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ORIGINAL ARTICLE

Effect of orange juice and tryptamine on the behavior and c-fos expression of Wistar rats

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Abstract

Recent reports have shown that commercial orange juice is rich in biogenic amines. Consumption of foods containing large amounts of biogenic amines increase hypertensive crisis and high levels of histamine and tyramine, which have been implicated as causative agents in a number of food poisoning episodes. In addition, accumulation of tryptamine in plasma may be associated with mood disorders. The aim of this study was to determine whether chronic administration of orange juice extract and tryptamine affects the behavior and c-fos expression in the rat. For this purpose, Wistar male rats were injected with saline solution, tryptamine or orange juice extract. Sucrose preference test and elevated plus maze were evaluated to determine hedonic and anxiety behavior, respectively. Rats treated with orange juice extract showed increased anxiety behavior and sucrose consumption, similar to those treated with tryptamine. In addition, dorsal raphe nucleus, accumbens nucleus, and hippocampus showed an increase of c-fos positive cells in rats treated with orange juice extract. In conclusion, the chronic and lengthy consumption of orange juice or their derivatives in the diet could be a factor responsible to induce mood disorders and may promote excess caloric consumption.

Keywords Orange juice · Tryptamine anxiety · Sucrose consumption

Introduction

Tryptamine is a biogenic amine (BA), and an organic base with low molecular weight that can be detected in raw and processed foods (Candete et al. 2007). Currently, nine different types of amines in fresh orange juice and five in orange soft drinks have been detected (Vieria et al. 2007). Low levels of BA in food are not considered a serious risk to human health, but when they are consumed in excessive amounts, they may cause distinctive pharmacological, physiological and toxic effects (Armagan 2007). In addition, the high concentration of BA in citric juices could be a health risk due to high consumption of fresh citrus juice in many countries of America (Vieria et al. 2007). The relationship between BA and human pathologies is known, they are involved in allergy and immune response (histamine), neurological disorders such as depression, schizophrenia or Alzheimer (serotonin, tyramine, tryptamine, noradrenaline), however many aspects related with these effects are not completely studied (Medina et al. 2003). Particularly, tryptamine (TRY) is a BA produced in the pineal gland by decarboxylation of tryptophan, and acts as neurotransmitter. Additionally, TRY is an alkaloid compound known for their psychotropic effects, which generates behavioral disorders. In vitro studies have reported that TRY is a potent enhancer of serotonin release (Shimazu and Miklya 2004).

Serotonin is a neurotransmitter related to mood control and whose deficiency is partly associated with the development of depression (Shimazu and Miklya 2004). Alterations in dopaminergic and serotonergic systems have been associated with social anxiety disorder (Stein and Stein 2008). In addition, the expression of the immediate-early gene (IEG) c-fos has been used as a

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marker for neural systems activation by a variety of stimuli (Frick et al. 2015), including activation of serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN), a brain area implicated in antidepressant/anxiolytic behavior (Krukoff et al. 1994).

Therefore, it is plausible to suggest that accumulation of BA in the orange juice could contribute to develop some behavioral disorders. In this sense, the objective of the present study was to determine the effect of chronic administration of orange juice extract on the behavior and c-fos expression in the Wistar rat.

Material and methods

Biological material

120 oranges (*Citrus sinensis* cv Valencia) from an orchard of El Chico, Veracruz, Mexico were used for the present study. They were divided in batches of 12 oranges. Juice extracted from every batch was centrifuged at 4500 rpm in refrigeration at 4 °C during 20 min and filtered with paper No. 4; the samples were stored at 4 °C until use.

Tryptamine determination by HPLC

Orange juice samples were centrifuged at 4500 rpm at 4 °C for 20 min and filtered through 0.45 μm HAWP membrane and tryptamine (TRY) concentration was determined by HPLC (Vieria et al. 2007).

