Journal of Ethnopharmacology 264 (2021) 113265

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Vertical exposition to *Luffa operculata* extract deregulates behavior and hypothalamus neurotransmitters in juvenile rats

Humberto V. Frias [a], Cinthia dos S. Alves [a], Jorge C. Flório [b], Eduardo F. Bondan [a], Leoni V. Bonamin [a], Cidéli P. Coelho [c], Maria M. Bernardi [a], Ivana B. Suffredini [a,d,*]

[a] Programa de Pós-Graduação em Patologia Ambiental e Experimental, Universidade Paulista - UNIP, Brazil
[b] Departamento de Patologia – VPT, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Brazil
[c] Universidade Santo Amaro - UNISA, Brazil
[d] Núcleo de Pesquisas em Biodiversidade, Laboratório de Extração, Universidade Paulista - UNIP, Brazil

ARTICLE INFO

Keywords:
Buchinha-do-norte
Neurochemistry
Prenatal exposition
Animal behavior
Open field
Light-dark box
Traditional medicine meso- and Southern America
Cognitive performance
Toxicology

ABSTRACT

Ethnopharmacological relevance: *Luffa operculata* (L.) Cogn (Cucurbitaceae) is a traditional plant popularly used in the abortion induction, against sinusitis and is toxic.
Aim of the study: To verify the influence of the aqueous extract obtained from the dry fruit of *L. operculata* (BNE) on the male rats vertically exposed to a subabortive dose of BNE, by evaluating alterations in behavior and neurochemical features in hypothalamus, striatum and frontal cortex, at a juvenile age, after receiving a stress challenge given by the use of the "New York subway stress" technique (NYS).
Materials and methods: Pregnant female rats (F0 generation) received 1.0 mg/kg BNE, or distilled water (100 mL/kg), by gavage, between gestation days GD17 and GD21. The pups were weaned at PND21 and were kept up to PND60 (juvenile age) in controlled environmental conditions. Four groups were obtained: control (CG), experimental (EG), stress control (SCG) and stress experimental (SEG) After being stressed, the animals were behavioral screened for in the open field (OF) and in light-dark box (LDB) apparatuses. They were euthanized, and the liver, kidneys and brain were removed for both macroscopic and microscopic analyses, and for quantification of vanillylmandelic acid (VMA), norepinephrine (NE), dopamine (DA) and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) and the serotonin (5-HT) and its metabolite 5-hydroxyindolylacetic acid (5-HIAA) were accessed in the hypothalamus, frontal cortex and striatum.
Results and discussion: although most of the behavior changes were due to the stress challenge, the rats spent more time in the dark side of the LDB and were less likely to explore the light side, indicating that the treatment with BNE induced to fear. Interferences of BNE over behavior were due to impairment of VMA, NE, 5-HT and DA and increasing of DOPAC in the hypothalamus, and an increase of 5-HIAA in the frontal cortex, indicating alterations in the hypothalamic-hypophysis-adrenal axis (HHAA). No macroscopic or histopathological changes were observed in the liver, kidneys, or brain, although GFAP was diminished in the SCG, as expected for stressed rats.
Conclusion: the vertical exposition of juvenile rats to BNE led to the manifestation of fear and to a down regulation of the hypothalamic-hypophysis-adrenal axis.

1. Introduction

The use of plants as a fertility regulator agent is done since ancient times, as they work as emmenagogues, local contraceptives or abortifacients (Kumar et al., 2012). Some of the ancient papers by Theophrastus, Pliny and Dioscorides were previously reviewed (Albert-Puleo, 1979) and described the use of scammony (*Convolvulus scammonia*) as an abortifacient plant, which were also used against headache, due to the presence of ergot alkaloids. In Brazil, the use of traditional plants in fertility control is not only restricted to indigenous or *quilombola* populations, but are also widespread in the urban adjacent population. Recently, 54 Brazilian species were reported to induce abortion, and among those, *Luffa operculata* (L.) Cogn. (Cucurbitaceae) (Yazbek et al., 2016) highlights.

The dried fruits of *L. operculata* is popularly known as *buchinha-do-norte* and the tea made with them can lead to the occurrence of uterus hemorrhage and end up with abortion (Revilla, 2002). The tea made

* Corresponding author. Av. Paulista, 900, 1 andar, Cerqueira César, São Paulo, SP, 01310-100, Brazil.
E-mail addresses: ivana.suffredini@docente.unip.br, ibsuffredini@yahoo.com.br (I.B. Suffredini).

https://doi.org/10.1016/j.jep.2020.113265
Received 2 June 2019; Received in revised form 15 July 2020; Accepted 6 August 2020
Available online 25 August 2020

Abbreviations

BNE	*buchinha-do-norte* extract
GD	gestation day
PND	post-natal day
CG	control group
EG	experimental group
SCG	stress control group
SEG	stress experimental group
NYS	New York Subway stress technique
OF	open field
LDB	light-dark box
NE	norepinephrine
CRH	corticotropin-releasing hormone
LC/NE	*locus ceruleus*-norepinephrine
DA	dopamine
5-HT	serotonin
DOPAC	3,4-dihydroxyphenylacetic acid
5-HIAA	5-hydroxyindolacetic acid
HVA	homovanillic acid
VMA	vanillylmandelic acid
GFAP	glial fibrillary acidic protein
NPY	neuropeptide Y
BDNF	brain-derived neurotrophic factor
COMT	catechol-O-methyltransferase
MAO	monoamine oxidase
ANOVA	analysis of variance

with the same plant organ is used to treat sinusitis as it is instilled through the nostrils (Menon-Miyake et al., 2005). The mechanism by which the tea provokes abortion is unclear, and yet to be related to the cucurbitanes commonly found in the Cucurbitaceae.

Recent findings reported for the first time the alterations in the histological structure of the testicles and in the behavior of adult male rats that received the aqueous extract of *L. operculata*, by gavage, for five consecutive days (Alves et al., 2018). The authors reported that testicles from sex-experienced adult male Wistar were macroscopically and microscopically affected by the direct administration of BNE. The testicle weights, relative weights, volume, cranial-caudal and lateral-lateral were increased, and the diameter of the seminiferous tubule and their lumens were augmented, while the parenchyma was diminished, including the number of Leydig cells. In this work, the authors reported anxiety-like behavior and diminishing in locomotion after the treatment. From these findings, new questions related to the influence of the administration of the tea to pregnant female rats leading to alterations in testicle and in the behavioral phenotype of their male offspring have emerged. Although the authors did not correlate the histological and behavioral findings to some chemicals present in *L. operculata*, the resemblance of the chemical structure of cucurbitanes that occur in the species and steroid hormones shows some perspective in the explanation of our findings. The occurrence of cucurbitacin B, dihydrocucurbitacin B (Lang et al., 2012), operculins A and B (Kawahara et al., 2004), neocucurbitacins A and B (Kawahara et al., 2001), ceramides, spinasterol and other sterols (Feitosa et al., 2011) and isocucurbitacin B (Matos and Gottlieb, 1967) was reported to *L. operculata*.

Despite the possible occurrence of testicle histological disarrangement in the males from the F1 generation, BNE may cause influence on the behavior of juvenile male rats, due to the possibility of cucurbitanes to cross the blood-brain barrier. The brain of rats, men and other mammalians develop under a constant and coordinate change in steroid hormone levels of the mother and of the fetus itself, in a very precise regulation of time and levels (Gore et al., 2014). The subsequent release of progesterone, 17-β-estradiol, prolactin, oxytocin and placenta lactogens, which reorganizes the mother brain plasticity guarantees the adequate fetus development as it extends to its adulthood, as well as the mother reproduction, parenting and nursing behaviors (Keller et al., 2019). Steroids can be considered as major brain modulators which directly interferes with its function, differentiation, growth and synapse formation, both for androgens (Rubinow and Schmidt, 1996) and estrogens (Keller et al., 2019). Also, the exposure to steroids, natural or mimics, in the early stages of brain development can cause permanent alterations in behavior (Miodovnik et al., 2012). Somehow, these alterations can influence the hypothalamic-pituitary-adrenal axis. Some exogenous compounds, known as endocrine-disrupting chemicals, or EDCs, may have a pivotal whole in the developing brain during pregnancy, once they can reach the fetus by crossing the placental barrier and disrupt the hormonal balance needed for the normal physiological functions to reach full cognition abilities (Gore et al., 2014).

In face of the findings reporting the deleterious influence of BNE on adult male rats, there is a lack of information regarding the influence of BNE on the male offspring vertically exposed to the extract. In order to have a more accurate prospection of behavior and neurotransmitter concentration alterations, a stress challenge was performed in the male rats at post-natal day 60 (PND60), which is a period that the rats are considered as late adolescents or young adults (Lupien et al., 2009), a period of time when rats can still be vulnerable to the influence of stress, which may end up with some degree of disruption in physical or mental health, in some grade (Holder and Blaustein, 2014), which can directly influence the maturation of cognitive behavior (Ariza Traslaviña et al., 2014). Also, behavior can be influenced by drugs interfering in the HHAA as the corticoids, which ends up reflecting with the stress levels (Spiga et al., 2014). For that reason, the prospection of the consequences of the BNE exposition to the male rats was conducted by introducing a stress challenge at PND60, in order to proportionate a condition to observe subtle alterations in both behavior and neurotransmitters quantification.

The present work aimed at the evaluation of the putative effects of prenatal exposure to sub-abortive dose of the aqueous extract from the fruits of *L. operculata* on juvenile male rats of the F1 generation, regarding to liver, kidneys and brain histological patterns and to evaluate the brain functional changes, comprising behavioral and neurochemical studies.

