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STATUS OF PRO- AND ANTIOXIDANT SYSTEM OF RATS UNDER CONDITIONS OF ENERGY DRINK CONSUMPTION

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The article is devoted to the study of the mechanism of the impact of energy drinks on the state of pro- and antioxidant system of erythrocytes of experimental animals. The study was carried out on male Wistar rats. We found that in the erythrocytes of experimental animals that consumed an energy drink for 30 days, the processes of free radical oxidation are activated, accompanied by the activation of lipo-peroxidation and peroxidation of proteins in experimental animals. Accumulation of the thiobarbituric acid active products and the content of diene conjugates can damage the lipid matrix of biomembranes, which in turn leads to disruption of the structural and functional capacity of erythrocyte cell membranes. High levels of protein carbonyl derivatives of erythrocytes may be due to disruption of both structural proteins and enzymes. Under such conditions, it is advisable to study the antioxidant defense of erythrocytes. The obtained results indicate an increase in catalase and superoxide dismutase activity in experimental animals compared with the control group, which may be due to the growth in the peripheral population of "early" forms of cells which are capable of active protein synthesis. This process can be considered as an adaptive synthesis of antioxidant enzymes. The study of the microelement status of rat erythrocytes under the conditions of energy consumption allowed to establish the development of dysmicroelementosis, which was accompanied by a decrease in the concentration of Copper and Ferrum.

Key words: laboratory rats, energy drink, catalase, superoxide dismutase, TBK-active products, diene conjugates oxidative modification of proteins.

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СТАН ПРО- ТА АНТИОКСИДАНТНОЇ СИСТЕМИ ЩУРІВ ЗА УМОВ СПОЖИВАННЯ ЕНЕРГОНАПОЮ

Стаття присвячена вивченню механізму впливу енергетичного напою на стан про- та антиоксидантної системи еритроцитів експериментальних тварин. Дослідження було проведено на щурах-самцях лінії Вістар. Нами встановлено, що в еритроцитах експериментальних тварин, які споживали енергетичний напій протягом 30 днів, активуються процеси вільнорадикального окиснення, що супроводжуються активацією процесів ліпопероксидації та пероксидації білків. Накопичення ТБК-активних продуктів та дієнових кон'югатів може призвести до пошкодження ліпідного матриксу біомембран, що в свою чергу зумовлює порушення структурно-функціональної здатності клітинних мембран еритроцитів. Високий рівень карбонільних похідних білків еритроцитів може бути зумовлений порушенням як структурних білків, так і ензимів. За таких умов доцільним є дослідження антиоксидантного захисту еритроцитів. Отримані результати свідчать про збільшення активності каталази та супероксиддисмутази у дослідних тварин порівняно з контрольною групою, що може бути зумовлене зростанням у периферичній популяції «ранніх» форм клітин, які здатні до активного білкового синтезу. Такий процес можна розглядати як адаптивний синтез антиоксидантних ферментів. Дослідження мікроелементного статусу еритроцитів щурів за умов споживання енергетика дозволило встановити розвиток дисмікроелементозу, що супроводжувався зниженням концентрації Купруму та Феруму.

Ключові слова: лабораторні щурі, енергетичний напій, каталаза, супероксиддисмутаза, ТБК-активні продукти, дієнові кон'югати, окисна модифікація білків.

The study is a fragment of the research project "Scientific substantiation and improvement of diagnosis and treatment of endocrinopathies based on the study of priority epipathogenetic factors and comorbid conditions" state registration No. 0120U105103.