High performance liquid chromatography (HPLC) analysis was performed with a HPLC apparatus (Varian, Mod. ProStar 210), using an Agilent C18 reverse phase column (4.6 × 250 mm, 0.45 μm, Agilent Techn, Wilmington, DE, USA). The UV detector was set at 225 nm (Varian model ProStar 210, Agilent Techn, Wilmington, DE, USA). Aliquots of TRY (20 μL) were gradient-eluted at 0.7 mL/min using 0.1 M acetate buffer containing 10 mM 1-octanesulfonic acid sodium salt, pH adjusted to 4.9 with acetic acid (A) and acetonitrile (B): 0–5 min (12% B), 5–15 min (13% B), 15–20 min (12% B). All assays were performed by triplicate as a pre-condition to equilibrate the chromatography system before sample injection. The results are expressed as mg of TRY/100 mL.

Animals, experimental groups and treatments

We used male Wistar rats (250–300 g body weight and 16th weeks age) obtained from our facility. Animals were housed in a temperature-controlled room (25 ± 1 °C) under a 12/12 h dark/light (07:00 lights on) cycle with ad-libitum access to food and water. All of the experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals in agreement with national guidelines (NOM-062ZOO-2003). A longitudinal study with five independent groups ($n = 8$ rats per group) was performed. Control group received an intraperitoneal (i.p.) injection of 1 mL/kg of vehicle (sterile water), while the rats of the orange juice extract groups received an i.p. injection of 0.125, 0.25 or 0.50 mg/kg of body

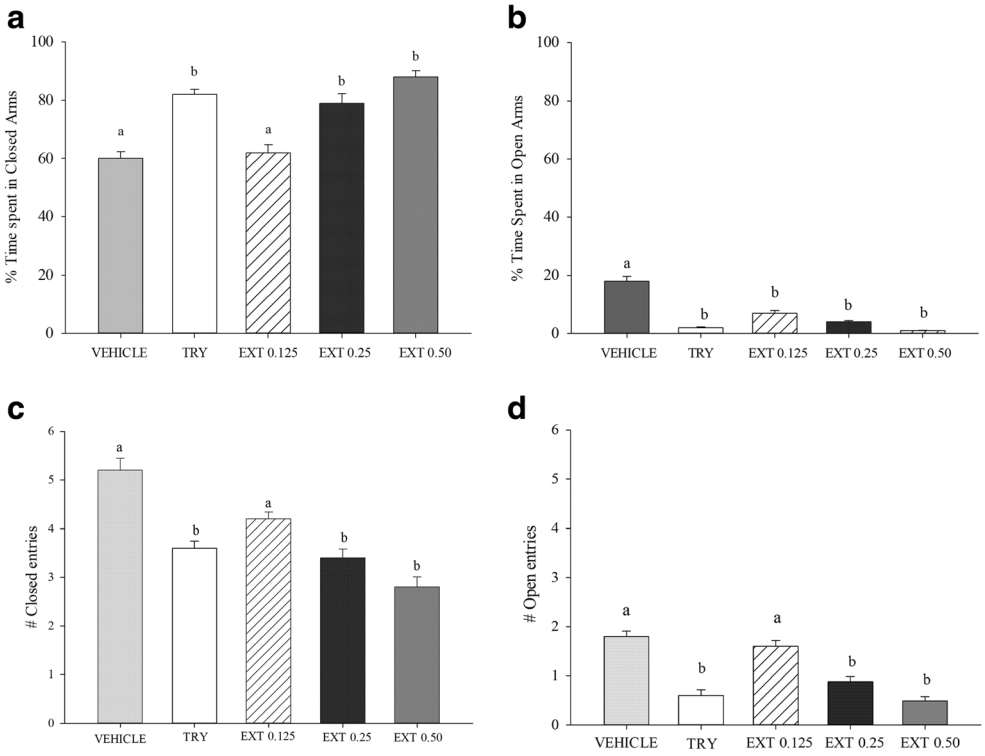

Fig. 1 Anxiolytic effect of orange juice extract in rats administered with orange juice extract (EXT) or tryptamine (TRY). **a**. Time of permanence in the closed arms. **b**. Time of permanence in the open arms. **c**. Number of entries in the closed arms. **d**. Number of entries in the open arms. The results are mean ± S.E.M. Significant difference compared with vehicle, b = $p < 0.05$

weight. Tryptamine group received an i.p. injection of 0.25 mg/kg. All injections were administered every 24 h over 56 days (Hale et al. 2012).