2. Materials and methods

2.1. Plant extract

The dried fruits of *buchinha-do-norte* (*L. operculata*) were obtained from Santos Flora (batch # BUCHO 01/0914, collect date 09/24/2014, validity: 09/24/2017, origin Brazil) and were prepared according to the popular use, as a decoct. Popular use describes that one dried fruit has to be added to a glass of water, and has to be boiled for 10 min. So, 15 dried fruits (34.677 g) were added to 5,100 mL of Milli-Q® boiling water, and were kept under infusion for 10 min. Then, the infusion was cooled, filtered, frozen (−70 °C) and lyophilized, resulting in the aqueous dried extract, the so-called BNE. The yield of BNE was 14.82 g, or 42.73%, in relation to the dry fruit weight (Alves et al., 2018).

2.2. Animals

2.2.1. Ethics

The study was performed as in accordance to the Guide for the Care and Use of Laboratory Animals National Institutes of Health, aiming good laboratory practices. The protocols were approved by the Ethics

Committee for Animal Use, from the Universidade Paulista - UNIP, (permit #043/2016). All efforts were made in order to minimize animal suffering and to maximize animal well-being.

2.2.2. Mating and birth protocol

Twenty six virgin female Wistar rats aged 12–14 weeks weighting 200 g were distributed in groups of three in propylene cages (45.5 X 34.5 × 20 cm), in an environment under a controlled temperature (22 °C ± 2 °C), controlled humidity (55–65% relative humidity) and under artificial lighting (12h light cycle and 12h dark, lights witched on at 07:00). They were housed for ten days for habituation. Also, six male Wistar rats of 20–23 weeks of age, weighting approximately 350g, and sexually experienced, were kept under the same conditions. The animals had free access to filtered water and irradiated food (BioBase®, Águas Frias, Brazil) during the study. Rats were donated from the School of Veterinary Medicine, University of São Paulo, Brazil. Three female rats were housed in the same cage, and one male rat was introduced to the cage and left to mate. The pregnancy determination was done by the evaluation of the presence of spermatozoids in the vaginal smear. Female dams were separated according to the identification of pregnancy, but as soon as their female companions got pregnant, they were again reunited, and remained together up to gestation day (GD)18. At GD18, each female dam was separated in an individual cage that was attached to a microisolator system (Tecniplast®, Buguggiate, Italy), under a controlled temperature (22 °C ± 2 °C), controlled humidity (55–65% relative humidity) and under artificial lighting (12h light cycle and 12h dark, lights witched on at 7h00), so as to give birth alone to avoid canibalism.

2.2.3. Litter standardization

At PND2, the reproductive performance was done to all litters by the use of a heat bed comfortably regulated to a physiological temperature. The number of females and males that were born in each litter was recorded. So, one male and one female pup were individually weighed, then the female and male sets of pups were weighed and finally the litter was also weighed. After that, the litters were standardized in 4 males and 4 females each (Udo et al., 2014) in order to avoid differences in the litters development. At PND21, the littermates were separated and co-housed by sex under the same conditions as their parents.

2.3. Treatment procedures

Dam female rats at GD17 up to GD21 were divided in two treatment groups: the control group (CG; n = 14) which received water and the

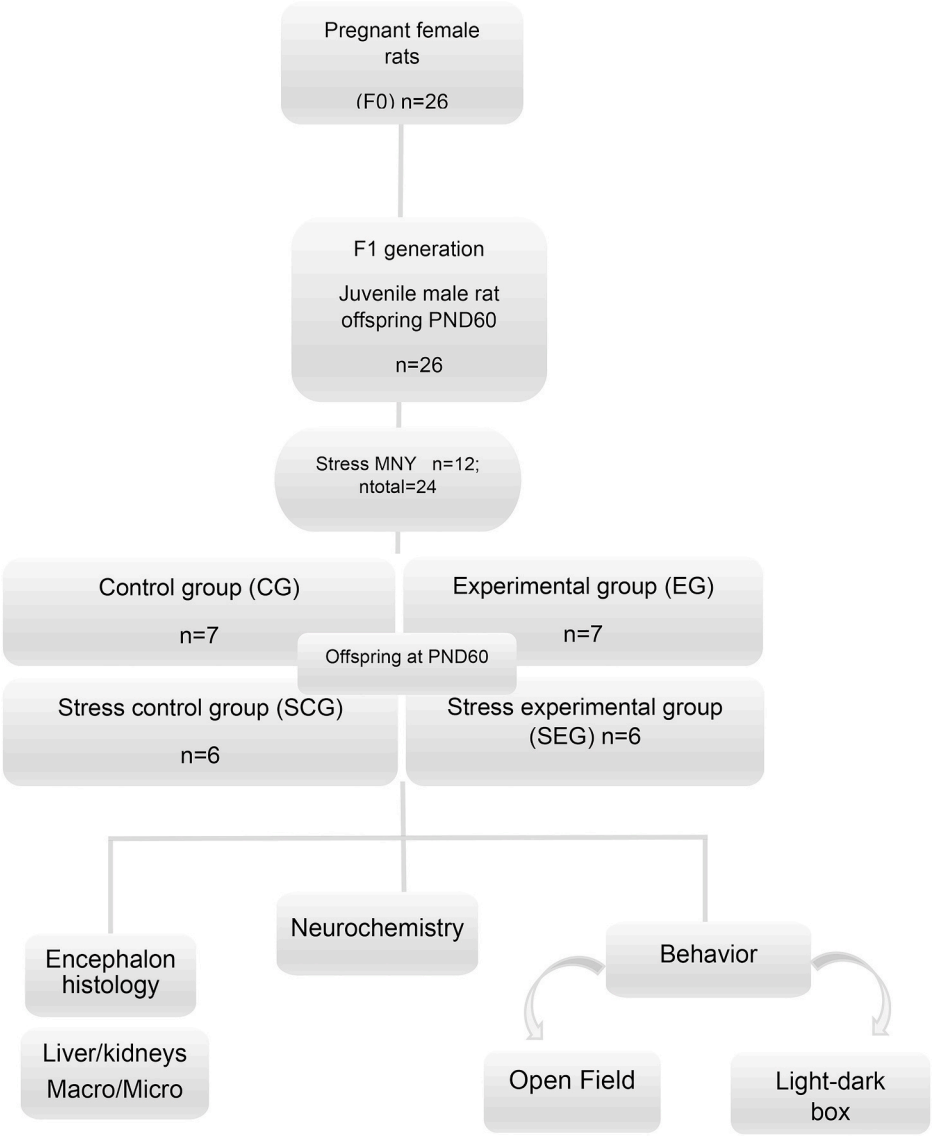


Fig. 1. Experimental design.

experimental group (EG; n = 12) which received BNE. Treatments were administered by gavage, at a dose of 1.0 mg/kg, a subabortive dose used in previous works (Alves et al., 2018). The dose of 1.0 mg/mL was determined based on the abortive dose of 4.0 mg/mL used in the abortive evaluation of *L. operculata* in previous reports (Barilli et al., 2007). The period of gestation spanning from GD17 to GD21 has been chosen due to the implication of the treatment over the brain development of the offspring. After given birth, the females kept their litters up to the weaning day at PND21. After separated, male and female pups were left to grow up to a juvenile age, at PND60, when they received a stress treatment induced by the New York Metro stress technique (NYS; Dhabhar and McEwen, 1997), in order to evince for alterations. The rats from F1 generation were divided in those which were born from the control dams (CG; n = 7), those which were born from the experimental dams (EG; n = 6), those that came from the CG which received stress treatment, named SCG (n = 7), and those which came from the experimental group which received stress treatment, named SEG (n = 6; Fig. 1).

At PND60, tests were performed exclusively with the male rats from the F1 generation, while the female rats were studied in a separate protocol, not included in the present study. The four male pups from each litter were designated to different analyses, so one was submitted to open field and light-dark box apparatuses tests, one was submitted to histochemical analyses of brains, livers and kidneys and one was submitted to neurochemical analyses of the hypothalamus, frontal cortex and striatum.

2.4. Experimental design

Fig. 1 reports the experimental design that was proposed for the present project. F1 male rats were divided into four groups, according to the treatment received by their mothers during pregnancy (CG and EG) and according to the stress that they received or not (SEG and SCG; Fig. 1).

2.4.1. Stress induction

The F1 generation male Wistar rats were submitted to the NYS procedure. This technique was first developed by New Yorker researchers and were based on the stress caused by the metro travelling during rush hours (Dhabhar and McEwen, 1997). Briefly, the animals are put into PVC plastic pipes in a way that they cannot move or escape, and the pipes were then placed on a shaker at 10 cycles per minute, for 1 h. Rats that were submitted to stress were designated as “stress groups”, so, four groups were obtained: control group (CG; n = 7), experimental group (EG; n = 7), stress control group (SCG; n = 6) and stress experimental group (SEG; n = 6), ntotal = 26. After that, the rats were submitted to the tests.

2.5. Open field evaluation

Open field (OF) was used in order to evaluate the influence of BNE 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 peripheral of the apparatus and number of fecal *boli* (Alves et al., 2018; Estork et al., 2016, 2014; Gusmão et al., 2013a, 2013b; Suffredini et al., 2017).

2.6. Light-dark box evaluation

The model was based on the aversion of rodents to clear spaces, generating a conflict between remaining in the dark side and the exploratory instinct impulsively 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 placed in a light-dark box (LDB) for evaluation of anxiety immediately after the evaluation in the open field. The animals were allocated in the dark side of the box (Takao and Miyakawa, 2006) and remained under experimental conditions for 5 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 (Alves et al., 2018; Crawley and Goodwin, 1980).

