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# Long-term administration of soft drink causes memory impairment and oxidative damage in adult and middle-aged rats

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ABSTRACT

*Introduction:* The consumption of soft drinks has increased considerably in recent decades, mainly cola soft drinks. Excessive consumption of cola-based soft drinks is associated with several diseases and cognitive decline, particularly memory impairment. Furthermore, diets with high sugar can promote insulin resistance, metabolic syndrome, and dyslipidemia.

*Aim:* Thus, the present study aimed to evaluate the effect of cola soft drink intake on behavioral alterations and oxidative damage in 2-, 8- and 14- month-old male Wistar rats.

*Methods:* The soft drink groups drank soft drink and/or water *ad libitum* during 67 days, the control groups ingested only water. Radial-arm maze and Y-maze were used to evaluate spatial memory, open-field to evaluate the habituation memory, and inhibitory avoidance to evaluate aversive memory. The behavioral tests started at the day 57 and finished at day 67 of treatment. At 68th day, the rats were killed; frontal cortex and hippocampus were dissected to the analysis of antioxidants enzymes catalase (CAT) and superoxide dismutase (SOD); and the oxidative markers thiobarbituric acid reactive substances (TBARS) and dichloro-dihydro-fluorescein diacetate (DCFH) were measured in the hippocampus.

*Results and discussion:* The cola-based soft drink intake caused memory impairment in the radial-arm maze, Y-maze task, and open-field in the 2- and 8-month-old rat, but not in the 14-month-old. There were no difference among groups in the inhibitory avoidance test. In the frontal cortex, soft drink intake reduced CAT activity in the 8-month-old rats and SOD activity in the 8- and 14-month-old rats. In the hippocampus, the soft drink increased CAT activity in 2- and 8-month-old rats, increased DCFH levels at all ages, and increased TBARS levels in 2-month-rats. Therefore, the results show that long-term soft drink intake leads to memory impairment and oxidative stress. The younger seems to be more susceptible to the soft drink alterations on behavior; however, soft drink caused alterations in the oxidative system at all ages evaluated.

## 1. Introduction

The excessive consumption of soft drinks has become a serious public health issue worldwide (Pomeranz, 2012). The consumption of soft drinks has increased considerably in recent decades, cola syrup-based beverages are responsible for the highest sales worldwide (Nielsen and Popkin, 2004; Vereecken et al., 2005). High consumption of sugar-sweetened beverages may contribute to weight gain and obesity and can have negative consequences on overall health (Qin et al., 2020; Vartanian et al., 2007).

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Soft drinks have been associated with mental health problems, including depression (Hu et al., 2019), stroke and dementia (Pase et al., 2017), cognitive decline, and memory impairment (Wong et al., 2017). Cola syrup-base beverages are rich in refined sugars, such as fructose, which leads to the formation of Advanced Glycation Endproducts (AGEs) endogenously that can disturb lipid synthesis, cause peripheral insulin resistance, inflammation, and oxidative stress (Aragno and Mastrocola, 2017).

Oxidative stress is characterized as an imbalance between the anti- and pro-oxidant systems, resulting in an increase in the number of oxidizing compounds, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Sies et al., 2017). Three main enzymes are responsible for the organism's defense against the pro-oxidant agents: superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). SOD is responsible for converting the superoxide anion into hydrogen peroxide, and subsequently, CAT and GPx convert hydrogen peroxide into less reactive compounds, such as water (Halliwell, 2007).

The increase of oxidative stress and formation of free radicals can cause DNA damage, mitochondrial mutation, and lesion, as well as membrane destruction by interacting with various cellular components (Grimm et al., 2011). A growing body of evidence supports that oxidative stress leads to the development of neurodegenerative diseases (Santos et al., 2016). A systematic review found that free radicals triggered the activation of multiple noxious pathways associated with the development of neurodegenerative diseases, including oxidation of nucleic acids, proteins, and lipids, mitochondrial dysfunction, glial cell activation, amyloid β deposition, and plaque formation, apoptosis, cytokine production and inflammatory responses (Yaribeygi et al., 2018).

Some studies have already related fructose intake with cognitive impairment in rodents (Kendig et al., 2013; Soares et al., 2013). However, in the present study, we evaluated cola-based soft drink that besides sugar (fructose, corn syrup, sucrose) it has ingredients like caffeine, phosphoric acid, and caramel color that can contribute to brain toxicity. Thus, the present study aimed to evaluate the impact of the chronic administration of a cola-based soft drink in Wistar rats at 2-, 8- and 14-month-old on memory and oxidative stress.

## 2. Materials and methods

### 2.1. Animals

The 2-, 8-, and 14-month-old male Wistar rats, weighing 250 g to 450 g were housed in 5 per cage with food and water or soft drinks *ad libitum*, in a light/dark cycle of 12 h (lights on at 06:00 a.m.) and temperature within 23 ± 1 °C. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and Brazilian Society for Neuroscience and Behavior recommendations for animal care. The Ethics Committee on Animal Use of Universidade do Extremo Sul Catarinense (protocol number 042/2015-02) approved this study.

