STUDIA UNIVERSITATIS BABEŞ – BOLYAI, BIOLOGIA, LIX, 2, 2014 (p. 89-98)

CHRONIC ADMINISTRATION OF RED BULL AFFECTS BLOOD PARAMETERS IN RATS

CAMELIA MUNTEANU [1], IOAN UŢIU[2], CORINA L. ROŞIORU[2] and CAMELIA LANG[2,✉]

SUMMARY. Energy drinks are commercial drinks conceived in order to improve physical and intellectual performance. Short term effects of energy drinks are known and do not seem to affect the state of health, but long term effects are less known and raise serious health questions. In this work we investigated if Red Bull affects morphologic and some of the biochemical parameters of the blood.

Twenty adult Wistar rats, weighing 186.6±3.15 g were organized into two groups. The Control (C) group received a standard diet and tap water. The animals in the energy drink (ED) group were orally administrated 1.5 ml/100 g b.wt. of Red Bull daily, for 4 weeks. After 4 weeks of treatment, the rats were killed by exsanguination and blood samples were collected for morphologic and biochemical parameter analysis. A significant reduction in red blood cell count was noticed in the ED group, while the hemoglobin concentration and the hematocrit slightly increased in the experimental group, as compared to the control. The concentrations of glucose and total cholesterol increased significantly after Red Bull administration, as did the activities of serum LDH, AST and ALT. These results indicate that a long-term consumption of energy drinks can affect certain morphological and biochemical blood parameters. Furthermore, these drinks may be risk factors for the development of a metabolic disease.

Keywords: blood, caffeine, energy drinks, Red Bull

Introduction

Red Bull is a commercial energy drink conceived in order to improve physical and intellectual performance. This beverage was launched in 1987, but in 1992 it was banned in France and other countries, in Europe and South America, due to its negative effects. In 2008, the commercialization of Red Bull restarted in France, as a result of growing pressure from the European Union (Reissing *et al.,* 2009). The stimulating properties of Red Bull stem from the combined action of its

[1] *Babeş-Bolyai University, School of Physical Education and Sport, 400376 Cluj-Napoca*
[2] *Babeş-Bolyai University, School of Biology and Geology, 400006 Cluj-Napoca*
✉ ***Corresponding author: Camelia Lang**, 5-7 Clinicilor Str., Babeş-Bolyai University, Cluj-Napoca*
E-mail: camelia_lang@yahoo.de

ingredients: taurine, caffeine, carbohydrates (glucose and sucrose), B12 vitamin, glucuronolactone etc. Each ingredient, taken separately, may have protective or therapeutic effects, if taken in appropriate dose.

Caffeine, the main ingredient of the energy drink, is an ergogenic compound (Zang *et al*., 2014). It improves physical activity (Davis and Green, 2009; Lara *et al.,* 2014) and stimulates diuresis and lipolysis, reduces intrahepatic lipid content and stimulates fatty acid β-oxidation in liver parenchimal cells (Sinha *et al*., 2014). It also stimulates the release of calcium from the sarcoplasmic reticulum, which is indispensable for muscle contraction. Furthermore, caffeine is a nonselective competitive inhibitor of phosphodiesterase enzymes (Echeverri *et al.,* 2010) and it could be an effective therapeutic agent against Alzheimers disease (Arendash and Cao, 2010).

Taurine is involved in the regulatory processes of a broad spectrum of biological functions. Electrophysiological studies conducted on mouse brain have shown that taurine induces long-term synaptic potentiation, which is believed to underline learning and memorizing (Ito *et al*., 2010). It is known that taurine has a cholesterol-lowering effect and decreases fat deposits. These beneficial effects are due to acceleration of cholesterol conversion into bile acids (Yamori *et al.,* 2010). It has also been demonstrated that taurine acts as an antioxidant and anti-inflammatory agent, in addition to its vasodilatory properties. In endothelial cells, taurine inhibits apoptosis, inflammation and oxidative stress (Maia *et al*., 2014). However, the effects of chronic administration of taurine in food supplements are controversial. It has been shown that taurine either improves the capacity of learning and memory (Lu *et al*., 2014), or it has no effect on these processes (Bichler *et al*., 2006).

