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ORIGINAL CONTRIBUTION

Energy drinks and their component modulate attention, memory, and antioxidant defences in rats

M. T. Costa Valle[1] · N. S. Couto-Pereira[2] · C. Lampert[2] · D. M. Arcego[2] · A. P. Toniazzo[2] · R. P. Limberger[3] · E. Dallegrave[4] · C. Dalmaz[1,2,5] · M. D. Arbo[6] · M. B. Leal[1,7]

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Abstract
Purpose This study aimed to evaluate the effects of the subchronic consumption of energy drinks and their constituents (caffeine and taurine) in male Wistar rats using behavioural and oxidative measures.
Methods Energy drinks (ED 5, 7.5, and 10 mL/kg) or their constituents, caffeine (3.2 mg/kg) and taurine (40 mg/kg), either separately or in combination, were administered orally to animals for 28 days. Attention was measured though the ox-maze apparatus and the object recognition memory test. Following behavioural analyses, markers of oxidative stress, including SOD, CAT, GPx, thiol content, and free radicals, were measured in the prefrontal cortex, hippocampus, and striatum.
Results The latency time to find the first reward was lower in animals that received caffeine, taurine, or a combination of both ($P = 0.003$; ANOVA/Bonferroni). In addition, these animals took less time to complete the ox-maze task ($P = 0.0001$; ANOVA/Bonferroni), and had better short-term memory ($P < 0.01$, Kruskal–Wallis). The ED 10 group showed improvement in the attention task, but did not differ on other measures. In addition, there was an imbalance in enzymatic markers of oxidative stress in the prefrontal cortex, the hippocampus, and the striatum. In the group that received both caffeine and taurine, there was a significant increase in the production of free radicals in the prefrontal cortex and in the hippocampus ($P < 0.0001$; ANOVA/Bonferroni).
Conclusions Exposure to a combination of caffeine and taurine improved memory and attention, and led to an imbalance in the antioxidant defence system. These results differed from those of the group that was exposed to the energy drink. This might be related to other components contained in the energy drink, such as vitamins and minerals, which may have altered the ability of caffeine and taurine to modulate memory and attention.

Keywords Energy drinks · Subchronic toxicity · Memory · Attention · Oxidative stress

✉ M. B. Leal
mirnablufrgs@gmail.com

1 Post-Graduate Program in Biological Sciences, Neuroscience, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

2 Post-Graduate Program in Biological Sciences, Biochemistry, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

3 Laboratory of Analysis and Research in Toxicology, Faculty of Pharmacy, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

4 Department of Farmacosciences, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil

5 Department of Biochemistry, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porte Alegre, Brazil

6 Laboratory of Toxicology (LATOX), Department of Analyses, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

7 Department of Pharmacology, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre 90050-17, Brazil

Introduction

Energy drinks were launched in the 1960s in Europe and Asia, but achieved worldwide recognition once the Red Bull® brand reached the US market in 1997. Such drinks

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were developed with caffeine to provide an energy boost and increase alertness. In 2006, more than 500 energy drink brands were marketed around the world [1]. The energy drink industry saw a growth of around 400% between 2003 and 2007 [2], with sales reaching $6.7 billion in 2010. The consumption of these beverages is higher among adolescents and young adults [3, 4], who represent about 50% of the market [3]. Studies have shown that 31% of adolescents between 12 and 17 years of age and 34% of young adults between 18 and 24 years of age reported regular consumption of energy drinks [5].

The main constituents of energy drinks are caffeine, taurine, guarana, sugar, sodium and vitamin B6 [6]. Some energy drink brands also include glucuronolactone, ginseng, ginkgo biloba, and others in their composition [7]. Excessive use of energy drinks may induce cardiac symptoms, such as ventricular and arterial arrhythmias [8].

Caffeine (1,3,7-trimetilxantine) is the main psychoactive component in energy drinks. It is usually present in high concentrations, which often results in excessive intake and toxicity symptoms [5, 9]. Although caffeine is associated with improvements in cognitive abilities, such as memory and concentration, its complete profile of effects, whether alone or in combination with other ingredients, are not entirely understood [6]. Caffeine is rapidly and fully absorbed when taken orally and it has an elimination half-life of about 4.5 h [9, 10]. In rats and humans, caffeine acts as a psychostimulant [11] by increasing alertness and anxiety and reducing fatigue [12]. Pharmacological studies indicate that the effects of caffeine on the central nervous system are mediated by antagonistic action at A_1 and A_2 adenosine receptor subtypes [13].

Another widely used component of energy drinks is taurine, which has numerous biological and physiological functions [7]. Taurine acts on the central nervous system, although the impact of high concentrations of taurine is not well understood [4, 5]. Taurine (2-aminoethyl sulfonic acid) is a sulfonated β-amino acid that is derived from diet or synthesized from cysteine, mainly in the liver. It is the most abundant amino acid, found primarily in the retina, skeletal muscles, cardiac muscles, heart, and liver. The metabolism of methionine and cysteine can also produce taurine in the central nervous system. Taurine is associated with a variety of physiological functions, including neuromodulation, neuroprotection, osmoregulation, cell membrane stability, and modulation of intracellular calcium levels [14, 15].

Some of taurine's effects are related to the modulation of N-methyl-D-aspartate (NMDA) receptors, which enhances memory function in rats [16]. Taurine can also affect neural excitability by elevating the expression of glutamic acid decarboxylase (GAD), increasing GABA levels, and down-regulating $GABA_A$ receptors, thereby altering the inhibitory GABAergic system [17]. Taurine acts as a $GABA_A$ agonist, therefore, exogenous taurine supplementation can improve age-related deterioration in GABAergic function and cognitive decline in learning and memory [18].

