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Short communication

Experimental poisoning of guinea pig (*Cavia porcellus*) with *Indigofera suffruticosa*

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ABSTRACT

Indigofera suffruticosa causes hemolytic anemia and hemoglobinuria in cattle. The plant was administered to six groups of two guinea pigs each, at the daily dose of 10 g/kg body weight, for periods of 2, 4, 6, 8, 10 and 15 days. The guinea pigs progressively developed reduced hematocrits and hemoglobin concentrations, and finally presented anemia, without hemoglobinuria. Urine passed by guinea pigs that had ingested the plant for more than 24 h acquired a turquoise blue pigmentation 8–10 h after urination. It is suggested that the anemia is caused by the aniline contained in *I. suffruticosa*.

Indigofera suffruticosa (indigo), a plant originally from the Antilles and Central America, was introduced and cultivated in Brazil on a large scale for the extraction of natural indigo dye for the textile industry. In the 1980s this natural dye was replaced by an artificially produced pigment (Alzugaray and Alzugaray, 1988). In Northeast Brazil it is currently considered a weed that after a few days of consumption causes hemolytic anemia, hemoglobinuria and decreased milk production in cattle (Barbosa Neto et al., 2001). In most experimental and spontaneous poisonings by *I. suffruticosa* the animals recover spontaneously while continuing to eat the plant (Barbosa Neto et al., 2001; Salvador et al., 2010), but in a recent outbreak a cow died as a result of acute hemolytic anemia (Salvador et al., 2010).

To determine the toxicity of *I. suffruticosa*, and establish an experimental model to study the poisoning in laboratory animals, the plant was fed to guinea pigs (*Cavia porcellus*). Sixteen male and female guinea pigs were randomly divided into eight experimental groups and one control group, composed of two animals each. The guinea pigs in Group 0 were killed at the start of the experiment without ingestion of the plant. Groups 1 to 6 received a daily amount of 10 g of fresh chopped *I. suffruticosa* (Fig. 1) leaves and fine branches per kg of live body weight (g/kg) divided in two doses of 5 g/kg, during 2, 4, 6, 8, 10 and 15 days, respectively. For the guinea pigs in Group 8, *I. suffruticosa* was replaced by fresh chopped *Pennisetum purpureum* in a daily dosage of 10 g/kg body weight, divided in two 5 g/kg portions.

The guinea pigs were kept in temperature-controlled rooms at 25 °C in individual 90 cm × 60 cm × 30 cm polyethylene cages with white background for observation of changes in the urine. The guinea pigs of all groups received water and commercial feed *ad libitum*. At the end of each experimental period the guinea pigs were euthanized with ethyl ether. Immediately after euthanasia, intracardiac blood was collected in vacuum tubes containing sodium ethylenediaminetetraacetate (EDTA) at 10% as anticoagulant for

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Fig. 1. *Indigofera suffruticosa.*

hematocrit and hemoglobin determinations. Hematocrits were determined after five minutes of microcapillary centrifugation at 10,000 g. Hemoglobin contents were determined by the colorimetric tests using commercial kits (Bioclin®, Quibasa Química Básica Ltda), and a spectrophotometer (Bioclin Systems II®, Quibasa Química Básica Ltda). Legal and ethical requirements of the Animal Care Committee of the Federal University of Campina Grande were followed in the animal experiments.

Portions of liver, kidneys, lungs, stomach, intestines, spleen, heart and central nervous system were fixed in 10% formalin, embedded in paraffin, cut into sections 5 μm thick and stained with hematoxylin-eosin for histopathological examination.

After the first 24 h of feeding *I. suffruticosa*, all guinea pigs from Groups 1–6 presented with apathy and their urine acquired a turquoise blue color 8–10 h after urination (Fig. 2). Apathy remained mild in the guinea pigs that ingested the plant for 2–10 days, but after 10 days it became slightly worse in the two guinea pigs that ingested the plant for 15 days. No variations were observed in the blue pigment present in the urine during the whole experimental period. No other clinical signs were observed.

The hematocrit decreased from 46% on day 2 to 33.5% on day 15 (Fig. 3). There was a gradual decrease in hemoglobin concentrations, from 12.75 g/dL on day 0 to 9.5 g/dL on day 15 (Fig. 4). Normal reference ranges for guinea pigs for these parameters are usually 37–48% and 11–15 g/dL, respectively (Noonan, 1994).

Necropsy revealed, especially in Groups 5 and 6, pale livers with marked lobular pattern and rounded edges. Histologic examination of the liver showed diffuse vacuolation of hepatocytes, which became more severe the longer the plant was ingested. In guinea pigs that ingested the plant for 10 and 15 days hepatocytes were severely vacuolated and enlarged (Fig. 5B), and some were necrotic (Fig. 5, inset). The other organs showed no significant lesions. No clinical signs and no lesions (Fig. 5A) were observed on guinea pigs from Group 8 (control).

The results from the current study demonstrate that the continued ingestion of *I. suffruticosa* by guinea pigs results in anemia, which presumably results from hemolysis. However, differently from what it is observed in spontaneous (Salvador et al., 2010) and experimental (Barbosa Neto et al., 2001) cases of *I. suffruticosa* poisoning in cattle, which causes intravascular hemolysis and hemoglobinuria, in guinea pigs there is no hemoglobinuria and hemolysis seems to be solely extravascular. The reason for this difference is not clear, but it is possible that it stems from metabolic and/or morphological peculiarities of the guinea pig red cell membrane. Circumstantial evidence for this is that in the experimental poisoning by *I. suffruticosa* in goats, extravascular hemolysis is similarly observed (R. M. T. Medeiros, unpublished). In goats it is known that the constitution of the red cell membrane is different enough to markedly alter the osmotic fragility and, as

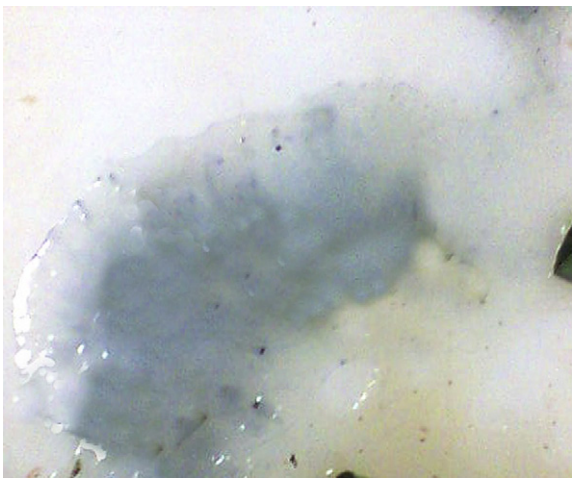

Fig. 2. Turquoise blue urine observed in the experimental poisoning by *Indigofera suffruticosa* in guinea pigs. The urine acquired the blue color 8–10 h after urination.