2.7. Neurochemical analysis

Rats from F1 generation were euthanized by rapid decapitation, the brains were removed and dissected in order to separate hypothalamus, frontal cortex and striatum, over a glass Petri dish laid over a bed of ice. Brain segments were weighed and stored in freezer at −80 °C until analyses. The brain areas were chosen due to their relation and regulatory role in the HHAA and associated behaviors. Each segment was dissected, weighed and stored at −80 °C until use. Each brain segment was defrost and added to a vial containing perchloric acid solution and homogenized with a sonicator, before being centrifuged (Felicio et al., 1996; Soto et al., 2013). Supernatants were taken and used in the quantification by high performance liquid chromatography coupled to electrochemical detector (HPLC-ECD; Felicio et al., 1996), a sample injector (15 and 20 Al valve) an integrator (Chromatopac, Shimatzu, Japan), a C-18 column (Shimpack; ODS, Kyoto, Japan). Dihydroxybenzylamine (DHBA) was used as internal standard. Samples were run for 18 min and the limit detection was 2.0 pg. Vanylmandelic acid (VMA), norepinephrine (NE), dopamine (DA) and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and its metabolite 5-hydroxyindolylacetic acid (5-HIAA) were quantified.

2.8. Histological and immunohistochemical analysis

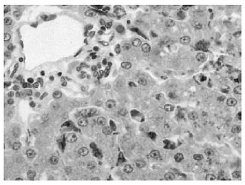
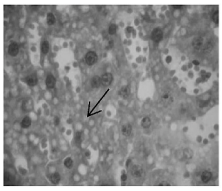
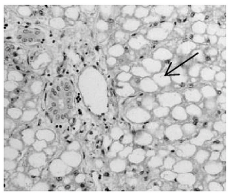
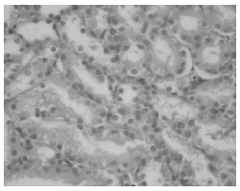
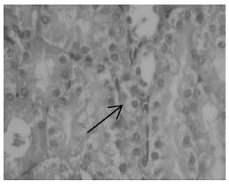
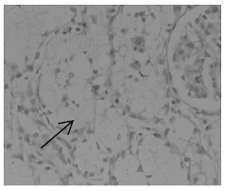
Brains were collected and fixed in 10% buffered formalin for 72 h. Coronal sections of each brain were made and the tissue samples were embedded in paraffin for processing for conventional histological procedures. GFAP immunohistochemistry was performed using the chain polymer-conjugated staining method (DAKO EnVision™ System). Polyclonal rabbit anti-GFAP immunoglobulin (1:50; Z033401, Dako, Glostrup, Denmark) was used as the primary antibody followed by the EnVision^{+} Kit (HRP. Rabbit. DAB+, K4011, Dako, Glostrup, Denmark). Ten sections (5 μm thick) per rat were made from each chosen area (frontal cortex, striatum and hypothalamus), and, from each individual section, ten photomicrographs were taken (40x objective, Nikon E200 microscope, equipped with a Nikon Coolpix digital camera linked to a liquid crystal display monitor, Kanagawa, Japan). Morphometric analysis was performed using the Image Pro-Plus 6 software (Media Cybernetics, Rockville, MD, USA), calibrated with digital color filters such that only positive cells were included and background staining was excluded from the measurement. The astrocyte index per area was used to represent the GFAP staining extent compared to the total area of the image (zero, as the complete absence of staining; and 1, as the total staining of the area).

The liver and kidney are organs involved in drug metabolism, and alterations in the tissue structures can indicate toxicity of BNE. Fragments of tissues measuring up to 0.5 mm were randomly harvested and fixed in 10% buffered formalin for 24 h at least. The histological procedures were made according to the conventional paraffin embedding and hematoxylin-eosin (HE) staining methods. Parameters were scored from 0 to 2 as shown in Table 1.

2.9. Phytochemical testing

A thin layer chromatography (TLC) technique was used in the

Table 1
Parameters established for the evaluation of histological alterations of liver and kidneys of male Wistar rats that underwent treatment with *Luffa operculata* fruit aqueous extract or control vehicle. Scores from 0 to 2 were given to each parameter.

Parameter	Score		
	0	1	2
Liver			
Vacuolization of cytoplasm	absent	moderate	severe
			
Kidneys			
Tubular vacuolization	absent	moderate	severe
			

evaluation of the presence of cucurbitacin derivatives in the lyophilized aqueous extract, BNE, diluted in H_2O and in ethanol. Solutions were prepared at a concentration of 10 mg/mL each. The chromatographic system was composed by the mixture of chloroform and methanol (95:10) as mobile phase, silica gel GF254 (Merck) as the stationary phase, vanillin phosphoric acid reagent (VP41; Wagner and Bladt, 1996) and plate heat for 10 min at 100 °C to visualize the spots. Visualization was made under U.V. 365 nm and normal illumination. Some traditional reactions were made to prospect for alkaloids, anthraquinones and cardiac glycosides.

2.10. Statistical analysis

The premises of randomness, independency and normality were adopted in the present work. The Shapiro-Wilk test was adopted to verify normality among data for all analyses. Levene's Test and Mauchly Test were used to verify homogeneity and sphericity of data, respectively, and data are available as Supplementary Material. Eventually size of effect (η^2) was calculated to some of the parameter factors. In order to verify differences in the parameters related to behavior in open field, light-dark box apparatuses, as well as the differences in the GFAP and neurochemistry after treatments with BNE and stress, data were evaluated by two-way ANOVA and Tukey's post-test, while data related to scores obtained from the liver, kidneys histological evaluation were conducted by Kruskal-Wallis and Dunn's post-test. Outliers were not considered so as data remained inside normality. Sample size was estimated based on the formula $n = 1+[2C^*(s/d)^2]$, $C=(z\alpha+z\beta)^2$, considering confidence interval of 0,95/2 (0.475), $z = 1.96$, and test power of 90% ($z\beta = 1.282$), maximum deviation of 0.2 (20%) and difference between groups of 0.5 (50%). Significances were considered if $\alpha < 0.05$ for all analyses (Zar, 1999; Eng, 2003).

3. Results

Sample size was estimated for all the analysis and results developed as follows: $n = 1+[2(1.96 + 1.282)^{2*}[(0.2/0; 5)2] = 4.36$, so, $n = 5$ animals per group is the minimum required for a better statistical resolution. In the present work, a minimum of six animals per group was considered for all analyses.

3.1. Behavioral studies of males from the F1 generation

A decreased locomotion frequency of SCG ($p = 0.0331$) and SEG ($p = 0.0393$) groups in relation to CG group was observed, and 33.62% of the variance is related to the stress factor ($F_{(1,21)} = 11.25$; $p = 0.0030$; Fig. 2A). The rearing frequency was reduced in the SEG group in relation to EG ($p = 0.277$) and to CG ($p = 0.0432$) groups and stress factor accounted for 34.69% of the variance ($F_{(1,22)} = 12.90$; $p = 0,0016$; Fig. 2B). Grooming was increased in both SCG and SEG groups when compared to the CG ($p = 0.0473$ and $p = 0.0187$, respectively) and EG ($p = 0.0377$ and $p = 0.01287$, respectively) groups and the stress factor accounted for 47.47% of the variance ($F_{(1,21)} = 19,29$; $p = 0,0003$; Fig. 2C).

In the light-dark box apparatus, CG group spent more time in the light dark of the apparatus than SCG group ($p = 0.0393$), the stress factor accounted for 24.63% of the variance ($F_{(1,20)} = 7.683$; $p = 0,0118$; Fig. 3A). Group SCG spent more time in the dark side than CG group ($p = 0.0344$), as well as group EG, in comparison to CG (0.0283) and the interaction between the stress and the treatment factors accounted for 26.83% of the variance ($F_{(1,21)} = 9,021$; $p = 0,0068$; Fig. 3B). SEG group showed higher latency to the light side when compared to EG ($p = 0.0287$) and CG ($p = 0.0008$) groups, and the stress factor accounts for 38.61% of the total variance ($F_{(1,21)} = 16.83$; $p = 0,0005$; Fig. 3C) while the treatment factor accounted for 15.51% of the variance ($F_{(1,21)} = 6.761$; $p = 0,0167$). The group SEG also showed more attempts to enter the light side in relation to CG group ($p = 0.0031$), and both treatment (14.33%, $F_{(1,20)} = 5.362$; $p = 0,0313$) and the stress factors accounted for the variance (32.51%; $F_{(1,20)} = 12.16$; $p = 0,0023$; Fig. 3D). The locomotion frequency in the light side was diminished in the SCG and SEG groups in relation to the CG ($p = 0.0015$ and $p = 0.0071$, respectively) and EG ($p = 0.0084$ and $p = 0.0329$, respectively) groups, and the stress factor accounted for 55.45% of the variance ($F_{(1,20)} = 26,76$; $p < 0,0001$; Fig. 3E). EG were the group showing more locomotion frequency in the dark side in relation to SCG group ($p = 0.0060$) and to the SEG group (0.0344), and both the stress and treatment factors accounted to the variance in 25.62% ($F_{(1,20)} = 9.273$; $p = 0,0064$; Fig. 3F) and in 14.03% ($F_{(1,20)} = 5.077$; $p = 0,0356$), respectively. CG group showed an augmented rearing frequency in relation to SCG group ($p = 0.0264$) and to SEG group ($p = 0.0130$), and the stress factor accounted for 25.7% of the total variance ($F_{(1,20)} = 8,996$; $p = 0,0071$; Fig. 3G) in the light side of the apparatus, while rearing frequency was established back into the normality in the