Behavioral tests

Elevated plus maze (EPM) is a behavioral test used to determine anxiety in the rats (Walf and Frye 2007). The EPM apparatus consisted of two open arms (length: 50 cm / width: 10 cm), perpendicular to two closed arms of equal dimensions with high walls of 40 cm. The EPM was elevated 50 cm above the floor, and located inside a room with constant background noise (50 dB) and controlled luminosity (30 lx at the level of the open arms of the maze). The behavioral tests were made in the last day of the treatment. The maze was cleaned with 20% ethanol before each test. Experimental sessions for the different groups were conducted between 14:00 and 16:00 h. The motor activity was video recorded during 20 min. Two independent observers who were blinded to the treatments recorded the dependent variables using a custom-software.

Sucrose consumption test

The method used was the two-bottle free-choice where the animals choose freely between water and water + sucrose (Frick et al. 2015). Each animal was placed in individual cage

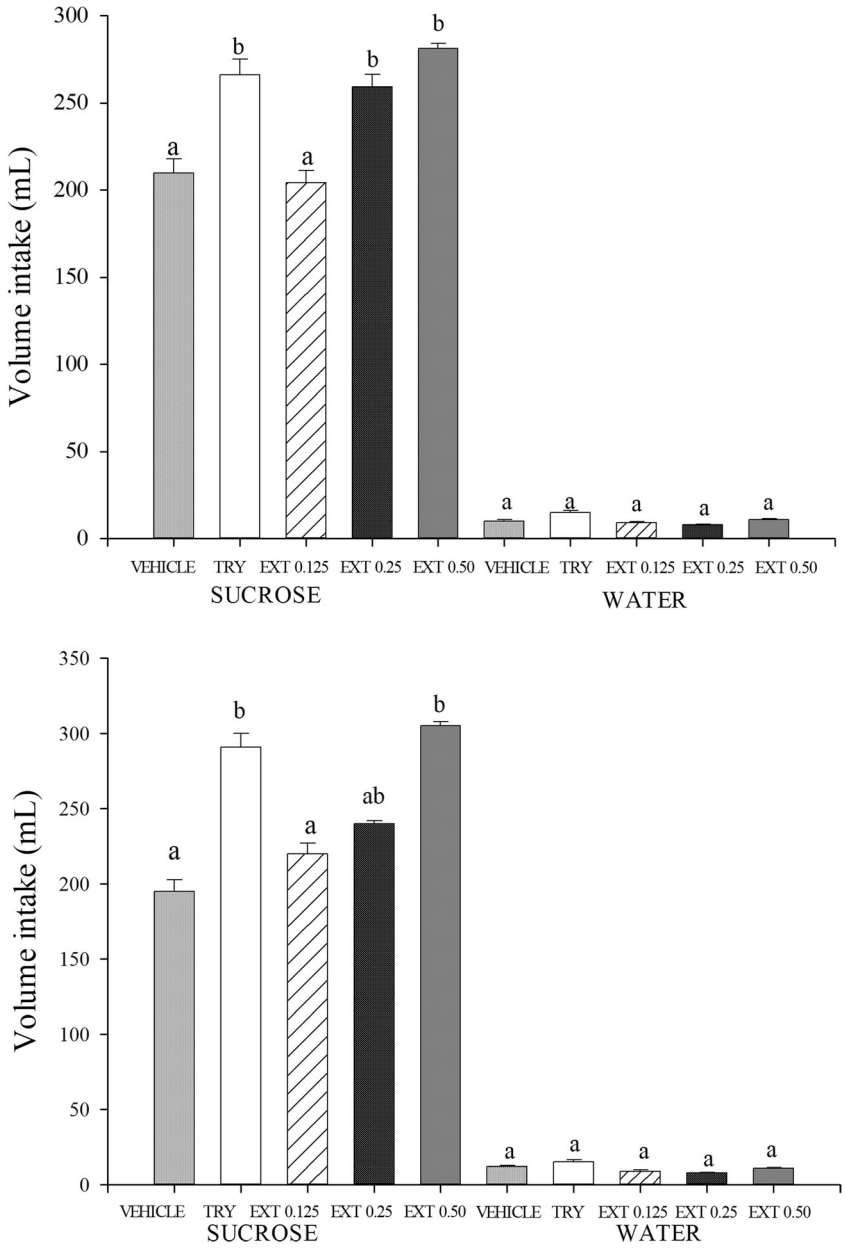

Fig. 2 Intake of sucrose and water in rats treated with EXT and TRY. Sucrose consumption was higher than water in five weeks (upper panel) and 8 weeks of treatment (lower panel). The results are presented as mean ± S.E.M. Significant difference compared with vehicle, b = $p < 0.05$

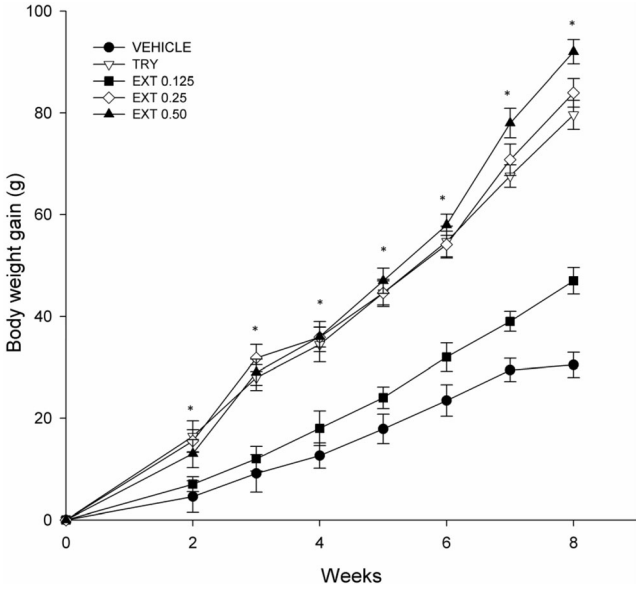

Fig. 3 Increase in body weight of the rats treated with different doses of EXT and TRY during 8 weeks. Closed circles represent the vehicle group, open triangle correspond to TRY. Square, diamond and closed triangle represent different doses of EXT. The results are mean ± S.E.M. Significant difference compared with the vehicle group, b = $p < 0.05$

and exposed to two solutions, one with plain water and the other with water plus sucrose (10%). Daily liquid consumption was measured every 24 h during two days at 5 and 8 weeks of the treatment.