As caffeine and taurine are widely used in energy drinks, there have been several studies that have examined their effects on physical performance. The combined administration of both components increased the distance travelled in physical endurance tests [19]. Caffeine also yields different effects on memory depending on the stage of memory processing. Rats that were administered caffeine had an improvement in memory retention [20]. Other studies have shown, however, that adenosine A_1 receptor agonists impair learning and memory in rodents, while the non-selective blockade of A_1 and A_2 adenosine receptors by caffeine facilitates this process [21]. In newborn rats that were injured with 6-hydroxydopamine (6-OHDA), attention improved after continuous treatment with caffeine [22].

Few studies have assessed the impact of energy drinks on the antioxidant system. In a study of human neuronal SH-SY5Y cells, caffeine, taurine, and guarana reduced basal-free radical generation. A combination of caffeine or taurine and guarana reduced the activity of superoxide dismutase (SOD) and catalase (CAT) in vitro, but no changes in glutathione peroxidase (GPx) activity were observed [23].

Oxidative stress occurs when there is an imbalance between the generation of oxidant compounds and antioxidant defence systems in the body [24]. Free radicals and reactive oxygen species (ROS) are constantly produced at low levels as part of normal and essential biological processes [25]. When they are produced in excess, however, they can cause cell injury, such as damage to lipids, proteins and nucleic acids [26], or even cell death. Due to its high oxygen consumption, abundant content of lipids, and insufficient levels of antioxidant enzymes relative to other tissues, the brain is particularly vulnerable to the damaging effects of free radicals and reactive species [27, 28].

Given that the consumption of energy drinks is high and that the central nervous system is vulnerable to toxicity, the aim of this study was to evaluate the subchronic effects of energy drinks and their constituents in rats. Models of attention and memory were used in this study, as the use of energy drinks is common among young adults seeking to improve attention and learning processes.

Materials and methods

Drugs and chemicals

Caffeine, taurine, dichlorofluorescein (DCF), 2-7-dichlorofluorescein diacetate (DCFH), and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Sigma-Aldrich (St. Louis, MO, USA). SOD RANSOD and GPx Ransel kits

were purchased from Randox Labs (Crumlin, UK). Energy drink (Red Bull®, Fuschl, Austria), caffeine, and taurine were solubilized in distilled water. For all experiments, 1-mL/kg distilled water was used as control. All treatments were administered by oral gavage.

Doses of energy drink (ED), caffeine (3.2 mg/kg) and taurine (40 mg/kg) were determined by calculating the relative doses that are typically ingested by consumers. In establishing a similarity to energy drinks, it was determined that the amount of caffeine and taurine contained in a 250 mL energy drink and consumed by a 70 kg adult is 1000-mg/can taurine and 80-mg/can caffeine. The volumes administered were equivalent to one can (5 mL/kg, ED 5), two cans (7.5 mL/kg, ED 7.5) and three cans (10 mL/kg, ED 10) [29]. The dose of 100-mL/kg ED contained 40-mg/kg taurine and 3.2-mg/kg caffeine [30]. In all experiments, these doses were administered either alone or in combination.

Animals and treatment

A total of 70 male adult (60 days old) Wistar rats ($n = 10$/group) obtained from Centro de Reprodução e Experimentação de Animais de Laboratório (CREAL-UFRGS) were used in this study. Rodents were kept in polypropylene cages (41 × 34 × 16 cm, 4 rats per cage) with free access to food and water on a 24-h light/dark cycle (lights on 7–19 h), in a controlled-temperature environment (22 ± 2 °C) with monitored humidity. The experiments were approved by the University Ethics Committee (Number 26689) and were carried out in accordance with current guidelines for the care of laboratory animals.

Treatments were based on subchronic toxicity test guidelines by the Organization for Economic Cooperation and Development (OECD 407). Treatment was administered orally for 28 days to the following groups of ten animals each: 1-mL/kg distilled water (control), ED 5, ED 7.5, ED 10, 3.2-mg/kg caffeine, 40-mg/kg taurine, and a combination 3.2-mg/kg caffeine + 40-mg/kg taurine (caffeine + taurine group). On the 29th day, the animals were euthanized by decapitation. The liver, kidney, and adrenals were weighed and examined for macroscopic alterations. The brain was also removed and the cortex, hippocampus, and striatum were dissected for oxidative stress analyses (Fig. 1). The sample size was determined with the Minitab program. The values used in the calculation were based on published studies that used these biochemical and behavioural techniques, as well as on our previous experience. Due to differences in the dispersion of data, the sample size for the behavioural experiments is usually higher than that for biochemical experiments.

Rota-rod performance test

The Rota-rod test was conducted on the 14th day of the daily treatment regimen as previously described by Schmitt, et al. [31]. The animals first completed a training session, which consisted of three trials in which the animals attempted to remain on a bar that was rotating at 18 rpm for at least 90 s. The test was conducted 1 h after training. The latency to fall from the rota-rod apparatus (Insight Equipment Ltda, Ribeirão Preto, Brazil) at 18 rpm over a 90-s time period was recorded.

