons showing vesiculated perikaryon and nuclear displacement. HE stain.

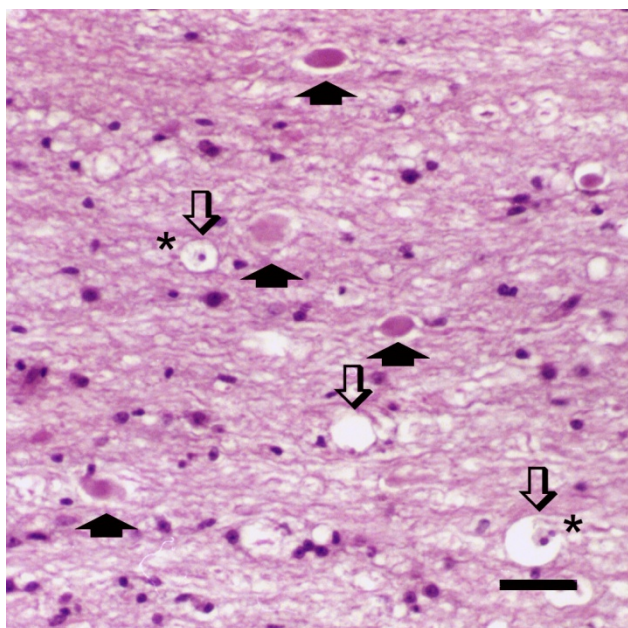


Figure 3. Cerebellar white matter of spontaneously poisoned heifer showing axonal spheroids (black arrows) and macrophages (asterisks) within some microcavitations (empty arrows). HE stain. Bar=70µm.

interposital, and lateral cerebellar nuclei, and more rarely found in the caudal colliculi (Riet-Correa et al. 1983), gracilis nucleus (Guaraná et al. 2011b), or in other brain stem nuclei (Odriozola et al. 2012). Degenerative changes in the cerebellar cortex suggest a progressive demyelination of white matter by Wallerian degeneration (Riet-Correa et al. 1983, Verdes et al. 2006), which was confirmed by Kluver-Barrera and Bielchowsky staining methods (Verdes et al. 2011).

Gangliosides and glycolipids are particularly abundant in neurons, requiring a continual

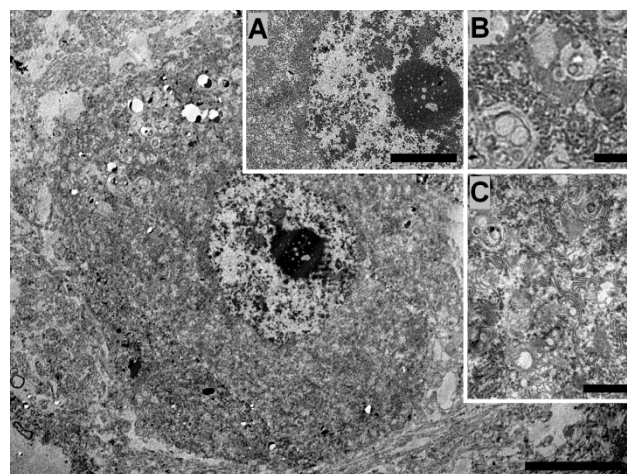


Figure 4. A TEM image of a Purkinje cell in a steer experimentally poisoned by *Solanum bonariense*. The selected neuron shows damage in organelles present in the perikaryon of the cell, but no signs of nuclear apoptosis can be seen. Bar = 10000 nm. A. Magnification of the nuclear area where disaggregation of granular and fibrillar elements can be observed in the nucleolus. Bar = 5000 nm; B. Magnification of the perikaryon of the same cell showing typical extensive vacuolation and vesicles filled with several membranous bodies. Bar = 1000 nm; C. Lamellar arrays of endoplasmic reticulum and myelin figures filled with membranous debris are observed. Bar = 500 nm.

