Experimental poisoning of goats by *Ipomoea carnea* subsp. *fistulosa* in Argentina: A clinic and pathological correlation with special consideration on the central nervous system

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*Ipomoea carnea* subsp. *fistulosa*, aguapeí or mandiyura, is responsible for lysosomal storage in goats. The shrub contains several alkaloids, mainly swansonine which inhibits lysosomal α-mannosidase and Golgi mannosidase II. Poisoning occurs by inhibition of these hydrolases. There is neuronal vacuolation, endocrine dysfunction, cardiovascular and gastrointestinal injury, and immune disorders. Clinical signs and pathology of the experimental poisoning of goats by *Ipomoea carnea* in Argentina are here described. Five goats received fresh leaves and stems of *Ipomoea*. At the beginning, the goats did not consume the plant, but later, it was preferred over any other forage. High dose induced rapid intoxication, whereas with low doses, the course of the toxicosis was more protracted. The goats were euthanized when they were recumbent.

Cerebrum, cerebellum, medulla oblongata, pons and colliculi, were routinely processed for histology. In nine days, the following clinical signs developed: abnormal fascies, dilated nostrils and abnormal postures of the head, cephalic tremors and nystagmus, difficulty in standing. Subsequently, the goats had a tendency to fall, always to the left, with spastic convulsions. There was lack in coordination of voluntary movements due to Purkinje and deep nuclei neurons damage. The cochlear reflex originated hyperreflexia, abnormal posture, head movements and tremors. The withdrawal reflex produced flexor muscles hypersensitivity at the four legs, later depression and stupor. Abnormal responses to sounds were related to collicular lesions. Thalamic damage altered the withdrawal reflex, showing incomplete reaction. The observed cervical hair bristling was attributed to a thalamic regulated nociceptive response. Depression may be associated with agonists of lysergic acid contained in *Ipomoea*. These clinical signs were correlated with lesions in different parts of the CNS.

**INDEX TERMS:** Poisonous plants, *Ipomoea carnea*, plant poisoning, goats, CNS lesions, transmission electron microscopy.

INTRODUCTION

Ipomoea sp. has been held responsible for severe episodes of intoxication in the tropics and subtropics. There are reports of spontaneous poisoning, especially in goats, in parts of Brazil, Sudan, India, Mozambique and Argentina (Damir et al. 1987, De Balogh et al. 1999, Rodriguez Armesto et al. 2004, Barbosa et al. 2006, Antoniassi et al. 2007, Armien et al. 2007, Barbosa et al. 2007, Ríos et al. 2008). The plant contains as main toxic component swainsonine which inhibits lysosomal enzyme α-mannosidase and mannosidase II Golgi apparatus (Colegate et al. 1979, Molyneux & James 1982). The intoxication induces enzymatic dysfunction and the accumulation of complex oligosaccharides in lysosomes. As a consequence, vacuolation becomes evident in different cells, mainly in neurons. The alteration of glycoprotein synthesis may be associated with endocrine dysfunction, cardiovascular and gastrointestinal injury, neural and immune disorders (Hueza et al. 2003, Armien et al. 2007).

The most frequent clinical signs observed in ruminants, within the first month after ingestion of Ipomoea, consist of: lethargy, muscle tremors, hyperexcitability, dilated pupils, opisthotonus, strabisimus, weakness of the hind limbs, severe incoordination, ataxia, wasting and recumbence; all primarily associated with dysfunction of the central nervous system. (Tokarnia et al. 2002, Haraguchi et al. 2003, Barbosa et al. 2006, Armien et al. 2007, Guedes et al. 2007).

Despite many reports on the poisoning of Ipomoea carnea, clinical signs and morphological lesions of poisoning have not been well characterized (Adam et al. 1973, Idris et al. 1973, Tartour et al. 1974, Damir et al. 1987, Nath & Pathak 1995, Schumaher-Henrique et al. 2003). The aim of this paper is to describe the epidemiology, clinical signs and pathology of the experimental poisoning by Ipomoea carnea subsp. fistulosa primarily affecting the CNS of goats.

