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Evaluation of the Cytotoxicity and Anticytotoxicity of *Brugmansia Suaveolens* in Mus Musculus Mice

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Abstract

Someone individuals search through alternative sources for substances that provide hallucinogenic effects, changes in mood and perception. The *Brugmansia suaveolens*, a plant commonly found in Brazil, is widely used, whether in religious cults and indigenous tribes. This plant has the ability to interact with the central nervous system inducing potent hallucinogenic effects to the user. However, there are few studies evaluating the toxicological risk of this plant. The present study aimed to evaluate possible cytotoxic / anticytotoxic activities based on the PCE/NCE ratio of hematopoietic bone marrow of mice. Fortyeight Mus musculus mice were divided into 8 groups and the *Brugmansia suaveolens* flowers aqueous solution was prepared for the tests. The ratio PCE/NCE in the doses (250, 500, 1000mg / kg), presented difference when compared with the negative control ($p < 0,01$). The test dose (500 and 1000mg / kg) of the anticytotoxicity group showed no difference ($p > 0.01$) in relation to the positive control. The *Brugmansia suaveolens* crude aqueous solution through the PCE/ NCE ratio showed cytotoxic activity at doses of 250, 500 and 1000mg / kg and no anticytotoxic activity at doses of 500 and 1000mg / kg.

Keywords: *Brugmansia suaveolens*, citotoxicity, hallucinogenic plants

Abbreviations: CAE: Crude Aqueous Extract; PCE: Polychromatic Erythrocites; NCE: Normochromatic Erythrocites

Introduction

The Psychoactive plants contain chemicals that presumably evolved as allelochemicals (capable of affecting the growth of other species), but target certain neuronal receptors when consumed by humans, altering perception, emotion, and cognition. Interestingly, people have been exploring alternative uses for plants containing psychoactive substances, such as medicines and in the context of religious rituals for their various effects, including reality-distorting hallucinogens, sleep-inducing sedatives, antidepressants and mind stimulants [1-3]. There is still a belief that these plants have medicinal properties and that, having been used for thousands of years, have proven efficacy and lack of side effects and health risks. However, certain plants are known to contain potentially hazardous substances. From the scientific point of view, research has shown that many of them contain potentially toxic or genotoxic substances and, for this reason, should be used with caution, respecting their toxicological risks [4,5]. In Brazil has a large number of plants with psychotropic properties and many of them can be found easily in the urban environment.

The *Brugmansia suaveolens* (*Solanaceae)* (Figure 1), or trumpet tree, also called in Brazil cartridge and milk's glass is a plant originating from tropical America and widely distributed in humid South American environments. It is commonly observed in gardens, backyards or even parks, as spontaneous and probably cultivated by former residents [6,7]. Usually the population uses fresh or dried flowers of B. suaveolens to produce a drink with psychoactive properties, which is used in mystical or religious rituals. However, another use for this plant, especially among the younger population, is intentional ingestion or cigarette smoking to induce hallucinations [8]. Although some studies show that B. suaveolens Crude Aqueous Extract (CAE) affects the neurotrans-

mitter system. Little information has been reported about its genotoxic, antigenotoxic, cytotoxic or anticytotoxic properties [9]. This study aimed to evaluate the possible cytotoxic / anticytotoxic effects of *B. suaveolens* flowers extract.

Figure 1: Brugmansia suaveonlens flowers.

Materials and Methods

Materials

Botanical material

The *B. suaveolens* flowers were collected in September of 2018 in the city of Santana do Araguaia, Pará.

Controls and reagents

a) Negative Control: Sterile H2O - Manufactured by Equiplex, Lot No. 1731060, valid for 07/2019.

b) Positive Control: Doxorubicin - Manufactured by Libbs Farmacmaceutica Ltda. Lot No. 17A0663, manufactured in January 2018, valid for 2 years.

c) Bovine fetal serum - Manufactured by Laborclin Farmacmaceutica Ltda. Lot No. 60428001, valid April for 1 year.

Animals

To execute the cytotoxicity and anticytotoxicity tests, 48 healthy male and female Mus musculus "out bread" mice from the Pontifical Catholic University of Goiás, presenting body weight between 30 and 40 grams were used. and age range from 45 to 60 days on the day of the experiment. The animals were housed in 30x20x13cm polypropylene cages, lined with shavings, according to international standards. Each cage accommodated a maximum of 06 (six) animals. They received water and feed ad libitum. The shave was changed every two days.

The mice were kept in an airy experiment room with an average temperature of 2°C, with ventilation system, light - dark cycle (light 7:00 - 19:00, dark 19:00 - 07:00). Thirty-six mice were required for cytotoxicity and anticytotoxicity tests, 6 for negative control and 6 for positive control.