Consumption of energy drinks has increased worldwide since they appeared on the market in 1987 [13]. The purpose of drinking such drinks is to improve physical endurance, increase physiological and cognitive reactions, reduce sleep needs. The main ingredients of energy drinks are caffeine, taurine, guarana, carbohydrates, sodium and vitamin B_6. Some energy drink brands also include glucuronolactone, ginseng, ginkgo biloba [9]. Due to the presence of these components, energy drinks eliminate the signs of

fatigue and because of this feature, many people use them to improve quality of life and increase productivity. Athletes, children (12 years old), teenagers (12–18 years old) and young adults (19 to 25 years old) mainly are the most prone to abuse these beverages [5, 7]. Knowing the composition of energy drinks and the fact that they contain psychoactive (caffeine, taurine, guarana) substances that have high stimulating properties, we can assume uncontrolled dosing of such substances and the accumulation of their therapeutic effect with manifestations of side effects. [12]. Excessive consumption of energy drinks can cause cardiovascular symptoms such as ventricular and atrial arrhythmias, hypertension [6].

Since erythrocytes are the one of the first to respond to the action of endogenous and exogenous factors, it is natural to be interested in studying the effect of energy drinks primarily on the peripheral erythron. Red blood cells are the main cells in the circulatory system, whose function is to transport oxygen. Erythrocytes have a high sensitivity to the action of chemicals, which allows to determine a certain specifics of their action. Therefore, the facts of changes in the metabolism of a number of substances that are components of erythrocyte membranes can be used as an indicator of physiological conditions of cell existence and as a factor in predicting the occurrence of such changes, which include irreversible degradation processes. In addition, the high concentration of polyunsaturated fatty acids in the erythrocyte membrane, the autooxidation of oxyhemoglobin (the source of reactive oxygen species in erythrocytes) make them a good model for estimating oxidative stress induced by xenobiotics [11]. Oxidative stress caused by xenobiotics leads to erythrocyte damage, which causes changes in cell morphology, in particular, changes in the conformation of membrane proteins, the formation of crosslinks of proteins, lipid peroxidation and, accordingly, hemolysis of erythrocytes [10].

It is known from literature that in red blood cells under the influence of various xenobiotics, in particular under the action of nitrates and nitrites [14], there is the development of oxidative stress by accumulation of products of lipid peroxidation, which in turn lead to loss of membrane integrity and cell death. Oxidative stress impairs oxygen transport and induces erythrocyte aging.

It has been established that the constant formation of prooxidants in living organisms is balanced by their inactivation by antioxidants, therefore continuous regeneration of antioxidant capacity is necessary to maintain homeostasis. Erythrocytes are protected from oxidative damage by a variety of biological mechanisms, including antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [15].

SOD (EC 1.15.1.1) – protects erythrocytes at an early stage, as it catalyzes the reaction of dismutation of superoxide radicals with the formation of hydrogen peroxide and oxygen. Depending on the conditions, SOD can act as a prooxidant by reacting with hydrogen peroxide and initiate the formation of superoxide anion and hydroxyl radical. Increased or decreased SOD activity may be the cause of pathological changes. In the first case, the changes occur as a result of increased cytotoxic action of hydrogen peroxide, which is formed as a result of dismutation of superoxide, in the second – due to insufficient protection against reactive oxygen species [2]. SOD in its structure contains zinc to maintain stability, and copper to maintain its activity [8].

CAT (EC 1.11.1.6) is a key enzyme which limits the formation of hydrogen peroxide and thus protects erythrocytes from the accumulation of not only peroxides but also other active forms of oxygen (AFO).

The purpose of the study was to investigate mechanisms of the impact of energy drinks on the state of pro- and antioxidant systems in erythrocytes in experimental animals.

Material and methods. The study was performed on male Wistar rats, which were in the vivarium under appropriate lighting conditions, temperature and standard diet. Experimental animals were divided into four groups: 1st group – received drinking water (control group); 2nd group – received an energy drink for a month and the collection of material was carried out on the 1th day at the end of the experiment; 3rd group – received an energy drink for a month and the collection of material was carried out on the 20th day at the end of the experiment; 4th group – received an energy drink for a month and the collection of material was carried out on the 30th day at the end of the experiment.

All animal experiments were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986), in accordance with the rules for keeping experimental animals established by European Parliament and Council Directive (2010/63/EU) and the Order No.134 of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 “On approval of the procedure for conducting tests, experiments on animals by research institutions”, as well as the recommendations of the First National Congress of Ukraine on Bioethics (2001). The material was taken on the 1st, 20th and 30th days after the completion of the experiment under anesthesia (intramuscular sodium thiopental, 60 mg/kg).

