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Effects of energy drinks on biochemical and sperm parameters in Wistar rats

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Abstract

Background: The present study evaluates the effects of energy drinks on the reproductive and biochemical parameters of adult male rats.

Methods: A total of 40 male rats (Wistar) were exposed to an energy drink mixed with the drinking water for a period of 120 days. The animals were divided into four groups and exposed to increasing therapeutic doses (DT) of an energy drink, based on allometric extrapolation, resulting in values (mL/day) per animal of 250 g: DT1 2.36 mL, DT3 7.47 mL, and DT6 14.16 mL. The control group (CTRL) consumed water only. During the treatment, the rats were assessed for signs of toxicity. After treatment, the animals were sacrificed and their organs were weighed. Sperm parameters (motility, concentration, and morphology) were evaluated. The biochemical markers alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactic dehydrogenase, urea, creatinine, creatine phosphokinase, and creatine kinase MB fraction were measured, in addition to total cholesterol and testosterone.

Results: There was a significant decrease ($p < 0.05$) in the concentration of sperm in the treated groups (DT1 8.5 ± 0.7; DT3 7.2 ± 0.9; DT6 8.4 ± 0.9) compared to the control group (12.3 ± 1.2). No difference was observed with respect to relative weights of the animals' organs, water consumption, signs of toxicity, behavioral changes, biochemical markers, and sperm motility and morphology.

Conclusion: The long-term consumption of energy drinks interferes negatively with sperm concentration, without affecting sperm motility and morphology or altering the hepatic, cardiac, or renal functions.

Keywords: Rats, Energy drink, Sperm parameters, Toxicity, Biochemical markers, Heart, Liver, Kidney, Spleen, Reproductive organs

Background

The consumption of energy drinks has increased worldwide, since their appearance on the market in 1987. Their purpose is to increase the physical stamina, promote faster responses, and higher mental concentration of the organism, diminishing sleep needs and keeping the body in a state of alert [1, 2]. In addition to water, energy drinks contain ingredients such as caffeine, taurine, guarana, glucuronolactone, vitamins, and carbohydrates. Carbohydrates provide nutrients for energy, and caffeine stimulates the central nervous system [1].

These drinks eliminate the signs of tiredness produced naturally by the body, and because of this feature, many people choose to use them to allow them to increase their workload or improve their performance. Among those people who tend to abuse of these types of drinks, one finds mainly athletes and students, who attempt to increase their concentration and their physical or mental abilities for hours [3]. Knowing the composition of energy drinks and the fact that they contain psychoactive substances with highly stimulating properties, an important factor to consider is the question of caffeine concentrations, which can vary between 50 mg per 250 mL can and 505 mg per 1 L bottle, which may result in poisoning or even overdose [4]. In addition to poisoning by caffeine, the consumption of energy drinks has been

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associated with seizures [5], strokes [6], and gonadotoxic effects [7].

A significant increase in the incidence of male infertility has been described in the literature worldwide, which generates questions about its causes. There are several substances present in our daily lives that exhibit potential interferences with biological functions, such as reproduction, embryonic development, growth, and metabolism [8]. For this reason, these substances are more frequently becoming the focus of research with the aim of observing their effects in the long term and their consequences for humans, even at very low concentrations [9].

The aim of this study is to evaluate the effect of energy drinks on hepatic, cardiac, and renal functions, as well as on sperm parameters of male Wistar rats. The biochemical markers in blood serum for the above functions and testosterone will be analyzed, together with the sperm parameters including motility, concentration, and morphology.

Methods

Animals and treatment

For this study, 40 male rats (Wistar) 60 days old and with an average weight of 250 g were obtained from the vivarium of the University of "Vale do Itajaí–UNIVALI, SC". This study was approved by the Ethics Committee on Animal Use (CEUA) under the number 031/14.

