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# Adverse effects of *Croton urucurana* B. exposure during rat pregnancy

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ABSTRACT

*Ethnopharmacological relevance:* Several women often use plant extracts during pregnancy without any concern about its possible toxic effects. The plant effects have been experimentally confirmed in animals and humans, while others require additional investigations.

*Aim of study:* To evaluated the effect of aqueous extract of *Croton urucurana* latex on the maternal-fetal repercussions in rats.

*Methods:* Pregnant rats were randomly distributed into four experimental groups: Control=treated with water (vehicle); Treated 200=treated with a dose 200 mg/kg; Treated 400= dose 400 mg/kg and; and Treated 800= dose 800 mg/kg. The rats were orally treated by *gavage* with *Croton urucurana* or vehicle (water) during whole pregnancy. At term of pregnancy, all rats were killed to obtain maternal blood and tissues samples and fetal weight and anomaly analyses.

*Results: C. urucurana* treatment (Treated 400 and Treated 800) showed elevated liver enzymatic activities, reduced fetal body weight and placental efficiency. The Treated 800 group presented increased maternal total protein and cholesterol levels, and heart relative weight. All treated groups presented reduced maternal body weight and food intake, and increased pre-implantation loss rate compared to those of Control group. In addition, the treatment contributed to increased skeletal and visceral anomalies with higher doses.

*Conclusion: Croton urucurana* treatment caused maternal toxicity, which contributed for impairment embryo fetal development. These results showed that the indiscriminate use of plants during pregnancy should be avoided to prevent potential risk on maternal health as well as their offspring.

## 1. Introduction

Medicinal plants have been widely used to treat a variety of diseases. However, the use of these plants during pregnancy may present health risks to the woman and also to her fetus (Moreira et al., 2014). Certain herbs, used as abortifacients can induce embryotoxicity, fetotoxicity and/or teratogenicity when embryonic death does not occur. *Croton urucurana* Baillon, popularly known as dragon blood, blood water, capixingui, urucuana, lucurana, tapexingui and tapixingui, is considered an abortive plant (Gurgel et al., 2002). *Croton* is a large and diverse genus of Euphorbiaceae that comprises at least 800 species of the tropics and subtropics (Webster, 1993). *C. urucurana* is widespread in wetlands and riparian areas and is commonly found in southern Brazil, northern Argentina, Paraguay and Uruguay. The *C. urucurana* tree has an open canopy and bright stem, and reaches up to 15 m (Babieri et al., 2014).

The indigenous culture believe that *C. urucurana* shows remarkable healing properties. This plant has been extensively used in folk medicine for treatment of cancer, rheumatism, lesions, ulcers, diarrhea infections (Rao et al., 2007). Three different products from *C. urucurana* species are primarily used - the red sap or latex, stem bark and the gum exudate (Simionatto et al., 2007). In male rats, Esmeraldino et al. (2005) found that the stem bark of *C. urucurana* aqueous extract showed anti hemorrhagic activity. Also in male rats, was observed an anti-diarrheal response after treatment with 600 mg/kg of *C. urucurana* latex (Gurgel et al., 2001), and antifungal activity against five different dermatophytes when using *C. urucurana* sap in an in vitro study (Gurgel et al., 2005). Cordeiro et al. (2012), testing the acute toxicity of this plant, demonstrated that a single dose of 2000 mg/kg of *C. urucurana* bark methanol extract produced no toxicity signs in female rats, whereas doses at 50, 100 and 250 mg/kg caused reduced gastric lesions in male rats. In 2016, these same

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authors found that the *C. urucurana* bark methanol extract exhibited anti-inflammatory and antinociceptive properties in male mice (Cordeiro et al., 2016).

In spite of the widespread use of *C. urucurana* by women, no studies on the use of this plant during pregnancy are available. Given that *Croton urucurana* is strongly suspected of causing maternal and fetal damages, the objective of this study was to assess the effect of *C. urucurana* latex aqueous extract on maternal reproductive toxicity and fetal development in laboratory animals.

## 2. Materials and methods

### 2.1. Drugs and chemicals

Drugs and chemicals secured for the study included sodium thiopental (Thiopentax® Cristália Chemical Ltda., Brazil), total proteins, triglycerides, total cholesterol and high-density lipoprotein (HDL)-cholesterol, aspartate transaminase (AST) and alanine transaminase (ALT) (Wiener® Rosário, Argentina). All reagents used in this study were acquired of Merk® (São Paulo, Brazil).

### 2.2. Extraction of plant materials

*Croton urucurana* Baillon specimens were collected from Barra do Garças, Mato Grosso State, Brazil, between April and May 2009, in the morning period. The plant was identified and authenticated by experts from Mato Grosso Federal University, Mato Grosso State, Brazil, where a voucher specimen (Number 05360) has been deposited.

