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# Assessment of the perinatal effects of maternal ingestion of *Ipomoea carnea* in rats

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## Abstract

It is believed that *Ipomoea carnea* toxicosis induces abnormal embryogenesis in livestock. Studies on rats treated with *I. carnea* aqueous fraction (AF) during gestation, revealed litters with decreased body weight, but the characteristic vacuolar lesions promoted by swainsonine, its main toxic principle, were observed only in young rats on postnatal day (PND) 7. However, these alterations could have resulted as consequence of swainsonine placental passage and/or damage or even ingestion of the contaminated milk by pups. Thus, this perinatal work was performed to verify the transplacental passage of swainsonine and its excretion into milk employing the cross-fostering (CF) procedure as a tool of study. Females were treated with AF or vehicle during gestation and after birth pups were fostered between treated and untreated dams. Pup body weight gain (BWG) and histopathology to observe vacuolar degeneration were performed on PND 3 and 7. In addition, swainsonine detection was performed in amniotic fluid and milk from rats treated with the AF during gestation or lactation. BWG was significantly lower only in pups from mothers treated with the plant and fostered to other treated mothers (AF–AF group of pups). The histopathology revealed that pups from treated mothers fostered to untreated ones showed the characteristic vacuolar lesions; however, the lesions from the AF–AF pups were more severe in both periods evaluated. Amniotic fluid and milk analysis revealed the presence of swainsonine excretion into these fluid compartments. Thus, the results from CF and the chemical analysis allowed concluding that swainsonine passes the placental barrier and affects fetal development and milk excretion participates in *I. carnea* perinatal toxicosis.


***Keywords:*** *Ipomoea carnea*; Swainsonine; Cross-fostering; Vacuolar degeneration; Amniotic fluid; Milk excretion

## Introduction

*Ipomoea carnea* Jacq. spp. *fistulosa* Choisy (Convolvulaceae) is a toxic plant widely distributed in Brazil (Tokarnia et al., 2000) and other tropical countries

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(Austin and Huáman, 1996). Two kinds of toxic principles were isolated from the plant, the nortropane alkaloids calystegines $B_1$, $B_2$, $B_3$ and $C_1$ and mainly the indolizidine alkaloid swainsonine (De Balogh et al., 1999; Haraguchi et al., 2003). The latter alkaloid has a known toxic mechanism of action, being a potent inhibitor of two distinct intracellular enzymes, the acidic or lysosomal α-mannosidase and the Golgi mannosidase II. However, with respect to calystegines $B_2$ and $C_1$, these nortropane alkaloids are only recognized as inhibitors of α-galactosidase and β-glucosidase enzymes, respectively (Asano et al., 1995). Recent studies conducted in our lab, comparing the histological effects of each calystegine, in the same concentration contained in *I. carnea* aqueous fraction (AF) administered to rats, did not show the characteristic vacuolar lesions observed in animals treated solely with swainsonine (Hueza et al., 2005). Thus, whether all these alkaloids together enhance the effects produced by swainsonine intoxication on *I. carnea* is unknown (Asano et al., 2000).

The inhibition of α-mannosidase results in lysosomal accumulation of incompletely processed oligosaccharides rich in α-mannosyl and β-*N*-acetyl glucosamine moieties inside vacuoles, which progresses to loss of cellular function and, ultimately, to cell death (Tulsiani et al., 1988). Histologically, cellular vacuolization of Purkinje cells, thyroid follicles, exocrine pancreas, liver and kidney cells has been observed.

Swainsonine inhibition of Golgi mannosidase II enzyme causes alteration of the *N*-linked glycoprotein process (Elbein, 1989), producing increased numbers of high-mannose, hybrid or complex types of oligosaccharide structures that participate in hormones, cytokines, membrane receptors and adhesion molecules (Stegelmeier et al., 1998). These molecular effects can alter hormonal and endocrine function (Stegelmeier et al., 1995), cause abnormal gastrointestinal (Pan et al., 1993), immunological (Karasuno et al., 1992) and reproductive function (Nelson et al., 1980).

In a previous perinatal study conducted in our laboratory with rats treated with *I. carnea* AF during gestation, aimed at determining the occurrence of vacuolar cell lesions on fetal and pup tissues on gestational day (GD) 21 and on postnatal day (PND) 1 and 7, respectively, it was verified that fetal and pup tissues did not permit the identification of any vacuolar lesion and/or alteration, since the natural morphology of cells from the different organs examined was not yet completely differentiated. Conversely, characteristic vacuolar degeneration promoted by swainsonine was detected in different tissues in samples collected from the offspring on PND 7 (Hueza et al., 2003). However, as the lesions were detected in the oldest pups, it could be hypothesized that these alterations were due by indolizidine alkaloid excretion into milk and not by transplacental passage. Corroborating with this assumption, a study conducted by James and Hartley (1977) showed that different tissues from kittens, calves and lambs treated with milk containing swainsonine presented typical vacuolar degeneration produced by this compound.

On the other hand, anecdotal information suggests that animals chronically intoxicated with *I. carnea* and other swainsonine-containing plants show enhanced occurrence of abortion, stillbirth and teratological effects (McIlwraith and James, 1982; Panter et al., 1999), suggesting a possible direct effect of swainsonine in fetal development. Thus, it is not possible to rule out the role of transplacental passage of swainsonine in producing toxicosis in the neonate. The purpose of the present investigation was to more clearly verify whether the possible transplacental passage of swainsonine is the causative agent of offspring toxicosis, or whether the excretion of swainsonine into the milk participates in the toxicosis development, for this reason the cross-fostering (CF) procedure was employed as a tool to better observe the vacuolar lesions in the pups and analytical methods to verify the presence of swainsonine in the amniotic fluid and milk.

## Materials and methods

### Plant material

*I. carnea* leaves were collected in May, 2004 from plants cultivated at the Research Center for Veterinary Toxicology (CEPTOX), University of São Paulo (USP), Pirassununga, Brazil. A voucher specimen was deposited in the Herbarium of the Instituto de Botânica de São Paulo, Brazil (SP-360911) and its authenticity was confirmed by the taxonomist Rosangela Simão Bianchini of the same Institute.

The AF resulting from the extraction of dry *I. carnea* leaves was obtained according to previously described methods (Hueza et al., 2003). Briefly, dry leaf sample was macerated in 96% ethanol. After total solvent evaporation under reduced pressure at 50°C, a dark green extract was obtained, which was suspended in water to remove the waxy residue and consecutively fractionated with *n*-butanol saturated with water. The remaining aqueous solution was lyophilized to give an AF, which, by previous assay, revealed the presence of the active principles.

In previous studies (Hueza et al., 2003, 2005; Schwarz et al., 2003) the dose of 15.0 g/kg of AF of *I. carnea* dried leaves was established in our lab to develop the model of *I. carnea* maternal toxicity in rats. However, a dose of 7.0 g/kg of AF was also employed in pregnant rats to avoid fetal losses and lack of material to chemical analysis of amniotic fluid.