Locomotor activity

Spontaneous locomotor activity was evaluated on the 15th day after the beginning of the daily treatment regimen as previously described by Maciel et al. [32]. The locomotor activity cage (Insight Ltda Ribeirão Preto, Brazil) consisted of a 50 cm × 48 cm × 50 cm box equipped with 6 bars, each with 16 infrared light sensors that detected the relative position of the animal in the box. The total distance travelled by the animal was measured for 15 min, with the first 5 min considered to be exploratory activity and the final 10 min recorded as the test session. The test was performed in a dark room with no noise.

Ox-maze test

The ox-maze test was carried out between the 16th and 26th day of treatment. The test was used to assess visual discrimination, learning, and memory, and was performed as previously described by Wood et al. [33] and Rojas et al. [34]. The apparatus consisted of a 60 cm × 60 cm × 30 cm Plexiglas box that contained a maze that consisted of four white Perspex blocks (10 cm × 10 cm × 5 cm). The blocks had a circular hole (diameter 2 cm × 2 cm) on each side, and each hole was located in the middle of four symbols

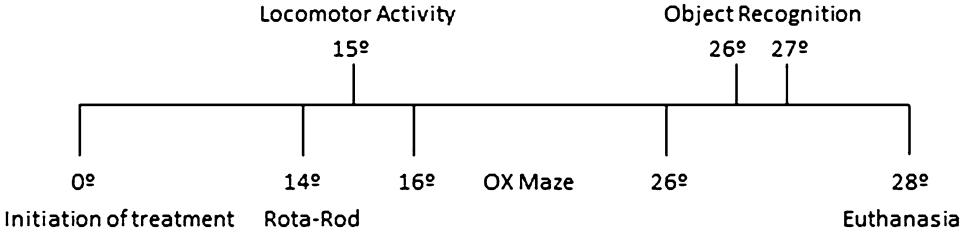

Fig. 1 Experimental design

(O, X, = , | |). To perform the test, an edible pellet (Fruit Loops, Kellogg's®, Brazil) was placed in one of the four holes on the blocks. The reward was always placed in the same place (on the block symbol O). The rats were placed on the maze and the latency to find the first reward, the number of times that the animal correctly smelled the holes of each block, and the time to complete the test were recorded for 10 min. The maze and the blocks were cleaned with 30% alcohol to prevent interference between the tests. Each circular hole received the smell of the reward to prevent the animal from orientating to the smell. The maze layout was alternated each day over the course of the 10-day experiment.

Novel object recognition memory

The object recognition test was conducted on the 27th and 28th days after the beginning of the treatment regimen. The short-term test was performed 90 min after training and the long-term test was performed 24 h later. This test was performed in the same arena that was used for the ox-maze test. Training was conducted by placing individual rats into the cages that contained two identical objects (objects A1 and A2; Duplo Lego toys) positioned in two adjacent corners, 9 cm away from the walls. Animals were left to explore the objects for 5 min. For the short-term memory (STM) test, the rats explored the cages for 5 min in the presence of one familiar object (object A) and one novel object (object B). For the long-term memory (LTM) test, the same rats explored the cages for 5 min in the presence of object A and a novel object (object C). All objects had similar textures, colours, and sizes, but had distinctive shapes. A recognition index was calculated for each animal as a ratio of TN/(TF + TN), where TF is the time spent exploring the familiar object (object A) and TN is the time spent exploring the novel object (object B or C). Between trials, the objects were washed with 10% ethanol solution. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the object was not considered exploration [32].

Assessment of markers of oxidative stress

Upon euthanasia, the brains were removed and the cortex, hippocampus, and striatum were quickly dissected and stored at −80 °C for the evaluation of markers of oxidative stress. Brain structures were homogenized in 10 vol (w:v) ice-cold 50-mM potassium phosphate buffer (pH 7.4) containing 1-mM EDTA. The homogenate was centrifuged at 1000 g for 10 min at 4 °C and the supernatant was collected for analysis.

Evaluation of the enzymatic activity of superoxide dismutase (SOD)

The superoxide dismutase activity was measured in the cortex, striatum, and hippocampus using the commercial kit RANSOD (RandoxLABs., USA) as described by Delmas-Beauvieux et al. [35]. This method employs xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a formazan derivative which is measured with a spectrophotometer at 492 nm and 37 °C. The inhibition of the chromogen production is proportional to the SOD activity in the sample. One unit of SOD causes 50% inhibition of INT reduction under the test conditions.

Evaluation of the enzymatic activity of catalase (CAT)

Catalase is an enzyme that is capable of degrading peroxides, including hydrogen peroxide (H_2O_2). Its activity is proportional to the H_2O_2 degradation rate, which is measured with a spectrophotometer at 240 nm and 25 °C [36]. CAT activity was calculated in the cortex, striatum, and hippocampus as micromoles consumed per min per mg of protein, with a molar extinction coefficient of 43.6 M/cm.

Evaluation of the enzymatic activity of glutathione peroxidase (GPx)

GPx activity was measured in the cortex, striatum, and hippocampus using a RANSEL kit (Randox Labs., USA), as previously described by Paglia and Valentine [37]. The enzyme GPx catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, oxidized glutathione (GSSG) is immediately converted into the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The reduction in absorbance at 340 nm can then be measured. GPx activity was calculated as nmol of oxidized NADPH per min per mg of protein and expressed as a percentage of control.