degradation and resynthesis of new molecules. Typical gangliosidoses are inherited lysosomal storage diseases characterized by an altered expression of lysosomal enzymes responsible to hydrolyze gangliosides, resulting in storage of these incomplete degradative by-products in neurons and others cells. This causes a lysosomal overload of this undigested material and results in neurological dysfunction and other diseases (Riet-Correa et al. 1983, Smith 2006, Guaraná et al. 2011b). A quantification of gangliosides in the cerebellum, especially in Purkinje cells, of normal and poisoned cattle is necessary to confirm that cerebellar cortical degenerations are associated with the accumulation of these substances. GD1 α , a sialoganglioside highly expressed in Purkinje cells, could be a potential candidate to confirm or refute this hypothesis (Furuya et al. 1996, Verdes 2006).

The axonal spheroids are the result of myelinated axon enlargement, with many heterogeneous membranous organelles filled with residual bodies and swollen mitochondria (Riet-Correa et al. 1983, Barros et al. 1987, Verdes et al. 2006). The progressive increase in the ratio of axoplasm to myelin confirms demyelination by Wallerian degeneration in affected axons (Barros et al. 1987, Verdes et al. 2006). The microtubules and

neurofilaments are markedly altered in affected Purkinje cells, some dendrites, and axonal spheroids (Barros et al. 1987, Verdes et al. unpublished results). Also, a great concentration of mitochondria is present in swollen axons, as well as in some swollen dendrites of Purkinje cells in the cerebellar molecular layer (Barros et al. 1987).

In cattle poisoned with *Solanum bonariense* (= *Solanum fastigiatum*), the lectin binding pattern of the stored material present in affected Purkinje cells demonstrates accumulation of β - (1-4)-D-N-acetylglucosamine, α -D-mannose, α -D-glucose, D-mannose, D-glucose, D-N-acetyl chitobiose, and N-acetyl lactosamine residues (Sant'Ana et al. 2011). A similar pattern is found in glycolipid storage diseases (Paulovich et al. 2002, Sant'Ana et al. 2011) or in those conditions detected in plant-induced α -mannosidosis, including poisonings by plants of the genera *Swainsona*, *Oxytropis*, *Astragalus*, *Sida*, and *Ipomoea*. However, in the latter cases, there is additional vacuolization in pancreatic, liver, and kidney epithelial cells (Alroy et al. 1984, 1985; Driemeier et al. 2000; Armien et al. 2007; Cholich et al. 2009; Sant'Ana et al. 2011).

Techniques to Identify the Pathogenetic Basis of These Cerebellar Cortical Degenerations

The decreased cytoskeletal components, particularly neurofilaments and neurotubules, observed by Barros et al. (1987) and the ribosomal disaggregation observed by Verdes et al. (2006) suggest that alteration of protein synthesis may occur in affected neurons. Protein synthesis alteration, as well as the concomitant cytoskeleton derangement, could play a role in the pathogenesis of these neurodegenerations with subsequent altered cell-specific axonal transport determining neuronal death (Verdes 2006, Verdes et al. 2006). The identification of the basic nature of these cytoskeletal alterations is an important step towards the understanding of underlying disease processes. To identify and characterize cytoskeletal alterations in the perikaryon of Purkinje cells in intoxicated cattle, immunohistochemistry against different cytoskeletal proteins is currently being used. Immunoreactivity for phosphorylated neurofilament protein β -tubulin and affinity reaction against phalloidin revealed an altered cellular distribution of these interconnected components of the neuronal cytoskeleton in poisoned cattle (Verdes et al. unpublished results). These preliminary results seem to indicate that the altered cytoskeleton is somehow

related to the miss-accumulation of membrane-bound cytoplasmic vesicles seen in affected neurons. Further investigation is needed to understand whether cytoskeletal alterations occur in the pathogenic cascade as a primary step leading to the vesicular miss-accumulations, or if they are a secondary/downstream event (Verdes et al. 2006).

