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Prolonged consumption of energy drinks does not affect the processes of memory, and increases the activity of transaminases, and cholesterol concentration – animal model study results

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A – Study Design, **B** – Data Collection, **C** – Statistical Analysis, **D** – Data Interpretation, **E** – Manuscript Preparation, **F** – Literature Search, **G** – Funds Collection

Summary **Background.** The consumption of energy drinks (ED) is popular among young people. There are concerns that it could be harmful for their health.
Objectives. The aim of the study was to analyze the influence of ED consumption on animals for 30 subsequent days on memory, weight gain and biochemical parameters (alanine transaminase-ALT, asparagine transaminase-AST, creatinine, cholesterol and glycated hemoglobin-HbA_{1C}).
Material and methods. The study was conducted on 32 mice (16 females and 16 males). The mice received standard feed for rodents *ad libitum*. The animals were randomly assigned to four groups (8 animals each): I – female controls provided with water *ad libitum*, II – females provided with ED *ad libitum*, III – male controls provided with water, and IV – males provided with ED. Every 7 days memory retention in a passive avoidance task, and fresh spatial memory in a Y-maze were checked. The results were analyzed with Statistica 10.0. $p < 0.05$ was considered statistically significant.
Results. The consumption of ED did not affect fresh spatial memory or memory retention in the experiment. Males drinking ED gained weight at a faster rate than control males. ED significantly increased the activity of AST in the blood sera of females and, to a lesser degree, of ALT in both males and females drinking ED. ED did not significantly affect the concentration of creatinine or HbA_{1C}, but significantly increased the concentration of cholesterol in the blood of males from group IV.
Conclusions. The prolonged consumption of ED does not affect memory processes, but increases the activity of transaminases and cholesterol concentration in blood sera in the mice model.
Key words: memory, cholesterol, energy drink, transaminases.

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Background

Energy drinks consumption is popular among young people. Energy drinks contain high doses of caffeine (up to 400 mg/0.5 L) and simple carbohydrates [1]. Advertisements of popular energy drinks persuade customers that ED are uplifting for both the body and the mind. Therefore, people forced to drive a car for a long time, performing work requiring long concentration, and young people studying for exams consume ED for a long time. There are concerns that these products may have a negative influence, especially when they are consumed along with alcohol [2]. The psychoactive effect of caffeine may lead to addiction to other substances affecting the central nervous system (CNS). Children choose sweet high-calorie food and drinks eagerly [3]. Parents and guardians are afraid that due to high consumption of high-sugar beverages their children will become obese [4]. Furthermore, there are reports emphasizing that consumption of high sugar beverages increases the risk of type 2 diabetes and cardiovascular diseases [5]. Energy drink consumption may lead to kidney and liver impairment [6, 7]. This is why many countries, including Poland, have taken action to withdraw high-sugar caffeine-containing beverages from schools, or to implement a high tax on them.

Objectives

This study aimed to evaluate the influence of prolonged energy drink consumption in chosen animals on the processes of memory, body weight, and blood chemistry (alanine transaminase – ALT, aspartate transaminase – AST, creatinine, cholesterol concentration, and glycated hemoglobin level-HbA_{1C}).

Material and methods

The study was conducted on 32 Albino Swiss mice (16 females and 16 males) aged 6 weeks at the beginning of the experiment. The animals were obtained from an authorized breeder. The experiment was conducted with the consent of the Local Ethics Committee, no. 1166/11 of 23.01.2015. For seven days the animals acclimatized to the conditions of the laboratory. During the entire experiment the animals were kept in standard laboratory conditions complying with Polish and European Union laws. Mice in the tested groups were provided with an energy drink (Burn®) instead of water. The caffeine concentration amounted to 32 mg/100 mL. Other components included: sugar – 13.3 g/100 mL, taurine – 0.4%, inositol – 0.01%, niacin – 6,5 mg/100 m:, pentetic

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acid – 1.5 mg/10 mL, vitamin B_6 – 0.21 mg/100 mL, vitamin B_{12} – 0.38 µg/100 mL, water, CO_2, citric acid, potassium sorbate, sodium benzoate, ascorbic acid, and guarana extract. The caloric value amounted to 236 kJ/100 mL or 56 kcal/100 mL. The energy drink was dissolved in water in order to obtain a concentration of 250 mg/L. All animals were fed with the standard *ad libitum* feed for rodents. The animals were randomly assigned to four groups (8 animals each): I – female controls provided with water *ad libitum*, II – females provided with ED *ad libitum*, III – male controls provided with water, IV – males provided with ED for the 30 following days. The animals were weighed every day. Every 7 days the animals were tested with a passive avoidance test and in a Y labyrinth.