The state of lipid peroxidation was assessed by the accumulation of an intermediate product – conjugated dienes (CD) and products that react with thiobarbituric acid (TBA-AP) [1]. The intensity of protein peroxidation (PP) was determined by the number of products of protein oxidative modifications (POM) by spectrophotometry. The optical density of the formed dinitrophenylhydrazones was registered in units on a spectrophotometer SPECORD M 40 (Germany) at wavelength of 356, 370, 430, 530 (nm) [3].

In erythrocyte hemolysates, the following was determined: the activity of CAT was determined by the ratio of the catalase number to the number of erythrocytes in 1 ml of test blood and expressed in the units by the method based on measuring the concentration of hydrogen peroxide degradation by catalase contained in the examined samples; SOD activity was determined by the level of the inhibition of NADH-dependent nitroblue tetrazolium (NBT) reduction and phenazinemetasulfate (PMS) [1]. The determination of trace elements in erythrocytes was carried out by the atomic adsorption method, using the "S-115PK" and "AA-7000 SHIMADZU" devices.

The research was conducted on the basis of the accredited Center for Bioelementology Ivano-Frankivsk National Medical University (accreditation certificate 037/19). The obtained digital data were statistically calculated using the program STATISTICA 7 taking into account the Student's *t*-test.

Results of the study and their discussion. Since changes in the structure and function of biomembranes are now considered as one of the universal links in regulating the adaptation of cells to adverse epigenetic conditions, the study of the lipoprotein complex of erythrocyte membranes under energy consumption deserves special attention.

As a result of our research, we found that in the body of experimental animals that consumed an energy drink, the processes of free radical oxidation are activated, accompanied by the accumulation of lipid and protein peroxidation products, particulary in erythrocytes (Table 1).

Table 1

The level of TBA- active products and CD of rats erythrocytes that consumed energy drink (M±m) (n=7)

Group of animals	Index	
	TBA-active products, nmol MDA/ml	Conjugated dienes, unit. act/ml
Control	0.67±0.04	0.20±0.02
1st day	0.81±0.06**	0.47±0.06**
20th day	0.76±0.04**	0.38±0.03$^{** \&}$
30th day	0.72±0.08$^{\&}$	0.27±0.02$^{** \#}$

Notes: * – p<0.05, ** – p<0.001 – reliability compared with intact group of animals; $^{\&}$ – p<0.05, $^{\#}$ – p<0.001 – reliability compared with 1st experimental group of animals.

The study of the level of TBK-active products in erythrocytes on the 1st and 20th days showed an increase in their content by 21 % (p<0.001) and 13 % (p<0.001) and a slight increase on the 30th day compared to control animals. An increase in CD content in erythrocytes was also observed on the 1st, 20th, and 30th days by 135 % (p<0.001), 90 % (p<0.001), and 35 % (p<0.001), respectively. The comparative analysis shows that after the withdrawal of energy there was a gradual decrease in the level of TBC-active products and CD in erythrocytes on the 20th and 30th days by 6–12 % (p<0.05) and by 24–74 % (p<0.05, p<0.001) respectively compared with the 1st experimental group of animals.

At the same time, we conducted a research of the protein components of erythrocytes by studying the oxidative modification of proteins (Table 2).

Table 2

The level of oxidative protein modifications of rats erythrocytes, which consumed energy drink, units (M±m) (n=7)

Group of animals	Neutral products		Alkaline products	
	ADNPH λ=356	KDNPH λ=370	ADNPH λ=430	KDNPH λ=530
Control	0.229±0.014	0.283±0.078	0.179±0.043	0.135±0.013
1st day	0.279±0.026*	0.366±0.05*	0.237±0.026*	0.182±0.016**
20th day	0.249±0.005$^{* \&}$	0.350±0.013*	0.215±0.001$^{* \&}$	0.164±0.002$^{** \&}$
30st day	0.244±0.001$^{* \&}$	0.342±0.018	0.202±0.001$^{\&}$	0.153±0.002$^{* \#}$

Notes: * – p<0.05, ** – p<0.001 – reliability compared with intact group of animals; $^{\&}$ – p<0.05, $^{\#}$ – p<0.001 – reliability compared with 1st experimental group of animals.