The animals were fed at will and were kept under cycling light/dark conditions of 12/12 h and controlled temperature (22 ± 2 °C). Their cages were equipped with wood shavings and paper towel as a way to provide an enriched environment. The animals were divided into four groups of ten individuals, allocated in three cages with three, three and four rats per cage, in accordance with the guide for the care and use of laboratory animals [10]. The dose of energy drink was administered through water bottles. The animals were exposed to increasing therapeutic doses (DT) of energy drink, based on allometric extrapolation, resulting in values per animal of 150 g: Group 1 received DT1 = 2.36 mL per day, group 2, DT3 = 7.47 mL, group 3, DT6 = 14.16 mL. The control group consumed only water. The energy drink (commercial name not disclosed) contained the following components according to the manufacturer: 80 mg of caffeine, 1 g of taurine, group B vitamins (B2, B3, B5, B6, and B12), 27 g of carbohydrates, and 50 mg of glucuronolactone.

The animals were not submitted to any restrictions or control regarding food consumption. The energy drinks were diluted with water in appropriate proportions and supplied trough water bottles suitable for rodents, which were controlled daily to ensure that the entire dose was ingested and supplying untreated water only after total consumption. The average daily consumption for a 70 kg adult human is 250 mL of energy drink; however, the manufacturer notes that the maximum dose consumed in a day should not exceed 400 mg of caffeine, i.e., 1250 mL, a value above which caffeine intoxication may occur. To determine the quantity of energy drink to be administered to one rat in order to have an equivalent exposition to that of an adult man drinking a can of 250 mL, we followed an allometric scale [11]. The doses were adapted according the average weight of the animals of each cage, according to the following formula:

$$\mathrm{DDR} = \mathrm{DDM} / (\mathrm{k} \times \mathrm{WM})^{0.75} \times (\mathrm{k} \times \mathrm{WA})^{0.75}$$

where DDR: daily dose for a rat, DDM: daily dose for a man, k: metabolic constant (= 70 for both man and rat), WM: weight of a man (70 kg), WA: weight of the animal.

During the 120-day treatment period, the animals were observed for signs and symptoms that could indicate systemic toxicity or decrease in well-being such as piloerection, behavioral changes, bent posture, changes in food and water consumption, and alterations in body weight. These aspects were evaluated daily when researchers interacted with the animals, during supply of energy drink, water, food, or cleaning of the cages.

The animals were weighed weekly, and the doses of energy drink adapted accordingly, using the above-mentioned formula.

After the treatment period, the animals were sacrificed in a CO_2/O_2 (from 30/70% to 100/0%) chamber. During the entire procedure, the animals were kept in observation, until they fell and the respiratory arrest was confirmed. Once these signs were clearly irreversible, the following biochemical, anatomical, and sperm analyses were performed.

Biochemical analyses

Blood was collected by cardiac puncture before total heart arrest using a 5-mL syringe and a 25 × 7 hypodermic needle. Serum was obtained by centrifuging the blood in tubes containing a coagulation accelerator, without anticoagulants, at 3000 RPM for 10 min. The chemical analyses were performed in serum samples using an automated analyzer for clinical chemistry (Roche Cobas Mira, São Paulo, Brazil). Diagnostic kits (Labtest®) were used for assessing the biochemical markers, such as the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (FAL), as well as urea, creatinine, creatine phosphokinase (CPK), and creatine kinase MB fraction (CK). Total cholesterol and testosterone were also analyzed.

Anatomical analysis

The liver, kidney, spleen, testes, seminal vesicles, and epididymal fat were individually weighed, and the relative weight of each organ/gland was calculated in respect to the final weight of the animal and expressed as a percentage.

Seminal analysis

Both vas deferens were dissected over a length of 1 cm starting close to the epididymis [12]. The cut pieces were placed for 10 min at 37 °C in 0.3 mL modified HTF medium (Irvine Scientific, Spectrun, Brazil) containing 10% fetal bovine serum (Cripion®, Brazil), in order to allow dispersion and capacitation of spermatozoa. These standardized conditions were rigorously respected for each animal, in order to diminish the risks of compromising sperm counts by performing different cuts in each case.