B vitamins have benefic effects on the nervous system. They are involved in the regulation of the cognitive processes and affective state (Duthie *et al*., 2002; Mattson and Shea, 2003). Cobalamin (vitamin B12) is indispensable for DNA synthesis (Gueant *et al.,* 2013; Zhao *et al*., 2014) and erythrocytes formation (Koury *et al*., 2004; Zhao *et al*., 2014). Vitamin B12 is a cofactor of methionine synthase in the synthesis of methionine, which is the precursor of the universal methyl donor S-adenosylmethionine (Gueant *et al*., 2013). It plays a crucial role in maintaining neurological function (Zhao *et al.,* 2014). It has been proven that niacin (vitamin B3) decreases total serum cholesterol and oxidative stress (Hamound *et al*., 2013). Niacin has been used in the treatment of dyslipidemia and cardiovascular disease for more than 50 years (Digby *et al.,* 2012). It decreases the levels of all atherogenic lipoproteins and increases the level of protective HDL.

Short term effects of the energy drinks are relatively well known and do not seem to affect the state of health, but the effects on the long term are less known and raise serious questions. There are several studies that reported moderate improvements in physical endurance and mental performance, including choice reaction time, concentration and memory after acute ingestion of energy drinks (Alford *et al*., 2001; Seidl *et al.,* 2000; Scholey and Kennedy, 2004).

The long term effects of energy drinks consumption are controversial. Only a few studies have shown that chronic ingestion of energy drinks are beneficial to one's health (Schrader *et al*., 2013). Other studies mentioned that chronic administration of energy drinks did not influence physical activity (Forbes *et al.,* 2008; Candow *et al*., 2009). Most investigations have reported adverse health effects of energy drinks due, most probably, to caffeine (Malinauskas *et al*., 2007; Kurtz *et al*., 2014). Central nervous system, cardiovascular, gastrointestinal and renal dysfunctions have been associated with chronic caffeine ingestion (Bichler *et al*., 2006; Maulinaukas *et al*., 2007; Clauson *et al*., 2008). Excessive caffeine consumption elevates blood levels of cholesterol and homocysteine (Dworzanski *et al*., 2011).

Animal studies have shown that chronic oral administration of energy drinks is associated with changes in blood chemistry and liver enzymes activities. For example, the concentrations of total cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL) and glucose increased after energy drinks consumption. The activities of transaminases increased in both rats and rabbits (Akande *et al*., 2011; Ebuehi *et al.,* 2011; Khayyat *et al*., 2014).

Long term studies should aim to better define maximum safe doses, the effects of chronic use and effects in at-risk population, and better document and track of adverse health effects (Seifert *et al*., 2011). Energy drinks may exacerbate risk factors for heart disease, since studies suggest that energy drinks may serve as a gateway to other forms of drug dependence (Higgins *et al*., 2010).

Knowledge of energy drink consumption effects on health is very important, especially given its prevalence among young people. Therefore, the aim of our study was to investigate if chronic administration of energy drinks affect morphologic and certain biochemical parameters of the blood.

Materials and methods

Chemicals. All reagents used in this study were of analytical grade and were purchased from Sigma-Aldrich Chemie GmbH, Germany, Nordic Invest S.R.L., Romania and S.C. BioZyme S.R.L, Romania. The Red Bull energy drink was bought from the local market.

Animals. The animals were twenty albino male Wistar rats, weighing 186.6±3.15 g, housed in the zoobase of the Department of Molecular Biology and Biotechnology, School of Biology and Geology, Babes-Bolyai University. They were kept in hygienic conditions, under 12/12 h light/dark cycle, received a standard diet (S.C. Siamond Prod. S.R.L., Cluj Napoca, Romania) and had *ad libitum* access to tap water. Rats were organized into two experimental groups. The control (C) group received a standard diet and tap water. The animals from the energy drink (ED) group were orally administered 1.5 ml/100 g b.wt. of Red Bull daily, for 4 weeks. The animals were gently handled, without causing them stress or pain, and after 4 weeks, they were killed under anesthesia by exsanguination.

