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Effects of *Buchenavia tomentosa* consumption on female rats and their offspring

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ABSTRACT. *Buchenavia tomentosa* Eichler is a common plant in Brazilian cerrado. Fruits of this plant are employed in human feeding and folk medicine. Cattle producers affirm that consumption of the fruits cause abortion in cows, and even death. Considering that the plant may be consumed by pregnant women and animals, the present study was undertaken to evaluate the possible toxic effects of the ingestion of *B. tomentosa* fruit (10% added to the diet), from the first to the twenty-first days of gestation, on reproductive parameters and on physical and neurobehavioral development of rats offspring. An increase in food consumption at pregnancy days 11 and 17, and weight increase at day 17 of pregnancy were observed. Besides that, we verified an increase in weight of male offspring on post natal day 1. Other parameters were not affected by plant consumption. These results indicate that the consumption of *B. tomentosa* at 10% during pregnancy cause slight toxicological effects. The changes verified in the present study indicate toxic action of the fruit possibly induced by flavonoids with hormonal action; however, further studies must be accomplished to corroborate this hypothesis.

Key words: teratology, developmental toxicology, reproductive toxicology,toxic plant.

RESUMO. Efeitos do consumo de *Buchenavia tomentosa* em ratas e em suas proles. *Buchenavia tomentosa* Eichler é uma planta típica do cerrado brasileiro. Os frutos desta planta são empregados na alimentação humana e medicina popular. Criadores de bovinos afirmam que o consumo desta planta produz aborto em vacas bem como a morte destes animais. Uma vez que a planta pode ser consumida pelo homem e animais, em idade fértil e inclusive gestantes, o presente estudo avaliou os possíveis efeitos tóxicos da ingestão de dieta com 10% de frutos de *B. tomentosa* do primeiro ao vigésimo primeiro dia de gestação sobre os parâmetros reprodutivos e sobre o desenvolvimento físico e neurocomportamental das ninhadas de ratos. Foram observadas elevações no consumo de alimentos nos dias 11 e 17 de gestação e no peso ao dia 17 de gestação. Aumento do peso dos filhotes machos foi verificado no primeiro dia após o nascimento. Outros parâmetros não foram afetados pelo consumo da planta. Estes resultados indicam que a ingestão, durante a gestação, de uma dieta acrescida com 10% de *B. tomentosa* causa efeitos tóxicos leves. As alterações verificadas no presente estudo indicam ação tóxica do fruto possivelmente induzidos por flavonoides de ação hormonal; no entanto, estudos detalhados devem ser conduzidos para garantir esta hipótese.

Palavra-chave: teratologia, toxicologia do desenvolvimento, toxicologia da reprodução, planta tóxica.

Introduction

Buchenavia tomentosa Eichler (*Combretaceae*) popularly known as merindiba, embiridiba and tarumarana is a very common plant in the Amazonian savanna (MARQUETE; VALENTE, 2005). In Brazil, the fruits of this plant are used in human feeding and folk medicine (NETO; CARNIELLO, 2007; POTT; POTT, 1986). Local cattle producers affirm that the consumption of the fruits cause abortion in cows, and even death.

The Combretaceae family comprises 20 genera and more than 600 species (GARCEZ et al., 2003). Combretum and Terminalia are the most species-rich genera, with over 200 species each (KATERERE et al., 2003). Other members of the Combretaceae family of plants, which include *Anogeissus leiocarpus, Pteleopsis suberosa, Thiloa glaucocarpa* are toxic to man and animals (ITAKURA et al., 1987; MAIGA et al., 2005). Plants of the *Buchenavia* genus contain flavonoid alkaloids, and part of the toxicity may be due to these substances. For instance, buchenavianine and capitavine are flavonoid alkaloids found in fruits, leaves and/or seeds of *B. macrophylla* and *B. capitata* (HOUGHTON, 2002). Flavonoids are capable to alter monooxygenasis activity and consequently the

steroidal hormone metabolism (LIMA et al., 2001). *In vivo* estrogenicity study of several flavonoids showed the ability of these chemicals to alter estrogen receptor alpha (ERα) distribution in the mouse uterus (BREINHOLT et al., 2000). Inhibitory effects of 15 flavonoids on the reduction of progesterone to 20α-hydroxyprogesterone in rat liver were observed (SHIMADA et al., 2005).