Sperm concentrations were determined using Makler counting chamber (Sefi Medical Instrument, Itajaí, Brazil). Observations were made using an Olympus microscope (Olympus, Brazil) at ×100 magnification. Results are expressed as a number of millions per milliliter. For motility determinations, a 20-μL aliquot of the sperm suspension was placed on a slide and covered with a coverslip 24 × 24 mm (KASVI, Curitiba, Brazil): 200 spermatozoa were counted and classified as motile or immotile. The results are expressed as the percentage of motile cells. For sperm morphology, smears were prepared using a 10-μL aliquot of the sperm suspension and stained with the hematological kit Panótico (NewProv®, Pinhais, Brazil). In all, 200 spermatozoa were classified as normal or abnormal (no hook, banana-shaped, triangular, or amorphous head).

Statistical analysis

Data were submitted to statistical analysis (Instat, GraphPad Software, USA) using ANOVA and Tukey's multiple mean comparison between the four groups. The significance level was set at $p < 0.05$.

Results

During the 120 days of treatment, the animals treated with the energy drink showed no signs of systemic toxicity, such as irritability, weight loss, piloerection, behavioral changes, bent posture, or diarrhea. There were also no changes in water consumption between the groups.

The relative weights of the organs and glands (liver, kidneys, spleen, testes, seminal vesicles, and epididymal fat) between the groups were similar, as well as the animals' weight gain (Table 1). All animals gained weight in a physiological manner throughout the experimental period (Fig. 1) and there no significant differences between groups.

The values measured in the serum for aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (FAL), creatinine phosphokinase (CPK), and creatine kinase fraction MB (CKMB) are presented in Table 2. Values found in the treated groups were not statistically different from those of the control group (Table 2).

There was a significant decrease ($p < 0.05$) in the concentration of sperm in the treated groups (DT1 8.5 ± 0.7; DT3 7.2 ± 0.9; DT6 8.4 ± 0.9) compared to the control group (12.3 ± 1.2). However, other sperm parameters (motility and morphology) were not significantly different between groups (Table 3).

Discussion

This study investigates the effects of energy drinks on the reproductive system of male rats and their potential effects on the several biochemical and biological parameters. The therapeutic dose (DT1) was calculated using an interspecific allometric scaling, based on the dose corresponding to one can of energy drink (250 mL) by an adult human. Higher treatment doses of 3× DT (DT3) and 6× DT (DT6) were also applied in order to investigate higher dosages where negative effects might be more clearly visible.

Table 1 Body gain (g) and organ/gland weights relative to the final weight (%) of the control group (CTR) and the energy-drink-treated groups (DT1, DT3, and DT6)

	CTR	DT1	DT3	DT6	*p* value
Body weight gain (g)	115.7 ± 6.0	100.2 ± 8.7	116.7 ± 6.0	119.3 ± 6.9	NS
Liver (%)	3.16 ± 0.12*	3.44 ± 0.14	2.98 ± 0.10	2.86 ± 0.15*	NS
Spleen (%)	0.17 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	NS
Kidneys (%)	0.59 ± 0.02	0.64 ± 0.03	0.54 ± 0.02	0.55 ± 0.03	NS
Testes (%)	0.82 ± 0.04	0.83 ± 0.07	0.69 ± 0.06	0.82 ± 0.04	NS
Seminal vesicles (%)	0.32 ± 0.02	0.35 ± 0.01	0.32 ± 0.02	0.32 ± 0.02	NS
Epididymal fat (%)	1.74 ± 0.13	1.89 ± 0.12	1.57 ± 0.14	1.89 ± 0.28	NS

Values represent means ± standard error of the mean. Data were evaluated by analysis of variance (ANOVA) and Tukey's test for comparison of the means. Level of significance was set at $p < 0.05$