In addition, the body weight of each experimental subject was recorded every week. Rats were individually weighed using a balance with underseat AND® capable of 1500 g, which was cleaned with 20% ethanol before each measurement.

C-Fos immunohistochemistry

Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and transcardially perfused with saline solution (0.9%), followed by paraformaldehyde (4%) in 0.1 M phosphate buffer pH 7.4 (PB). Brains were removed, postfixed overnight and then equilibrated to a gradient of sucrose solutions (10, 20 and 30%). Coronal sections of 40 μm of thick from accumbens nucleus, hippocampus and dorsal raphe nucleus were obtained using a cryostat (Hyrax C25, ZEISS, Waldorf, Germany). Tissue was washed in PB four times and then exposed 10 min to 0.5% hydrogen peroxide to neutralize endogenous peroxidase. Sections were washed four times with PB and incubated for 1 h in 3% normal goat serum to block non-specific labeling. Tissue sections were then incubated for 48 h at 4 °C with the anti-Fos antibody (ABT6973; Santa Cruz Biotechnology, Santa Cruz, CA, USA), diluted 1:2000 in 3% normal goat serum with 0.3% Triton X-100 (Sigma, St Louis, MO, USA). Tissue sections were washed four times with PB and incubated for 2 h with a biotinylated goat anti-rabbit antibody (81–6140, Invitrogen, Carlsbad, California, USA), diluted 1:250 with 0.3% Triton X-100 in PB. After 4 washes with PB, slices were incubated with the avidin-biotin-HRP complex (1:250, Elite Kit, Vector Laboratories, Burlingame, CA, USA) for 1 h. Peroxidase activity was visualized by reaction with a solution of 0.06% diaminobenzidine (D-8001, Sigma St Louis, MO, USA) in the presence of nickel sulfate (1%), cobalt chloride solution (1%) (Sigma A1827 and 202,185, respectively), and 0.01% hydrogen peroxide. Sections were mounted onto gelatin-subbed slides, dehydrated, and cleared in xylene then coverslipped with Permount. Control sections were processed as above but with the primary antibody omitted.