MATERIALS AND METHODS

Plant identification and toxicological analysis. Ipomoea carnea subsp. fistulosa was collected in the Capital Department, Corrientes Province, Argentina in the spring. A sample of the plant was deposited at the Institute of Botany, Faculty of Agricultural Sciences, UNNE (Corrientes, Argentina), with the number CTES-395. Taxonomic identification was made by Lic. Roberto Salas Taxonomist of the Institute.

Experimental design. The experimental group consisted of five 1-3-year-old goats which had not previously been exposed to the plant. Each of these goats received 50g/kg/day of fresh leaves and stems of I. carnea, for 21 days. Equal numbers of goats were used as control, and received only alfalfa. Both groups received water ad libitum. During the experiment, all goats were submitted to a detailed examination of the central nervous system, including state of consciousness and sensory, facies attitudes, standing, walking and lyingdown, and reflexes. The goats were euthanized when they were recumbent and not able to eat or drink for themselves.

Histopathology. For sacrifice, animals were anesthetized with a combination of xylazine hydrochloride 2% and ketamine hydrochloride, and then, subjected to a drain "on target" and immediately proceeded to the taking of tissue, for histopathological analysis (Ethics Commission local, Faculty of Veterinary Sciences, UNNE). The CNS was serially sectioned and cut at level of thalamus, cerebellum, colliculus, pons, and medulla oblongata. Specimens were fixed in 0.2% neutral buffered formalin at room temperature, embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin (HE) and examined under a light microscope.

Transmission electron microscopy. Representative sections were fixed by immersion in 2% glutaraldehyde, post-fixed in osmium tetroxide and embedded in epoxy resin. Semi-thin sections (1 µm thick and stained with 1% toluidine blue in 1% borax) were evaluated for the presence of intracytoplasmatic storage vacuoles and abnormal staining. Ultra-thin section (60-80 nm) of selected areas, were stained with uranyl acetate and 2% lead citrate and examined in a JEOL transmission electron microscope.

Lectin histochemistry. Representative sections of the thalamus, cerebellum and colliculi were submitted to lectin histochemistry as follows: after dewaxing, sections were treated with 0.3% hydrogen peroxide in methanol for 30 min at room temperature (to Inhibit the endogenous peroxidase), rinsed several times in 0.01M PBS (pH 7.2), and treat with 0.1% bovine serum albumin in PBS for 15 min. The sections were then incubated for 1 hour at room temperature with biotinylated lectin with different specificity. Nine lectins, with different specificity were employed: Con A (Concanavalina ensiformis, binding specificity a-D-Man and a-D-Glc), DBA (Dolichos biflorus, binding specificity a-D-GalNaC), SBA (Glycine max, binding specificity a-D-GalNaC, a-D-GalNaC and a and b-Gal), PNA (Arachis hypogaea, binding specificity b and a and a GalNaC), RCA-I (Ricinus communis-I, binding specificity b-D-Gal and a-D-Gal), UEA-I (Ulex europaeus-I, binding specificity a-L-Fuc), WGA (Triticum vulgaris, binding specificity a-D-GlcNAc and NeuNaC) and sWGA (succinyl WGA, binding specificity b-D-GlcNAc and NeuNaC) LCA (Lens culinaris, binding specificity a-D-Glc, a-D-Man) (Vector Laboratories, Burlingame, CA, USA).

The optimal lectin concentration was 30 mg/ml in PBS for all lectins, except for PNA, which was applied at a concentration...
of 10 mg/ml. Later, the slides were incubated with avidin-biotin-peroxidase complex (ABC) (Vector) for 45 min. The horseradish peroxidase was activated by incubation for 1-2 min with a commercial diaminobenzidine kit (Dako Cytomation, Carpinteria, CA, USA). Specimens were rinsed in water, dehydrated with graded ethanol solutions, cleared in xylene and mounted in Permount (Fisher Scientific International Inc., Liberty Lane Hampton, NH, USA). Controls for lectin staining included: Exposure to horseradish-peroxidase and substrate medium without the lectin, and blocking by incubation with the appropriate blocking sugars (0.1 to 0.2M in PBS) for 1 h at room temperature before applying to the sections. The intensity of lectin binding was scored subjectively from 0 (none) to 3 (strongly positive).