Evaluation of free radical production by the chemical oxidation of dichlorodihydrofluorescein (DCFH)

Cortex, striatum, and hippocampus homogenates were incubated with 100-µM diacetate 2-7-dichlorofluorescein (DCFH-DA) at 37 °C for 30 min. The DCFH-DA is then cleaved by cellular esterases to form DCFH and oxidized by reactive oxygen/nitrogen species. The formation of the dichlorofluorescein (DCF) derivative was monitored by fluorescence (SpectraMax M5) using wavelengths of 488 nm (excitation) and 525 nm (emission). The amount of reactive

species was quantified using a DCF standard curve and the results were expressed as nmoles of DCF formed per mg protein [38].

Determination of the total thiol content

This assay is based on the reduction of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) by thiol groups into the yellow oxidized compound TNB. The absorption of TNB was measured with a spectrophotometer at 412 nm.

Statistical analysis

Relative body weight was analysed with a repeated measures ANOVA. Results from the rota-rod test, the locomotor activity test, and the measurement of oxidative stress biomarkers were analysed with a one-way ANOVA and Bonferroni's post hoc test. The results from the ox-maze test were analysed with a repeated measures ANOVA and a one-way ANOVA. Comparisons between groups in the novel object recognition memory test were performed with a Kruskal–Wallis test. Values are presented as mean ± SEM. A value of $P < 0.01$ was considered statistically significant. All statistics were performed using SPSS version 18 software.

Results

After 28 days of treatment, there were no significant signs of toxicity or mortality in the rats. There also was no alteration in body weight gain or in the relative mass of the liver, kidney, and adrenals. There were no differences between treatment groups in performance on the rota-rod test ($df = 6$; $F = 1.177$; $P = 0.342$, one-way ANOVA/Bonferroni), which indicates that there was no impairment in motor control. Similarly, there were no differences in exploratory activity ($df = 6$; $F = 2.089$; $P = 0.067$, one-way ANOVA) or locomotor activity ($df = 6$; $F = 1.816$; $P = 0.110$, ANOVA).

In the ox-maze memory test, the latency time to find the first reward was lower in the groups treated with ED10, caffeine, taurine, and a combination of caffeine and taurine when compared with the control group ($df = 6$; $F = 3.773$; $P = 0.003$; one-way ANOVA/Bonferroni, Fig. 2). The latency time of the ED 10, caffeine, taurine, and caffeine + taurine-treated groups was compared to that of the control group ($df = 6$; $F = 3.773$; $P = 0.03$; one-way ANOVA/Bonferroni) until the 8th day of the task. The groups that were treated with caffeine, taurine, and caffeine + taurine took less time to complete the task than the control group ($df = 6$; $F = 5.526$; $P = 0.0001$; one-way ANOVA/Bonferroni, Fig. 3). The time that the animal remained at the correct block reward after having found and consumed the reward was also recorded, as this suggested that the animal was awaiting additional reward. The caffeine and taurine-treated groups remained at the block for less time than the control group ($df = 6$; $F = 3.104$; $P = 0.01$; one-way ANOVA/Bonferroni, Fig. 4). Furthermore, the object recognition memory test showed that treatment with a combination of caffeine and taurine improved short-term memory relative to controls ($df = 6$; $P < 0.01$, Kruskal–Wallis, Fig. 5a). There were no significant differences in long-term memory (Fig. 5b).

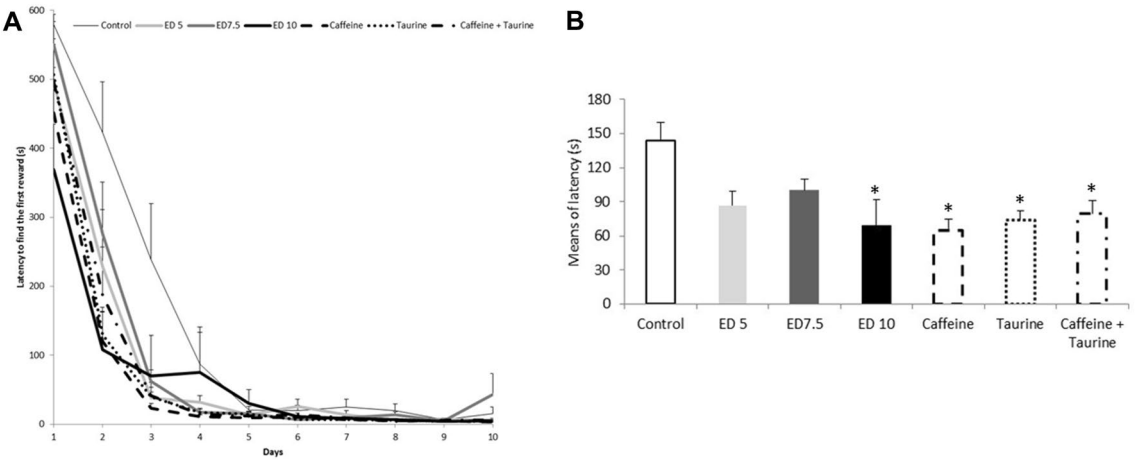

Fig. 2 Effects of the oral energy drinks 5 mL/kg (ED 5), 7.5 mL/kg (ED 7.5) e 10 mL/kg (ED 10), caffeine (3.2 mg/kg), taurine (40 mg/kg) and the combination of caffeine 3.2 mg/kg + taurine 40 mg/kg subchronic treatments (28 days) of rats in the latency time to find the first reward in the ox-maze test. The test was performed from the 18th to the 28th day after the beginning of the treatments. **a** Data represents mean daily latency ± SEM during the 10 days of testing (a, b = $P < 0.03$ repeated measures ANOVA); **b** Data represents mean ± SEM, considering the mean of the total test period ($n = 10$). *$P < 0.003$ by ANOVA/Bonferroni