### 2.3. Preparation of extract aqueous of Croton urucurana latex

To obtain the latex was held vertical section of approximately 1.60 m long in the trunk of the tree, and then several other form of "V" cuts were made. Trees were chosen with a diameter less than 20 cm. The latex was frozen and kept in a freezer until the time of use. On the day of the experiment, the lyophilized latex was diluted in filtrated water in a proportion of 200 mg per ml of water.

### 2.4. Experimental animals

Female and male Wistar rats weighing approximately 240 and 260 g, respectively, were obtained from CEMIB (Multidisciplinary Center for Biological Research) - Campinas State University (UNICAMP). These animals were adapted and maintained in Vivarium of Laboratory of System Physiology and Reproductive Toxicology, Mato Grosso State, Brazil, under standard laboratory conditions (22 ± 3 °C, 12-h light/dark cycle), with pelleted food (Purina rat chow, Purina®, São Paulo, Brazil) and tap water ad libitum. The animals were cared for in accordance with the principles of the Guide for Care and Use of Experimental Animals. The local Committee of Ethics in Animal Experimentation approved all experimental procedures of this study (Protocol number 23108.026432/12-0).

### 2.5. Mating procedure and experimental groups

All female rats were mated overnight to male rats. The morning when sperm was found in the vaginal smear was designated as day 0 of pregnancy. The mating procedure consisted for 15 consecutive days, which comprises approximately three-oestral cycle, however non-mated female rats in this period were considered infertile and removed of the study (Santos et al., 2015). After mating period, the pregnant rats were distributed into four experimental groups (n minimum=12 animals/group): Control = treated with water (vehicle); Treated 200= treated at dose 200 mg/kg; Treated 400= treated at dose 400 mg/kg; and Treated 800= treated at dose 800 mg/kg. The rats were treated with *C. urucurana* or vehicle (water) in the morning period by intragastric route (*gavage*) during pregnancy (from gestational day 0–21).

### 2.6. Course of pregnancy

Maternal weight, food and water intake were measured in the beginning and end of pregnancy, at approximately 9 a.m. At day 21 of pregnancy, the rats were lethally anesthetized by sodium thiopental. The gravid uterus was dissected to count dead and live fetuses, resorption (embryonic death), implantation sites, and corpora lutea numbers. The number of undetectable implantation sites was determined by Salewski (1964). The rate of pre-implantation loss was calculated as: Number of corpora lutea – Number of implantations×100/ Number of corpora lutea and for post- implantation loss rate was calculated: Number of implantations – Number of live fetuses×100/ Number of implantations (Volpato et al., 2015).

### 2.7. Analysis of maternal biochemical parameters

After maternal whole blood collection (day 21 of pregnancy), an aliquot was centrifuged at 3500 rpm for obtaining serum. The serum samplØs were then stored at −20 °C for measurement of total protein, triglycerides, total cholesterol and high-density lipoprotein (HDL)-cholesterol using colorimetric assay by commercial kits. Very low-density lipoprotein (VLDL)-cholesterol levels were determined by mathematical estimation (Knopfholz et al., 2014). Aspartate transaminase (AST) and alanine transaminase (ALT) activities were determined using commercial kits.

### 2.8. Maternal organ relative weight

The relative weight of heart, liver and kidneys of each rat was calculated by ratio of weight of each organ (grams) and body weight at day 21 of pregnancy. The result was expressed in grams/100 g body weight.

### 2.9. Evaluation of the placental and fetal weight

The fetuses and placentas were weighed to calculate the placental efficiency (fetal weight/placental weight). The mean birth weight of the control pups (Control) was 5.16 ± 0.50 g. Newborns in the experimental groups whose birth weights did not diverge more than ± 1.7×standard deviation (SD) from the Control mean (i.e., those that were within the 4.81–6.01 g range) were classified as appropriate for pregnancy age (APA). Those whose weights were at least 1.7×SD greater than the Control mean birth weight were classified as large for pregnancy age (LPA). Those whose birth weights were at least 1.7×SD lower than the Control mean birth weight were classified as small for pregnancy age (SPA) (Damasceno et al., 2012).

### 2.10. Analysis of external and internal (visceral and skeletal) anomalies

The fetuses were evaluated in a microscope with respect to incidence of external anomaly. After external analysis, half of the fetuses were fixed in Bodian´s solution and serial sections were prepared as described by Wilson (1965) for visceral examination. The remaining fetuses were prepared for examination of the bones by the staining procedure of Staples and Schnell (1964).

### 2.11. Statistical evaluation

For comparison of the mean values among the experimental groups, one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison test was used. The proportions were calculated by the Fisher´s Exact test. For fetal data, the litter was used as statistical