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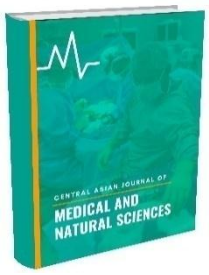

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Effects of Energy Drinks on Biochemical and Sperm Parameters in Albino Rats

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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. 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.

KEYWORDS: Albino Rats, Energy drink, Biochemical markers, Heart, Liver, Kidney, Spleen, Reproductive organs .

The consumption of energy drinks has increased world- wide, since their appearance on the market in 1987. Their purpose is to increase the physical stamina, promote faster responses, and higher mental concentra- tion of the organism, diminishing sleep needs and keep- ing 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 carbo- hydrates. 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 en- ergy drinks and the fact that they contain psychoactive substances with highly stimulating properties, an import- ant factor to consider is the question of caffeine concen- trations, which can vary between 50 mg per 250 mL can and 505 mg per 1 L

bottle, which may result in

caffeine, the consumption of energy drinks has been associated with seizuresstrokes [6], and gonadotoxic effects [7].

A significant increase in the incidence of male infertility has been described in the literature worldwide, which gener- ates 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 func- tions and testosterone will be analyzed, together with the sperm parameters including motility, concentration, and morphology.

MethodsAnimals 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 cyc- ling 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 allo- metric 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 (com- mercial 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 con- sumed 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 equiva- lent 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 ani- mals of each cage, according to the following formula: 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 re- searchers 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 sacri- ficed 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 irrevers- ible, 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 hypoder- mic 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 dehydro- genase (LDH), and alkaline phosphatase (FAL), as well as urea, creatinine, creatine phosphokinase (CPK), and creatine kinase MB fraction (CK). Total cholesterol and testosterone were Anatomical analysis

The liver, kidney, spleen, testes, seminal vesicles, and epididymal fat were individually weighed, and the rela- tive 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 stan- dardized 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 milli- liter. 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 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 tox- icity, such as irritability, weight loss, piloerection, behav- ioral 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 ani- mals' weight gain (Table 1). All animals gained weight in a physiological manner throughout the experimental period (Fig. 1) and there no significant differences be- tween groups.

The values measured in the serum for aspartate amino- transferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (FAL), cre- atinine 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

There was a significant decrease ($p < 0.05$) in the con-centration 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 differ- ent between groups

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 param- eters. 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.With regard to weight gain, all animals behaved simi- larly 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 sub- stance 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 com- pounds present in the energy drink, especially to caf- feine. 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 publi- cations 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 consump- tion should be considered with caution [14]. In our 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 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 pre- liminary stage, with the exception of caffeine, whose mechanism and mode of action on the body is almost completely elucidated. The effects of guarana, for ex- ample, are still poorly understood, although it is recog- nized 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 in- gredients contained in energy drinks, as well as, for the cumulative effects of these substances with other prod- ucts 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, al- bumin, and total protein [19].

The microscopic evaluation of sperm concentration, motility, and morphology is an essential step for predict- ing the reproductive potential of the males of any spe- cies. In our study, sperm motility was not significantly different between groups (Table 3), which suggests that the energy drink did not measurably influence this par- ameter under our conditions. This finding is in contrast to studies demonstrating the beneficial potential of caf- feine on sperm motility in animals and humans [20, 21] with reduced fecundability among males, but not among females [27].Testosterone levels did not show any difference be- tween the control and treated groups 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 indi- vidual characteristics [26, 28]. To better understand the reason for the decrease in sperm concentrations, meas- uring LH and FSH levels might help to determine which mechanism is responsible (endocrine versus sperm matur- ation defect). The length of treatment was approximately two spermatogenic cycles, which in rodents lasts approxi- mately 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 observa- tions 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 un- changed. 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 en- ergy drink either directly on spermatogenesis, through endocrine hormones or other metabolic pathways.

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