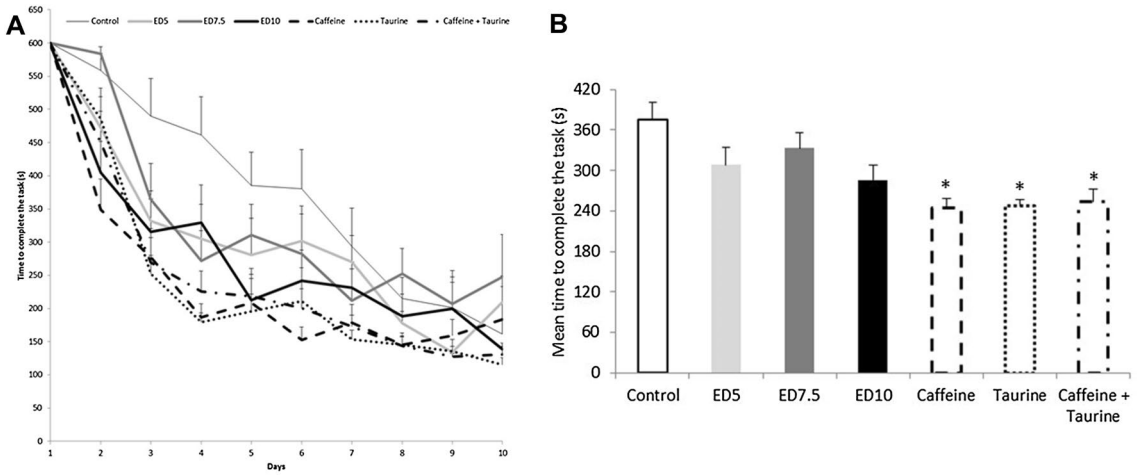

Fig. 3 Effects of the oral energy drinks 5 mL/kg (ED 5), 7.5 mL/kg (ED 7.5) e 10 mL/kg (ED 10), caffeine (3.2 mg/kg), taurine (40 mg/kg) and the combination of caffeine 3.2 mg/kg + taurine 40 mg/kg subchronic treatments (28 days) of rats in the time to complete the task in the ox-maze test. The test was performed from the 18th to the 28th day after the beginning of the treatments. **a** Data represents mean daily time to complete the task ± SEM during the 10 days of testing (a, b = $P < 0.001$ repeated measures ANOVA); **b** Data represents mean ± SEM, considering the mean of the total test period ($n = 10$). *$P < 0.0001$ by ANOVA/Bonferroni

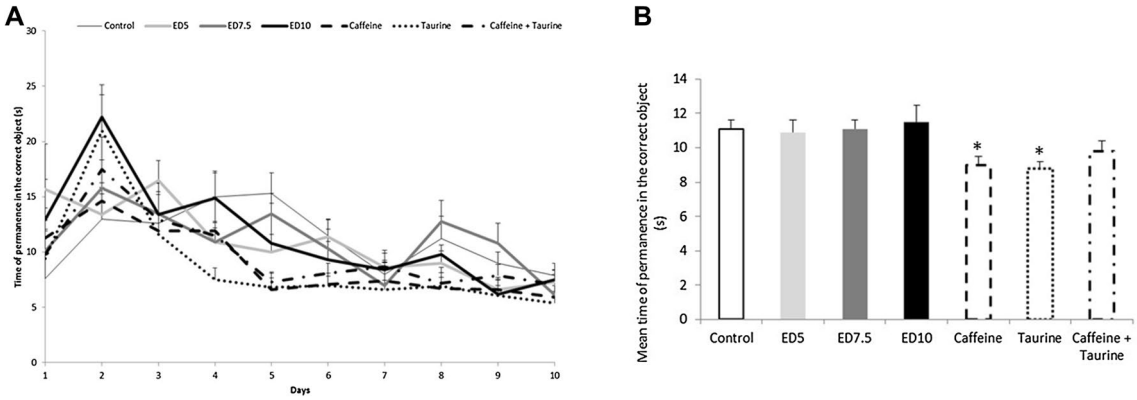

Fig. 4 Effects of the oral energy drinks 5 mL/kg (ED 5), 7.5 mL/kg (ED 7.5) e 10 mL/kg (ED 10), caffeine (3.2 mg/kg), taurine (40 mg/kg) and the combination of caffeine 3.2 mg/kg + taurine 40 mg/kg subchronic treatments (28 days) of rats in the time of permanence in the correct object in the ox-maze test. The test was performed from the 18th to the 28th day after the beginning of the treatments. **a** Data represents mean daily time of permanence ± SEM during the 10 days of testing (a, b = $P < 0,01$ repeated measures ANOVA); **b** data represents mean ± SEM, considering the mean of the total test period ($n = 10$). *$P < 0.01$ by ANOVA/Bonferroni