Tannins and terpenes are also present in plants from this genus. These chemical compounds were isolated from green leaves of *Buchenavia oxicarpa* (HERNES; HEDGES, 2004; PEÑUELAS et al., 1996). Numerous reports are available about the influence of tannins on rats, causing systemic effects such as metabolism and blood disturbances (HERNES; HEDGES, 2004; SALUNKHE et al., 1990), and reproductive toxicity (BURGÜER et al., 1999). Terpenes produce several reproductive effects such as developmental toxicity and estrous cycle irregularity (DELGADO et al., 1993; RASIER et al., 2006; SATO et al., 2003; WASUNTARAWAT et al., 1998).

Due to the importance of this fruit in human feeding by traditional communities, and possible toxic risks, the present study was undertaken to determine the possible toxic effects of 10% *B. tomentosa* dried fruit ingestion on female rats and their offspring.

Material and methods

Plant

Buchenavia tomentosa fruits were collected in the region of Araguaína (Tocantins State, Brazil) and identified by Taciana Barbosa Cavalcanti and Bruno Machado Teles Walter from Empresa Brasileira de Pesquisa Agropecuária – Embrapa/Brasília. The voucher specimens are kept in the HT Herbarium/University of Tocantins (registration: HT 2095).

Buchenavia tomentosa unripe fruits were sliced and oven dried at 50°C. The fruits were then milled and stored in plastic dark bags, to avoid humidity and light.

Each week, regular chow was milled and mixed with 10% *B. tomentosa* dried milled fruits, using a 'V' homogenizer apparatus and stored in plastic bags in a controlled room temperature (22–25°C), according to the methodology described by Maruo et al. (2003a and b). During the experimental period, metal vessels were filled with the diet, weighted and attached to the animal cages. The next morning the recipients were weighted again and the difference of weight was taken as the measure of food consumption.

Concentration of 10% *B. tomentosa* was established to avoid nutritional disturbances capable to disrupt gestation.

Animals

In this experiment, we employed outbreed male and female Wistar-Han rats, three- to four-month-old, obtained from the School of Veterinary Medicine and Zootechny biotery, University of Tocantins, Brazil. The animals were housed in groups of four or five in plastic cages (40 x 50 x 20 cm) at temperature and humidity-controlled room (20 ± 3°C; 45-65%) with a 12h light/dark cycle (lights on at 06:00h). The animals had free access to a standard laboratory chow (Labina®, Purina, Brazil) and water.

The animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council, USA (ILAR, 1996).

Every morning, 0.5 mL of 0.9% saline was introduced into the vaginas of the females, and immediately aspirated. The material was observed under the light microscope, and the phase of the estrous cycle was determined through cytology. Two females from the same group were placed overnight with one young untreated male rat, previously determined to be fertile. The minimal body weight considered appropriate for mating was fixed at 190 g. The onset of pregnancy was confirmed by the presence of spermatozoa in vaginal smears on the next morning, designated as gestation day (GD) 1. Pregnant rats were housed individually in polycarbonate cages with stainless-steel wire lids, and randomly divided into experimental and control group.

Experimental rats (n = 14) received *B. tomentosa* (10% added in the diet) throughout the pregnancy (GD01 to GD21) as the only source of nutrition and water *ad libitum*. The control group of 14 female rats received the regular powdered diet, for the same time period.

Reproductive parameters and offspring studies

The groups were weighed over the pregnancy period (GD01 until parturition), and at postnatal day (PND) 1, 7, 14 and 21. Food and water consumption during pregnancy and lactation (measured daily), length of gestation, litter size, litter weight, presence of external malformation, and sex ratio were also assessed. All pregnant rats were allowed to give birth and nurture their offspring normally. No cross-fostering procedure was used. At PND 1 all the litters were externally examined and sexed. The litters were organized

into groups of eight pups each, four males and four females, and the remaining pups were culled.

Physical development

Each day, beginning on PND 01, all pups from each litter were examined for the following physical parameters of development: development of primary coat of downy hair (PND 1–3), ear unfolding (PND 2–5), development of fur (PND 3–6), incisor eruption (PND 6–12), ear opening (PND 11–17), and eye opening (PND 11–17). The days required for the appearance of these landmarks were recorded until all pups in the litter were positive for that developmental parameter. The mean day of appearance of each of the above parameters was calculated. The pups were weighed at PND 1, 7, 14 and 21. All observations were made in the morning.

Reflex development

The following reflex tests were assessed in all pups from each litter: surface righting reflex (a normal ventral position assumed after the animal is placed on its back, observed for 15 s, postnatal days 2 to 7), negative geotaxis (to turn at least 90, after being placed face-down in a platform inclined at 45; the maximum time allowed for the pup to turn was 30 s, PD 7 to PD 12), and grasping reflex (registering the day of absence of involuntary bending of the fingers in response to tactile stimulation on the palm, PD2 to PD12).