Another feature that suggests the occurrence of metabolic stress in degenerating Purkinje cells is the altered immunohistochemical pattern of conjugated ubiquitin that indicates the inhibition of the ATP-dependent hydrolytic ubiquitin-proteasome system, a non-lysosomal hydrolytic pathway responsible for digesting misfolded proteins that is altered in human neurodegeneration (Glickman and Ciechanover 2002, Lindsten et al. 2002, Klimaschewski 2003, Korhonen and Lindholm 2004, Verdes 2006). Different reports suggest that CbD28k is a specific marker of Purkinje cells in both normal and degenerative cerebellums (Ishikawa et al. 1995, Haworth et al. 2006, Verdes et al. 2010). Moreover, in the cerebellum where rapid-firing neurons experience a high level of calcium influx, intracellular calcium is mainly regulated by calcium binding proteins (CaBPs). For example, some excitotoxic degenerative Purkinje cells may have a disruption of calcium-buffering systems.

Calbindin D 28k (CbD28k) is a CaBP highly expressed in Purkinje cells (Ishikawa et al. 1995, Haworth et al. 2006, Verdes et al. 2010). Its expression in a variety of acute and chronic disorders seems to have a neuroprotective role against degeneration (Krebs 1998, Bastianelli 2002, Clowry and McHanwell 2004), specifically against calcium-mediated excitatory amino acid neurotoxicity by reducing levels of intracellular free calcium (Ishikawa et al. 1995). This does not seem to be the case in *Solanum* spp. poisoning because CbD28K preserves its immunoreactivity in cell bodies, axons, and dendrites. The immunoreactivity of CbD28K is similar in both experimentally and naturally poisoned animals as well as in controls. Degenerative axons or spheroids in the cerebellar white matter are also CbD28k positive; neurons of the deep cerebellar nuclei are CbD28k negative; and the surrounding synaptic terminals are positive (Verdes et al. 2010).

Finally, using TUNEL apoptotic reagents to define if programmed cell death pathways are activated in Purkinje cells of *Solanum* spp. poisoned cattle, Verdes et al. (2010) demonstrated that apoptosis is not activated. Van der Lugt et al. (2010) reached similar conclusions with bovines intoxicated by *S. kwebense*. In the *S. bonariense* cases, the

absence of typical apoptotic signs in the nuclei of affected Purkinje cells using TEM (figure 3) also ruled out the occurrence of apoptosis. This would suggest that the changes observed in degenerative neurons were probably more indicative of necrosis, although this hypothesis must be confirmed by more specific methods (Verdes et al. 2010).

Botanical and Toxicological Aspects

S. bonariense L. (= *Solanum fastigiatum* Willd.)

Solanum bonariense L., as described by Linnaeus in 1753, was probably based on a specimen from Buenos Aires, Argentina; and *S. fastigiatum* was proposed by Willdenow in 1809, based on a specimen grown in Berlin, Germany, of unknown origin. Dunal (1852) defined a variety of typical *Solanum fastigiatum* Willd. (var. *fastigiatum*), describing *Solanum fastigiatum* var. *acicularium*. The differences between typical *S. fastigiatum* Willd., and its variety *acicularium* are not clear except that the variety is said to be much spicier, but this may not be a character of much taxonomic significance in these plants (Morton 1976, Chiarini et al. 2007). Both species are morphologically very similar (Morton 1976, Lombardo 1983, Riet-Correa et al. 1983), and recently Chiarini et al. (2007) and Wagstaff (2008) suggested that *S. fastigiatum* is a synonym of *S. bonariense* (figure 5).

S. bonariense is widely distributed in southern Brazil, northeastern Argentina, and Uruguay (Morton 1976, Lombardo 1983, Simões et al. 1998, Chiarini et al. 2007, Sabir and Rocha 2008). It also occurs as a naturalized weed in the Mediterranean region of Europe after its introduction as an ornamental plant (Pascual-Villalobos 1999, Bruneton 2001, Rita 2002). Plant extracts from European specimens have demonstrated insecticide activity (Pascual-Villalobos 1999).

