

## Elimination of the tremorgenic toxin of *Ipomoea asarifolia* by milk<sup>1</sup>

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**ABSTRACT.**- Lopes J.R.G., Riet-Correa F., Cook D., Pfister J.A. & Medeiros R.M.T. 2014. **Elimination of the tremorgenic toxin of *Ipomoea asarifolia* by milk.** *Pesquisa Veterinária Brasileira* 34(11):1085-1088. Hospital Veterinário, Centro de Saúde e Tecnologia Rural, Universidade Federal de Campina Grande, Patos, PB. 58708-110, Brazil. E-mail: [rmtmed@uol.com.br](mailto:rmtmed@uol.com.br)

With the aim to determine if the tremorgenic toxin of *Ipomoea asarifolia* is eliminated in milk, three groups of Swiss female mice received, immediately after giving birth until weaning, a ration containing 20% or 30% of dry *I. asarifolia*. All the offspring of the females that received 20% or 30% *I. asarifolia* showed tremors 2-4 days after birth. The offspring of the females that received 20% *I. asarifolia* recovered 4-7 days after weaning. The offspring of the females that received 30% of the plant in the ration died while showing tremors before weaning or up to two days after weaning. It is concluded that the tremorgenic compound of *I. asarifolia* or its toxic metabolites are eliminated in milk, and that lactating mice may be used as a model for the determination of the toxic compound(s) in this plant.

INDEX TERMS: Poisonous plants, *Ipomoea asarifolia*, tremorgenic toxins, toxins in milk, plant poisoning.

**RESUMO.**- [Eliminação da toxina tremorgênica de *Ipomoea asarifolia* pelo leite.] Com o objetivo de determinar se a toxina tremorgênica da *Ipomoea asarifolia* é eliminada pelo leite, três grupos de camundongos fêmeas da linhagem Swiss receberam, imediatamente após o parto até o desmame, ração contendo 20% ou 30% de folhas secas de *I. asarifolia*. Todos os filhotes das fêmeas que receberam 20% ou 30% de *I. asarifolia* apresentaram tremores 2-4 dias após o nascimento. Os filhotes das fêmeas que receberam 20% de *I. asarifolia* se recuperaram 4-7 dias após o desmame. Os filhotes das fêmeas que receberam 30% da planta na ração morreram antes do desmame ou até dois dias após o desmame, ainda apresentando tremores. Conclui-se que o componente tremorgênico de *I. asarifolia* ou seus metabólitos são eliminados no leite, e que camundongos fêmeas em lactação podem ser usados como um modelo para a determinação do(s) composto(s) tóxico(s) desta planta.

TERMOS DE INDEXAÇÃO: Plantas tóxicas, *Ipomoea asarifolia*, toxinas tremorgênica, toxinas no leite, plantas tóxicas.

### INTRODUCTION

*Ipomoea asarifolia* R. et Schult. (common name: salsa), family Convolvulaceae, is a plant native to South and Central America. In Brazil it is very common in the Amazon region, in the Northeast, and along the coast, from northern Brazil to the southern states of Rio de Janeiro and São Paulo (Kissmann & Groth 1992). *I. asarifolia* is a tremorgenic plant that causes poisoning in sheep (Döbereiner et al. 1960, Guedes et al. 2007), goats (Döbereiner et al. 1960, Medeiros et al. 2003, Guedes et al. 2007), cattle (Döbereiner et al. 1960, Barbosa et al. 2005) and buffalo (Barbosa et al. 2005). Clinical signs caused by *I. asarifolia* are similar than those caused by the indole-diterpene mycotoxins produced by different fungi (di Menna et al. 2012, Cawdell-Smith et al. 2010), but the tremorgenic toxin of the plant is unknown.

In the states of Paraíba (Alves & Nascimento 2010, Agra et al. 2007) and Piauí (Franco & Barros 2006) Brazil, *I. asarifolia* is popularly known as a medicinal plant and is used in the treatment of dermatological diseases, used to wash the affected areas. In studies conducted in Nigeria, *I. asarifolia* showed antioxidant (Ene-OjoAtawodi & Onoalapo 2010), analgesic and anti-inflammatory action (Lawal et al. 2010, Jegede et al. 2009).

<sup>1</sup> Received on October 10, 2014.

Accepted for publication on October 22, 2014.

