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Luffa operculata fruit aqueous extract induces motor impairments, anxiety-like behavior, and testis damage in rats

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ABSTRACT

Ethnopharmacological relevance: *Luffa operculata* (L.) Cogn., Cucurbitaceae (*buchinha-do-norte*), aqueous extract (EBN) is popularly used to relieve symptoms of sinusitis and as abortive.
Aim of the study: As neurotoxicity and toxicity studies on the male reproductive system are scarce, the present study aimed at quantitatively addressing the question.
Materials and methods: Male adult rats were observed in the open field (OF) and in the light-dark box test (LDB) to evaluate locomotion and anxiety. Macroscopical and microscopical alterations on the rats' testes were also studied. The rats were divided into two groups, control (GC) and experimental (GE). GE received 1.0 mg/kg *per day* of EBN, orally, for five consecutive days, whereas GC received water. On the 6th day, each animal was evaluated in OF and in LDB for 3 min in each apparatus. After that, the left testicles were studied.
Results: In the OF, GE showed decreased locomotion, increased immobility time and decreased grooming and remained for less time in the center of the apparatus. In LDB, GE showed significant difficulty in moving into the light side of the device and remained longer in the dark side, exhibiting less displacement on both sides and less transitions between sides. Testicle weights, relative weights, testicular volume, cranial-caudal and lateral-lateral axes presented an increase in relation to the GC. Microscopic changes were observed in parenchyma, lumen and diameter of seminiferous tubules. Leydig cell numbers were decreased in GE.
Conclusions: The administration of EBN induced anxiety-like behavior, impaired locomotion and altered the testes morphology of rats.

1. Introduction

The use of plants for the treatment, curing and prevention of diseases is one of the oldest forms of medicinal practice. In the early 1990s, the World Health Organization (WHO) reported that 65–80% of the population in developing countries depended on medicinal plants as their only means of access to basic health care (Santos Junior et al., 2017). Upper respiratory tract infections (URI's) are one of the most common problems, particularly in children, resulting in significant morbidity worldwide (Pitrez, 2003). Sinusitis, for example, is a URI defined as a bacterial infection of the paranasal sinuses.

In order to circumvent the side effects of medicines, people consume medicinal plants. For the treatment of sinusitis, for example, different plant preparations are used by the population, such as the decoction of sunflower seeds (*Helianthus anuus* L.), eucalyptus leaves (*Eucalyptus citriodora* L.), infusion of *capim-de-são-josé* (*Cymbopogon martinii* (Roxb.) JF Watson), lavender (*Lavandula angustifolia* Miller), garlic (*Allium sativum* L.) and lemon (*Citrus limon* (L.) Burman F.) (Grandi et al., 1989). However, many of these plants have important side effects. Garlic, for example, should not be consumed by people with blood clotting problems (Chan et al., 2007). There is a recurrent questioning about the use of medicinal plants, since a belief in both the safety and consumption of products of natural origin is widespread. Although frequently in use, teas prepared with certain plants can cause toxic effects and drug interactions (Stoddard et al., 2015). The efficacy and safety of medicinal plants should, however, be demonstrated.

When instilled into the nostrils, a decoction made with the dried fruits of *buchinha-do-norte* (*Luffa operculata* (L.) Cogn. is also used in the treatment of sinusitis, but is also known for its abostiuve properties (Revilla, 2002). Besides the popular name *buchinha-do-norte*, L. *operculata* is also known in Brazil as *cabacinha, buchinha, bucha-dos-paulistas*, *purga-de-João-Pais* (Duke and Vasquez, 1994; Mengue et al.,

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2001). The plant is commonly found in the tropical Americas and is cultivated mainly in the Northeast and North of Brazil. Its fruit is ovoid-oblong, with a brownish appearance when dry, and a spongy texture formed by reticulated tissue, sometimes being used as a "vegetal sponge" in baths. The fruit is also known in folk medicine for its purgative effect. Dried fruits are indicated for use against "rhinitis and sinusitis", being administered through inhalation or a nasal aqueous solution to be instilled through nostrils, which can cause severe nasal irritations and bleeding (Mengue et al., 2001). It is in its use as a homeopathic and alternative medicine that it is indicated for treating sinusitis. It provokes a profuse release of mucus, which relieves nasosinusal symptoms; its use is also widely diffused among both women and men (Menon-Miyake et al., 2005a). Few reports describe unpleasant symptoms, such as nasal irritation, epistaxis and anosmia (Menon-Miyake et al., 2005b). Also, antibacterial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *S. pyogenes* have been reported Scalia et al., 2015).

Although the main side effect of consuming the teat made with the dried fruits of L. *operculata* relates to pregnant women (Revilla, 2002), there is a lack of information concerning its importance in terms of the male reproductive system, or even its influence over male behavior, as they too consume the medicinal plant in order to combat sinusitis. Male infertility affects over 10% of reproductive age couples worldwide; luckily, most cases can be treated and reverted. The causes of male infertility may be of a genetic, endocrine, immunological and environmental origin. In theory, chemical agents can damage spermatogenesis at any stage, from spermatogonia to mature spermatozoa (Parada et al., 2004). Some plants are known for their impairment of the male reproductive system, such as cannabis, neem, species from *Gossipum* genera, and even some commonly consumed foods such as papaya, garlic, rosemary, curcuma (D'Cruz et al., 2010).