Solanum bonariense leaves and roots are used in Brazilian medicine as a tonic or for the treatment of fever, anemia, erysipelas, hepatitis and other liver disease conditions, spleen disorders, uterine tumors, irritable bowel syndrome, chronic gastritis, and other digestive problems such as sluggish digestion, bloating, and flatulence (Riet-Correa et al. 1983, Sabir and Rocha 2008). The “jurubeba” leaf tea is a very common household remedy throughout Brazil for hangovers. The aqueous extract of the plant has antioxidant and hepatoprotective activity in mice with liver damage (Sabir and Rocha 2008). Phytochemical analysis has shown the presence of rutin, flavonoids, and glycosides in leaves (Higa et

al. 2006). Specimens collected in farms affected by spontaneous cerebellar disease of cattle in Uruguay

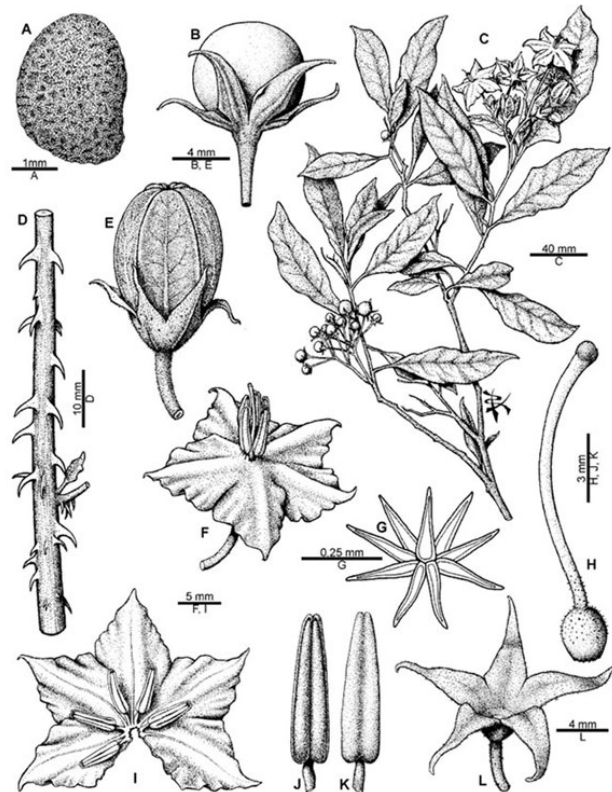


Figure 5. *Solanum bonariense* (D: Chiarini & Marini 642; A-C, E-L: Barboza et al. 1568). A: seed; B: fruit; C: branch with flowers and fruits; D: part of stem showing prickles; E: flower bud; F: flower; G: trichome sessile on the leaf lower surface; H: gynoecium; I: open corolla; J, K: anthers, ventral and dorsal views respectively; L: calyx. Courtesy of Chiarini et al. 2007, with permission of Gayana Botánica.

did not contain swainsonine or calystegines (R. Molyneux 2005, personal communication), but some specific alkaloids were obtained from alcoholic fractions of leaves (Ruiz-Diaz et al. 2004, M. Haraguchi 2012, personal communication). A complete botanical description of *S. bonariense* and some interesting details about its synonymy are given by Chiarini et al. (2007).