*$p < 0.01$

NS not significant

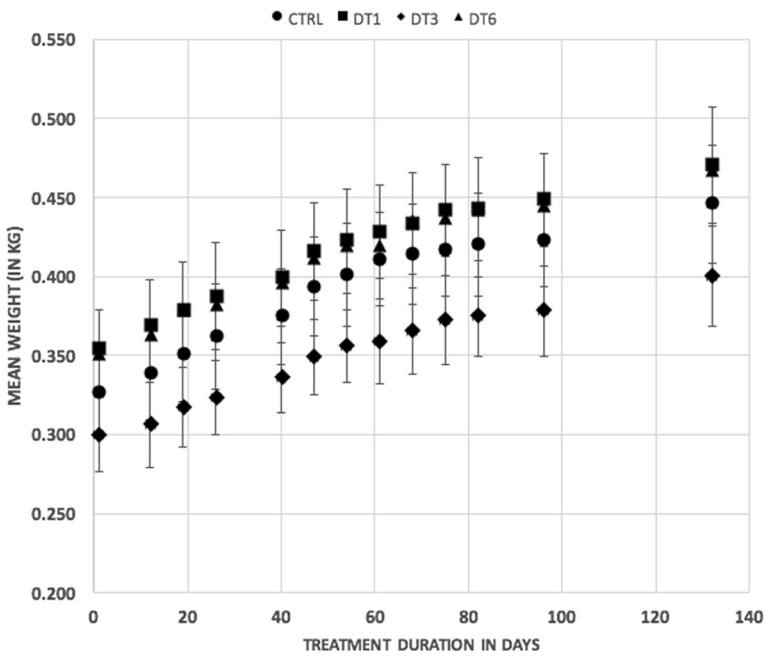

Fig. 1 Mean animals' weights during treatment. Mean animals' weights (in kg) in the four groups during the treatment period (in days). *Error bars* indicate the standard deviation. CTRL (*black circle*) was not significantly different from the treated groups, DT1 (*black square*), DT3 (*black diamond*), DT6 (*black triangle*). Treatment started on day 1

With regard to weight gain, all animals behaved similarly and showed the expected physiological gain over the treatment period (Fig. 1). This is unlike what one could expect from human studies, where the intake of sugar-added beverages has been shown to contribute to weight gain and eventually obesity [13]. However, one cannot neglect the thermogenic effect of caffeine, a substance present in large concentrations in energy drinks, which may have been responsible for some level of weight control, especially at higher doses.

Signs of toxicity may be associated with several compounds present in the energy drink, especially to caffeine. At high levels, caffeine may cause adverse health effects by altering the functioning of the cardiovascular system, causing an imbalance in calcium, and increasing the risk of cancer and even death [14]. Although publications in this field are contradictory, the evidence suggests that due to a lack of sufficient studies on the long-term effects of caffeine intake, caffeine consumption should be considered with caution [14]. In our

Table 2 Biochemical parameters of the control group (CTRL) and the energy-drink-treated groups (DT1, DT3, and DT6)

Biochemical parameters	CTR	DT1	DT3	DT6	*p* value
AST (U/L)	113.5 ± 14.5	95.9 ± 9.3	89.9 ± 8.7	86.5 ± 11.9	NS
ALT (U/L)	58.9 ± 3.4	52.8 ± 4.7	55.2 ± 3.3	56.9 ± 5.10	NS
FAL (U/L)	152.8 ± 24.8	163.1 ± 26.5	133.5 ± 24.2	145.2 ± 26.4	NS
Cholesterol total (mg/dL)	85.7 ± 3.2	90.6 ± 5.2	80.3 ± 2.7	93.4 ± 8.0	NS
LDH (U/L)	632.4 ± 141.3	475.9 ± 114.7	325.0 ± 65.0	336.7 ± 43.2	NS
Glucose (mg/dL)	145 ± 8.8	152.8 ± 9.2	163.9 ± 8.4	142.6 ± 7.5	NS
Urea (mg/dL)	41.4 ± 1.9	39.5 ± 1.8	40.3 ± 1.2	37.8 ± 1.9	NS
Creatinine (mg/dL)	0.46 ± 0.03	0.42 ± 0.03	0.42 ± 0.03	0.45 ± 0.07	NS
CKMB (U/L)	200.4 ± 20.0	150.4 ± 13.8	150.7 ± 8.2	198.6 ± 23.2	NS
CPK (U/L)	376.4 ± 154.9	357.6 ± 207.1	266.8 ± 128.3	120.3 ± 18.4	NS
Testosterone (ng/dL)	181.4 ± 22.5	284.8 ± 45.6	293.5 ± 98.8	226.2 ± 31.8	NS