Table 1 Total number of c-fos positive cells in the brain after chronic administration of tryptamine (TRY) and orange juice extract (EXT: 0.25 mg/mL) in the rat

Nucleus	Vehicle	TRY	Extract (0.50 mg/mL)	p
Accumbens Core	102.18 ± 4.57	65.30 ± 3.01	67.37 ± 8.01	0.001
Accumbens Shell	111.68 ± 5.32	54.55 ± 3.76	57.20 ± 6.58	0.001
Dorsal raphe	24.73 ± 2.22	15.25 ± 0.60	17.90 ± 1.09	0.05
Hippocampus CA1	22.71 ± 1.12	13.41 ± 0.76	14.02 ± 0.70	0.001
Hippocampus CA2	30.14 ± 2.04	19.08 ± 0.67	20.02 ± 0.39	0.001
Hippocampus CA3	24.03 ± 1.35	16.16 ± 0.80	18.86 ± 0.51	0.001

The results are mean ± S.E.M

Quantification of Fos positive cells in brain sections

Fos-immunoreactivity (Fos-ir) was identified as a black-purple precipitate from the DAB-nickel/cobalt reaction in the cell nucleus. All slides were coded and Fos-ir nuclei were counted in both hemispheres by two observers blind to the experimental condition of subjects with aid of a rectangular grid (6440μm^2), using a Nikon microscope (Eclipse E200, Melville, New York, USA) with a 40X objective. Six tissue sections per structure were analyzed with the aid of an imaging analysis freeware (ImageJ 1.34, NIH, Bethesda, Maryland, USA).

Statistical analysis

The data were analyzed by two-way repeated measures analysis of variance (ANOVA), with treatment (different groups) as the between-subject factor and days of treatment as the within-subjects factor. ANOVA were followed by the Student Newman-Keuls post hoc test. The data are expressed as the mean ± standard error of each variable. A significant level of $P < 0.05$ was accepted.

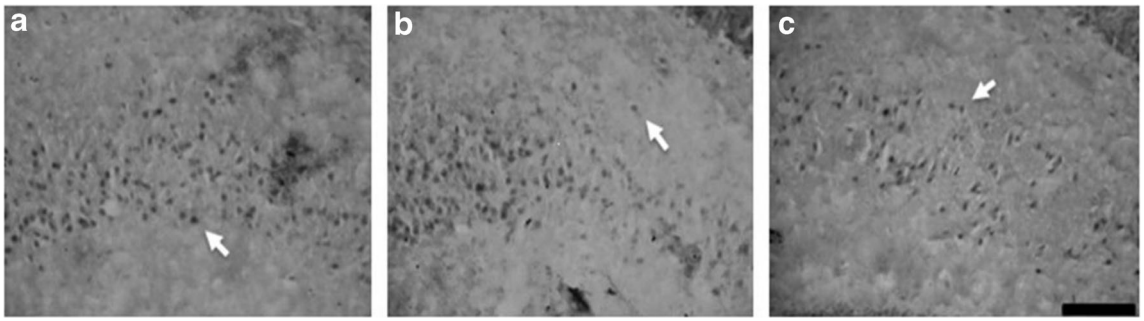

Fig. 4 Positive c-Fos cells in the hippocampus after chronic orange juice administration. Panel **a**: CA3 field of the hippocampus belonging to the vehicle group, panel **b**: orange juice extract group, panel **c**: tryptamine group. White arrow indicated c-Fos positive cells. 20x. Magnification bar 15 μm