As can be seen from the above data, a probable increase in the level of aldehyde derivatives (356 nm) was observed on the 1st, 20th and 30th days by 22 % (p<0.05), 9 % (p<0.05), 7 % (p<0.05) and neutral

ketone derivatives (370 nm) by 29 % ($p<0.05$), 24 % ($p<0.05$) and 21 %, respectively, as well as an increase in the level of aldehyde derivatives (430 nm) by 32 % ($p<0.05$), 20 % ($p<0.05$), 13 % and ketone derivatives of the main character (530 nm) by 35 % ($p<0.001$), 22 % ($p<0.001$) and 13 % ($p<0.05$), respectively, compared with intact animals.

At the same time, it should be noted that on the 20th and 30th days there was a significant decrease in the level of aldehyde derivatives by 11 % ($p<0.05$), 10 % ($p<0.05$), respectively, and a slight decrease in neutral ketone derivatives compared with the 1st experimental group animals. A decrease in the level of aldehyde and keto derivatives of the main character was also observed on the 20th and 30th days by 10 % ($p<0.05$), 15 % ($p<0.05$) and 10 % ($p<0.05$), 16 % ($p<0.001$) respectively compared with the 1st experimental group.

Maintenance of red blood cells homeostasis in particular, and the body as a whole, is determined by the state of antioxidant protection of erythrocytes. Therefore, we studied the activity of superoxide dismutase and catalase. SOD in the blood as a primary antioxidant supports and controls the level of free radicals and thus creates the conditions for the normal use of the body's oxygen environment (fig. 1).

The obtained data indicate a significant increase in SOD activity in erythrocytes on the 1st day by 10 % ($p<0.05$) and a decrease on the 20th and 30th days by 25 % ($p<0.001$) 21 % ($p<0.05$) in experimental animals compared with the control group. After the end of the energy drink intake, a decrease in the activity of this enzyme was observed on the 20th and 30th days by 32 % ($p<0.001$) and 28 % ($p<0.001$) compared with the 1st experimental group of animals.

Catalase is a highly efficient enzyme that does not require energy for activation. Superoxide dismutase with catalase and other antioxidant enzymes protects the body from highly toxic oxygen radicals. The results of the study indicate changes in the activity of erythrocyte CAT (fig. 2).

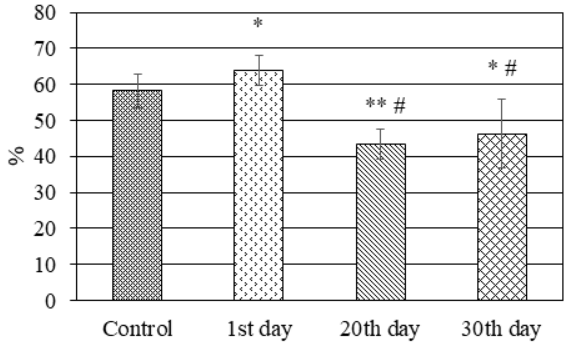

Fig. 1. The effect of an energy drink on the activity of superoxide dismutase of erythrocytes of laboratory rats (M±m) (n=7)

Notes: * – $p<0.05$, ** – $p<0.001$ – reliability compared with intact group of animals; # – $p<0.001$ – reliability compared with 1st experimental group of animals.