The passive avoidance test is performed by placing the mouse into a device consisting of two segments: a dark one and an illuminated one. The animal is placed in the illuminated segment and observed for 180 s. If the animal shelters in the dark section, it receives an electric shock of 0.2 mA for 2 s delivered by the panels located in the floor. The next day, the same animal is placed in the illuminated section again and observed for 180 s. If the animal shelters in the dark section, it means that it does not remember the negative stimulus of the previous day. On the other hand, if it remains in the illuminated section, it remembers the electric shock and therefore avoids it. The latency is measured for 180 s, when the mouse remains in the device.

Spatial fresh memory is tested in the Y-shaped labyrinth. The animal remains in the device for 8 minutes. The branches of the labyrinth the animal enters are recorded. An animal that has a good memory should not enter a branch that it has already been to. In this test the percentage of logical (not repeated) alterations (crossings) is measured.

On the last day of the experiment the blood of the animals was sampled for lab testing (AST, ALT, creatinine, total cholesterol, HbA_{1C}). Then the animals were euthanized with CO_2.

The results were analyzed with Statistica 10.0. The results were presented as a mean ± standard error of measurement (SEM). $p < 0.05$ was regarded as statistically significant. Comparative analysis of evaluated variables was performed with nonparametric tests (for the passive avoidance test) and parametric tests (for other results). For the constant quantitative characteristics of normal distribution the parametric T-student test was used in order to compare the results of the two groups. For the passive avoidance test the quantitative characteristics were analyzed with nonparametric tests. The independent trials were confronted with the U Mann–Whitney test.

Results

Energy drink consumption did not significantly influence memory retention at any stage of the study in comparison with controls (Tab. 1). Energy drink consumption did not have any significant influence of fresh spatial memory (Tab. 2). Everyday weighing did not reveal any statistically significant differences in body weight increase dynamics between the tested animals (Tab. 3). After 30 days of ED consumption females presented a significantly increased serum AST activity in comparison to the other groups, $p < 0.05$ (Tab. 4). After 30 days of the experiment, the serum ALT activity in females and males was slightly elevated in comparison to controls, $p > 0.05$. ED consumption significantly influenced neither serum creatinine levels nor HbA_{1C} percentage in the evaluated animals. After 30 days of ED consumption the serum cholesterol level was significantly higher in males ($p < 0.05$) than in male controls. Serum cholesterol concentration in females drinking ED did not significantly differ from the female controls.

Table 1. The effects of energy drinks

Latency; mean ± SEM [s]	Female control group	Female ED	Male control group	Male ED
Day 2	174 ± 4	177 ± 3	160 ± 18	158 ± 17
Day 9	179 ± 1	168 ± 12	160 ± 10	169 ± 7
Day 16	168 ± 12	167 ± 10	175 ± 5	166 ± 7
Day 23	180 ± 0	179 ± 1	180 ± 1	180 ± 0
Day 30	180 ± 0	180 ± 0	180 ± 0	180 ± 0

Table 2. The effect of energy drink (ED) on the fresh spatial memory of mice tested in the Y labyrinth, $p > 0.05$

% of logic alterations; mean ± SEM	Female control group	Female ED	Male control group	Male ED
Day 1	64 ± 2	61 ± 2	56 ± 1	53 ± 5
Day 8	59 ± 3	62 ± 1	61 ± 1	65 ± 0.5
Day 15	63 ± 3	56 ± 4	61.5 ± 3	58 ± 1
Day 22	63 ± 2	66 ± 3	68 ± 2	65 ± 1
Day 29	65 ± 2	69 ± 3	61 ± 1	72 ± 1

Table 3. The effect of an energy drink (ED) on an increase in mouse body weight, $p > 0.05$

Body weight; average [g]	Female control group	Female ED	Male control group	Male ED
Day 1	20.9	21.2	20	24.1
Day 8	21.6	20.6	21.6	24
Day 15	24.1	22	25.4	26
Day 22	24.5	22.7	25.3	27
Day 29	25	23.5	26.6	29.3

Table 4. The effects of energy drinks (ED) on laboratory results of mice blood, $*p < 0.05$