Assays. Blood was collected from the jugular vein and processed for hematological and biochemical examinations. Counts of red and white blood cells were carried out using a Bürker-Türk haemocytometer. The hematocrit was determined by the microhematocrit technique and the results were expressed as volume percentage (%) of red blood cells in blood. Haemoglobin concentration was measured with Drabkin reagent (Drabkin *et al*., 1935). Glycaemia was determined using Somogy-Nelson method (Somogy, 1945; Nelson, 1944). Serum cholesterol was assayed using ferric chloride (Zlatkins *et al*., 1953). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assessed according to the method described by Reitman and Frankel (1957), and lactate dehydrogenase (LDH) activity according to Bergmeyer and Bernt (1974).

Data analysis. The results are presented as mean ± standard error (SE). The data were analyzed for statistical significance using unpaired Students *t* test. A value of $p < 0.05$ was considered significant.

Results and discussion

The aim of our study was to investigate if energy drinks affects morphologic and certain biochemical parameters of blood in rats. Blood serves as a transport medium, carrying essential nutrients to cells and removing metabolic waste products. The biochemistry of the blood plays a key role in maintaining health.

As seen in Fig. 1a, the red blood cell count decreased significantly after energy drink administration (ED 7.45±0.39 *vs* control 9.09±0.27; $p<0.01$). Perhaps energy drinks, due to a high content of carbohydrates increased osmotic pressure (Gottlieb *et al*., 2006). On the other hand, Khayyat *et al.* (2014) mentioned that depletion of red blood cells count could be attributed to disturbed haematopoiesis, destruction of erythrocytes, reduction in the rate of their formation and/or their enhanced removal from circulation. Destruction of red blood cells reflects a failure of hepatocellular functions that could be caused by caffeinated energy drink consumption (Akande *et al*., 2011; Khayyat *et al*., 2014).

Interestingly, the hematocrit (ED 45.98±0.46 *vs* control 44.96±0.49) (Fig. 1b) and haemoglobin concentration (ED 20.76±0.63 *vs* control 18.83±0.65) (Fig. 1c) increased after Red Bull intake. These changes may be a result of dehydration caused by caffeine (Pennay *et al.,* 2011). Treatment with Red Bull led to a significant decrease in the total white blood cell count (ED 8.85±0.22 *vs* control 9.90±0.27; $p<0.05$) (Fig. 1d). This modification may also suggest the existence of a moderate dehydration (Nakyinsige *et al*., 2013).

Red Bull increased significantly the plasma glucose concentration (ED 133.81±6.53 *vs* control 115.86±3.22, $p<0.05$) (Fig. 2a). The same effect was seen by Ebuehi *et al*. in rabbits (2011). The increment might result from the fact that the

glucose content of Red Bull is high. Regarding that, Red Bull chronic consumption could have adverse effects, because it is showed that consumption of sweetened beverages is associated with health risk as metabolic syndrome and type 2 diabetes (Malik *et al*., 2010).