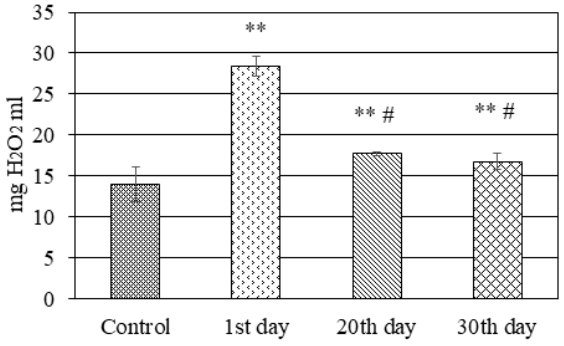

Fig. 2. Effect of energy drink on catalase activity of erythrocytes of laboratory rats (M±m) (n=7)

Notes: * – $p<0.05$, ** – $p<0.001$ – reliability compared with intact group of animals; # – $p<0.001$ – reliability compared with 1st experimental group of animals.

We found that under the influence of the energy drink, there was an increase in the activity of CAT in erythrocytes, especially on the 1st day – by 103 % ($p<0.001$) compared with the control group. After withdrawal of energy drink, enzyme activity remains significantly high on the 20th and 30th days of observation – by 38 % ($p<0.001$) and 41 % ($p<0.001$), respectively, with the 1st experimental group of animals.

It is well known that metabolic processes are under the control of trace elements that act as activators or inhibitors of enzymes. Essential microelements, such as Copper and Ferrum, play an important role in energy exchange, stimulate the processes of tissue respiration, hematopoiesis, and also correct the level of free radical oxidation processes. The study of the influence of an energy drink on the content of copper in the erythrocytes of experimental animals is presented in fig. 3.

We found that the content of the regulatory trace element Copper decreases on the 1st, 20th and 30th days by 1.8 ($p<0.001$), 1.2 ($p<0.001$) and 1.1 ($p<0.05$) times, respectively, compared with the control group. After the completion of the energy drink intake, a gradual and significant increase in the level of this trace element was observed on the 20th and 30th days by 1.4 ($p<0.001$) and 1.7 ($p<0.001$) times, respectively, compared with the 1st experimental group of animals.

The results of the study of the influence of an energy drink on the level of Ferrum in erythrocytes of experimental animals are presented in fig. 4.

The concentration of Ferum under the conditions of consumption of an energy drink decreased in erythrocytes throughout the entire period of the study: on the 1st day – by 3 ($p<0.001$) times, on the 20th

day – by 1.4 ($p<0.001$) times and on the 30th day – 1.2 ($p<0.001$) times compared with the control group of animals. After withdrawal of energy drink, there is a significant increase in the Ferrum level on the 20th and 30th days by 2.2 ($p<0.001$) and 2.6 ($p<0.001$) times, respectively, compared with the 1st experimental group of animals.

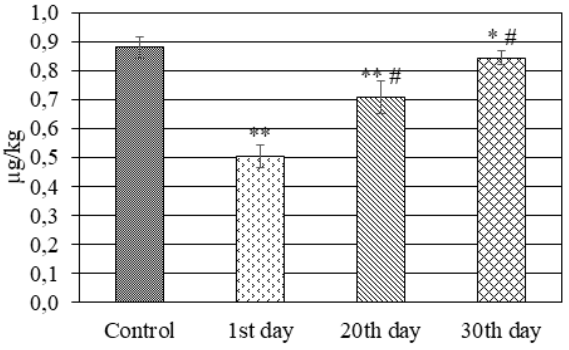

Fig. 3. Dynamics of changes in Cu content in erythrocytes of laboratory rats under conditions of energy drink consumption (M±m) (n=7)

Notes: * – $p<0.05$, ** – $p<0.001$ – reliability compared with intact group of animals; # – $p<0.001$ – reliability compared with 1st experimental group of animals.

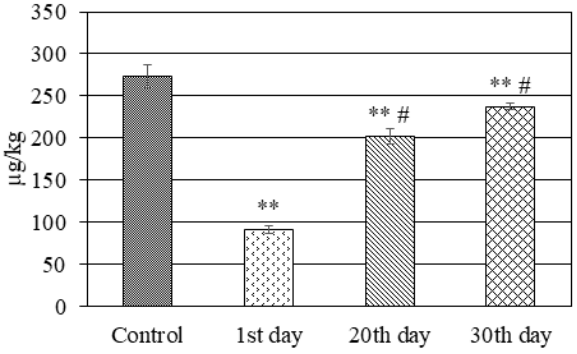

Fig. 4. Dynamics of changes in Fe content in erythrocytes of laboratory rats under conditions of energy drink consumption (M±m) (n=7)

Notes: * – $p<0.05$, ** – $p<0.001$ – reliability compared with intact group of animals; # – $p<0.001$ – reliability compared with 1st experimental group of animals.