### 2.2. Treatment

Animals were randomly assigned into two groups experimental groups according to the treatment (control water or soft drink). The control group received just water *ad libitum* and the soft drink group received a cola-based soft drink and water *ad libitum*. Soft drinks (Coca-cola®) and water were available daily in separate bottles during the treatment (67 days). The animals drank both soft drinks and water *ad libitum*. The volume consumed was evaluated daily in the first week and then on the 30th and 67th days of the experiment. The ratio between the initial volume in the bottle (900 mL) and the remaining liquid in the bottle was measured after 24 h of consumption. The volume of water or soft drink consumed was quantified and the value was divided by five (number of animals in the cage). The 2-month-old rat ended the treatment at 4-month-old, the 8-month-old animals at 10-month-old, and the 14-month-old ended the treatment at 16-month-old. The behavior tests started on the 57th day of treatment. The animas were killed on 68th day, 24 h after the last soft drink consumption. The body weight and blood glucose levels were measured on the first, 30th, and 67th days of the experiment. The glycaemia of the rats was analyzed using the G-Tech (SD Biosensor Inc., Republic of Korea) and G-Tech strips (SD Biosensor Inc., Republic of Korea).

### 2.3. Radial arm maze test

The radial arm maze test evaluates spatial memory. The radial arm maze apparatus has 8-arms, which are numbered from 1 to 8 (48 × 12 cm), extending radially from a central area (32 cm diameter), placed 50 cm above the floor. On the 57th day of treatment, each animal was placed in the apparatus for 10 min to explore, then, returned to its cage (habituation). On the second day (first day of the test), a reward (cereal) was placed in four of the eight arms of the apparatus, geometric shapes were positioned in the straight arms where the food was placed (visual cues). The animals were placed in the center of the apparatus and allowed to explore for 10 min or until to find the 4 pieces of cereal. The entry into arms without food (total errors to find food) and the time that each animal took to find the 4 pieces of cereal were recorded (latency to find food). The same test was held over four consecutive days, 1 trial per day (Foyet et al., 2011; Garcez et al., 2017; Hritcu et al., 2012).

### 2.4. Y-maze test

Y-maze test is performed to evaluate short-term spatial memory. The Y-maze apparatus consisted of three arms made of black plastic, which were joined in the middle to form a "Y" shape. The inside of the arms ("start", "other" and "novel") were all identical. This test is based on the rodent's innate curiosity to explore novel areas, locating them via spatial clues (spatial memory). Briefly, on the 64th day of treatment, the rats were placed into the "start arm" to explore the maze with one of the three arms closed off (novel arm), for 5 min (training trial). After a 2-hour interval, the rat returned to the Y-maze and was placed in the same start arm, however, this time with all three arms opened. The rat was allowed to freely explore all three arms of the maze for 5 min (test trial). The time spent in each arm was recorded (Dellu et al., 1997). For analyses are compared only the time in the "novel" and in the "other" arm to avoid bias. Because the "start" arm is the starting point, consequently, the animal spends more time there.

### 2.5. Open-field test

Long-term retention of habituation to a novel environment is a non-associative, non-aversive type of learning, measured by the decrease in the exploratory activity. This apparatus consists of a 45 cm × 60 cm brown plywood arena surrounded by 50 cm high wooden walls and containing a frontal glass wall. The floor of the open field was divided into nine rectangles (15 cm × 20 cm each) by black lines. On the 65th day of treatment, the rats were gently placed on the left rear quadrant and left to explore the arena. To investigate the spontaneous locomotor activity, the numbers of horizontal (crossings) and vertical (rearings) activities performed by each rat during 5 min observation period were counted by an expert observer. Twenty-four hours after the training session, one new exposition (test session) to the open field was carried out for 5 min. The number of crossing and rearing performed in the test session compared to the training session was used to evaluate the habituation memory (Vianna et al., 2000).

### 2.6. Inhibitory avoidance test

The apparatus used in this test was an acrylic box in which the floor consisted of parallel stainless-steel bars and a platform that was 7 cm

wide and 2.5 cm high. The animals were placed on the platform, and their latency to step down onto the grid with all four paws was measured. Immediately after stepping down onto the grid, the animals received a foot shock of 0.4 mA for a period of 2 s. In the test sessions carried out 24 h after training, no foot shock was given, and the step-down latency (maximum of 180 s) was used as a measure of memory retention to evaluate aversive immediate memory (IM) 5 s after training, short-term memory (STM) 1.5 h after training, and long-term memory (LTM) 24 h after training (Roesler et al., 1999).

### 2.7. Biochemical analysis

The animals were killed by decapitation on the 68th day of the experimental protocol, and the brain was removed for dissection of the frontal cortex and hippocampus. All samples were immediately frozen in liquid nitrogen and stored in the −80 °C freezer until analysis.

#### 2.7.1. Oxidative stress parameters

The quantification of thiobarbituric acid reactive substances (TBARS), formed as a byproduct of lipid peroxidation; 2,7-dichlorodihydrofluorescein diacetate (DCF-DA) that quantitatively assess reactive oxygen species (mainly $H_2O_2$); and the activity of the antioxidant enzymes, SOD and CAT were evaluated in the hippocampus and/or frontal cortex from rats. TBARS were determined according to the method of Esterbauer and Cheeseman. A calibration curve was performed using 1,1,3,3-tetramethoxypropane, and each curve point was subjected to the same treatment as supernatants. Values of TBARS were calculated as nanomoles of TBARS per milligram of protein (Esterbauer and Cheeseman, 1990). DCFH oxidation reactive species production was assessed using 2′,7′-dihydrodichlorofluorescein diacetate (DCF-DA). A calibration curve was performed with standard DCF (0.25–10 mM), and the levels of reactive species were calculated as picomoles of DCF formed per milligram of protein (LeBel and Bondy, 1992). CAT activity (EC 1.11.1.6) was assayed according to Aebi (1984), by measuring the $H_2O_2$ absorbance decrease at 240 nm. The specific activity was expressed as nanomoles per minute per milligram of protein. SOD Activity (EC 1.15.1.1) was determined according to Bannister and Calabrese (Bannister and Calabrese, 1987), using a spectrophotometric assay based on superoxide-dependent oxidation of epinephrine to adrenochrome at 32 °C. SOD specific activity is represented as nanomoles per minute per milligram of protein. Protein levels were determined by Lowry protein assay (Lowry et al., 1951) and bovine serum albumin was used as standard.