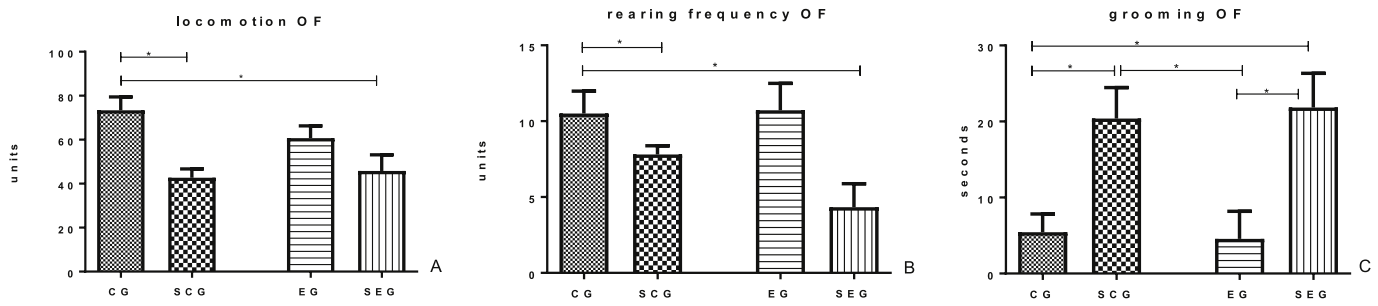


Fig. 2. Results obtained from open field apparatus analysis of the prenatal exposure to *Luffa operculata* of male Wistar rats at PND60. **A.** Locomotion frequency; **B.** Rearing frequency; **C.** Grooming. Two-way ANOVA and Tukey's post-test were used after analysis by Shapiro-Wilk normality test, Levene's Test for homogeneity and Mauchly Test for sphericity; significance at $\alpha < 0.05$.

dark side ($p > 0,05$; Fig. 3H). Finally, none of the groups showed alterations in the grooming in both the light and dark sides (Fig. 3I and J; $p > 0.05$). The lack of statistical differences in the grooming realized in the dark side of the apparatus were not observed as was in the OF apparatus, although it seems that a difference could have been observed. In order to verify the robustness of data, the eta squared (η^2) was calculated to establish the size of the effect reported to the stress, BNE and their interaction. Results were $\eta^2 = 0.86$, $\eta^2 = 0.07$ and $\eta^2 = 0.08$, respectively, and indicate that the differences can be more likely related to the stress factor other than to the BNE or even the interaction between them, although no statistical differences were effectively observed.

3.2. Neurochemistry studies of males from the F1 generation

In the hypothalamus, a decrease in VMA levels in EG group in relation to SCG group ($p = 0.0364$) and to the EG group ($p = 0.0135$) was observed, and both the stress factor (17.46%, $F_{(1,15)} = 5.139$; $p = 0,0386$; Fig. 4A) and the interaction between stress and treatment factors (28.87%, $F_{(1,15)} = 8.501$; $p = 0,0107$) accounted for the variance. VMA levels in the striatum and in the frontal cortex did not show statistical differences ($p > 0.05$). The NE hypothalamus level was significantly diminished in SEG group compared to EG group ($p = 0.0365$) and SCG group ($p = 0.0158$), and BNE factor accounted for 15.33% of the total variance ($F_{(1,17)} = 4.731$; $p = 0,0440$; Fig. 4B) while the interaction between factors stress and treatment factors accounted for 24.64% of the total variance ($F_{(1,17)} = 7.607$; $p = 0,0134$). NE levels in the striatum and in the frontal cortex did not show statistical differences ($p > 0.05$).

The DA level in the hypothalamus was reduced in SEG group in relation to CG group (0.0280) and EG group ($p = 0.0095$) and both the stress (38.58%; $F_{(1,15)} = 11.65$; $p = 0,0038$) and treatment (15.47%; $F_{(1,15)} = 4.673$; $p = 0,0472$; Fig. 4C) factors responded to the variance. There were no statistical differences in the DA level in the striatum, however, the eta squared (η^2) was calculated to establish the size of the effect reported to the stress, BNE and their interaction. Results were $\eta^2 = 0.12$, $\eta^2 = 0.85$ and $\eta^2 = 0.03$, respectively, and indicate that the differences can be more likely strongly related to the BNE factor and weakly related to the stress, but not related to the interaction between them. DA levels in the frontal cortex did not show statistical differences ($p > 0.05$). DOPAC level in the striatum was diminished in SEG group in relation to the CG group ($p = 0.0009$), to the SCG group ($p = 0.0013$) and to the EG group (0.0299), and both the treatment (48.42%; $F_{(1,16)} = 22.67$; $p = 0,0002$) and the interaction between stress and treatment (9.926%; $F_{(1,16)} = 4.649$; $p = 0,0466$; Fig. 4D) factors interfered with the variance DOPAC levels in the hypothalamus and in the frontal cortex did not show statistical differences ($p > 0.05$).

The level of 5-HT in the hypothalamus in the SEG group was impaired in relation to the CG group ($p = 0.0418$) and to the EG group ($p = 0.0445$), and the treatment accounted for 17.68% of the variance ($F_{(1,15)} = 4.762$; $p = 0,0454$; Fig. 4E). In the frontal cortex, 5-HT was diminished in SEG in relation to SG ($p = 0.0479$) and the treatment accounted for 26.38% of the total variance ($F_{(1,15)} = 6.391$; $p = 0.0232$; Fig. 4F). 5HT levels in the striatum did not show statistical differences ($p > 0.05$). 5HIAA level in the hypothalamus were high in the EG group in relation to the CG group (0.0230) and to the SCG group (0.0488), and the treatment accounted for 39.72% of the total variance ($F_{(1,14)} = 11.32$; $p = 0.0046$; Fig. 4G). 5HIAA level in the striatum was higher both in the EG and the SEG groups in relation to the CG group ($p = 0.0070$ and 0.0273, respectively) and to the SCG group ($p = 0.0002$ and $p = 0.0011$, respectively), and the treatment accounted for 64.8% of the total variance ($F_{(1,14)} = 37.24$; $p < 0.0001$; Fig. 4H). Finally, the 5HIAA level in the frontal cortex was higher in the SEG group in relation to the CG group ($p = 0.0396$) and to the SCG group ($p = 0.0388$), and the treatment accounted for 35.99% of the total variance ($F_{(1,14)} = 10.52$; $p = 0.0048$; Fig. 4I).

3.3. Histological studies of the brain, liver and kidneys of males from the F1 generation

The SCG group presented a decreased GFAP expression in the hypothalamus in relation to the CG group (0.0297) and stress accounted for 4.70% of the total variance ($F_{(1,87)} = 4,463$; $p = 0.0375$), while there were no significant differences ($p > 0.05$) between groups in the frontal cortex and striatum, as shown in Fig. 5.

No differences among medians were observed for any of the liver parameters ($p > 0,05$; Fig. 6A), and no alterations in the three parameters analyzed in the kidneys ($p > 0,05$; Fig. 6B).

3.4. Phytochemical findings

The analysis of the aqueous and the ethanolic solutions of BNE by TLC resulted in the presence of red spots at Rfs 0.18, 0.27, 0.34, 0.42 and 0.62 and a yellow-green spot in the application area for the aqueous solution of BNE, and in the red spots at Rfs 0.27, 0.62 and 0.75 for the ethanolic solution (Fig. 7). According to the referenced literature (Wagner and Bladt, 1996), the presence of 23-cucurbitacin derivatives is visualized as red spots in the plate, while yellow-green spots indicate the presence of 23,24-dihydrocucurbotacin derivatives.

4. Discussion

In a previous work, it was reported that aqueous extracts obtained from *L. operculata* fruits affected the behavior of male Wistar rats after oral administration of 1.0 mg/kg for five consecutive days (Alves et al., 2018). In the present study, the subabortive dose of 1.0 mg/kg was administered to pregnant Wistar rats between gestation days GD17 and GD21 in an effort to understand how the male offspring is affected by the vertical exposition to the abortive plant. In order to prospect for changes in male rats vertically exposed to BNE, parameters observed in the OF and in the LDB, complemented by the analysis of sera neurochemicals quantification, macroscopic and microscopic alterations in the liver,