Analysis of blood samples and histopathological stains

At PND 21 (weaning day), the mother and the offspring were anesthetized with a mixture of intra-peritoneal xylazine (KENSOL 2%®, König, 3 mg kg^{-1}) and ketamine (KETALAR 5%®, Pfizer, 10 mg kg^{-1}). Blood samples were taken from the hepatic vein into tubes. Serum biochemical parameters included urea nitrogen, total proteins, albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Ovaries, uterine horns, kidneys, lung and liver were removed, weighed, analyzed, and specimens were fixed in 10% neutral buffered formalin. The specimens were routinely processed, embedded in paraffin, and 5 µm thick sections were cut and stained with haematoxylin and eosin for light microscopic evaluation.

Statistical analysis

The Student *t*-test was used to analyze the data. A significant level of $p < 0.05$ was indicative of significant difference between groups for all comparisons made.

Results and discussion

Reproductive Parameters

As shown in Figure 1, continuous feeding of 10% *B. tomentosa* added in the diet during pregnancy increased body weight on GD 17. Additionally, the exposed group increased the food consumption on GD 11 and 17 (Figure 2).

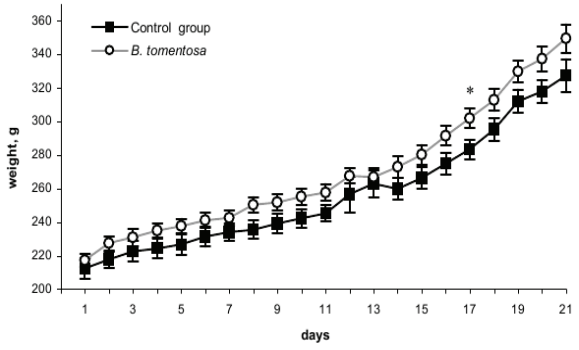

Figure 1. Weight of female rats that received a diet containing *B. tomentosa* 10% or just chow (control) during pregnancy. Data expressed as mean ± S. E. M. *p < 0.05, t test.

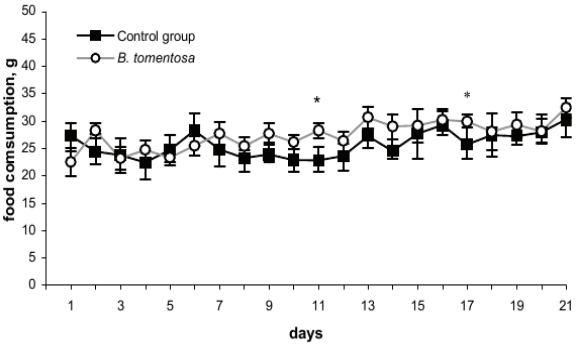

Figure 2. Food consumption of female rats that received a diet containing *B. tomentosa* 10% or just chow (control) during pregnancy. Data expressed as mean ± S. E. M. *p < 0.05, t test.

No differences were detected in the weight gain of control and experimental groups, nor differences in water consumption, gestation length, litter size, presence of external malformation and sex ratio of newborn pups (Table 1).

Table 1. Mean weight (g ± S. E. M.), size (number of animals ± S. E. M) and sex ratio (number of animals ± S. E. M) of litters of rats that received a diet containing *B. tomentosa* 10% or just chow (control) throughout pregnancy.

Parameters		Control	*B. tomentosa* 10%
Litter weight		63.21 ± 3.39	78.39 ± 4.49
Litter size		10.08 ± 0.59	11.21 ± 0.57
Sex size	male	5.41 ± 0.47	5.10 ± 0.68
	female	5.91 ± 0.97	6.00 ± 0.61

p > 0.05, t test.

Offspring development, blood sample analysis and histopathological stains

The weight of male offspring from the group of female rats that received the *B. tomentosa* during gestation was increased on PND 1, as shown in Figure 3.