Values represent means ± standard error of the mean. Tested parameters (units in parenthesis): aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (FAL), creatinine phosphokinase (CPK), and creatine kinase fraction MB (CKMB). Data were evaluated by analysis of variance (ANOVA) and Tukey's test for comparison of the means. Level of significance was set at $p < 0.05$
NS not significant

Table 3 Sperm parameters of the control group (CTRL) and the energy-drink-treated groups (DT1, DT3, and DT6)

Sperm parameters	CTR	DT1	DT3	DT6	*p* value
Concentration (10^6/mL)	12.3 ± 1.18	8.5 ± 0.67*	7.2 ± 0.90*	8.4 ± 0.90*	*
Motility (%)	65.6 ± 1.13	64.63 ± 2.34	65.3 ± 7.40	67.6 ± 1.68	NS
Normal morphology (%)	96.2 ± 0.49	96.2 ± 0.53	95.4 ± 0.75	96.3 ± 0.72	NS

Values represent means ± standard error of the mean. Data were evaluated by analysis of variance (ANOVA) and Tukey's test for comparison of the means. Level of significance was set at $p < 0.05$
*Significantly lower than the control ($p < 0.05$)
NS not significant

study, no signs of systemic toxicity were observed during the 120 days of exposure to the energy drink. There were no changes in behavior and in ingestion of liquids in the treated groups, a fact that may have been altered in view of the amount of glucose supplied.

Energy drinks have not been evaluated experimentally, despite their routine use by many young people and adults. The literature is limited to studies on these drinks' individual components, most of these at a preliminary stage, with the exception of caffeine, whose mechanism and mode of action on the body is almost completely elucidated. The effects of guarana, for example, are still poorly understood, although it is recognized that products with high amounts of guarana have physiological effects similar to those of caffeine. The same scarcity of studies exists for taurine and other ingredients contained in energy drinks, as well as, for the cumulative effects of these substances with other products such as alcohol or drugs [15].

All of the biochemical markers tested were identical between the treated and the control groups. A normal value for total cholesterol could be explained by the presence of the amino acid taurine, the function of which is to maintain the solubility of cholesterol by binding it to certain bile salts, therefore improving its ability to be digested [16]. However, according to Du et al. [17], caffeine would induce a dose-dependent increase in total cholesterol, HDL, and LDL. According to a study conducted by Onuegbu et al. [18], on the biochemical profiles of healthy men and women who consumed 2 g of coffee, daily for 30 days, it was observed that some markers were high, such as AST, ALT, FAL, and total proteins. Another study shows that the energy drinks also affect the concentration of creatinine, uric acid, albumin, and total protein [19].

The microscopic evaluation of sperm concentration, motility, and morphology is an essential step for predicting the reproductive potential of the males of any species. In our study, sperm motility was not significantly different between groups (Table 3), which suggests that the energy drink did not measurably influence this parameter under our conditions. This finding is in contrast to studies demonstrating the beneficial potential of caffeine on sperm motility in animals and humans [20, 21] or that of taurine, which might, in the long term, have a protective effect through its antioxidant properties [22]. Nevertheless, there is a lack of research on the effects of energy drinks on sperm parameters, which might help us to sort out the individual and cumulative effects of the various compounds included in such beverages.