### 2.8. Statistical analysis

Statistical analyses were performed using Statistica software 8.0 (StatSoft Inc., Tulsa, USA). Mauchly's test of sphericity was used (assumption of violated sphericity) and two-way repeated-measures analysis of variance (ANOVA) test was undertaken to assess radial arm maze task data following the Tukey post hoc test. The Y-maze and open-field data were analyzed by paired Student's *t*-test. Wilcoxon was used to analyze the inhibitory avoidance test, data are reported as mean ± interquartile range. Data from oxidative stress were analyzed by two-way ANOVA followed by Duncan's post hoc test. Repeated measures two-way ANOVA followed by Duncan's post hoc test was performed to evaluate the data of body weight and blood glucose levels. The data were reported as mean ± SEM. Statistical significance was considered for values of $p < 0.05$.

## 3. Results

Table 1 shows the blood glucose and body weight. The one-way repeated measures ANOVA revealed significant differences in glycaemia [F (10,78) = 5.26; $p < 0.01$] and weight [F (10,170) = 41.06; p < 0.01] among groups. Duncan's post-hoc test showed that glucose levels in the 2-month-old control group (water) were lower on the 30th and 67th days independent of the treatment. 8- and 14- month-old animals did not show alteration in their blood glucose levels. Duncan's post hoc test showed both 2-month-old rats gained weight on the 30th and 67th day, independent of the treatment. The 8- and 14-month-old animals had a weight gain compared to the 2-month-old animals compliant with the normal aging weight gain; it was independent of the treatment with soft drink.

**Table 1**
Effect of chronic administration of cola-based soft drink during 67 days in 2-, 8- and 14-month-old rats in the blood glucose levels and body weight. The table shows the blood glucose levels ($n = 10$) and the body weight (n = 10–12) of the animals. Data were expressed as mean ± SEM. *$p < 0.05$ when compared within the same group with the first day of testing and $^{\#}p < 0.05$ compared to the 2-month-old at the same day of treatment.

| Blood glucose (mg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 day | | 30 days | | 67 days | |
| | | Mean | SEM | Mean | SEM | Mean | SEM |
| 2-month-old | Water | 131 | 6 | 115* | 6 | 98* | 5 |
| | Soft drink | 140 | 7 | 110* | 4 | 108* | 3 |
| 8-month-old | Water | 94 | 5 | 93 | 5 | 98 | 3 |
| | Soft drink | 96 | 6 | 86 | 4 | 104 | 6 |
| 14-month-old | Water | 109 | 5 | 109 | 5 | 110 | 4 |
| | Soft drink | 101 | 4 | 110 | 3 | 114 | 3 |
| Body weight (g) | | | | | | | |
| 2-month-old | Water | 256 | 11 | 292* | 11 | 357* | 13 |
| | Soft drink | 256 | 9 | 291* | 9 | 386* | 11 |
| 8-month-old | Water | 424# | 14 | 426# | 13 | 445# | 9 |
| | Soft drink | 422# | 12 | 429# | 14 | 442# | 13 |
| 14-month-old | Water | 513# | 11 | 512# | 11 | 507# | 10 |
| | Soft drink | 545# | 12 | 545# | 15 | 556# | 12 |

Fig. 1 shows the radial arm maze data. Two-way repeated-measures ANOVA revealed a significant difference in the interaction between the factors age and treatment when evaluated latency (time) to find food [F (2,67) = 20.705; $p < 0.0001$) (Fig. 1A) and total errors to find food [F (2,67) = 12.29; p < 0.0001] (Fig. 1B). Tukey post hoc test showed that 2-month-old rats treated with water reduced the time to find food (Fig. 1A) over days 2, 3, and 4 when compared to the first day of the test, indicating learning of spatial localization of the food. The same was observed in the 8-month-old rats treated with water (control). However, the 2- and 8-month-old rats treated with soft drink did not reduce the time to find food along the days of the test, indicating spatial memory impairment. At 14-month-old, the animals did not learn the localization of the food when latency was observed, however, it was independent of the treatment, showing either age or soft drink could impair the spatial memory of these animals.

Fig. 1B shows the number of total errors to find the food on the radial arm maze. Tukey post hoc test showed that 2-month-old control (water) rats did not show spatial memory damage, reducing the number of errors over the days of the test. However, the 2-months-old that received soft drink did not show reduction of errors over the days, indicating cognitive impairment. However, the 8-month-old control (water) group did not reduce the entrances in the wrong arms. Maybe it is because they had committed a few errors since the first test day. Furthermore, the 8-month-old rats treated with soft drink decreased the number of errors only on the last test day. Lastly, 14-month-old control animals (water) reduced the mistakes in finding food over the test days, while those treated with soft drink did not. These results indicate that soft drink can cause spatial memory impairment.