Solanum paniculatum L. (1762)

Solanum paniculatum L. was described by Linnaeus (1762) as a neotropical weed of very common occurrence in Brazil, Paraguay, Bolivia, and Argentina. It is used in folk medicine and for culinary purposes. Many species of the genus *Solanum* are known by local people as “jurubeba,” but the species *S. paniculatum* is considered the authentic “jurubeba” (Corrêa 1984). The infusion prepared with “jurubeba” is a very common household remedy used throughout Brazil for

hangovers, because it exhibits antisecretory gastric properties (Vieira et al. 2010). Extracts of all parts of this plant are used as anti-inflammatory, antioxidant, molluscicidal, diuretic, and hepatoprotective agents. Many steroidal compounds have been isolated from this species; these alkaloids include jurubebine, jubebine, and solanine. The phytochemical analysis of *S. paniculatum* extracts showed variation according to the plant parts. Fructose, glucose, and galactose were detected in the fruits, and solanine was isolated from its roots and stems. Saponins were also identified in the roots of this species, including isojuripidine, isojurubidine, isopaniculidine, and jurubidine. Jurubidine, a sugar-free steroid obtained via acid hydrolysis of the glucoside jurubine, was also isolated from *S. paniculatum* roots. The alkaloids jurubebine and jubebine were identified in leaves and fruits (Vieira et al 2010).

The mouse is currently used as an animal model to study different pharmacological effects and to characterize folk medicine uses (Mesia-Vela et al. 2002, Vieira et al. 2010). No toxic signs were observed in mice following administration of different aqueous extracts up to 2 g/kg BW intraduodenally, which promoted antiulcer activity (Mesia-Vela et al. 2002).

Discussion

Different hypotheses about the pathogenic mechanism of this intoxication have been proposed for *Solanum* species. Riet-Correa et al. (1983) suggested that these plants cause an induced lysosomal storage disease, probably an acquired gangliosidosis or a neuropilidosis. In the case of normal Purkinje cells, the ganglioside GD1 α appears to be highly expressed (Furuya et al. 1996). Its quantification in normal and poisoned cattle could be an interesting tool to clarify if these cerebellar cortical degenerations are acquired gangliosidoses (Verdes 2006).

The main feature of the intoxication is the selective damage to Purkinje cells. Barros et al. (1987) suggested that the disease is not a typical storage one, instead proposing a physico-chemical interaction between the active principle of the plant and normal lipids within neurons with concomitant formation of a complex that would be less susceptible to enzymatic degradation than normal lipidic compounds. Lectin histochemical patterns seem to support this hypothesis (Paulovich et al. 2002, Sant'Ana et al. 2011).

Ultrastructural studies in Purkinje cell perikarya also show alteration of the endoplasmic reticulum, a

decrease in the number of neurofilaments and microtubules (Barros et al. 1987), ribosomal disaggregation, accumulation of electron-dense vesicles, and swollen mitochondria (Verdes et al. 2006). Similar alterations of the cytoskeleton and accumulation of vesicles are observed in axonal spheroids of granular layer and subcortical white matter (Barros et al. 1987, Verdes et al. 2006) and confirmed by immunohistochemistry (Verdes et al. unpublished results). These findings are in agreement with the hypothesis that alteration of protein synthesis and subsequent axonal transport disruption could play a role in the development of axonal spheroids and the pathogenesis of these cerebellar cortical degenerations (Verdes et al. 2006). Another fact that confirms the neuronal metabolic stress in these neurodegenerations is the presence of altered immunostaining against ubiquitin, supporting the hypothesis of inhibition of the ATP-dependent hydrolytic ubiquitin proteasome system (Verdes 2006). Degenerating Purkinje cells preserve calcium homeostasis and progressively die by necrosis (Verdes et al. 2010).

Available botanical, phytochemical, and clinicopathological evidence of this poisonous plant genera supports the view that both *S. bonariense* and *S. fastigiatum* are synonyms (Chiarini et al. 2007, Wagstaff 2008) or at least closely related species. Future work should address the use of sheep and goats as practical experimental models to study cerebellar cortical degeneration in ruminants, given the susceptibility of these domestic species and their smaller size.

The risk of human poisoning from *Solanum* spp. should also be considered and better characterized. The toxic compounds of *Solanum* spp. are still largely unknown, yet these plants are widely used as traditional medicines. The use of *Solanum* spp. in folk medicine is not recommended (Riet-Correa et al. 1983, Guaraná et al. 2011b).

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