Results

The TRY concentration in orange juice was 0.105 ± 0.02 mg/100 mL. This value was used to estimate the dose of TRY administrated to the Wistar rats. After fifty-six days of treatment with concentrated orange juice or TRY, rats were subjected to the elevated plus maze to test for anxiolytic behavior. The percentage of time spent in the closed arms was higher in the groups treated with orange juice extract and TRY compared with vehicle group (vehicle: 60.38 ± 3.62; TRY: 84.1 ± 2.8; orange juice extract (EXT) 0.125: 63.7 ± 3.9; EXT 0.25: 81.6 ± 4.5; EXT 0.50: 88.4 ± 2.3, $F_{4,59} = 11.13$ $p < 0.05$, Fig. 1a). In contrast, the percentage of time spent in the open arms was lower in the rats treated with the EXT and TRY compared with rats injected with vehicle ($F_{4,59} = 3.32$ $p < 0.05$, Fig. 1b). The rats injected with EXT 0.25 (3.58 ± 0.11) or TRY (3.33 ± 0.19) did not show differences in the number of entries into the closed arms (Fig. 1c), but there were differences with the vehicle group (5.25 ± 0.27), EXT 0.125 (4.17 ± 0.15), and EXT 0.50 (2.58 ± 0.21), $F_{4,59} = 6.83$ $P < 0.05$. Total entries into the open arms was lower in rats injected with EXT 0.25 (0.92 ± 0.09), EXT 0.50 (0.42 ± 0.05) and TRY (0.55 ± 0.12) compared to vehicle (1.8 ± 0.11) (Fig. 1d, $F_{4,59} = 3.01$ $p < 0.05$). Administration of EXT 0.125 did not induce any significant change (Fig. 1d).

In order to evaluate the hedonic threshold in the rats treated with TRY, we measure sucrose consumption. The sucrose solution consumption was higher than water in the five groups, rats injected with orange juice extracts and TRY consumed more sucrose solution at 5 weeks of treatment than vehicle rats (vehicle: 209.8 ± 2.2 mL, EXT 0.125: 204.2 ± 2.8 mL, EXT 0.25: 259.3 ± 2.1 mL, EXT 0.50: 281.1 ± 3.1 mL, TRY: 266.0 ± 2.7 mL, $F_{4,119} = 17.09$ $p < 0.05$, Fig. 2a). It was also observed interaction between treatment with the type of liquid ($F_{4,119} = 15.34$ $P < 0.05$). This effect remains until week 8 of orange juice extracts (EXT 0.125: 223.1 ± 6.1 mL, EXT 0.25; 249.7 ± 2.4 mL and EXT 0.50: 307.2 ± 6.6 mL) and TRY (298.0 ± 2.5 mL) administration ($F_{4,119} = 31.01$ $p < 0.05$, Fig. 2b). Interestingly, TRY and EXT 0.50 administered rats for 8 weeks, consumed more sucrose solution than rats treated with other concentrations of EXT, and it was observed interaction too ($F_{4,119} = 31.27$ $p < 0.05$, Fig. 2).

Rats injected with orange juice EXT 0.25, EXT 0.50 or TRY show higher weight gain than rats of the vehicle group during the course of the experiment (vehicle: 26.17 ± 1.4 g; TRY: 80.4 ± 4.6 g; EXT 0.125: 37.2 ± 2.6 g, EXT 0.25: 84.6 ± 3.5 g, EXT 0.50: 92.0 ± 2.4 g, $F_{4,39} = 18.19$ $p < 0.05$, $F_{7,39} = 34.51$ $p < 0.05$, Fig. 3).

Regarding to the c-Fos immunoreactivity, the results show that the total number of Fos positive cells in the accumbens nucleus (in the shell and core regions) increased after TRY or EXT (0.25 mg/mL) treatment ($F_{2,48} = 5.75$ $p < 0.001$, Table 1). Also in the dorsal raphe nucleus, both TRY and EXT increased the number of c-Fos positive cells compared with rats that received vehicle only ($F_{2,48} = 9.08$ $p < 0.05$, Table 1). In addition, TRY and EXT treatment increased the number of Fos positive cells in the CA regions of the hippocampus compared to the vehicle group ($F_{2,48} = 30.38$ $p < 0.001$, Table 1, Fig. 4).