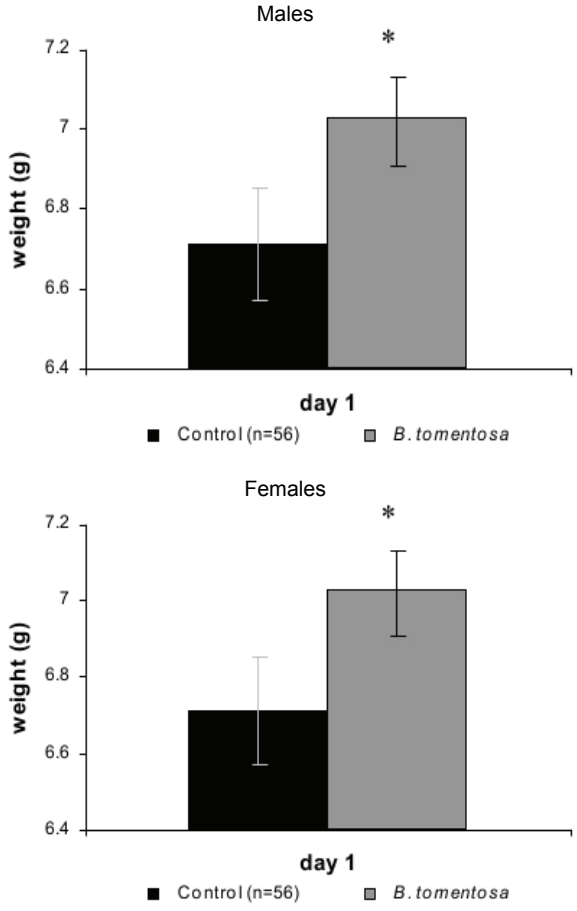

Figure 3. Weight on PND 1 of male and female offspring rats that received a diet containing 10% of *B. tomentosa* or just chow (control) during pregnancy. Data expressed as mean ± S. E. M. *p < 0.05, t test.

However, female pups were not affected, and no alteration on male pups was observed in the other evaluating days. Differences in weight gain of the offspring, and food and water consumption were not evidenced.

Physical parameters of male and female pups from rats exposed to *B. tomentosa* 10% during pregnancy were not affected, as listed in Table 2.

There were no significant differences in neurobehavioral tests (Table 3, Figures 4 and 5) as well as organs weight, and blood sample analysis. Macroscopic and microscopic observations revealed no treatment-related effects on any organ evaluated (data not shown).

Table 2. Physical development (mean day of complete appearance ± S. E. M) of thet litters of rats that received a diet containing 10% *B. tomentos*a throughout pregnancy.

Parameters	Males		Females	
	Control (n = 56)	*B. tomentosa* 10% n = 56)	Control (n = 56)	*B. tomentosa* 10% (n = 56)
Primary coat of downy hair	2.43 ± 0.17	2.36 ± 0.13	2.43 ± 0.17	2.36 ± 0.13
Ear unfolding	2.91 ± 0.25	2.64 ± 0.17	2.79 ± 0.19	2.57 ± 0.17
Development of fur	3.69 ± 0.24	4.57 ± 0.25	3.83 ± 0.27	4.43 ± 0.31
Incisor eruption	8.64 ± 0.23	8.08 ± 0.24	8.34 ± 0.23	8.25 ± 0.32
Ear openings	12.69 ± 0.95	13.21 ± 0.35	13.50 ± 0.36	13.18 ± 0.26
Eye openings	14.50 ± 0.31	14.00 ± 0.17	14,43 ± 0,23	13.58 ± 0.26

p < .0.05, t test.

Table 3. Grasping reflex (mean day of absence ± S. E. M) of the litters of rats received a diet containing *B. tomentosa* 10% or just chow (control) throughout pregnancy.

Males		Females	
Control (n = 56)	*B. tomentosa* 10% (n = 56)	Control (n = 56)	*B. tomentosa* 10% (n = 56)
8.21 ± 0.46	8.28 ± 0.32	7.71 ± 0.47	7.28 ± 0.59

*p > .0.05, t test.

The toxic effects of a test substance basically can be defined by physical examination, daily observations, visual examination, determination of food and water consumption, body and organ weights, hematology, urinalysis, biochemical organ function test and pathology studies (STEVENS; GALLO, 1989).

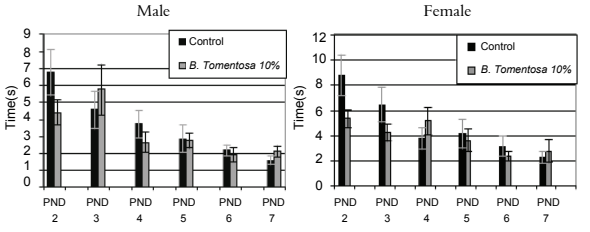

Figure 4. Righting reflex of the litters of rats that received a diet containing *B. tomentosa* 10% or just chow (control) throughout pregnancy. Data expressed as mean ± S. E. M. p > .0.05, t test.