RESULTS

Plant identification and toxicological analysis. The plant was identified as Ipomoea carnea subsp. fistulosa, belonging to the family Convolvulaceae, commonly known in northeastern Argentina as “aguapeí” or “mandiyura” (Fig.1).

Experimental design. Ipomoea poisoning in goats occurred after the ingestion of 50g of fresh leaves/per kg body weight, per day, over a period of 21 days, with a total consumption of 20 kg of plant per goat. Clinical signs were similar in all affected animals which were not observed in control animals. After the first week of study, behavioral changes were observed; including the great preference for consumption of the plant, which was observed even when the goats had other available food (Fig.2A). Towards the end of the experiment, the goats showed depression. Despite depression and weakness, they were still eating in lateral decubitus (Fig.2B). From the day nine, all intoxicated goats showed abnormal fascies, dilated nostrils, abnormal carrying of the head and cephalic tremors, resembling denial and affirmation, with nystagmus. Additionally, there was difficulty to keep a standing position and to sit up, and but this was achieved only after several attempts (Fig.3). At the beginning of clinical signs, the goats walked with weakness of the hind limbs, or both hind limbs rigid, ataxia, and difficulty in keeping a normal lift. Subsequently, the rigidity of the limbs increased, with difficulty in maintaining the balance. Consequently, they fell always to the left, displaying marked seizures. Other signs were walking in circles, paddling and opisthotonus (Fig.4). After eleven days of intoxication, vestibulocochlear deficit was manifested in response to sound stimuli, with reflexes, posture and coordination accompanied by abnormal head movements and tremors. Also, the withdrawal reflex was altered to the end of the experiment, showing an incomplete reaction of the flexor muscles and hypersensitive to the stimulus on the...
Histopathology. The main histological lesions were observed in neurons of the thalamus and the Purkinje cells. The perikaryon was distended with foamy cytoplasm by the presence of small vacuoles. In other sections of the CNS, it was mild to moderate vacuolation.

Transmission electron microscopy. Ultrastructurally, the cytoplasmic vacuolation was evident in neurons and astrocytes in the thalamus and Purkinje cells, most of which were optically empty and delimited by a membrane. Membrane fragments, reticular or dense granules and amorphous substances were observed in some vacuoles (Fig.5).

Lectin histochemistry. With regard to the lectin histochemical, the vacuolated neurons and astrocytes in the thalamus and Purkinje cells reacted strongly to LCA and moderately to Con-A, sWGA and WGA. No reaction was observed with the other lectins. Additionally, cellular staining was absent in slides from control goats (Fig.6A,B).

**DISCUSSION AND CONCLUSION**

Clinical signs in experimentally *Ipomoea carnea*-intoxicated goats, were mainly neurological disorders characterized by depression, weakness, tremor, nystagmus, dysmetria, ataxia, spastic paresis, increased base of support, abnormal postures, signs similar to those described by other authors in poisoning by *Ipomoea sp.* (Tokarnia et al. 2000, Armien et al. 2007).


First signs were observed after one week of intoxication. In our study, we administered a high dose, which induced a rapid and early toxicity. Doses as low 5g/kg induce clinical signs after 100 days into the poisoning (Srilatha et al. 1993a,b). At the beginning of the experiment, the animals resisted *Ipomoea* consumption but after a week, it was accepted up to the point that they preferred *Ipomoea* leaves to alfalfa hay. Preference for the consumption has also been commonly reported in poisoning by plants containing swainsonine such as locoweeds (Ralphs et al. 1990), *I. car-
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