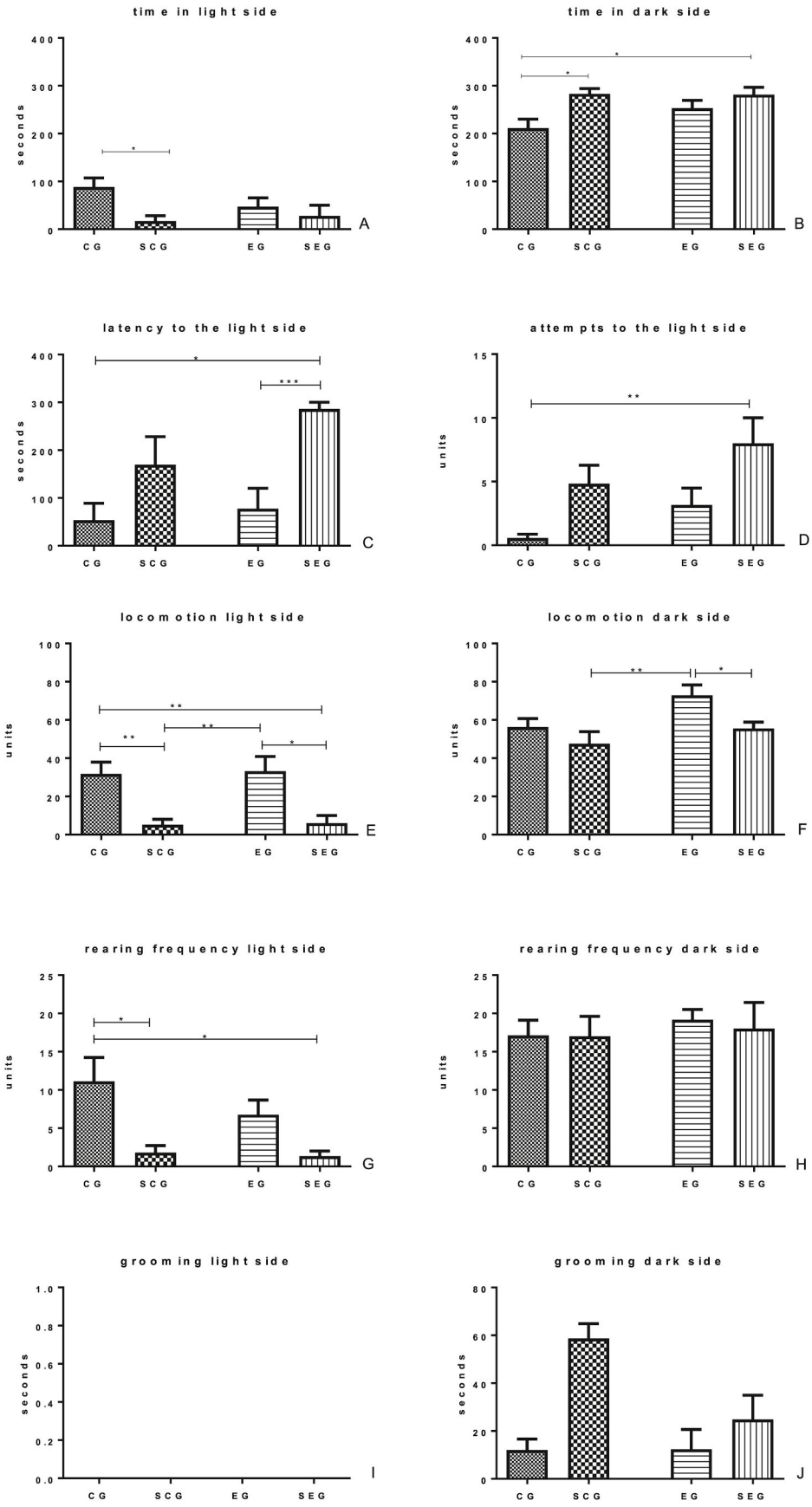


Fig. 3. Effects of prenatal exposure to *Luffa operculata* in male rats at PND60 in the light-dark box. **A**. Time in the light side; **B**. Time in the dark side; **C**. Latency to the light side; **D**. Attempts to the light side; **E**. Locomotion frequency in the light side; **F**. Locomotion frequency in the dark side; **G**. Rearing frequency in the light side; **H**. Rearing frequency in the dark side; **I**. Grooming in the light side; **J**. Grooming in the dark side. Two-way ANOVA and Tukey's post-test were used after Shapiro-Wilk normality test, Levene's test for homogeneity and Mauchly test for sphericity; significance at $\alpha < 0.05$.

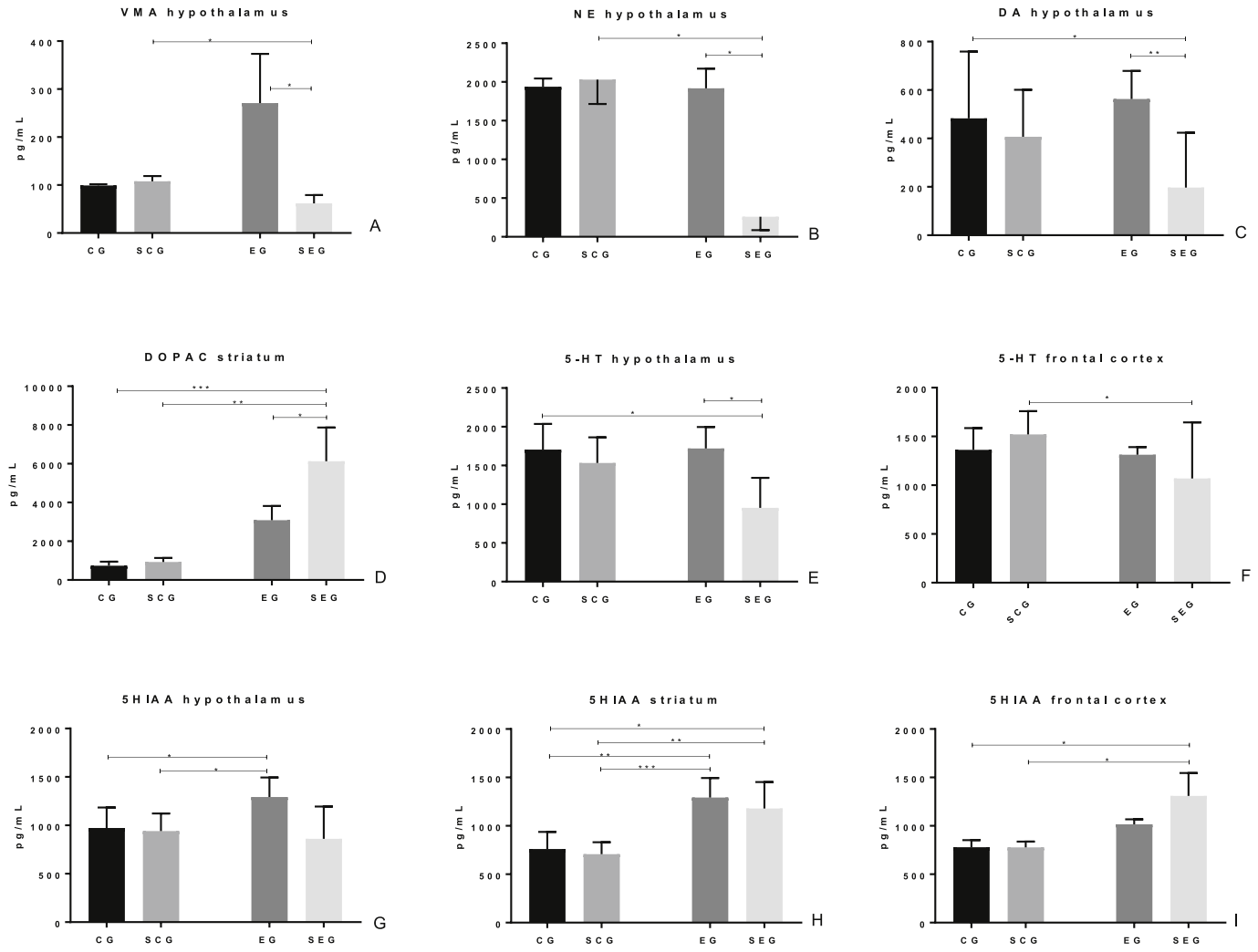


Fig. 4. Neurochemical analyses of hypothalamus, frontal cortex and striatum from juvenile male rats from F1 generation prenatally treated with *Luffa operculata*. Vanillylmandelic acid (VMA), norepinephrine (NE), dopamine (DA), DOPAC (3,4-dihydroxyphenylacetic acid), serotonin (5HT) and 5-HIAA (5-hidroxyindolacetic acid) (5-HIAA **A**. VMA in hypothalamus; **B**. NE in hypothalamus; **C**. DA in hypothalamus; **D**. DOPAC in striatum; **E**. 5-HT in hypothalamus; **F.** 5-HT in frontal cortex; **G**. 5HIAA in hypothalamus; **H**. 5HIAA in striatum; and **I**. 5HIAA in frontal cortex. Two-way ANOVA and Tukey's post-test were used after Shapiro-Wilk normality test was applied and normality was found for all parameters; significance if $\alpha < 0.05$.

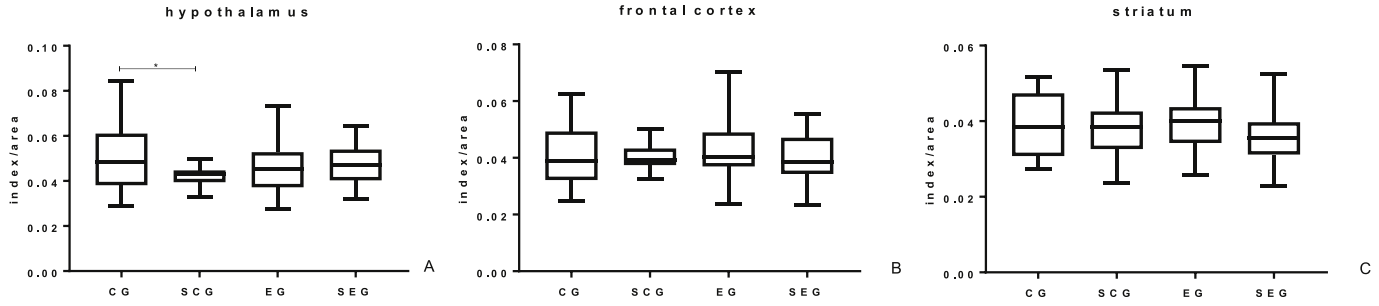


Fig. 5. GFAP expression in male juvenile rats of the F1 generation prenatally exposed to *Luffa operculata* in **A**. Hypothalamus; **B**. Frontal cortex; **C**. Striatum. Two-way ANOVA and Tukey's post-test were used after Shapiro-Wilk normality test was applied and normality was found for all parameters, significant was considered different if $\alpha < 0.05$.

kidneys and in the brain were made.