Methodologies Used

Flowers extract preparation

The collected flowers were dried in a greenhouse at 37ºC and kept for 5 days. After drying, the material was macerated with the aid of a pistil mortar to obtain a powder. The solution was obtained by decoctioning 30g of B. suaveolens dried flower powder in 300mL of distilled water for 5 minutes. A 40mg / g extract of distilled water was obtained and used for the studies [9]. Evaluation of citotocity and anticitotocity activities for the micronucleus test and Poliychromatic erythrocites/ Normochromatic erythrocites (PCE/NCE) ratio *In vivo* tests were performed on mice through the micronucleus test. The experimental procedure of this study focused mainly on analyzing the doses of 1000, 500 and 250mg / kg of B. suaveolens CAE, which were injected into mice via intraperitoneal. After 24 hours, the mice were submitted to thiopental anesthesia (30mg / kg), then euthanized by cervical dislocation and the femurs removed. The proximal femoral epiphysis was cut, and the hematopoietic bone marrow aspirated with fetal bovine serum. After homogenization of the bone marrow in the serum, it was centrifuged at 1000rpm for five minutes. The supernatant was discarded, and the laminar preparation was made with the

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precipitate. Then, after drying the slides, they were stained in buffered Giemsa solutions.

Slide analysis was performed using a standard Nikon optical microscope to detect possible alterations and/ or chromosomal losses (micronucleus) in the polychromatic erythrocytes of animals submitted to different treatments. The cells were visualized in a 100x immersion objective, using three slides for each animal, evaluating 2,000 PCE per slide. The average of three slides was used as results. For cytotoxicity evaluation, PCE and NCE were counted. The PCE/NCE ratio was determined according to Schmid [10]. In the evaluation of the anticytotoxic activity of this plant the solution of B. suaveolens was administered simultaneously with Doxorubicin - DXR, which already has known genotoxic action, the experimental procedure followed was similar to the methodology previously described. Also included was the positive control.

Statistical Analysis

Statistical analysis of the present study was performed with the aid of BioEstat v. 5.3, developed by the Federal University of the state of Pará (UFPA), the data were tabulated with the aid of Excel 2016 software, developed by the company Microsoft. To verify if the data followed the normal distribution patterns, the Kolmogorov-Smirnov test with Reliability Index - 99% CI was performed. The cytotoxic action analysis was evaluated by comparing the EPC and ENC frequencies of each group treated with B. suaveolens SAB with the negative and positive group by the ANOVA test, complemented by the Tukey test. Values of p <0.01 were also considered significant.

Ethic

The plant exsicata was deposited in the Herbarium of the Federal University of Goiás - UFG, Goiânia - Goiás, with the registration number # 65798.This study was reviewed and approved by CEUA - Animal Use Ethics Committee of PUC / GO under protocol number 8878200617 for the period from 08/2017 to 12/2018. After the euthanasia process, the carcasses of the animals that participated in the experiment were discarded to be incinerated. A commercial company was hired to provide services in this area.

Results

The results of the PCE/NCE ratio for cytotoxic and anticytotoxic activities are shown in Table 1. The PCE/NCE ratio for cytotoxicity at B. gentleolens (250, 500 and 1000mg / kg) doses of B. suaveolens when compared to the negative control showed a significant difference (p <0.01). Related the tests used in the anticytotoxicity group, there was similarity in the PCE/NCE ratio and the positive control (DXR). The 250mg / kg dose showed a significant reduction (P <0.01) in the EPC / ENC ratio when compared to the positive control.

Table 1: Bone marrow PCE/NCE ratio of mice treated within 24 hours with three doses of Brugmansia suaveolens CAE

		Micronucleus Test			
Groups	Treatments	Number of Animals	PCE/2000	NCE/2000	PCE/NCE ratio
1	Negative control *	6	94[a]	106a	0,89
2	Positive control **	6	68[b]	132[b]	0,52[b]
3	CAE of B. suaveolens (1000mg.kg p.c.)	6	72[b]	128[b]	0,56[b]
4	CAE of B. suaveolens (500mg.kg p.c.)	6	76[b]	124[b]	0,61[b]
5	CAE of B. suaveolens (250mg.kg p.c.)	6	77[b]	123[b]	0,63[b]
6	CAE of B. suaveolens (mg.kg p.c.) + DXR (2mg/kg)	6	70c	130c	0,54[c]
7	CAE of B. suaveolens (500mg.kg p.c.) + DXR (2mg/kg)	6	75[c]	125[c]	0,60[c]
8	CAE of B. suaveolens (250mg.kg p.c.) + DXR (2mg/kg)	6	88[d]	112[d]	0,79[d]

a: (P> 0.01) there was no significant difference when compared to the negative control;
b: (P <0.01) there was significant difference when compared to negative control.
c: P> 0.01 there was no significant difference when compared to the positive control;
d: P <0.01 significant difference when compared to the positive control.
* Negative control: vehicle substance (sterile H2O) (0.1ml / 10g p.c.); ** Positive Control: (Doxorubicin: 2mg / kg).