The present study aimed to verify the effects of repeated oral administration of *buchinha-do-norte* aqueous extract (EBN) upon the motor and anxiety-related behaviors of male rats and to verify if it causes macroscopical and microscopical damages in the testes.

2. Material and methods

2.1. Plant extract

Plant material, which consisted of the dried fruit of *buchinha-do-norte* (L. *operculata*), was obtained from Santos Flora (lot # BUCHO 01/0914, collect date 24/09/2014, validity: 24/09/2017, origin Brazil). The plant extract was prepared according to its popular use, as an infusion. One dried fruit was added to 300 mL of boiling water. For the pharmacological assays, 15 dried fruits (34.677 g) were added to 5100 mL of Milli-Q boiling water, and kept under infusion for 10 min. After this period, the infusion was cooled, filtered, frozen (-70 °C) and lyophilized, resulting in the aqueous extract of L. *operculata*, here called EBN. The yield of EBN was 14.82 g, or 42.73%, in relation to the dry fruit weight.

2.2. Animals

Animal experiments were conducted with the approval of the Committee of Ethics in the Use of Animals (UNIP#043/2016). Twenty-one adult male Wistar rats (*Rattus novergicus*), weighing approximately 350 g, 20–23 weeks of age, were used. The rats were kept 5 to a box in polypropylene cages (45.5 × 34.5 × 20 cm), in a microisolator system (Tecniplast, Buguggiate, Italy), under a controlled temperature (22 °C ± 2 °C), controlled humidity (55–65% relative humidity) and under artificial lighting (12 h light cycle and 12 h dark, lights being switched on at 7h00) for a week in order to adapt to the laboratory conditions. The animals had free access to filtered water and irradiated feed (BioBase, Águas Frias, Brazil) for all experiments. The animals were kept in a bed made of sterilized wood. The bed was changing on a weekly basis, in order to minimize interference with their behavior.

2.3. Treatment administration

After lyophilized, EBN was diluted in water and administered at 1 mg/kg to the experimental group animals (GE), whereas water was administered to the control group animals (GC), for five consecutive days, by gavage. The dose used in the current work was adjusted to 1/3 of the dose used in the evaluation of the plant drug as abortifacient to female rats (Barilli et al., 2007).

2.4. Open field evaluation

Open field (OF) was used in order to evaluate the influence of EBN over the locomotion and anxiety of the animals (Broadhurst, 1960). Initially, each animal was placed in the central circle of the arena and was evaluated for 3 min according to the following parameters: frequency of locomotion, immobility time, rearing frequency, number and time of grooming, time spent in the center of the apparatus, time spent on the borders of the apparatus and number of fecal *boli* (Bergamini et al., 2017; Estork et al., 2016, 2014; Gusmão et al., 2013a, b).

2.5. Light-dark box evaluation

Immediately after the evaluation in the open field, the animals were placed in a light-dark box (LDB) for evaluation of anxiety. The model was based on the aversion of rodents to clear spaces, generating a conflict between remaining in the closed and dark space and the exploratory instinct leading to the light side. The device consisted of an acrylic box divided into a dark compartment and a light and bright compartment separated by a door. The animals were allocated to the dark side of the box (Takao and Miyakawa, 2017) and remained under experimental conditions for 3 min (Shimada et al., 1995). The parameters evaluated during this period were latency to transit to the light side; number of attempts to enter the light side; time spent in the dark side and in the light side; light and dark sides locomotion and rearing frequency; and number of fecal *boli* in the dark and in light sides (Crawley and Goodwin, 1980).

2.6. Body weight gain

The animals were weighed before EBN administration, and on the 6th day, before hystopatological assays.

2.7. Macroscopic and histopathological evaluation of testis

Immediately after euthanasia, the left testicle of each animal was taken and weighed in an analytical scale. Both cranial-caudal and lateral-lateral axes were measured with a caliper rule, and those measurements were applied in the ellipsoid formula $V = 4/3 \cdot \pi ab^2$, where a is the semiprolate axis (cranial-caudal) and b is the semioblate axis (lateral-lateral), in order to calculate testes volumes. Also, the testes' relative weight was obtained; the rats' body-weight being obtained on the same day testes were weighed.

The testes were prepared according to a technique described elsewhere (Freitas et al., 2002). They were briefly conditioned in Bouin solution for 50 h, being transferred to ethanol 70% until histological processing. The testicles were dehydrated by the ethanol/xylol battery, which was followed by paraffin packing. Slices of 4–5 μm were obtained and added to blades, one slice per blade, and then the paraffin was removed using the xylol/ethanol battery. Slices were divided into two groups: one dyed with hematoxylin and the other with PAS (periodic acid of Schiff).

The morphometrics were made through the use of a photo-microscope to quantify Leydig cells and seminiferous tubules. Leydig cells can be seen in the external region among seminiferous tubules. Ten regions