Discussion

Orange juice is an excellent source of vitamin C, potassium, thiamin and other nutrients. Synephrine, octopamine, and tyramine were the first amines to be reported in the orange juice (Vieria et al. 2007). More recently, putresine, spermidinem, spermine, and TRY have been also reported in the orange fruit. Some studies have reported the chemopreventive properties of orange juice associated with its effect on metabolic enzymes and its anti-inflammatory, cytoprotective/apoptotic, hormonal, cell signaling-modulating, antioxidant, and antigenotoxic effects (Franke et al. 2013). Also, orange juice provides several kinds of minerals and vitamins necessary to healthy state (Lee et al. 2014).

Currently, there are few studies that have analyzed the effect of fruit extracts on the behavior. In this sense, several authors have reported the effect of different extracts derived from leaves or roots on behavioral aspects or cellular changes,

both in humans and animal models (Okamoto et al. 1997; Tohda et al. 2000; Tassoni et al. 2004).

In our study, the levels of TRY present in the orange juice extract were higher than any other substance previously reported (Vieria et al. 2007). TRY is one of the precursors of serotonin, a main neurotransmitter related to mood disorders. Serotonin mediates many essential behaviors such as anxiety-like behavior and cognitive rigidity (Shimazu and Miklya 2004; Stein and Stein 2008). Therefore, is plausible to suggest that accumulation of TRY by daily consumption of orange juice could be one of the causes that induce mood disorders. Several studies report that multiple anxiety or stress-related behavioral tests alter activation of serotonergic neurons in regions of the dorsal raphe (Zhao et al. 2002). Open-field exposure in rats results in an increase of c-Fos expression (Blazevic et al. 2012). Our results show an increase of c-Fos positive cells in dorsal raphe nucleus of rats treated with TRY and orange juice extract suggesting that serotoninergic system is active.

The results from the elevated plus maze show that administration of orange juice extract for 8 weeks induced an anxious behavioral pattern in the Wistar rat, this effect was dose-depend. The same result was found with TRY administration. TRY is one of the substances at higher concentration in the orange juice and may be responsible for inducing mood disorders when it is daily consumed for long time. In the same way, rats treated with TRY or orange juice extract increased their body weight, and it has been reported that anxiety and stress stimulate eating in the absence of hunger, which could facilitate overweight (Bouwknecht et al. 2007).

It is well known that rodents prefer sugar solution intake than plain water. Sucrose consumption is related to motivational processes, in particular with the dopaminergic system. Our results show that rats treated with orange juice extract or TRY preferring sucrose solution intake over water. This result suggests that the orange extract and TRY modify the sensitive of the reward system. Given the role of the NAC in behavior reinforcement, the NAc shell is the region of the accumbens that is purely limbic, closely related to the amygdala and the hippocampus, with a role in information processing of stimuli associated with the reinforcing effect of hedonic consumption substances like sucrose (Di Chiara 2002; Mestre et al. 2016). In addition, the NAc shell is the region most susceptible to the motivational mechanisms in drugs consumption (Ito et al. 2004; Robbins and Everitt 1999). In both regions of the NAc, the number of c-Fos positive cells was higher in TRY and EXT conditions than vehicle rats, suggesting that the TRY contained in the orange juice has an effect on the reward system.

However, more studies will be necessary to understand how this kind of substances present in some citrus could alter the behavior. In conclusion, the results of this study support the hypothesis that chronic treatment with tryptamine present in the extract of orange juice, induce anxiety, body weight gain, and increases the hedonic threshold in male Wistar rats.

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Compliance with ethical standards

Conflict of interests There are no conflicts of interest to declare.

References

Armagan O (2007) A review current analytical methods for the determination of biogenic amines in foods. Food Chem 103:1457–1486

Blazevic S, Colic L, Culig L, Hranilovic D (2012) Anxiety-like behavior and cognitive flexibility in adult rats perinatally exposed to increased serotonin concentrations. Behav Brain Res 230(1):175–181

Bouwknecht JA, Spiga F, Staub DR, Hale MW, Shekhar A, Lowry CA (2007) Differential effects of exposure to low-light or high-light open field on anxiety-related behaviors: relationship to c-Fos expression in serotonergic and non-serotonergic neurons in the dorsal raphe nucleus. Brain Res Bull 72(1):32–43