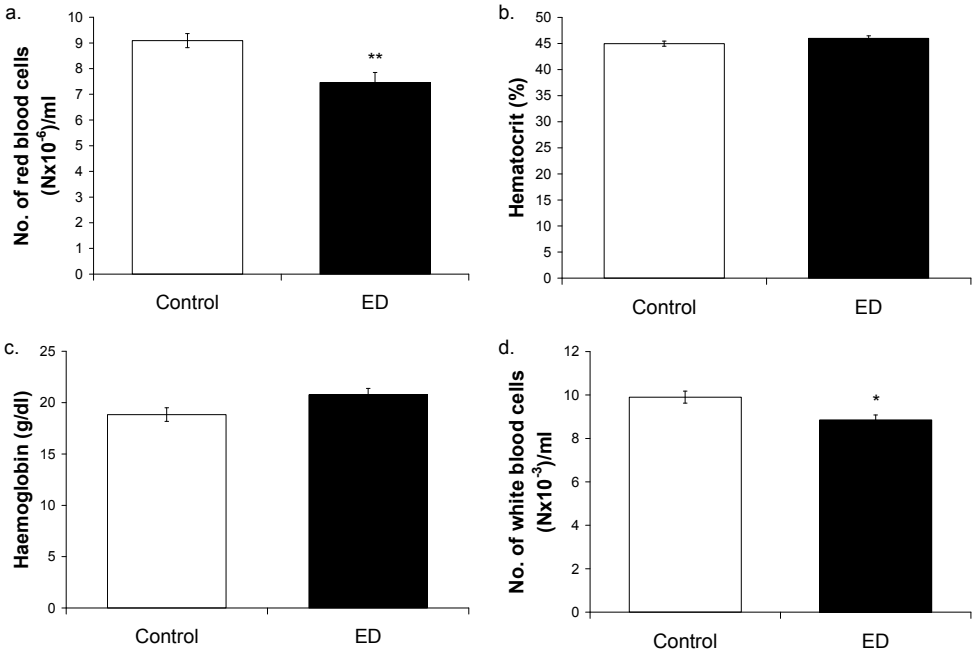

Figure. 1. Erythrocyte number ($p<0.01$) (a), hematocrit (b), haemoglobin concentration (c) and white blood cell count ($p<0.05$) (d) in the two experimental groups. n=10 in each group. The results are expressed as mean ± SE.

Energy drinks may, in fact, be a risk factor for metabolic syndrome development due to their high sugar content. In this study, we have observed that the experimental groups had a similar evolution concerning body weight: all animals gained continuous weight (Fig. 2b), but the difference between the beginning and the end of experiment was higher in ED group (ED from 143.82 to 176.35 *vs* control from 155.8 to 177.43). This finding is confirmed by Ugwuja (2014), who also attributed it to the high sugar content.

As depicted in Fig. 2c, serum total cholesterol increased slightly after the energy drink administration (ED 155.95±5.33 *vs* control 150.06±6.13). This result seems surprising since Red Bull contains taurine and niacin, two ingredients that are

commonly used for lowering lipids concentration (Yamori *et al*., 2010; Hamound *et al*., 2013). However, Red Bull contains caffeine which elevates the blood level of cholesterol and homocysteine (Dworzanski *et al*., 2011).

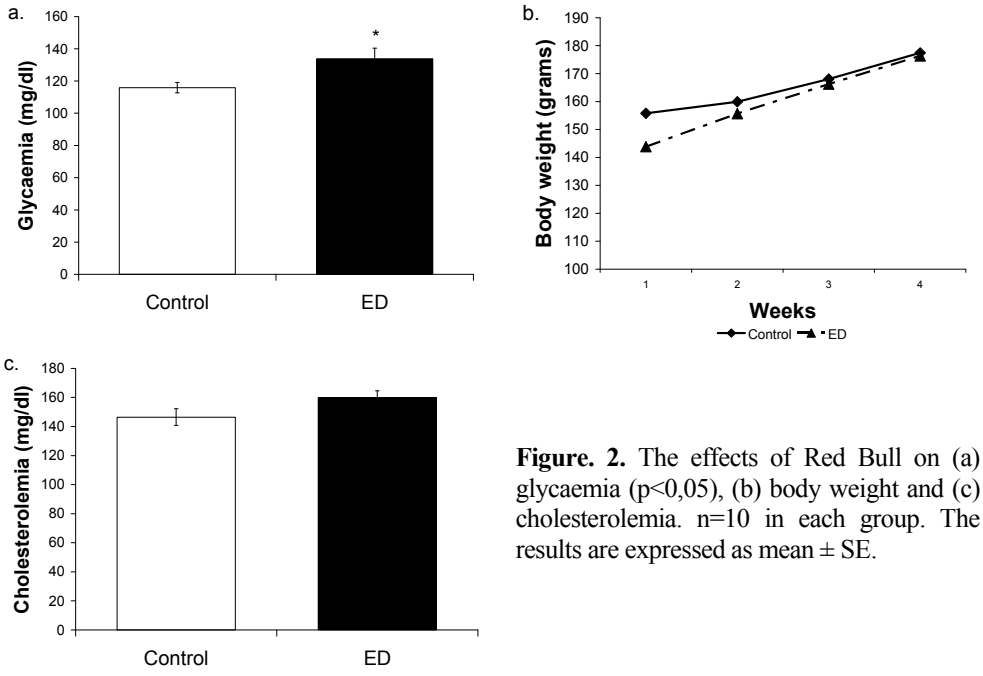

Figure. 2. The effects of Red Bull on (a) glycaemia ($p<0,05$), (b) body weight and (c) cholesterolemia. n=10 in each group. The results are expressed as mean ± SE.