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In an experiment with rats *I. asarifolia* was administered to females during pregnancy and lactation; the dams showed no alterations, but the pups presented behavioral changes, however tremors were not observed (Silva et al. 2012). Behaviour changes without tremors were also observed in weaned mice ingesting *I. asarifolia* (Lopes et al. 2014). In sheep the poisoning has been observed in nursing lambs that remain confined, without ingesting the plant, while their mothers ingested the plant while grazing without showing signs of disease (Araújo et al. 2008, Freitas et al. 2011). These observations suggest that the tremorgenic compound of the plant is eliminated in milk. However, this hypothesis was not confirmed in experiments in sheep (Araújo et al. 2008) and goats (Freitas et al. 2011) that ingested *I. asarifolia* before and/or after lambing without causing clinical signs in the offspring.

This experiment was performed to determine if the tremorgenic toxin of *I. asarifolia* is eliminated through the milk in lactating mice, causing tremors in the offspring.

## MATERIALS AND METHODS

Fifteen Swiss mice *Mus musculus*, 12 females and 3 males, aged 40 days were housed in plastic cages with metal lids, measuring 30x20x13cm. All cages were kept in rooms with controlled temperature of 22-25°C, using a natural light cycle, from approximately 5:00am to 5:45pm. The animals were housed in cages with four females and one male per cage. After confirmation of pregnancy, by the increasing abdominal size and weight gain in females, they were transferred to individual cages. After parturition the females were kept with their offspring until 21 days post parturition.

The ration given to the animals was prepared with the leaves of *Ipomoea asarifolia* collected in a place (S 07° 04' 00.9" and W 037° 16' 48.8") in the municipality of Patos, Paraíba state. A voucher specimen of the plant was authenticated and deposited in the Center for Health and Rural Technology herbarium (CSTR #4756) of the Federal University of Campina Grande. The plant material was dried in the shade for about 10 days, milled through a 1-mm screen, and mixed with milled commercial feed for mice (PRESENCE®) in the proportions of 20% (Group 2) and 30% (Group 3). This mixture was mixed with 200 mL of water and 10% corn starch to obtain proper consistency, and then pellets were manually made using disposable 20 mL syringes. The pellets were dried in the shade at room temperature. Control mice of Group 1 received only the commercial ration which was also milled and prepared in the same manner.

Food and water intake of the dams and weight of the dams and offspring were determined on the day of birth and every 5 days until weaning, at 21 days. Statistical analysis was performed by using a one-way ANOVA with a significance of  $P < 0.05$ . Each morning the pups were observed for the presence of tremors; the pups were not handled in any way during this observation period. After the end of the experiment the surviving animals were euthanized with isoflurane.

The experiment was approved by the ethical committee on animal experimentation of the UFCG, process CEP 69-2013.

## RESULTS

Food and water intake and weight of dams and offspring showed no significant differences ( $P > 0.05$ ) between groups.

All pups of the two experimental groups showed tre-

**Table 1. Effect of the tremorgenic toxin of *Ipomoea asarifolia* on the offspring of mice. Number of pups, latency to the start of the tremors after birth, and recovery after weaning at 21 days**

Groups	Number of the female	Number of pups	Onset of tremors after birth	End of tremors after weaning
1 (Control)	I	7	NT <sup>a</sup>	NT
	II	7	NT	NT
	III	6	NT	NT
	IV	7	NT	NT
2 (20% <i>I. asarifolia</i> )	I	5	3 days	6 days
	II	6	4 days	7 days
	III	5	2 days	5 days
	IV	5	2 days	4 days
3 (30% <i>I. asarifolia</i> )	I	2	3 days	Death <sup>b</sup>
	II	3	2 days	Death <sup>c</sup>
	III	7	2 days	Death <sup>d</sup>
	IV	6	3 days	Death <sup>e</sup>

<sup>a</sup> NT = No tremors, <sup>b</sup> All offspring died 1 day after weaning, still showing tremors, <sup>c</sup> All offspring died at 7 days after birth, <sup>d</sup> All offspring died at 4 days after birth, <sup>e</sup> All offspring died 2 days after weaning, still showing tremors.

mors 2-4 days after birth (Table 1). The tremors, manifested mainly by lateral continuous movements of the head and with less intensity in the front legs, were observed when the pups were moving, but not when they were at rest. From the 12th - 15th day of life, when the pups started walking, tremors, mainly in the head and tail, were observed as they walked, but not at rest. The offspring of group 2 recovered 4-7 days after weaning, while the offspring of group 3 died before or after weaning (Table 1). No clinical signs were observed in the dams. No significant lesions were observed in the pups at necropsies nor histologically using Hematoxylin-Eosin stained sections of the central nervous system.