Similarly, normal sperm morphology did not show statistical differences between the control and the treatment groups (Table 3). The predominant characteristic of spermatozoa classified as abnormal was the absence of a hook. This might illustrate damages to DNA packing and integrity, which could directly influence the reproductive potential of the treated animals. It would be interesting to perform an assessment of DNA fragmentation in follow-up studies, in addition to fertility tests of the treated males in order to rule out the possible transmission of genetic or epigenetic alterations to the offspring [23].

The only sperm parameters that showed a negative sensitivity towards energy-drink administration was the sperm concentration (Table 3). Due to its gonadotoxic and pro-oxidant properties, caffeine possesses preferential targets on Sertoli cells and spermatogonia and appears to cause little harm to spermatids, differentiating and mature spermatozoa [7, 24]. At high caffeine concentrations, these effects would result in a reduction of sperm concentrations in a similar fashion as other compounds do either directly or through the endocrine system. If the damages to sperm cells occur during or after spermatogenesis, the effects may be reversed once exposure to the harmful substance is discontinued, unless spermatogonia are also damaged and azoospermia eventually occurs [24]. The significant decrease in sperm count observed in this study suggests that the energy drink might have damaged spermatogonia and/or Sertoli cells, but this can only be confirmed histologically and by a withdrawal experiment in an attempt to see if sperm concentrations return to the level of the control when energy drink administration is discontinued. Any damage to Sertoli cells will result in a decrease of inhibin B and an increase in the levels of FSH [25, 26], both hormones that might help to determine the mechanisms of action of energy drinks in further studies. Interestingly, in a preconception cohort, a recent study showed that caffeinated soda and energy drink intake were associated

with reduced fecundability among males, but not among females [27].

Testosterone levels did not show any difference between the control and treated groups (Table 2). Due to the high stability of testosterone in the blood, the levels of this hormone may take time to fall depending on the severity of the cellular injury of the Leydig cells and individual characteristics [26, 28]. To better understand the reason for the decrease in sperm concentrations, measuring LH and FSH levels might help to determine which mechanism is responsible (endocrine versus sperm maturation defect). The length of treatment was approximately two spermatogenic cycles, which in rodents lasts approximately 54 days [29], thus, this treatment might also have to be extended. The protocol of the study did not include dosages of LH and FSH because a reduction in sperm concentration was not anticipated. Furthermore, the serum volume collected did not allow for more analyses than those that were performed. The fact that T was not altered by the treatment suggests that LH was not affected either. The most important hormone remains FSH which should be analyzed, together with histological observations of the testes in further studies.

Conclusion

The energy drinks, when consumed on a long-term basis and in high concentrations, interfere negatively with sperm concentration in rats, while motility, morphology, water consumption, and signs of toxicity remain unchanged. It has been ruled out that energy drinks, in the respective doses, may result in hepatic, renal, and/or cardiac damage. Further studies on a larger cohort are needed to specifically locate the mode of action of energy drink either directly on spermatogenesis, through endocrine hormones or other metabolic pathways.

Abbreviations

CTRL: Control group; DT1, DT3, DT6: Treated groups with respectively 1, 3, and 6 therapeutic doses of energy drink per day

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Availability of data and materials

The datasets used and analyzed during the current study can be obtained from the corresponding author on reasonable request.

Authors' contributions

DS and ES performed all animal handling during the experiment, collected the data, and wrote the manuscript. RAS contributed to the animal handling and provided technical guidance. AEDN performed all biochemical tests at LEAC (Laboratório Escola de Análises Clínicas da UNIVALI). DT advised on welfare and accommodation conditions of the animals during the whole treatment period. APS accompanied the development of the project and contributed to the translation into English and to the revisions of the manuscript. VLLA supervised the project, analyzed the dataset, and contributed to all steps of the experiment up to the manuscript finalization. All authors read and approved the final manuscript.

Ethics approval

This study was approved by the Ethics Committee on Animal Use (CEUA) of the UNIVALI University under the number 031/14.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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