4.1. Behavior analyses

The exploratory behaviors as locomotion and rearing were evaluated in the OF and LDB apparatuses. In the OF, locomotion frequency and rearing frequency are generally used as measures of locomotor system activity but are also measures of exploratory behavior and anxiety. The high frequency of such behaviors indicates greater locomotion and exploration and is associated with a lower level of anxiety (Gusmão et al., 2013b; Heiming and Sachser, 2010). One of the most frequently used behavioral tests in rodents is the open field test, a conflict test based

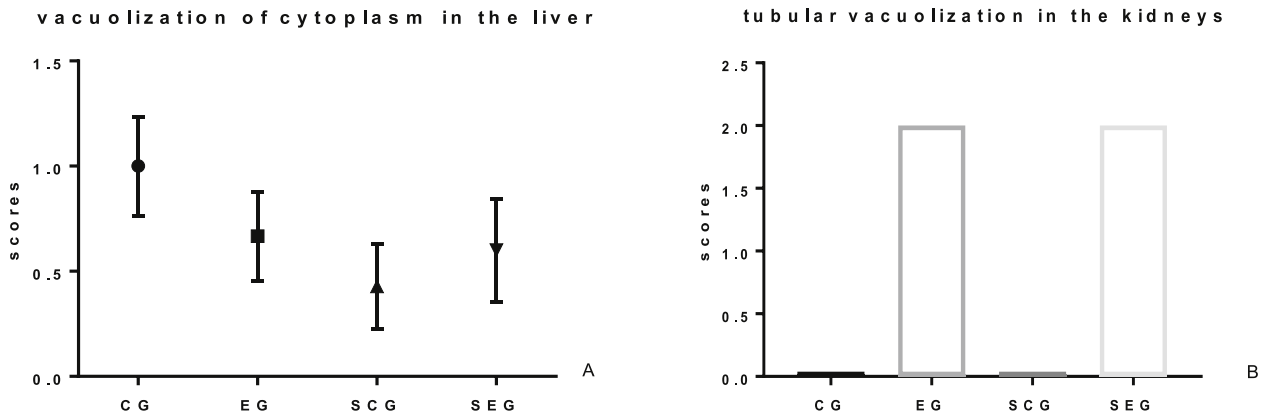


Fig. 6. Histopathological analyses of juvenile male rats F1 livers and kidneys after receiving a vertical administration of *Luffa operculata*. **A**. Vacuolization in liver cytoplasm; **B**. Tubular vacuolization in the kidneys. Kruskal-Wallis and Tukey's post-test were used considering significant if difference between means were $\alpha < 0.05$.

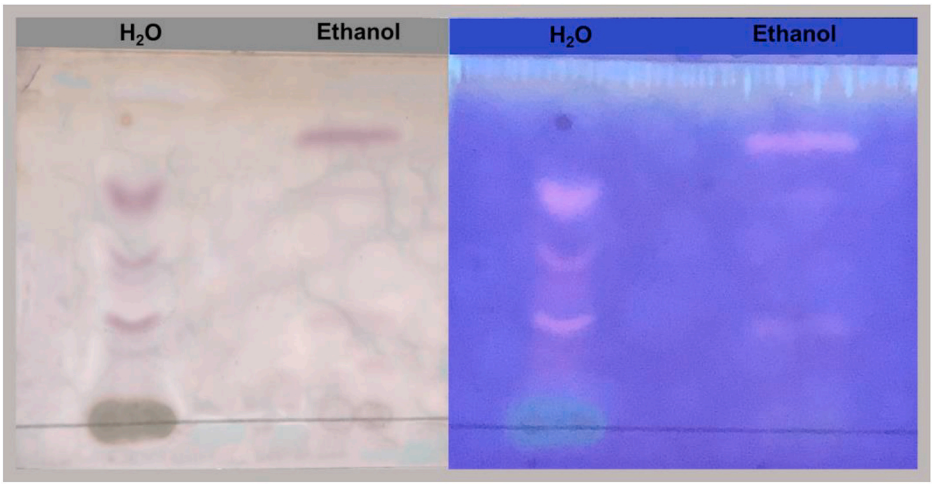


Fig. 7. Thin layer chromatography analysis of the *Luffa operculata* aqueous extract diluted in water and in ethanol. Red spots indicate cucurbitacin derivatives.

on the opposed drives to explore new environments and fearful avoidance of open areas (Campos et al., 2013; Prut and Belzung, 2003).

In the OF, the locomotion and rearing parameters were decreased in both stress groups, and the variance was explained only by the stress factor, indicating that BNE administration has not directly interfered with motor/exploratory behavior in the open field. Although the same observations for locomotion and rearing were again obtained in LDB, as the stress factor prevailed as the main cause of differences among groups, a more accurate analysis could be done in LDB, due to a high sensibility of the equipment in behavior evaluation. Despite the effective influence of the stress induction over rat behavior, the BNE administration has influenced the latency to the light side, the locomotion in the dark side and the time spent in the dark side, suggesting fear.

The grooming behavior is observed in several species at many levels of development (Bolles, 1960; Sachs, 1988; Spruijt et al., 1992; Valentine and Glorioso, 1978), and it is primarily involved with hygiene and caring for the body surface. In rodents, typical grooming behavior shows a general pattern of cephalocaudal progression (paw licking – nose/face wash – body wash – tail/genitals wash) (Kalueff et al., 2015). However, grooming behavior can serve other functions, such as stimulation of the skin, thermoregulation, chemo-communication, social interaction, and arousal, and it can be associated with stress (Kalueff et al., 2010; Sachs, 1988).

Recently, the evaluation of grooming in rodents has become an important tool to assess the neural circuits involved in this particular expression of innate behavior, which has been evolutionarily conserved as it is related to hygiene, social communication, excitation, but also can be related to pathologies that include repetitive behaviors; it also can be considered as an indirect index of behavioral changes when associated with other behavioral parameters (Kalueff et al., 2016). Usually, rodents show increased grooming during both low and high levels of stress. Low levels of stress can increase spontaneous grooming, and it occurs as a transition between other activities (Kalueff and Tuohimaa, 2005); while elevated levels of stress can increase grooming activity as a response to novel environments (Jolles et al., 1979; Spruijt et al., 1992). Nonetheless, in the present study, there was increased grooming behavior associated with low levels of locomotion and rearing frequencies in both groups submitted to the stress treatment, but not to the BNE treatment. In the LDB, no alterations in grooming were observed in the light side or the dark side of the apparatus. Nonetheless, the results obtained from the eta squared analyses indicated that the grooming alterations that was seen in the OF apparatus may have an important meaning in the BNE administration, even if the significance was nor observed in the LDB apparatus. Further analyses reporting the influence of BNE on the grooming must be made.

In the LDB apparatus it became clear that the vertical exposition to BNE have somehow interfered in the male rats' behavior related to exploration and fear, as the parameters reported as the attempts to the light side and as the latency to the light side showed that the SEG group took more time and attempted more to enter the light side of the

apparatus than the other groups. Despite the influence of the stress challenge ended up with interfering with the responses of many of the parameters in the LDB and in the OF, the BNE has somehow been involved in some of the parameters alterations as well, in a subtle way. The involvement of BNE in the behavior of male rats vertically exposed to BNE was more profoundly analyzed by the quantification of the neurochemicals in the hypothalamus, striatum and frontal cortex, which are brain zones involved with behavior and with the HHAA.

4.2. Neurochemical findings

In the present work, the statistic alterations involving the VMA, NE, DA, DOPAC, 5-HT and 5HIAA were mainly influenced by the interaction of both BNE and stress factors and by the BNE itself. It means that not only the BNE compounds can cross the placental barrier and expose the fetus, but also that BNE compounds can cross the blood-brain barrier and interfere with neurochemical balance in the hypothalamus, striatum and frontal cortex of the offspring, influencing the behavior.

4.3. The correlation of behavioral phenotype and neurochemicals

Although the stress induction has been proposed as an evaluation tool to verify the sensitization of the juvenile rats to the vertical exposition to BNE, it has strongly influenced the alterations that were observed in most of the parameters that were analyzed in OF and in LDB. However, data related to the locomotion and time spending in the dark side of the apparatus had been influenced by the vertical exposition to BNE and to the stress. Those findings are in accordance with the lack of differences on striatal DA and its metabolite, DOPAC in the central nervous area related to the control of the motor/exploratory behavior (Graybiel et al., 1994; Onn et al., 2000). Stressful experiences have a great impact in brain function and can lead to both short and long-term behavioral alterations (Lupien et al., 2009; Szafarczyk et al., 1993). The observation of the stress-related anxiety in rodents typically relies on species-specific behaviors such as increased risk assessment, the reduction of exploration, seeking shelter, escape, burying or defecation (Bailey and Crawley, 2009). In our study, we observed impairment in locomotion, in rearing frequencies and somehow in the grooming, which was mainly influenced by the stress but somehow by the BNE, as explained before.

In this concern, the stress system coordinates the adaptive responses of the organism to stressors of any kind. The main components of the stress system are the corticotropin-releasing hormone (CRH) and *locus ceruleus*-norepinephrine (LC/NE)-autonomic systems and their peripheral effectors, the HHAA, and the limbs of the autonomic system (Tsigos and Chrousos, 2002). Stress releases the CRH hormone, which regulates the expression of behavioral, endocrine and autonomic responses to stress through activation of forebrain noradrenergic systems (Chrousos and Gold, 1992).

There was significant change in 5-HT in the hypothalamus, frontal cortex and striatum, as there was increase of 5-HIAA in the hypothalamus, striatum and frontal cortex. The 5-HIAA quantification is an indirect measure of 5-HT turnover, which may have caused DA release in the striatal region, leading to increased grooming behavior in juvenile rats via 5-HT receptors, probably stimulated in the hypothalamus. Since activation of the central dopaminergic system can generate stereotypy, such as grooming (Roffler et al., 1971), there may be a trigger of 5-HT dependent release of DA, leading to stereotypic behavior. Although not well characterized in the present study, it is a clear result that reports the influence of the vertical exposition to BNE over the F1 male rats.

4.4. Toxicity to liver, kidneys and brain

Although no significant differences were observed in the hepatic parameters analyzed in the present study, the etiology of death of animals exposed to cucurbitacin E and cucurbitacin E glycoside is described as follows: animals die from congestion of the pancreas, intestine, kidneys and liver. The lungs become swollen and have a large amount of fluid and it is possible to find large amounts of fluid in the thorax and abdomen (Montesano et al., 2018), also, the authors report that the LD50 to rats are 340 and 40 mg/kg, respectively.