Fig. 2 shows the Y-maze data. The results were analyzed by the Student's *t*-test for dependent samples comparing the time spent within the “other” and “novel” arms of the apparatus. Spatial memory damage was not observed in 2-month-old [t (1,12) = 3.88; $p < 0.05$] and 8-month-old control animals [t (1,10) = 2.37; p < 0.05]. However, the

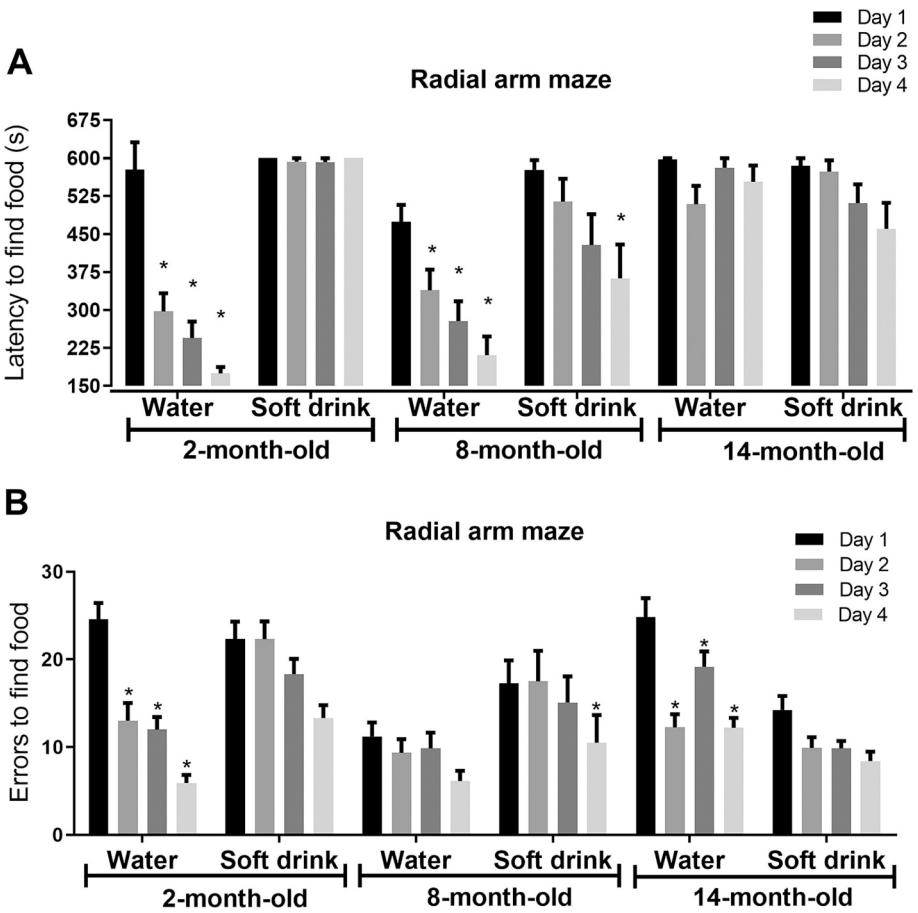


**Fig. 1.** Effect of chronic administration of cola-based soft drink during 67 days in 2-, 8- and 14-month-old rats in the long-term spatial memory evaluated on the radial arm maze. The figure shows latency to find food (A) and number of errors to find food (B). $n = 10–12$ animals per experimental group. Data were expressed as mean ± SEM. * $p < 0.05$ when compared with the first day of test within the same experimental group.

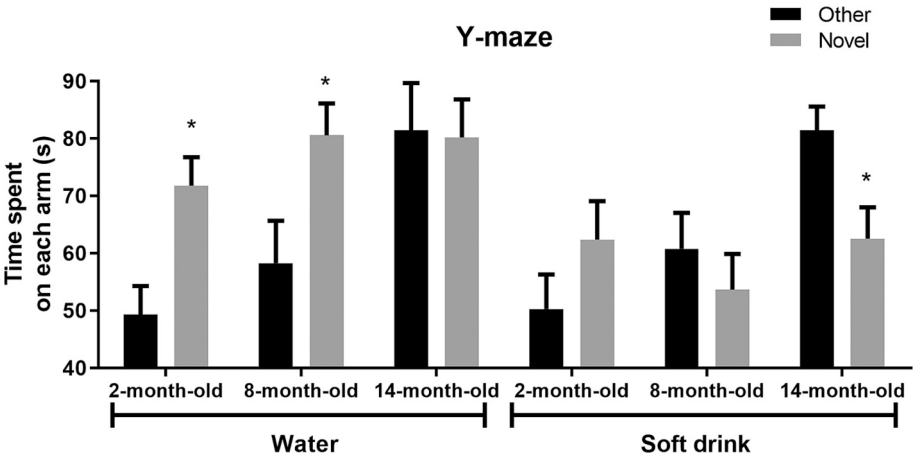


**Fig. 2.** Effect of chronic administration of cola-based soft drink during 67 days in 2-, 8- and 14-month-old rats in the short-term spatial memory evaluated on Y-maze. After 2-h interval, the rats returned to the Y-maze and was recorded the time (seconds) spent in each arm. Data are expressed as mean ± SEM of 9–12 animals per group, * $p < 0.05$ when compared to the "other" arm.

2-, 8- and 14-month-old animals treated with soft drink did not spend more time in the novel arm as expected, indicating spatial memory impairment. The 14-month-old soft drink group spent even less time in the novel arm when compared to the other arm.

Fig. 3 shows the open-field data. The 2-, 8-, and 14-month-old control rats reduced the number of crossings (Fig. 3A) and rearings (Fig. 3B) in the test, indicating they recognized the environment since they reduced the exploratory activity in the test compared to the training session. However, the 2- and 8-month-old rats treated with soft drink did not reduce the number of crossings or rearing in the test session, showing that soft drink impaired the non-associative habituation memory to a novel environment.