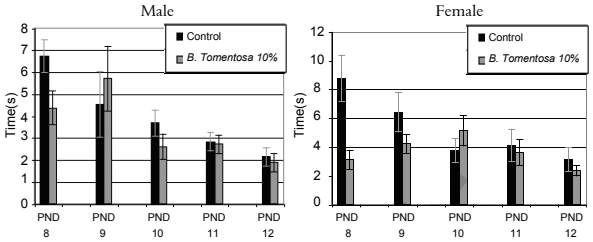

Figure 5. Negative geotaxis reflex of the litters of rats received a diet containing *B. tomentosa* 10% or just chow (control) throughout pregnancy. Datta expressed as mean ± S. E. M. p > .0.05, t test.

In the present study, the administration of 10% of *B. tomentosa* in the diet during pregnancy produced an increase in food consumption on GDs 11 and 17, and on body weight on GD 17. These alterations may

reflect metabolic changes produced by active compounds in the fruit. Among all the possible substances present in the plant, phytoestrogens may be capable to produce this kind of alteration.

Nonsteroidal estrogenic substances from plants, called phytoestrogens, although capable of causing infertility in animals grazing heavily on estrogen-containing fodder, are rarely involved in human problems. However, signs of estrogen imbalance, such as uterine bleeding and abnormal menstrual cycle, have been reported under unusual circumstances (STOB, 1983).

Phytoestrogens bind to the same intracellular receptors as those that bind estradiol, and produce similar physiologic responses, including hypertrophy of the accessory glands in males, antigonadotropic effects by feedback control in the hypothalamic-pituitary-gonadal axis, and gonadal-altered levels in mammals, of both sexes (STOB, 1983; TAPIERO; MATHÉ, 2002). Plants with estrogenic activity produces embryotoxicity, and sex-related differences in toxicity (MARUO et al., 2003a and b).

Flavonoids and particularly isoflavones which are abundant in soybean are important phytoestrogens with chemical structures that resemble those of estrogens, with weak estrogenic activities (IMAI et al., 2004).

In Buchenavia species, the following flavonoid alkaloids were isolated: Buchenavianine from *B. macrophylla* fruits and leaves, N-demethylbuchenavianine from *B. macrophylla* leaves, *O*-Demethylbuchenavianine, *O*,*N*-Demethylbuchenavianine, *N*-Demethylcapitavine 5,7-Dihydroxy-6-(*N*-methyl-20-piperidinyl) flavanone from *B. macrophylla* fruits, and capitavine and 5,7,49-Trihydroxy-6-(*N*-methyl-20-piperidinyl) flavone from *B. capitata* seeds (HOUGHTON, 2002; TAHARA; IBRAHIM, 1995).

Flavonoids are known by modifying the functions of endocrine glands, modulating the physiological aspects of steroid effects (BARNES, 1998).

Weight increase in the experimental male pups was observed at PND 1, and can be partially explained by the possible presence of agents with hormonal action in the plant.

Flavonoids possess inhibitory action on the transformation of androstenodione to estrone and testosterone to estradiol, being able to compete with steroids interacting with certain monoxigenases, modifying its activity and the metabolism of steroid hormones (LIMA et al., 2001). In rats liver study, the inhibitory capacity of several flavonoids on the reduction of the progesterone in a biological inactive form, 20-α-hidroxyprogesterone was demonstrated (SHIMADA et al., 2005).

Fetal exposure via the mother to bisphenol A, a plastic policarbonate constituent with low estrogenic action, at low doses produced an increase in pre-pubescent weight of males and females to the 22 days of age (HOWDESHELL; VOM SAAL, 2000). In the same way, the treatment of females with raloxifene, a selective estrogen receptor modulator, during four weeks before the mating, had induced increase of the weight of younglings at the postnatal day 1 (HOYT et al., 1998).

Thus, the weight increase verified in the present study indicates a toxic action of the fruit, possibly induced by hormonal action compounds, however, detailed studies must be lead to corroborate this hypothesis.

On the other hand, other physical parameters and neurobehavioral tests of male and female pups from rats exposed to *B. tomentosa* 10% during pregnancy were not affected indicating that although male pups weight was enhanced on post-natal day 1, suggesting fetal development interference; no anatomical and no reflex impairment was induced at this level dose. The organs weight, blood sample analysis and histopathological evaluation were not affected by the plant also suggesting neither anatomical or tissue injury.

In summary, the results indicate that exposure to *Buchenavia tomentosa* (10% dried fruits added) in the diet during pregnancy induces slight toxicity in the mothers and the male offspring. These data might be important to evaluate the safety of *B. tomentosa* during pregnancy, since this fruit is currently employed on the feeding of Brazilian traditional communities.

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Conflict of Interest statement

The authors declare that there are no conflicts of interest.

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