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Experimental intoxication of guinea pigs with *Ipomoea carnea*: Behavioural and neuropathological alterations

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ABSTRACT

Ipomoea carnea is a toxic plant that affects goats, with symptoms being characterised by nervous disorders and death. Swainsonine and calystegines are the principal toxic components isolated from *I. carnea*, which also yields lysergic acid derivatives. The aim of this study was to improve the clinical characterisation of experimental intoxication by *I. carnea* in guinea pigs through the evaluation of behavioural changes and to perform a thorough histopathological analysis of the affected CNS. Leaves of *I. carnea* were administered to guinea pigs. Open-field gait analysis and monoamine levels were measured. The poisoned animals exhibited increased vocalisation, lethargy, and a reduction in the locomotion frequency after the fourth week of intoxication, as demonstrated in the open-field test. Significant differences were observed in hind-limb gait width by the last week of intoxication. After 65 days, the guinea pigs were euthanised, necropsied, and examined using light and electron microscopy. At the end of the experiment, plasma serotonin decreased. In contrast, dopamine decreased, and noradrenaline increased in urine. Brain sections were evaluated with conventional histological methods and immunohistochemistry (IHC), as well as by transmission electron microscopy (TEM). Vacuoles were observed throughout the brain, but they were particularly prominent in the brainstem. In addition, there were PAS-negative regions, and the Nissl substance was dispersed or absent, which was confirmed with the Kluver-Barreda stain. Moderate microgliosis was observed by immunohistochemistry. In the medulla oblongata, numerous ubiquitin-positive spheroids together with neuronal degeneration were observed in the nucleus gracilis/cuneatus. Furthermore, vacuoles were observed in astrocytes, oligodendrocytes, and endothelial cells by TEM. Our results showed that the behavioural effects may have been caused by alterations in the brain in conjunction with changes in monoamine levels. This research confirms the utility of this model for studying the pathogenesis of plant-induced lysosomal storage diseases.

1. Introduction

Ipomoea carnea subsp. *fistulosa* (Convolvulaceae) is a toxic plant found throughout the northeastern region of Argentina and in other tropical and subtropical countries (Austin and Huaman, 1996). Poisoning occurs when various animal species, such as goats, sheep, and cattle, eat this

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plant, especially in drought periods, when it is one of the few plants that stay green (Riet-Correa and Mendez, 2000).

Prolonged ingestion of this and similar plants induces neurodegeneration characterised by neuropathy and other clinical manifestations. The animals exhibit a variety of clinical signs, such as addiction to consumption of the plant, depression, and loss of body weight, and neurological deficits, such as abnormalities of gait, difficulty standing, abnormal posture, symmetrical ataxia, and paraparesis (Barbosa et al., 2006; de Balogh et al., 1999; Dorling et al., 1980; Driemeier et al., 2000; James et al., 1970; James and Panter, 1989; Molyneux et al., 1995; Ríos et al., 2012; Rodriguez Armesto et al., 2004; Stegelmeier et al., 1999).

These toxic effects are attributed to the polyhydroxylated alkaloids swainsonine and the calystegines A_3, B_1, B_2, B_3, and C_1. Swainsonine is a powerful inhibitor of lysosomal α-mannosidase and Golgi mannosidase II. Calystegines inhibit the lysosomal α-galactosidase and β-glycosidase (Colodel et al., 2002; de Balogh et al., 1999; Haraguchi et al., 2003; Hueza et al., 2005; Molyneux et al., 1995). The inhibition of lysosomal α-mannosidase leads to the intralysosomal accumulation of incompletely processed oligosaccharides. These enzymes are essential for the proper functioning of all animal cells, and their inhibition results in a clinical, biochemical, and morphological presentation similar to inherited-α-mannosidosis (Dorling et al., 1978; James et al., 2004). These alkaloids were isolated from *I. carnea* found in Corrientes, Argentina with HPLC-MS (Cholich et al., 2009).

Furthermore, several authors reported the presence of lysergic acid derivatives in *Ipomoea* (Daló and Moussatché, 1978; Sandoval et al., 2010).