In brain structures that are related to memory (i.e., the prefrontal cortex (Table 1) and the hippocampus (Table 2), there was a reduction in the activity of antioxidant enzymes in most of the treated groups when compared with the control group. In the caffeine + taurine group, there was a significant reduction in CAT activity in the hippocampus ($df = 6$; $F = 3.435$; $P < 0.01$; one-way ANOVA/Bonferroni), as well as GPx activity in the prefrontal cortex ($df = 6$; $F = 7.425$; $P < 0.0001$; one-way ANOVA/Bonferroni). There was also a significant increase in the production of free radicals in the prefrontal cortex and hippocampus of these animals ($df = 6$; $F = 9.457$; $df = 6$; $F = 7.543$ $P < 0.0001$; one-way ANOVA/Bonferroni). In the groups treated with caffeine and taurine alone, there was a significant reduction in GPx activity in the prefrontal cortex, hippocampus, and striatum ($df = 6$; $F = 7.425$; $P < 0.0001$; $df = 6$; $F = 5.660$; $P < 0.001$; $df = 6$; $F = 11.156$; $P < 0.0001$; one-way ANOVA/Bonferroni) (Table 3). Moreover, SOD activity was significantly increased in the prefrontal cortex in the caffeine + taurine group and in the hippocampus of the groups treated with caffeine and taurine alone or in combination ($df = 6$; $F = 7.377$; $P < 0.0001$; $df = 6$; $F = 8.484$; $P < 0.0001$; one-way ANOVA/Bonferroni). SOD activity was significantly reduced, however, in the striatum of animals treated

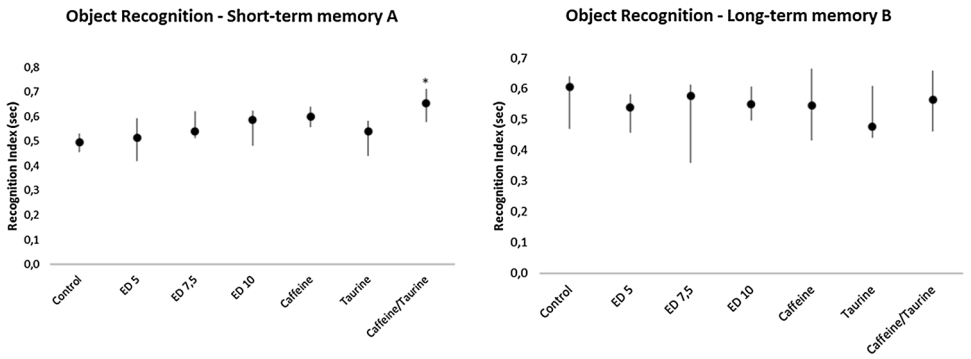

Fig. 5 Effects of the oral energy drinks 5 mL/kg (ED 5), 7.5 mL/kg (ED 7.5) e 10 mL/kg (ED 10), caffeine (3.2 mg/kg), taurine (40 mg/kg) and the combination of caffeine 3.2 mg/kg + taurine 40 mg/kg subchronic treatments (28 days) of rats in object recognition task. The test was performed from the 27th to the 28th day after the beginning of the treatments. **a** Short-term memory was evaluated 1 h 30 min after training. **b** Long-term memory was evaluated 24 h after training. "Recognition index" expressed by the ratio TN/TF + TN, TF = time spent exploring the familiar object and TN = time spent exploring the novel object. Data expressed as median (interquartile ranges), ($n = 10$). *$P < 0.01$; Kruskall–Wallis

Table 1 Oxidative stress biomarkers in prefrontal cortex of rats after 28-day oral treatment with energy 5-, 7.5- and 10-ml/kg energy drinks (ED), 3.2-mg/kg caffeine, 40-mg/kg taurine, or 3.2-mg/kg caffeine + 40-mg/kg taurine (Caf/Tau) ($n = 5$–8 animals/group)

	SOD (SOD/mg protein)	CAT (micromol/min/mg protein)	GPx (pmol NADPH oxidized/min/mg protein)	SOD/CAT + GPx	DCF (nmoles DCF per mg of protein)	Total thiols (nmol TNB/mg protein)
Control	10.7 ± 0.7	2.5 ± 0.2	160.6 ± 18.8	0.8 ± 0.7	0.7 ± 0.0	81.8 ± 2.2
ED 5	10.4 ± 1.6	1.9 ± 0.2	154.4 ± 18.1	0.1 ± 0.0	0.8 ± 0.0	72.7 ± 2.7
ED 7.5	7.0 ± 0.6	1.9 ± 0.1	100.2 ± 6.9*	0.1 ± 0.0	0.7 ± 0.0	74.0 ± 2.0
ED 10	7.4 ± 1.1	2.4 ± 0.2	107.1 ± 4.0*	0.7 ± 0.6	0.9 ± 0.0	75.8 ± 4.1
Caffeine	11.5 ± 1.0	1.6 ± 0.2	81.2 ± 4.1*	0.1 ± 0.0	0.8 ± 0.0	71.7 ± 3.3
Taurine	6.6 ± 0.5	2.1 ± 0.2	101.0 ± 7.4*	1.4 ± 0.6	0.9 ± 0.0	75.1 ± 2.6
Caf/Tau	14.1 ± 0.9*	2.3 ± 0.2	99.5 ± 6.5*	4.4 ± 0.6*	1.3 ± 0.1*	69.5 ± 3.2

Results expressed as mean ± standard error of the mean ($n = 5$–8 animals/group)

* Significantly different from control group ($p < 0.0001$) by ANOVA/Bonferroni

Table 2 Oxidative stress biomarkers in hippocampus of rats after 28-day oral treatment with energy 5-, 7.5- and 10-ml/kg energy drinks (ED), 3.2-mg/kg caffeine, 40-mg/kg taurine, or 3.2-mg/kg caffeine + 40-mg/kg taurine (Caf/Tau)