Candete JM, De las Rivas B, Marcobal A, Muñoz R (2007) Molecular methods for detection of biogenic amines producing bacteria on foods. Int J Food Microbiol 117:258–269

Di Chiara G (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 137:75–114

Franke SI, Guecheva TN, Henriques JA, Prá D (2013) Orange juice and cancer chemoprevention. Nutr Cancer 65(7):943–953. https://doi.org/10.1080/01635581.2013.817594

Frick A, Ahs F, Engman J, Jonasson M, Alaie I, Bjorkstrand J, Örjan F, Faria V, Linnman C, Appel L, Wahlstedt K, Lubberink M, Fredrikson M, Furmark T (2015) Serotonin synthesis and reuptake in social anxiety disorder: a positron emission tomography study. JAMA Psychiatry 72:794–802. https://doi.org/10.1001/jamapsychiatry.2015.0125

Hale MW, Shekhar A, Lowry CA (2012) Stress-related serotonergic systems: implications for symptomatology of anxiety and affective disorders. Cell Mol Neurobiol 32:695–708

Ito R, Robbins TW, Everitt BJ (2004) Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. Nat Neurosci 7:389–397

Krukoff TL, Harris KH, Linetsky E, Jhamandas JH (1994) Expression of c-fos protein in rat brain elicited by electrical and chemical stimulation of the hypothalamic paraventricular nucleus. Neuroendocrinology 59:590–602

Lee SG, Yang M, Wang Y, Vance T, Lloyd B, Chung SJ, Koo SI, Chun OK (2014) Impact of orange juice consumption on bone health of the U.S. population in the national health and nutrition examination survey 2003-2006. J Med Food 17(10):1142–1150. https://doi.org/10.1089/jmf.2013.0072

Medina MA, Urdiales JL, Rodríguez-Caso C, Ramírez FJ, Sánchez-Jiménez F (2003) Biogenic amines and polyamines: Similar Biochemistry for different physiological missions and biomedical applications. Crit Rev Biochem Mol Biol 38(1):23–59. https://doi.org/10.1080/713609209

Mestre ZL, Melhorn SJ, Askren MK, Tyagi V, Gatenby C, Young L, Mehta S, Webb MF, Grabowski TJ, Schur EA (2016) Effects of anxiety on caloric intake and satiety-related brain activation in women and men. Psychosom Med 78(4):454–464. https://doi.org/10.1097/PSY.0000000000000299

Okamoto A, Sugi E, Koizumi Y, Yanagida F, Udaka S (1997) Polyamine content of ordinary foodstuffs and various fermented foods. Biosci Biotechnol Biochem 61(9):1582–1584

Robbins TW, Everitt BJ (1999) Drug addiction: bad habits add up. Nature 398:567–570

Shimazu S, Miklya I (2004) Pharmacological studies with endogenous enhances substances: tryptamine, and their synthetic derivatives. Prog Neuro Psychopharmacol Biol Psychiatry 28:421–427

Stein MB, Stein DJ (2008) Social anxiety disorder. Lancet 371:1115–1125. https://doi.org/10.1016/S0140-6736(08)60488-2

Tassoni A, Germana MA, Bagni N (2004) Free and conjugated polyamine content in Citrus sinensis Osbeck, cultivar Brasiliano N.L.92, a navel orange, at different maturation stages. Food Chem 87(1):537–541

Tohda C, Kuboyama T, Komatsu K (2000) Dendrite extension by methanol extract of Ashwagandha (roots of Withania somnifera) in SK-N-SH cells. Neuroreport 11:1981–1985

Vieria SM, Theodoro KH, Gloria MBA (2007) Profile and levels of bioactive amines in orange juice and orange soft drink. Food Chem 100:895–903

Walf AA, Frye CA (2007) The use of elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2(2):322–328. https://doi.org/10.1038/nprot.2007.44

Zhao J, Nakamura N, Hattori M, Kuboyama T, Tohda C, Komatsu K (2002) Withanolide derivatives from the roots of Withania somnifera and their neurite outgrowth activities. Chem Pharm Bull 50:760–765