Abreviations - CAE: Crude Aqueous Extract, PCE: Poliychromatic erythrocites, NCE: Normochromatic erythrocityes.

Discussion

The results obtained through the micronucleus test showed citotoxic activities of B. suaveolens SAB in the three doses administered (250, 500 and 1000mg/kg). It was possible to observe significant difference in the frequency PCE/NCE ratio when compared to the negative group. Even being a plant commonly known in the brazilian territory, few studies have been carried out using B. suaveolens, not completely proving the extent of its effects (positive and negative) on the organism. Dickel et al. [9] carried out a study with aspects similar to the present report, in which, through tests using Wistar rats, it was able to identify the cytotoxic and neurotoxic capacity of the plant and also related it to possible damage to the genetic material and the promotion of oxidative stress. contribute to the pathogenicity of some diseases by damaging important biomolecules. The results obtained in the PCE/NCE ratio may be justified by the high concentration of alkaloids that B. suaveolens has, which promotes the protection of the plant against external agents such as insects and pests and which has known toxic capacity. Griffin & Lin [11] observed the toxic capacity of these compounds, especially scopolamine and hyoscine,

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but also atropine and hyoscine that can be lethal to cells even at low concentrations, such as the 250mg / kg dose that was administered for the experiments of the present study.

In contrast, the 250mg/kg dose of the anticytotoxicity group showed anticytotoxic activity, unlike the cytotoxicity group, another compound may be responsible for this result. Flavonoids, which are also present in the plant, are reported in the literature and were characterized by Amado et al. [12] and Hung et al. [13] as tumor cell inhibitors because they have antioxidant pharmacological properties, enabling the control of cell proliferation thus playing an important role. in the blockade of oncogenesis. No further studies were found that tested the cytotoxic and anticytotoxic potential of B. suaveolens under similar conditions to this experiment. Therefore, there is a need for further studies regarding the possible activities of this hallucinogenic plant, such as genotoxic, embryotoxic and mutagenic studies in order to better understand these processes.

Conclusion

Brugmansia suaveolens CAE through the PCE/NCE ratio showed:

a) Cytotoxic activity at doses of 250, 500 and 1000mg/ kg.

b) Did not show anticytotoxic activity at doses of 500 and 1000mg / kg.

c) presented anticytotoxic activity at the dose of 250mg / kg.

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References

1. Spinella M (2001) The psychopharmacology of herbal medicine: plant drugs that alter mind, brain, and behavior. London: MIT Press.
2. Ratsch C (2005) The encyclopedia of psychoactive plants: ethnopharmacology and its applications. Rochester: Park Street Press.
3. Van Wyk B, Wink M (2014) Phytomedicines, herbal drugs and poisons; Royal Botanic Gardens. The University of Chicago Press, Chicago and London.
4. Veiga JVF, Pinto AC (2005) Plantas medicinais: cura segura? Química Nova 28(3): 519.
5. De Bona AP, Batitucci MCP, Andrade MA, Riva JAR, Perdigão TL (2012) Estudo fitoquímico e análise mutagênica das folhas e inflorescências de Erythrina mulungu (Mart. ex Benth.) através do teste de micronúcleo em roedores. Revista Brasileira de Plantas Medicinais 14(2): 344-351.
6. Oliveira F, Akisue G, Akisue MK (1991) Farmacognosia. Atheneu Editora: Rio Janeiro, p. 95.
7. Soares EL, De C (2008) A família Solanaceae no Parque Estadual de Itapuã, Viamão, Rio Grande do Sul, Brasil. Revista Brasileira de Biociências 6(3): 177-188.
8. Oliveira RB, Godoy, SAP, Costa FB (2003) Plantas tóxicas. Conhecimento e prevenção de acidentes. Editora Holos, Ribeirão Preto, pp. 34-77.
9. Dickel OE, Aguiar RB, Geracitano L, Monserrat JM, Barros DM (2010) Efeitos comportamentais e neurotóxicos do extrato aquoso de Brugmansia suaveolens em ratos. Revista Brasileira de Farmacologia 91(4): 189-199.
10. Schmid W (1975) The micronucleus test. Mutation Research 31: 9-15.
11. Griffin WJ, Lin, GD (2002) Chemotaxonomy and geographical distribution of tropane alkaloids. Phytochemistry 53(6): 623- 37.
12. Amado NG (2011) Flavonoids: potential wnt/beta-catenin signaling modulators in cancer. Li
13. fe Science 89(15-16): 545-54.
14. Hung JY (2009) Didymin, a dietary flavonoid glycoside from citrus fruits, induces fas-mediated apoptotic pathway in human non-small-cell lung cancer cells *in vitro* and *in vivo*. Lung Cancer 68(3): 366-374.

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