	SOD (SOD/mg protein)	CAT (micromol/min/mg protein)	GPx (pmol NADPH oxidized/min/mg protein)	SOD/CAT + GPx	DCF (nmoles DCF per mg of protein)	Total thiols (nmol TNB/mg protein)
Control	5.7 ± 0.3	2.2 ± 0.2	90.6 ± 4.3	0.06 ± 0.0	1.3 ± 0.0	70.1 ± 2.0
ED 5	6.7 ± 0.4	2.4 ± 0.2	106.4 ± 11.3	0.07 ± 0.0	1.4 ± 0.0	70.3 ± 4.3
ED 7.5	7.0 ± 0.7	1.8 ± 0.2	81.4 ± 5.7	0.07 ± 0.0	1.3 ± 0.0	67.1 ± 2.8
ED 10	7.7 ± 0.3	1.7 ± 0.0*	84.5 ± 6.5	0.6 ± 0.5	1.4 ± 0.0	77.0 ± 2.4
Caffeine	8.5 ± 0.7*	1.6 ± 0.2*	60.3 ± 5.3*	0.1 ± 0.0	1.3 ± 0.0	70.1 ± 2.2
Taurine	10.3 ± 0.4*	2.2 ± 0.1	62.8 ± 8.9*	1.3 ± 0.8	1.4 ± 0.0	68.8 ± 2.0
Caf/Tau	8.3 ± 0.5*	1.6 ± 0.0*	96.2 ± 5.3	5.3 ± 0.4*	1.7 ± 0.0*	71.5 ± 1.1

Results expressed as mean ± standard error of the mean ($n = 5$–8 animals/group)

* Significantly different from control group ($p < 0.0001$) by ANOVA/Bonferroni

Table 3 Oxidative stress biomarkers in striatum of rats after 28-day oral treatment with energy 5-, 7.5- and 10-ml/kg energy drinks (ED), 3.2-mg/kg caffeine, 40-mg/kg taurine, or 3.2-mg/kg caffeine + 40-mg/kg taurine (Caf/Tau)

	SOD (SOD/mg protein)	CAT (micromol/min/mg protein)	GPx (pmol NADPH oxidized/min/mg protein)	SOD/CAT + GPx	DCF (nmoles DCF per mg of protein)	Total thiols (nmol TNB/mg protein)
Control	14.2 ± 0.7	2.0 ± 0.2	181.6 ± 9.3	0.1 ± 0.0	1.4 ± 0.0	76.9 ± 2.9
ED 5	12.3 ± 0.6	2.0 ± 0.1	118.5 ± 21.7*	0.6 ± 0.5	1.5 ± 0.1	76.9 ± 3.4
ED 7.5	13.5 ± 0.8	2.1 ± 0.3	125.5 ± 7.5*	0.8 ± 0.7	1.5 ± 0.1	76.2 ± 2.4
ED 10	9.9 ± 1.2*	2.0 ± 0.2	134.2 ± 6.0*	0.1 ± 0.0	1.5 ± 0.0	73.5 ± 1.9
Caffeine	9.1 ± 0.5*	2.4 ± 0.3	122.2 ± 5.3*	0.1 ± 0.0	1.6 ± 0.0	81.6 ± 3.3
Taurine	9.0 ± 0.7*	2.6 ± 0.2	95.7 ± 8.4*	1.7 ± 0.8	1.6 ± 0.0	78.6 ± 1.7
Caf/Tau	15.5 ± 1.2	2.7 ± 0.3	81.6 ± 7.8*	5.6 ± 1.8*	1.6 ± 0.0	80.2 ± 1.3

Results expressed as mean ± standard error of the mean (n = 5–8 animals/group)

* Significantly different from control group ($p < 0.0001$) by ANOVA/Bonferroni

with ED10 or caffeine or taurine alone ($df = 6$; $F = 8.539$; $P < 0.0001$; one-way ANOVA/Bonferroni). CAT activity was also significantly reduced in the hippocampus only of ED10, caffeine, and caffeine + taurine-treated animals. Furthermore, there was an increase in the ratio of SOD to CAT + GPx in all three structures of the group that were treated with the combination of caffeine and taurine. This imbalance in enzymatic activities was accompanied by an increase in the production of free radicals in the prefrontal cortex and hippocampus in the caffeine + taurine-treated group. The total thiol content did not change in response to any of the treatments.

Discussion

Given the excessive consumption of energy drinks and the fact that their toxicological risks are largely unknown, we sought to evaluate their effects on behaviour and oxidative stress. Behavioural and neurochemical measures were used in this study to examine the effects of subchronic exposure to energy drinks and their main constituents. Animals that were treated with energy drinks showed improved attention with no changes in locomotion. There was also an imbalance in the activity of antioxidant enzymes with no alterations in free radical production. Animals that were treated with a combination of caffeine and taurine, which are both present at high doses in energy drinks, showed improved memory and attention, as well as an increase in oxidative imbalance.