Congenital α-mannosidosis has been reported in humans, cattle, cats, mice, and guinea pig, and this disease is characterised in all species by progressive neurological deterioration and premature death (Auclair and Hopwood, 2007; Crawley and Walkley, 2007; Robinson et al., 2008). Plant-induced α-mannosidosis has been experimentally studied in goats, cattle, and sheep, as well as in rats and mice (Van Kampen and James, 1969; Riet-Correa and Mendez, 2000; Hueza et al., 2005; Armién et al., 2007; Stegelmeier et al., 2008). Nevertheless, it was found that small rodents are poor models for neuronal storage diseases. Vacuolation in various tissues but not central nervous tissue were obtained with the aqueous fraction of *I. carnea* and, to a lesser degree, with swainsonine alone, but no effect was found with the individual calystegines (Hueza et al., 2005). Stegelmeier et al. (2008) reproduced neuronal storage defects in mice but with high doses of pure swainsonine administered subcutaneously by osmotic minipumps.

Recently, we proposed that *I. carnea*-induced toxicosis in guinea pig could be a useful model for studying the pathogenesis of plant-induced storage diseases (intoxications with the genera *Ipomoea, Swainsona, Astragalus, Oxytropis, Sida*, and others) and for plants with similar effects that are still awaiting characterisation (Cholich et al., 2009).

The aim of this study was to improve the clinical characterisation of the disease in intoxicated guinea pigs, especially by the evaluation of behavioural changes and assaying monoamine levels, and to perform a thorough histopathological study of the guinea pig CNS by light microscopy and transmission electron microscopy (TEM).

2. Materials and methods

2.1. Preparation of plant material

The plant was identified as *I. carnea* subsp. *fistulosa* from the Convolvulaceae family, known in Northern Argentina with the common Guarani names of "mandiyura'" or "aguapeí". Polyhydroxy alkaloids were detected as chemical constituents of these plants, including swainsonine, calystegine B1, calystegine B2, calystegine C1, and trace amounts of calystegines A3 and B3. The swainsonine concentration was 0.02%, whereas the mean levels of calystegine were 0.05% (Cholich et al., 2009).

I. carnea leaves were dried al 37 °C for 72 h and milled. The leaves were mixed homogeneously with commercial guinea pig pellets, previously hydrated with water for making "small balls". A 50:50 (vol/vol) mixture of pellets and dry, milled leaf matter was used in this study.

2.2. Clinical study

Eight 4-week-old male Hartley guinea pigs (253 ± 22 g) were supplied by the Bioterio of the Faculty of Veterinary Sciences, UNNE. The animals were housed individually in a temperature-controlled room (23 ± 2 °C) with relative humidity between 35 and 65%. Lights in the animal room were on from 6 AM to 6 PM. The animals were divided at random into two groups: experimental group ($n = 4$), each received 5 "small balls" (10 g each) per day (total swainsonine, 1.25 mg). In the control group ($n = 4$), each animal received 50 g commercial pellets per day.

Both groups received both fresh alfalfa (*Medicago sativa*) and water *ad-libitum*. Through the consumption period, the animals were weighed weekly, and the daily intake of small balls and commercial pellets was calculated based on subtraction of food on the next day.

The present study was approved by the Ethics at Biosafety Committee of Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, Argentina.

2.3. Neurological examinations

2.3.1. Open-field studies

This type of study is commonly used to assess locomotion and exploration in laboratory animals. Each animal was individually placed in the centre of a box (120 × 120 × 50 cm) in which the floor was divided into 16 units of 30 cm^2 each, and the following parameters were measured over a period of 5 min: locomotion frequency (number of floor units entered with all feet), rearing frequency (number of times the animal stood on its hind legs), immobility time (total number of seconds with no movement) and defecation (number of faecal pellets). Hand-operated counters and stopwatches were employed to score these behaviours. To minimise possible influences of circadian changes on open-field behaviours, control and experimental animals were alternated. The device was washed with a 5% alcohol/water solution before the