Fig. 4 shows the inhibitory avoidance test. There were no difference among groups in the inhibitory avoidance test, since any group showed

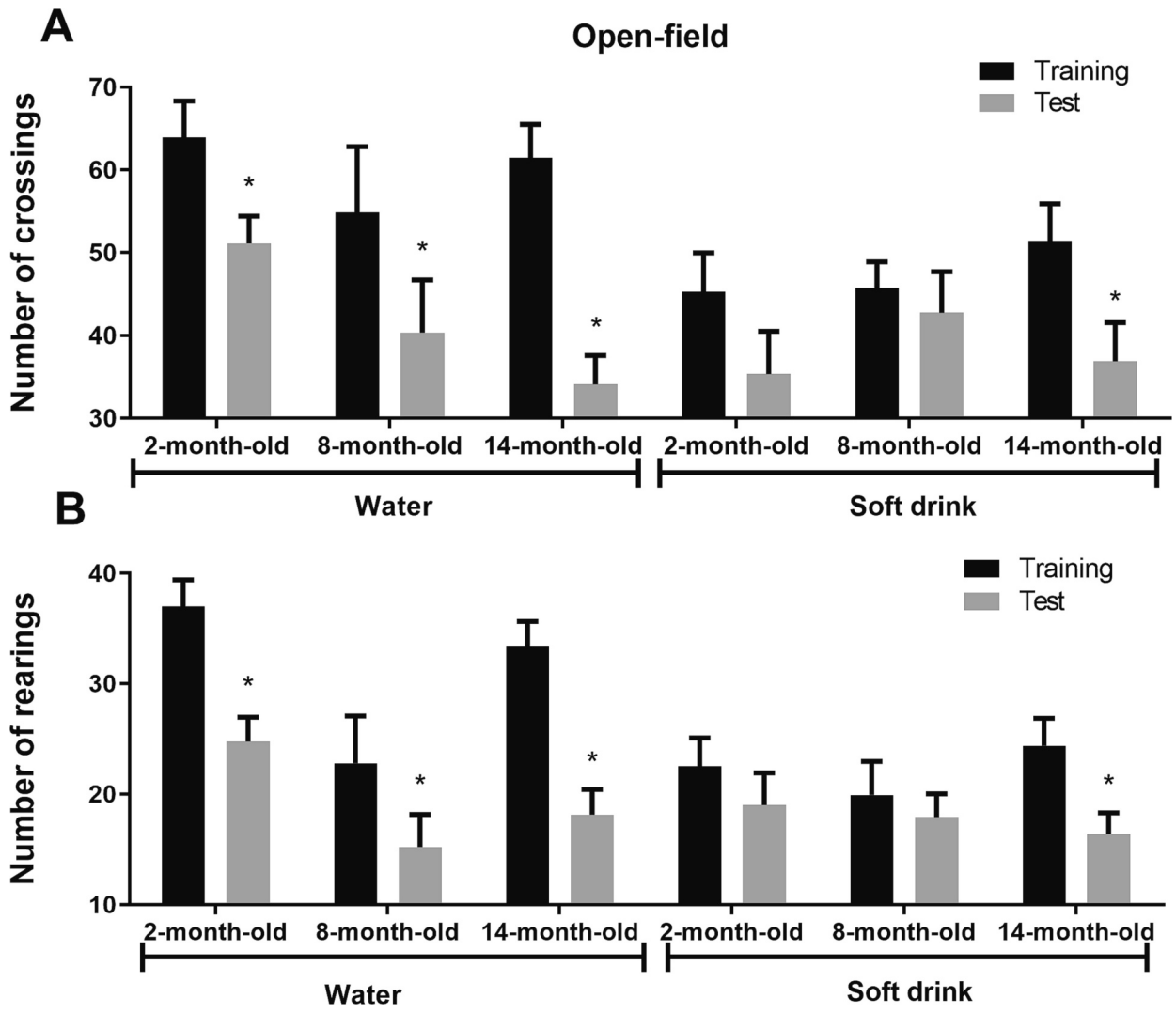


**Fig. 3.** Effect of chronic administration of cola-based soft drink during 67 days in 2-, 8- and 14-month-old rats in the retention of habituation memory to a novel environment evaluated on open-field. The figure shows the number of crossings (A) and rearings (B). Data are expressed as mean ± SEM of 10–15 animals per group, * $p < 0.05$ when compared to the "training" session.

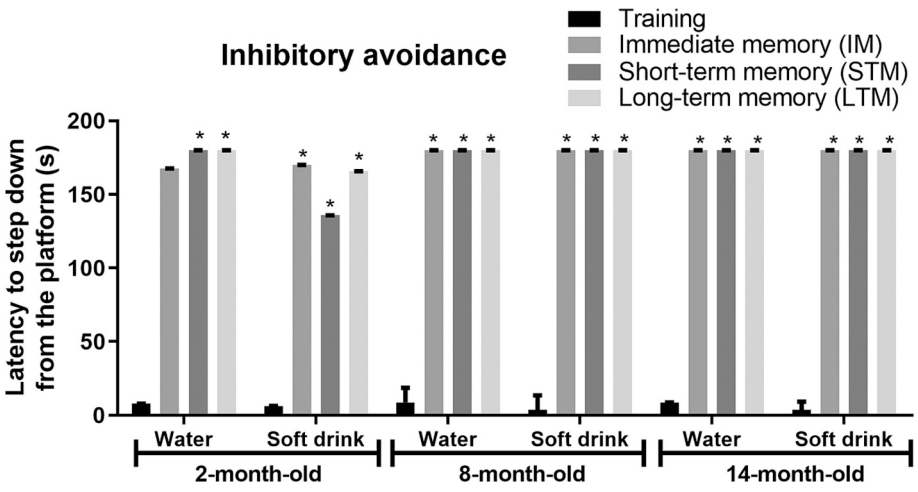


**Fig. 4.** Effect of chronic administration of cola-based soft drink during 67 days in 2-, 8- and 14-month-old rats in the aversive memory evaluated by the performance of the animals in the inhibitory avoidance task. Latency to step down from the platform (seconds) are presented as median ± interquartile range of 10–12 animals per group. Evaluation of the immediate memory (IM), short-term memory (STM) and long-term memory (LTM) data are expressed as median ± interquartile range. There was no aversive memory impairment in any experimental group.

aversive memory impairment.