As for the brain, it is known that, in pathological conditions, astrocytes rapidly develop morphological and functional changes that affect neuronal activity (Sofroniew and Vinters, 2010). In response to central nervous system insults, these glial cells present a hypertrophic or reactive status called astrogliosis (Sofroniew, 2009, 2015; Sofroniew and Vinters, 2010), in which increased expression of the specific astrocyte protein GFAP occurs (Middeldorp and Hol, 2011; Brenner, 2014; Yang and Wang, 2015).

Nevertheless, several reports demonstrate that exposure to various stressors induce an approximate 20% decrease in GFAP content (Banasr and Duman, 2008; Simard et al., 2018). Thus, as found in our study, the decreased hypothalamic expression of GFAP in the stressed rats from the SCG group is in accordance to these reports, although interestingly no differences were noted between stressed and non-stressed rats from the experimental groups (SEG and EG).

4.5. Phytochemical findings

The absence of alkaloids, cardiac glycosides and anthraquinones were observed in the present study, as the presence of triterpenes and steroids and phenolic compounds, such as flavonoids and tannins, were confirmed in the *buchinha-do-norte* extract. Although there is reports connecting the presence of alkaloids in Cucurbitaceae species as momordicin alkaloids (Omokhua-Uyi and Van Staden, 2020), or in the species named *Citrullus colocynthis* (L.) Schrad. (Hussain et al., 2014), there is no report concerning the occurrence of alkaloids in *L. operculata*. The occurrence of cardiac glycosides has been reported for Cucurbitaceae species, as reported in a revision made with *Momordica balsamina* (Thakur et al., 2009), but retrieving the original paper by Bot and collaborators (2007), the authors report to have detected steroidal ring other than cardiac glycosides, which is in agreement with our findings. The occurrence of tannins was considered positive in the present work, and is supported by the occurrence in cantaloupe melon (Vella et al., 2019). In this case, the analyses made by Vella and colleagues are more precise, due to its comparison to gallic acid and ellagic acid contents, despite having tested for condensed tannins. Anthraquinones are reported to occur in *Luffa* species, as in *L. acutangula* (Vanajothi and Srinivasan, 2016) but in our report, we did not observe the presence of this class of compounds. The presence of flavonoids was confirmed by positive Shinoda's reaction which is related to the chromophore nucleus composed by the C6–C3–C6 flavonoid resonant nucleus. The presence of the steroid nucleus reported for cucurbitanes was accessed by the analysis in TLC, using vanillin-phosphoric acid reagent to visualize the red and/or yellow-green spots corresponding to cucurbitacin derivatives.

5. Conclusions

The juvenile rats that were vertically exposed to a sub-abortive dose of the aqueous extract from the dried fruits of *Luffa operculata* led to fine but significant changes in behavior indicating the expression of fear when facing a stress challenge. Also, it became clear that the exposition to BNE interfered with the hypothalamic-hypophysis-adrenal axis, which was implied in the behavioral changes that were observed. No relevant systemic toxic lesions were seen in liver or in the kidneys.

Acknowledgements

The authors want to thank FAPESP – Fundação de Amparo à Pesquisa do Estado de São Paulo for grants #2017/03470–9 and 2019/12202–3, and to UNIP Scholarships for HVF and CSA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.113265.

References

Albert-Puleo, M., 1979. The obstetrical use in ancient and early modern times of convolvulus scammonia or scammony: another non-fungal source of ergot alkaloids? J. Ethnopharmacol. 1, 193–195. https://doi.org/10.1016/0378-8741(79)90006-0.

Alves, C.S., Frias, H.V., Kirsten, T.B., et al., 2018. *Luffa operculata* fruit aqueous extract induces motor impairments, anxiety-like behavior, and testis damage in rats. J. Ethnopharmacol. 222, 52–60. https://doi.org/10.1016/j.jep.2018.04.044.

Ariza Traslaviña, G.A., de Oliveira, F.L., Franci, C.R., 2014. Early adolescent stress alters behavior and the HPA axis response in male and female adult rats: the relevance of the nature and duration of the stressor. Physiol. Behav. 133, 178-189. https://doi.org/10.1016/j.physbeh.2014.05.031.

Banasr, M., Duman, R.S., 2008. Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biol. Psychiatr. 64, 863–870.

Barilli, S.L.S., Santos, S.T., Montanari, T., 2007. An experimental investigation of *Luffa operculata* (L.) Cogn. (buchinha-do-norte) as abortifacient plant. Reprod. Clim. 22, 165–168.

Bailey, K.R., Crawley, J.N., 2009. Anxiety-related behaviors in mice. In: Buccafusco, J.J. (Ed.), Methods of Behavior Analysis in Neuroscience, second ed. CRC Press/Taylor & Francis, Boca Raton.

Bolles, R.C., 1960. Grooming behavior in the rat. J. Comp. Physiol. Psychol. 53, 306–310.

Bot, Y.S., Mgbojikwe, L.O., Nwosu, C., Ambiku, A., Damshak, D., 2007. Screening of the fruit pulp extract of *Momordica balsamina* for anti HIV property. Afr. J. Biotechnol. 6, 47–52.

Brenner, M., 2014. Role of GFAP in CNS injuries. Neurosci. Lett. 565, 7–13. https://doi.org/10.1016/j.neulet.2014.01.055.

Broadhurst, P.L., 1960. Experiments in psychogenetics: application of biometrical genetics to the inheritance of behavior. In: Eysenck, H.J. (Ed.), Experiments in Personality, vol. 1. Routledge & Kegan Paul, London, pp. 1–256.

Campos, A.C., Fogaça, M.V., Aguiar, D.C., Guimarães, F.S., 2013. Animal models of anxiety disorders and stress. Br. J. Psychiatry 35 (Suppl. 2), S101–S111. https://doi.org/10.1590/1516-4446-2013-1139.

Chrousos, G.P., Gold, P.W., 1992. The concepts of stress and stress system disorders. JAMA, J. Am. Med. Assoc. 267, 1244–1252. https://doi.org/10.1001/jama.1992.03480090092034.

Crawley, J., Goodwin, F.K., 1980. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol. Biochem. Behav. 13, 167–170. https://doi.org/10.1016/0091-3057(80)90067-2.

Dhabhar, F.S., McEwen, B.S., 1997. Acute stress enhances while chronic stress suppresses cell-mediated immunity *in vivo*: a potential role for leukocyte trafficking. Brain Behav. Immun. https://doi.org/10.1006/brbi.1997.0508.

Eng, J., 2003. Sample size estimation: how many individuals should be studied? Radiology 227, 309–313. https://doi.org/10.1148/radiol.2272012051.

Estork, D.M., Gusmão, D.F., Paciencia, M.L.B., et al., 2014. First chemical evaluation and toxicity of casinga-cheirosa to Balb-c male mice. Molecules 19, 3973–3987. https://doi.org/10.3390/molecules19043973.

Estork, D.M., Gusmão, D.F., Paciencia, M.L.B., et al., 2016. Casinga-cheirosa organic extract impairment over balb-c male mice behavioral phenotype. Braz. J. Pharmacogn. 26, 216–224. https://doi.org/10.1016/j.bjp.2015.10.004.

Feitosa, C.R.S., Silva, R.C., Braz-filho, R., et al., 2011. Characterization of chemical constituents of Luffa operculata (Cucurbitaceae). Am. J. Anal. Chem. 2, 989–995.

Felicio, L.F., Florio, J.C., Sider, L.H., et al., 1996. Reproductive experience increases striatal and hypothalamic dopamine levels in .prezgnant rats. Brain Res. Bull. 40, 253–256. https://doi.org/10.1016/0361-9230(96)00008-1.

Gore, A.C., Martien, K.M., Gagnidze, K., Pfaff, D., 2014. Implications of prenatal steroid perturbations for neurodevelopment, behavior, and autism. Endocr. Rev. 35, 961–991. https://doi.org/10.1210/er.2013-1122.

Graybiel, A.M., Aosaki, T., Flaherty, A.W., et al., 1994. The basal ganglia and adaptive motor control. Science 23 (265), 1826–1831. https://doi.org/10.1126/science.8091209.

Gusmão, D.F., Estork, D.M., Paciencia, M.L.B., et al., 2013a. Preliminary evaluation of the acute toxicity related to *Abarema auriculata* to mice and investigation of cytotoxicity of isolated flavonones. Pharmacologyonline 1, 113–127.

Gusmão, D.F., Estork, D.M., Paciencia, M.L.B., et al., 2013b. Influence of the intraperitoneal administration of antitumor *Abarema auriculata* extract on mice behavior. Braz. J. Pharmacogn. 23, 903–912. https://doi.org/10.1590/S0102-695X2013000600007.

Heiming, R.S., Sachser, N., 2010. Consequences of serotonin transporter genotype and early adversity on behavioral profile - pathology or adaptation? Front. Neurosci. 4, 187. https://doi.org/10.3389/fnins.2010.00187.

Holder, M.K., Blaustein, J.D., 2014. Puberty and adolescence as a time of vulnerability to stressors that alter neurobehavioral processes. Front. Neuroendocrinol. 35, 89–110.

Hussain, A.I., Rathore, H.A., Sattar, M.Z., et al., 2014. *Citrullus colocynthis* (L.) Schrad (bitter apple fruit): a review of its phytochemistry, pharmacology, traditional uses and nutritional potential. J. Ethnopharmacol. 155, 54–66. https://doi.org/10.1016/j.jep.2014.06.011.

Jolles, J., Rompa-Barendregt, J., Gispen, W.H., 1979. ACTH-induced excessive grooming in the rat: the influence of environmental and motivational factors. Horm. Behav. 12, 60–72. https://doi.org/10.1016/0018-506X(79)90027-8.

Kalueff, A.V., Stewart, A.M., Song, C., Berridge, K.C., Graybiel, A.M., Fentress, J.C., 2016. Neurobiology of rodent self-grooming and its value for translational neuroscience [published correction appears in Nat. Rev. Neurosci. 2016 Feb;17(2):118]. Nat. Rev. Neurosci. 17, 45–59. https://doi.org/10.1038/nrn.2015.8.