One of the behavioural tests that was used in this study was the ox-maze task, which is used as an assessment of memory and attention [34]. It was originally designed to brain train R6/2 mice, which serve as a mouse model of Huntington's disease, and as a cognitive task that requires visual discrimination and spatial memory to find a food reward [33]. In our test, exposure to caffeine and taurine, alone or in combination, resulted in better performance in the major parameters that were assessed. In addition, the combination of caffeine and taurine yielded improvements in short-term memory during the object recognition memory test. These results are in agreement with other studies that have already demonstrated that both taurine [16], and caffeine [39], play a major role in tasks that require attention, memory and faster performance in a short period of time. Caffeine consumption at moderate (200 mg) and high (400 mg) doses led to an increase in alertness, learning, memory, and mood states, and improved performance in visual attention tasks [40] [41]. In this study, caffeine (3.2 mg/kg) was able to improve memory and attention. Brunyé et al. [42] has suggested that the effect of caffeine on attention is due to its antagonist activity at A_1 and A_2 adenosine receptors in areas with high dopaminergic innervation. The administration of taurine, the other component of energy drinks that was examined in this study, resulted in improvements in memory and attention without affecting locomotion. Taurine has previously been used in behavioural tests, and may be related to the recovery of memory deficits induced by alcohol, pentobarbital, sodium nitrate, and cycloheximide in mice, without any observable effects on other behaviours, such as coordination, exploratory activity, and locomotion [43].

The central nervous system is particularly vulnerable to oxidative stress-induced damage and was a primary point of consideration in this study. Tissue homeostasis is dependent on a balance between antioxidant enzyme activity and the production of reactive species. An important antioxidant defence mechanism is the dismutation of superoxide anion (O^{2-}) to form hydrogen peroxide (H_2O_2) and O_2 by SODs, as well as the transformation of H_2O_2 to H_2O by GPx or to O_2 and H_2O by CAT, which prevents the formation of the hydroxyl radical. GPx and CAT play an essential role in scavenging hydrogen peroxide and, in conjunction with SOD, mediate antioxidant protection [28, 44, 45]. An excess of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can lead to tissue damage in a variety of ways.

These compounds can directly damage proteins, DNA, and lipids and therefore affect all cellular functions [46]. Among the brain structures that are involved in the formation and retention of memory, the hippocampus and the prefrontal cortex play a central role, whereas the striatum, which is part of the basal ganglia, is involved in storing and retrieving learned motor behaviour [47].

Chronic ingestion of coffee and caffeine modulates the brain's antioxidant system in rats by reducing lipid peroxidation, increasing the concentration of glutathione, and increasing the activity of the antioxidant enzymes glutathione reductase and SOD [48]. This may be due to the fact that caffeine is acting as an acceptor of hydroxyl radicals [49], which would prevent lipid peroxidation and inhibit oxidative damage [50]. In contrast, subchronic caffeine exposure in our studies led to a reduction in GPx activity in the prefrontal cortex, hippocampus, and striatum, and also led to a reduction in the activity of CAT in hippocampus and the activity of SOD in the striatum. These brain structures were, therefore, more susceptible to oxidative damage. According to Martini et al. [51], studies that have examined coffee or caffeine consumption have found that caffeine can increase glutathione levels and enhance cellular protection, especially when it is consumed regularly. However, the effects of both chronic and acute coffee consumption are inconsistent with regards to the total plasma antioxidant capacity, antioxidant enzymes, lipid damage, and damage to proteins. This may provide an explanation as to why the results of our study differ from others in which caffeine and its analogue theobromine led to the in vitro production of oxygen radicals [52–54]. Given this, the focus of many new studies has been on the dosage that produces free radicals and the damaging effects that it has on the body.

Taurine has been studied in in vitro models, where it has been shown to act as a scavenger of reactive oxygen (peroxyl radical and anion superoxide) and nitrogen (nitric oxide and peroxynitrite) species [55]. According to Jong et al. [56], taurine serves as a regulator of mitochondrial protein synthesis by enhancing electron transport chain activity and protecting mitochondria against excessive superoxide generation. Taurine administration (50, 250, and 500 mg/kg) over 60 days in Wistar rats altered GPx and GR activities as well as GSH levels [57], which indicates that taurine yields antioxidant effects. In the present work, taurine reduced GPx activity in all three of the brain structures that were examined. SOD activity was also reduced in the striatum, while CAT activity was reduced in the hippocampus. Based on our results and on the previous literature, it is reasonable to conclude that the dosage of taurine or the time of administration can affect the enzymatic antioxidant system and consequently interfere with its antioxidant profile.

In this work, the combination of caffeine and taurine induced an oxidative imbalance in both the hippocampus and the prefrontal cortex. This may have been due to a reduction in the activity of antioxidant enzymes and a consequent increase in free radicals. This, in turn, could render the brain more susceptible to oxidative damage. In a previous study, human SH-SY5Y cells were incubated with components of energy drinks, particularly guarana (3.125–50 mg/mL), alone or in combination with caffeine (0.125–2 mg/mL) or taurine (1–16 mg/mL). This treatment produced an antioxidant effect, which may have resulted in a reduction in cellular enzymatic antioxidant defence mechanisms (e.g., the activity of SOD and CAT) because there was a reduction in the generation of free radicals [23].

In summary, the subchronic exposure of male Wistar rats to a combination of caffeine and taurine revealed signs of neurotoxicity that were evidenced by a reduction in antioxidant enzymatic defences. This occurred at the same doses that increased attention and memory, which are the effects that are desired by consumers. Oxidative damage was not observed in rats that received energy drinks. The protective effect observed in the energy drink groups may have been related to the presence of other components in the formulation that alter the ability of caffeine and taurine to contribute to oxidative damage. In fact, it is known that the association of substances with similar or opposite actions can antagonize, increase, or potentiate their pharmacological actions, which could, therefore, produce several unexpected reactions. Taking the possible interference of other constituents in the behavioural and neurochemical effects of energy drinks into consideration, we conclude that it is important to control the ingredients that are present in commercial products and the safety of these formulations.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflict of interests.

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