The analysis of the obtained results indicates the activation of free radical oxidation processes in the erythrocytes of experimental animals that consumed the energy drink. The accumulation of TBC-active products and CDs can lead to damage to the lipid matrix of biomembranes, which in turn leads to a violation of the structural and functional capacity of erythrocyte cell membranes [14].

Accumulation of erythrocyte POM products can lead to disorders of both structural proteins and enzymes. According to the latest data, carbonyl derivatives of proteins are early indicators of damage to organs and tissues by active oxygen metabolites and allow to quantify the degree of damage, so determining their level is important in early diagnosis of damage by various factors [4].

The achieved data deserve special attention, because the oxidative modification undergoes, first of all, proteins that contain a metal-binding site containing metal ions with variable valence, primarily iron and copper ions, which act as inducers of free radical reactions [10]. Intensification of POM can be a consequence of a violation of the functioning of the body's protective systems. First of all, metalloenzymes such as superoxide dismutase and catalase undergo oxidative modification [15].

Erythrocytes have a more powerful antioxidant system than other cells in the body, as they perform a role related to oxygen transport. In the process of transportation, erythrocytes are exposed to AFO, which significantly disrupts their structure and functional capacity [2]. The study of antioxidant protection of erythrocytes, which were among the first to respond to various influences, showed an increase in the activity of the studied enzymes, which may be due to growth in the peripheral population of “early” forms of cells capable of active protein synthesis. This process can be considered as an adaptive synthesis of antioxidant enzymes. After the end of energy consumption, a decrease in SOD activity was observed, which may be related to the damaging effect of free radicals on copper-containing metalloenzymes [8]. Determination of Copper content in the erythrocytes of the experimental groups of animals showed a tendency to decrease this element relative to the comparison group against the background of a decrease in the Ferrum level throughout the study period. The study of the content of microelements made it possible to establish the development of dysmicroelementosis in the body of experimental animals under the conditions of energy drink.

Conclusions

1. The conducted studies indicate the activation of free radical oxidation processes in the erythrocytes of laboratory animals after taking an energy drink, which was accompanied by the accumulation of lipid and protein peroxidation products, in particular: an increase in the level of TBK-active products in erythrocytes on the 1st and 20th days by 21 %($p<0.001$) and 13 % ($p<0.001$) and CD by 135 % ($p<0.001$), 90 % ($p<0.001$), as well as an increase in the level of neutral and basic aldehyde derivatives by 7–35 % ($p<0.05$) and ketone derivatives of a neutral and basic character by 13–35 % ($p<0.05$), respectively, compared with the intact animals.

2. The study of antioxidant protection confirms the activation of erythrocyte enzymes in the early observation period: on the 1st day after a 30–day intake, the activity of SOD and CAT increases by 10 % and 103 %, respectively, compared with the intact animals. However, in the late period of the experiment, a multidirectional nature of changes is observed: the activity of SOD is significantly lower by 21 % ($p<0.001$), CAT remains elevated by 20 % compared to the control group of animals.

3. In experimental animals, the development of dysmicroelementosis is observed, which is characterized by a decrease in the level of essential microelements Copper and Ferrum on the 1st, 20th and 30th days by 1.8 ($p<0.001$), 1.2 ($p<0.001$), 1.1 ($p<0.05$) and 3 ($p<0.001$), 1.4 ($p<0.001$), 1.2 ($p<0.001$) times compared with the control group of animals.

4. The obtained results give grounds for asserting that the consumption of an energy drink leads to a violation of the homeostasis of red blood cells in laboratory animals, which in turn affects the viability of cells and the supply of tissues with oxygen.

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