CAT and SOD activity were evaluated in the frontal cortex and hippocampus, while DCFH and TBARS levels in the hippocampus. Fig. 5 shows the data obtained from the CAT (Fig. 5A and B), SOD (Fig. 5C and D), DCFH (Fig. 5E) and TBARS (Fig. 5F). Duncan's post hoc test showed significant differences of CAT activity in the frontal cortex [F (5,22) =

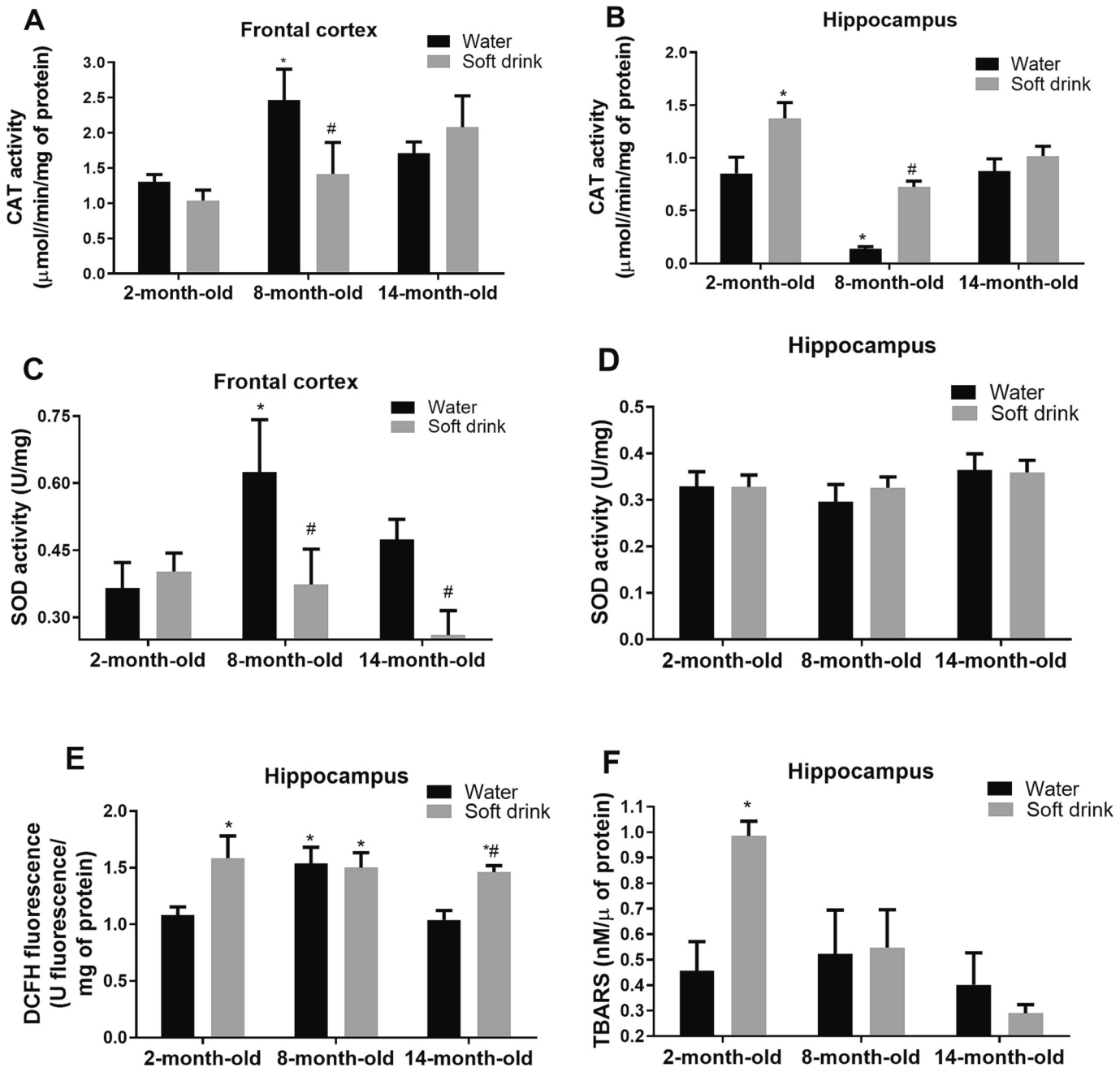


**Fig. 5.** Effect of chronic administration of cola-based soft drink during 67 days in 2-, 8- and 14-month-old rats in the antioxidant enzymes and oxidative damage markers. The figure shows CAT activity in the frontal cortex (A) and hippocampus (B); SOD activity in the frontal cortex (C) and hippocampus (D); the byproduct of lipid peroxidation, DCFH (E), and TBARS (F) levels in the hippocampus. Data are expressed as mean ± SEM of 3–6 animals per group *p < 0,05 when compared to the 2-month-old control group (water) and #p < 0,05 when compared to the control group (water) within the same age.