The liver enzyme markers (LDH, ALT and AST) activities are shown in Fig. 3a, b and c. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), together with lactate dehydrogenase (LDH) are common markers of plasma membrane integrity. Their concentration in the plasma is normally low, as they are considered "liver enzymes", although they can be also found in lower concentrations in other tissues. A rise of the serum transaminases is usually considered a sign of altered plasma membrane structure, causing the "leaking "of enzymes outside the cells.

LDH (ED 0.3153±0.0164 *vs* control 0.2433±0.0229; $p<0,05$), ALT (ED 130.01±12.44 *vs* control 87.71±5.57) and AST (ED 281.48±16.46 *vs* control 183.16±10.19; $p<0.001$) activities were elevated in the sera of the rats after Red Bull consumption. This is in agreement with the results of Ankade and Banjoko (2011), Ebuehi *et al*. (2011) and Khayyat *et al*. (2014). Moreover, Bukhar *et al*. (2012) mentioned that there is a relation between energy drink administration and the concentration of liver enzymes in normal and hyperglycemic rats.

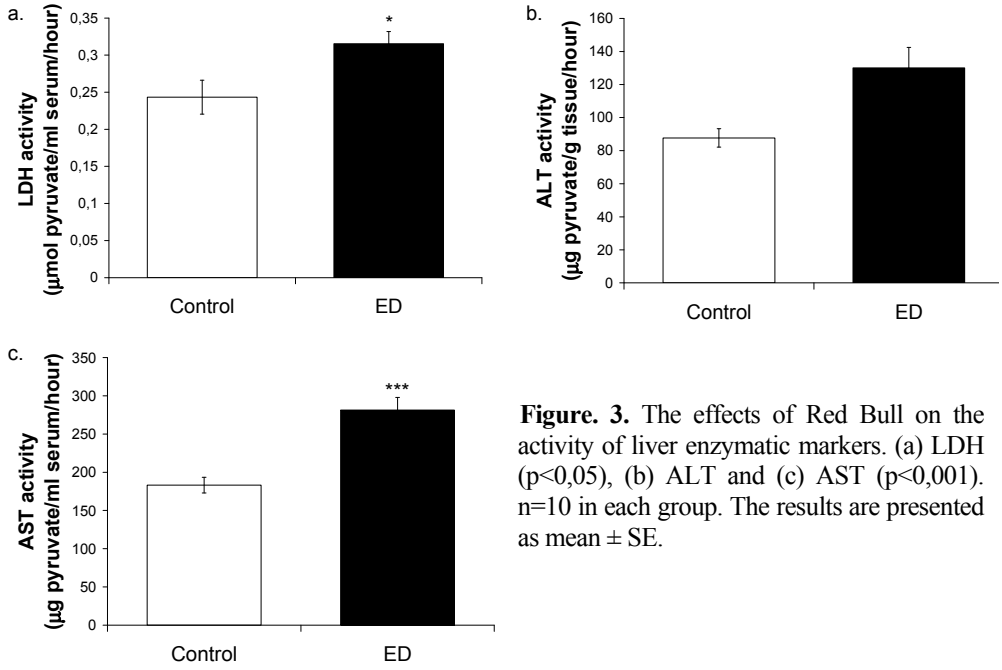

Figure. 3. The effects of Red Bull on the activity of liver enzymatic markers. (a) LDH ($p<0,05$), (b) ALT and (c) AST ($p<0,001$). n=10 in each group. The results are presented as mean ± SE.

Conclusions

The results of the present study indicate that energy drinks such as Red Bull can affect certain morphological and biochemical blood parameters. Furthermore, these drinks may be a risk factor for the development of metabolic diseases. Further research is needed and a raise of awareness in young people and athletes about the potential long term effects of energy drinks consumption.

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