Mean ± SEM Day 30	Female control group	Female ED	Male control group	Male ED
AST [U/L]	343 ± 118	823 ± 453*	472 ± 129	532.8 ± 131
ALT [U/L]	88.3 ± 43	144 ± 82	122 ± 78	163 ± 74
Creatinine [mg/dl]	0.3 ± 0.07	0.25 ± 0.04	0.3 ± 0.02	0.3 ± 0.1
HbA_{1C} [%]	3.7 ± 0.14	3.7 ± 0.2	3.9 ± 0.3	3.9 ± 0.3
Cholesterol [mg/dL]	64.5 ± 1.1	77 ± 14	65 ± 16	97.8 ± 14*

Discussion

The experiment did not prove that the animals consuming energy drinks had better memory than the controls. However, experiments on humans proved that Red Bull significantly improves response time, memory and concentration [8]. Studies performed on American and Australian students drinking ED showed that these people presented more dangerous behaviors, such as: they often drove mechanical vehicles after alcohol consumption, did not fasten seatbelts, were eager to fight, to engage in dangerous sexual behaviors, and to use drugs [9–11]. However, the presented study did not prove any significant behavioral changes in evaluated animals in comparison to the controls. It is possible that proving the psychoactive and/or precognitive effect of

caffeine requires special circumstances, such as: advanced age, lack of sleep, or stress exposure. Wadhwa et al. proved that caffeine improves memory in sleep-deprived rats by preventing synapsin I and synaptophysin expression in the hippocampus [12]. Caffeine is an A1 adenosine receptor blocker. Bagga et al. discovered the neuroprotective effect of caffeine in a mouse model of Parkinson's disease. According to these researchers, caffeine inhibits inflammation of the nervous system by decreasing the extracellular concentration of glutamate in mouse brains [13]. Also, Panza et al. demonstrated that consumption of caffeine-rich beverages prevents dementia in humans. The authors emphasize that this effect is more visible in women than in men [14]. On the other hand, Xu et al. proved that caffeine assists in the repair of gray matter damage in the mouse nervous system by decreasing A1 adenosine receptor expression [15]. Thus, the neurological effect of caffeine is related to its influence of the adenosine receptor and hippocampal protein expression.

The influence of ED was analyzed separately for female and male animals in the presented experiment, because there are reports regarding the especially strong correlation between ED consumption and elevated risk for metabolic syndrome in women [16]. The ED dose used in the experiment provides a dose of 250 μM of caffeine/L, which is cardiotoxic for humans [17]. According to many researchers, consumption of caffeine-containing beverages increases the risk of cardiovascular diseases because it increases blood pressure and contributes to the increase in homocysteine blood levels [18, 19]. At the same time, many authors emphasize that caffeine in drinks has an anti-oxidative effect, and therefore its consumption prevents type 2 diabetes and liver disorders [18, 19].

The study detected no significant influence of ED on body weight increase or HbA_{1C}. A randomized study on obese children lasting two years gave similar results [4]. Obese children consuming low-calorie beverages gained weight at the same rate as children drinking high-sugar beverages. Perhaps the negative influence of high carbohydrate concentration in ED was balanced by a protective effect of caffeine on the liver and carbohydrate metabolism. According to American data of 2006, children and adults in this country consume daily approximately 172–175 kcal in sweet beverages alone [5], and in Mexico the consumption of such beverages doubled between 1999 and 2006. Unfortunately, a similar tendency is also visible in Europe.

The presented experiment proved that after 30 days of ED consumption cholesterol levels in males were significantly higher than in male controls. Similar observations were made during experiments performed on people. The reason for the increase in blood cholesterol concentration in mammals consuming coffee-derived beverages is hakaveol and kafestrol – diterpenes contained in coffee beans [18, 19].

According to American data, consumption of ED and caffeine-rich diet supplements are a cause of 23 000 emergency unit admissions per year [20]. Most of the time this concerns people aged between 20 and 34, or children who consumed such products when unsupervised. 0.1% of such cases result in hospital admission. Mostly, young people report complaints related to the consumption of diet pills or ED, often combined with alcohol. In many cases, this causes arrhythmia, chest pain and tachycardia. According to Canadian data, energy drink overdose is also possible. The most common symptoms include: restlessness, insomnia, tremor, tachycardia, and psychophysical stimulation. 90 such cases were reported in this country in one year. Unfortunately, there have also been deaths related to caffeine overdose [21, 22].

Conclusion

Prolonged energy drink consumption does not influence the processes of memory, whereas it increases transaminase activity and cholesterol concentration in the mouse model.

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Conflict of interest: The authors declare no conflict of interests.

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