2.91; $p < 0.05$) (Fig. 5A) and hippocampus [F (5,22) = 7.99; $p < 0.001$] (Fig. 5B) among groups. There was an increase in CAT activity in the frontal cortex of rats received water, but not in the rats received soft drink in the 8-months-old. But, CAT activity was reduced in the 8-month-old rats treated with soft drink when compared to the control group (water) of the same age. In the hippocampus of 2-month-old rats that received soft drink there was an increase in CAT activity ($p < 0.05$) compared to the 2-month-old control group (water). CAT activity decreased in the hippocampus of 8-month-old control rats, but not in the rats treated with soft drink ($p < 0.05$) when compared to the 2-month-old control. In addition, CAT activity showed an increase in the 8-month-old rats treated with soft drink ($p < 0.05$) when compared to the control group (water) of the same age. Moreover, there are no statistical differences in CAT activity in the frontal cortex (Fig. 5A) or the hippocampus (Fig. 5B) in the 14-month-old rats.

It was observed an increase in SOD activity in the frontal cortex (Fig. 5C) in 8-month-old rats that received water ($p < 0.05$) when compared to the control 2-month-old, but not in the same age animals that received soft drink. In addition, SOD activity was reduced in the 8- and 14-month-old rats treated with soft drink ($p < 0.05$) when compared to the control group (water) of the same age. There was no statistical difference in the SOD activity among groups in the hippocampus [F (1,5) = 0.55; $p = 0.73$] (Fig. 5D). The byproduct of lipid peroxidation, DCFH [F (5,19) = 3.51; $p = 0.02$] increased in the 2- and 14-month-old animals that received soft drink (Fig. 5E), also in the 8-month-old that received water and soft drink. In addition, in the hippocampus, TBARS

[F (5,20) = 7.03; $p = 0.001$] increased in 2-month-old rats that received soft drink ($p < 0.001$) when compared to the control group (water) within the same age (Fig. 5F).

## 4. Discussion

In the present study, we evaluated the effect of soft drink intake on memory impairment and oxidative system in 2-, 8- and 14- month-old male Wistar rats. There was no alteration in the blood glucose levels in the animals received soft drink compared to the control that received water. However, there was an increase in the glyecemia in 3 to 4-month-old rats when compared to the 2-month-old rats, after 30 and 67 days of treatment with soft drink or water. In a study by Kendig et al. (2013) mice 21 and 56-day-old received a solution prepared with 10 % sucrose diluted in water for 2 h per day for 28 days. Blood glucose values revealed that younger animals tended to have larger readings compared to adult animals. Moreover, corroborating with the present study, there were no significant differences when comparing the respective control groups (Kendig et al., 2013). Another study conducted with 4-month-old animals that received 35 % sucrose for 9 weeks showed that sucrose intake increased postprandial glycemia values when compared to the control group (Soares et al., 2013). However, fasting blood glucose levels remained unchanged compared to the control group (Soares et al., 2013). Perhaps we could have observed alterations in the glyecemia if we had conducted a longer treatment or the evaluation of the post-prandial glycemia in the present study.

The rats that ingested soft drink did not gain weight compared to the animals that received water. However, the animals showed normal weight gain related to the age from 2-month-old. Some studies have shown that chronic consumption of soft drinks causes changes in the lipid profile of rats, which includes an increase in plasma levels of triglycerides. In addition, a decrease in the consumption of solid foods was observed, however, with no change in body weight (Otero-Losada et al., 2011; Otero-Losada et al., 2013). Another study was conducted with 2-month-old rats receiving a solution containing 20 % fructose *ad libitum* for 8 months. Weight assessments showed that animals with a high sugar diet had a significant difference from the third month of dietary change when compared to the control group (Stranahan et al., 2008). Soares et al. (2013) evaluated 4-month-old animals that received 35 % sucrose for 9 weeks. The results demonstrated that the sucrose intake had no influence on the weight values compared to the control group (Soares et al., 2013). Based on these studies, the duration of treatment (67 days), in the present study, was not sufficient for the rats to gain weight.

Based on our results, the radial arm maze showed soft drink caused spatial memory impairment at all ages since the animals treated with soft drink did not perform the same as the control group (water) compared to the respective age. A study conducted with male rats at 2-month-old administered with a control diet (fructose 0 %) or high fructose diet (60 %) for 18 weeks, showed that the high fructose diet group presented impairment in spatial memory retention during the Morris water maze test (Ross et al., 2009). The study carried out by Budni et al. (2016) showed that after 4 and 6 weeks of treatment with D-galactose sugar (100 mg/kg), the 4-month-old rats presented spatial memory damages when evaluated through the radial arm maze test. Another study conducted with 2-month-old male Sprague-Dawley rats treated with fructose for 8 weeks, showed that fructose caused spatial memory damage when evaluated in the barnes maze test (Agrawal et al., 2016).

The Y-maze behavioral test is based on the natural tendency of rodents to explore novelty, in this case, the novel arm, guided by spatial clues (Conrad et al., 1997; Dellu et al., 2000; Martin et al., 2003). In this test was possible to observe that soft drink impaired the spatial memory of the 2- and 8-month-old rats. Corroborating with the present data, 4-month-old Wistar rats that ingested 35 % of sucrose *ad libitum* for 9 weeks, also showed memory impairment in the Y-maze (Soares et al., 2013). Moreover, 2 h of daily access to 10 % of sucrose caused spatial memory impairment in young male rats evaluated by the T-maze test, which resembles the Y-maze, indicating that diets rich in sucrose cause an impact on spatial and working memory processes (Wong et al., 2017). One of the mechanisms that could be involved in the reduction of the brain-derived neurotrophic factor (BDNF) signaling. A study showed rats treated with a diet rich in saturated fat and refined sugar presented a decrease in the levels of BDNF, synapsin I, growth-associated protein 43 (GAP-43), and cyclic AMP-response element-binding protein CREB in the hippocampus, mainly structure involved in the spatial memory. This study mentioned that the impairment in this signaling might deprive neurons of their natural protection from aging, insults, or disease (Molteni et al., 2002).