## DISCUSSION

The results obtained in this study with *Ipomoea asarifolia* suggest that the tremorgenic toxin of this plant or its toxic metabolites are excreted by milk. The presence of clinical signs of toxicity in the offspring, but not in their mothers, is probably due to higher resistance of the adults compared with the newborns. With penitren A, the main tremorgenic toxin of *Lolium perenne*, it was demonstrated that 1-3-day-old mice dosed intraperitoneally were more susceptible than 11-week-old mice (Lu et al. 2008). In a previous work, it was observed that dry *I. asarifolia* mixed 20-30% in the food of 40-day-old mice causes changes in equilibrium and motor coordination, but without tremors (Lopes et al. 2014). These results also suggest that newborn mice can be used as an experimental model to study the tremorgenic compound of *I. asarifolia*. The cause of death of the pups from Group 3, that ingest the highest amount of plant (30% in the food), was not determined, but the fact that all offspring of each dam died in the same day suggest that the death was due to the toxin present in the milk.

The tremorgenic compounds toxic to livestock include

indole-diterpenes comprising the penitrem A, B and C produced in *Lolium perenne* by the endophyte *Neotyphodium lolii* (Di Menna et al. 2012) and by various species of *Penicillium* (Botha et al. 1996), paxilline produced also by *Neotyphodium lolii* (di Menna et al. 2012), and paspalitrem and paspaline produced by *Claviceps* spp. (Cawdell-Smith et al. 2010). Other tremorgenic substances produced by fungi include verruculogen produced by *Aspergillus fumigatus* and *Penicillium* spp., roquefortine produced by *Penicillium roqueforti*, fumitremorgen A and B produced by *Aspergillus fumigatus*, and flavostremorgen produced by *Aspergillus flavus* (Radostits et al. 2007). However, there are no references reporting the elimination of these tremorgenic substances in milk by lactating animals. A toxic substance that is eliminated by milk is tremetol, present in snakeroot, which causes milk sickness characterized by trembling, vomiting, and severe intestinal pain that affects individuals who ingest milk from cows that has fed with this plant (Radostits et al. 2007).

The ergot alkaloids have been reported to be present in a number of morning glory species including *I. asarifolia* (Hoffman & Tschertner 1961, Eich 2008). Research has shown that a clavicipitaceous epibiotic fungal symbiont, *Periglandula*, is associated with the Convolvulaceae that produce ergot alkaloids including *I. asarifolia* (Kucht et al. 2004, Markert et al. 2008, Steiner et al. 2011). More recently, it was shown that *I. asarifolia* contains the indole-diterpenes and that the *Periglandula* species associated with it contains the biosynthetic genes for both the ergot alkaloids as well as the indole diterpenes (Scharl et al. 2013). These reports demonstrating the presence of the ergot alkaloids and the indole diterpenes suggest that they may be responsible for the tremorgenic syndrome in animals poisoned by *I. asarifolia*. However other reports have suggested that a leaf lectin may be responsible for the observed toxicity of *I. asarifolia* (Salles et al. 2011). More research is needed to determine the tremorgenic compounds in *I. asarifolia*. There is also a possibility that the milk may represent a detoxication mechanism, and that the dams are unaffected because they have excreted the toxin.

The presence of a tremorgenic toxin in *I. asarifolia* which is excreted in milk is also a concern for public health, because in northeastern Brazil the cattle and goat dairy industry is very important. The risk of consuming milk contaminated by the toxin of the *I. asarifolia* is likely much greater on small farms, which are very common in northeastern Brazil, in which milk is produced mainly for household consumption.

## CONCLUSIONS

The results indicate that the tremorgenic toxin of *Ipomoea asarifolia* is excreted in the milk of lactating mice, causing tremors in the offspring.

This finding suggests that there may be a risk also for humans consuming contaminated milk from lactating cattle or goats that consume *I. asarifolia*.

Further work will be necessary to verify this finding in mice, and to determine the toxic compounds in milk.

**Acknowledgments.**- This work was supported by the National Institute for Science and Technology for the Control of Plant Poisonings, CNPq, grant 573534/2008-0.

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