Kalueff, A.V., Stewart, A.M., Song, K.C., et al., 2015. Neurobiology of rodent self-grooming and its value for translational neuroscience. Nat. Rev. Neurosci. 17, 45–59. https://doi.org/10.1038/nrn.2015.8.

Kalueff, A.V., Laporte, J.L., Bergner, C.L., 2010. Neurobiology of Grooming Behavior. [s. l.]. Cambridge University Press, Cambridge.

Kalueff, A.V., Tuohimaa, P., 2005. Mouse grooming microstructure is a reliable anxiety marker bidirectionally sensitive to GABAergic drugs. Eur. J. Pharmacol. 508, 147–153. https://doi.org/10.1016/j.ejphar.2004.11.054.

Kawahara, N., Kurata, A., Hakamatsuka, T., et al., 2001. Two novel cucurbitacins, neocucurbitacins A and B, from the Brazilian folk medicine “Buchinha” (*Luffa operculata*) and their effect on PEBP2alphaA and OCIF gene expression in a human osteoblast-like Saos-2 cell line. Chem. Pharm. Bull. 49, 1377–1379. https://doi.org/10.1248/cpb.49.1377.

Kawahara, N., Kurata, A., Hakamatsuka, T., et al., 2004. Two new cucurbitacin glucosides, opercurins A and B, from the Brazilian folk medicine "Buchinha" (*Luffa operculata*). Chem. Pharm. Bull. 52, 1018–1020.

Keller, M., Vandenberg, L.N., Charlier, T.D., 2019. The parental brain and behavior: a target for endocrine disruption. Front. Neuroendocrinol. 54, 100765. https://doi.org/10.1016/j.yfrne.2019.100765.

Kumar, D., Kumar, A., Prakash, O., 2012. Potential antifertility agents from plants: a comprehensive review. J. Ethnopharmacol. 140, 1–32. https://doi.org/10.1016/j.jep.2011.12.039.

Lang, K.L., Silva, I.T., Zimmermann, L.A., et al., 2012. Synthesis and cytotoxic activity evaluation of dihydrocucurbitacin B and cucurbitacin B derivatives. Bioorg. Med. Chem. 20, 3016–3030.

Lupien, S.J., McEwen, B.S., Gunnar, M.R., et al., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10, 434–445. https://doi.org/10.1038/nrn2639.

Matos, F.J.A., Gottlieb, O.R., 1967. Isocucurbitacina B, constituinte tóxico de *Luffa operculata*. An. Acad. Bras. Ciências 39, 245–247.

Menon-Miyake, M.A., Saldiva, P.H.N., Lorenzi-Filho, G., Ferreira, M.A., Butugan, O., Oliveira, R.C., 2005. Efeitos da *Luffa operculata* sobre o epitélio do palato de rã: aspectos histológicos. Rev. Bras. Otorrinolaringol. 71, 132–138. https://doi.org/10.1590/S0034-72992005000200003.

Middeldorp, J., Hol, E.M., 2011. GFAP in health and disease. Prog. Neurobiol. 93, 421–443. https://doi.org/10.1016/j.pneurobio.2011.01.005.

Miodovnik, A., Diplas, A.I., Chen, J., Zhu, C., Engel, S.M., Wolff, M.S., 2012. Polymorphisms in the maternal sex steroid pathway are associated with behavior problems in male offspring. Psychiatr. Genet. 22, 115–122. https://doi.org/10.1097/YPG.0b013e328351850b.

Montesano, D., Rocchetti, G., Putnik, P., et al., 2018. Bioactive profile of pumpkin: an overview on terpenoids and their health-promoting properties. Curr Opin Food Sci 22, 81–87.

Omokhua-Uyi, A.G., Van Staden, J., 2020. Phytomedicinal relevance of South African Cucurbitaceae species and their safety assessment: a review. J. Ethnopharmacol. 259, 112967. https://doi.org/10.1016/j.jep.2020.112967.

Onn, S.P., West, A.R., Grace, A.A., 2000. Dopamine-mediated regulation of striatal neuronal and network interactions. Trends Neurosci. 23, S48–S56. https://doi.org/10.1016/S1471-1931(00)00020-3.

Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. Pharmacol. 463, 3–33. https://doi.org/10.1016/S0014-2999(03)01272-X.

Revilla, J., 2002. Plantas úteis da bacia Amazônica 1. INPA/SEBRAE, Manaus, p. 391.

Roffler-Tarlov, S., Sharman, D.F., Tegerdine, P., 1971. 3,4-Dihydroxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid in the mouse striatum: a reflection of intra-and extra-neuronal metabolism of dopamine? Br. J. Pharmacol. 42, 343–351. https://doi.org/10.1111/j.1476-5381.1971.tb07118.x.

Rubinow, D.R., Schmidt, P.J., 1996. Androgens, brain, and behavior. Am. J. Psychiatr. 153, 974–984. https://doi.org/10.1176/ajp.153.8.974.

Sachs, B.D., 1988. The development of grooming and its expression in adult animals. Ann. N. Y. Acad. Sci. 525, 1–17. https://doi.org/10.1111/j.1749-6632.1988.tb38591.x.

Shimada, T., Matsumoto, K., Osanai, M., et al., 1995. The modified light/dark transition test in mice: evaluation of classic and putative anxiolytic and anxiogenic drugs. Gen. Pharmacol. 26, 205–210. https://doi.org/10.1016/0306-3623(94)00148-G.

Simard, S., Coppola, G., Rudyk, C.A., Hayley, S., McQuaid, R., Salmaso, N., 2018. Profiling changes in cortical astroglial cells following chronic stress. Neuropsychopharmacology 43, 1961–1971.

Sofroniew, M.V., 2009. Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci. 32, 638–647. https://doi.org/10.1016/j.tins.2009.08.002.

Sofroniew, M.V., Vinters, H.V., 2010. Astrocytes: biology and pathology. Acta Neuropathol. 119, 7–35. https://doi.org/10.1007/s00401-009-0619-8.

Sofroniew, M.V., 2015. Astrocyte barriers to neurotoxic inflammation. Nat. Rev. Neurosci. 16, 249–263. https://doi.org/10.1038/nrn3898.

Soto, A.M.S., Kirsten, T.B., Reis-Silva, T.M., et al., 2013. Single early prenatal lipopolysaccharide exposure impairs striatal monoamines and maternal care in female rats. Life Sci. 92, 852–858. https://doi.org/10.1016/j.lfs.2013.03.003.

Spiga, F., Walker, J.J., Terry, J.R., et al., 2014. HPA axis-rhythms. Comp. Physiol. 4, 1273–1298. https://doi.org/10.1002/cphy.c140003.

Spruijt, B.M., Van Hooff, J., Gispen, W.H., 1992. Ethology and neurobiology of grooming behavior. Physiol. Rev. 72, 825–852. https://doi.org/10.1152/physrev.1992.72.3.825.

Suffredini, I.B., Paciencia, M., Díaz, I.C., et al., 2017. Mice behavioral phenotype changes after administration of Anani (*Symphonia globulifera*, clusiaceae), an alternative Latin American and African medicine. Phcog. Mag. 13, 617–626. https://doi.org/10.4103/pm.pm_168_17.

Szafarczyk, A., Ixart, G., Gaillet, S., et al., 1993. Stress. Neurophysiologic studies. Encephale 1, 137–142.

Takao, K., Miyakawa, T., 2006. Light/dark transition test for mice. J. Vis. Exper. 1, 1–3. https://doi.org/10.3791/104.

Thakur, G.S., bag, M., Sanodiya, B., et al., 2009. Momordica balsamina: a medicinal and nutraceutical plant for health care management. Curr. Pharmaceut. Biotechnol. 10, 667–682. https://doi.org/10.2174/138920109789542066.

Tsigos, C., Chrousos, G.P., 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J. Psychosom. Res. 53, 865–871. https://doi.org/10.1016/S0022-3999(02)00429-4.

Udo, M.S.B., Sandini, T.M., Reis, T.M., et al., 2014. Prenatal exposure to a low fipronil dose disturbs maternal behavior and reflex development in rats. Neurotoxicol. Teratol. 45, 27–33. https://doi.org/10.1016/j.ntt.2014.05.010.

Valentine, B.D., Glorioso, M.J., 1978. Grooming behavior in diplura (insecta: apterygota). Psyche 85, 191–200. New York.

Vanajothi, R., Srinivasan, P., 2016. An anthraquinone derivative from *Luffa acutangula* induces apoptosis in human lung cancer cell line NCI-H460 through p53-dependent pathway. J. Recept. Signal Transduct. Res. 36, 292–302. https://doi.org/10.3109/10799893.2015.1108335.

Vella, F.M., Cautela, D., Laratta, B., 2019. Characterization of polyphenolic compounds in cantaloupe melon by-products. Foods 8, 196. https://doi.org/10.3390/foods8060196. Published 2019 Jun 6.

Wagner, H., Bladt, S., 1996. Plant Drug Analysis, second ed. Springer Berlin Heidelberg, Berlin, p. 384.

Yang, Z., Wang, K.W., 2015. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci. 38, 364–374. https://doi.org/10.1016/j.tins.2015.04.003.

Yazbek, P.B., Tezoto, J., Cassas, F., et al., 2016. Plants used during maternity, menstrual cycle and other women's health conditions among Brazilian cultures. J. Ethnopharmacol. 179, 310–331. https://doi.org/10.1016/j.jep.2015.12.054.

Zar, J.H., 1999. Biostatistical Analysis, fourth ed. Prentice-Hall Inc, New Jersey, p. 929.

Ivana Barbosa Suffredini. http://lattes.cnpq.br/8246665424444562.