No data were found in the literature regarding soft drink intake and spatial memory assessed by radial maze or Y-maze. Thus, for the first time, the results of the present study indicate that chronic intake of cola-based soft drinks induces spatial memory impairment.

In addition, soft drink caused memory impairment in the habituation memory to a novel environment assessed by open-field test only at 2- and 8-month-old, but not in the 14-month-old rats. Therefore, we speculate that the chronic consumption of soft drink can be more harmful to the memory of younger than middle-aged animals. A clinical trial showed that the acute treatment with 25 g of glucose improved memory and cognitive functions supported by the hippocampus (e.g. episodic memory), however, only the older adults (mean = 38.4 years), but not the younger adults (mean = 21.8 years) (Meikle et al., 2004). Evidence suggests a relationship between the effect of glucose on tasks that are related to hippocampal function such as episodic memory and spatial memory (Riby, 2004; Smith et al., 2011). Moreover, a recent study, demonstrates that glucose administration can attenuate cognitive performance deficits in older adults with impaired glucose regulation with clear dissociation in the effects of glucose by age (Peters et al., 2020).

Although the younger seems to be more susceptible to the soft drink alterations on memory, the chronic intake of soft drink caused biochemical alterations at all ages evaluated. Among the antioxidant defenses, we highlight the enzyme SOD which is responsible for the dismutation of superoxide anions, the most abundant form of ROS, and CAT, which neutralizes hydrogen peroxide (Gutteridge and Halliwell, 2018; Halliwell, 2001; Halliwell, 2006). In the present study, CAT activity increased in the hippocampus from 2- and 8-month-old rats treated with soft drink probably to respond to the increase in the DCFH, since there was an increase in DCFH in the same brain structure. CAT activity had no alteration in the frontal cortex or hippocampus in 14-month-old rats. This age-related change in the CAT activity was not expected in middle-aged rats according to the literature (Siqueira et al., 2005). However, there was an increase in the DCFH levels in the hippocampus of a 14-month-old treated with a soft drink, DCFH reacts with intracellular hydrogen peroxide to give the DCF evaluated, consequently, it is expected CAT would increase to protect against this damage. Therefore, no change in the CAT activity in the 14-month-old rats treated with soft drink might have contributed to the oxidative damage observed in this study. In addition, CAT increased in the frontal cortex from 8-month-old treated with water, but soft drink decreased the activity of this enzyme, probably an adaptative response to oxidative damage, however, as the frontal cortex was not evaluated to oxidative species we can not confirm.

The SOD activity increased in the frontal cortex in 8-month-old rats treated with water. The soft drink treatment from 8- and 14-month-old rats reduced the SOD activity when compared to water group of the same age. Oxidative stress is characterized by the imbalance between the production of ROS and/or RNS and the tissue antioxidant defenses (Betteridge, 2000). The decrease in the activity of the enzymes caused by soft drink can be harmful since there was an increase in the DCFH and TBARS levels in the hippocampus from 2-month-old rats treated with soft drink, DCFH levels in 14-month-old rats who received soft drink, and in 8-month-old animals received soft drink and water.

Corroborating to our results Lebda et al. (2017) found increased malondialdehyde levels (a biomarker of oxidative stress) and decreased antioxidant enzymes (CAT and SOD) in the brain tissue homogenate from wistar rats treated with Coca-cola daily for 60 days. The alterations are difficult to compare because they did not evaluate each brain structure, however, is possible to observe that chronic soft drink intake can cause oxidative damage.

In addition, the same study found that soft drink intake increased apoptosis-related genes (Lebda et al., 2017) that can lead to neurodegeneration and cognitive impairment. Increased oxidative stress has been associated with a decline in executive function in a healthy population (Hajjar et al., 2018) and dementia (Luca et al., 2015). Thus, the oxidative imbalance caused by the soft drink in the present study might be one of the mechanisms that caused the memory impairment observed in the rats in the present study. Considering our limited financial sources, we have used the minimum number of animals to take into account our experience in the procedures described for the power and sample size calculation, in addition to a literature review.

In this study, we didn't compare gender. Therefore, it is possible to be a limitation of this research. Indeed, there are sex differences in spatial learning, memory, and long-term hippocampal potentiation in Wistar rats (Safari et al., 2021). No studies compare soft drinks' chronic consumption in male and female rats. A study proved that brain insulin signaling differs between genders, suggesting sex differences in sensitivity to leptin and insulin (Clegg et al., 2003). Abbott et al. (2016) found males might be at more risk of high sugar diet-induced cognitive deficits, suggesting that endogenous estrogens may boost memory performance in females. In addition, another study found men are more sensitive than women to the acute anorexigenic effect of central nervous insulin signaling. In contrast, insulin's beneficial impact on hippocampus-dependent memory functions is more pronounced in women (Benedict et al., 2008). These studies show gender differences in central nervous insulin signaling and memory functions. In our research, we do not evaluate both sexes because of the number of animals and physical space in the animal house of our institution, since we have used different ages, which involves many rats. However, future studies on female are needed to compare any potential sex differences.

## CRediT authorship contribution statement

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## Declaration of competing interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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