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Erythrocyte indices under conditions of energy drink consumption in Ukraine

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Abstract

Background: The consumption of energy drinks, especially among young people, is constantly growing worldwide despite warnings about their safety. Therefore, determining the consequences of their impact on the human body and well-being is an actual issue. This work aimed to study the state of endogenous intoxication and the antioxidant system of erythrocytes in experimental animals that consumed energy drinks.

Methods: The study was conducted on rats that consumed energy drinks for a month. Samples for analysis were taken on the 10th day after the end of the experiment. The biochemical methods of analysis were used to assess the state of endogenous intoxication by the erythrocyte intoxication index and the content of middle mass molecules (MMM), the state of oxidative modification of proteins, and the state of the antioxidant system by the fermentative activity of catalase (CAT) and superoxide dismutase. Statistical methods were used to determine the reliability of the study results.

Results: It was found that the value of the erythrocyte intoxication index significantly increased by 1.8 times ($p<0.001$), the content of MMM254 by 1.2 times ($p<0.001$), and the content of MMM280 – by 3 times ($p<0.001$), indicating the development of endogenous intoxication. The activation of the processes of oxidative modification of proteins is proved by the increase in the oxidative modification of the protein index by 1.1-1.3 times. It was shown that the enzymatic activity of superoxide dismutase decreased by 1.1 times, and CAT activity increased by 1.3 times ($p<0.001$). A 1.5-fold decrease in the ratio between superoxide dismutase and CAT enzymes was also found.

Conclusions: The results indicate damage in the antioxidant system, a decrease in the effectiveness of antioxidant protection, and the development of oxidative stress. The results obtained in this work may help study the potential adverse health effects of energy drinks consumption.

Keywords: erythrocytes; endogenous intoxication; oxidative stress; antioxidant system; oxidative modification of proteins

Introduction

Energy drinks (EDs) are part of the broader category of soft drinks, which includes carbonated beverages, fruit and vegetable juices, bottled water, sports drinks, beverage concentrates, and ready-to-drink tea and coffee. Most EDs contain a combination of caffeine, sugar, B vitamins, minerals, and amino acids. These components work synergistically to provide a quick energy boost. For instance, caffeine is a central nervous system stimulant, enhancing alertness and reducing fatigue, while sugars offer a rapid energy source. B vitamins play essential roles in metabolism, aiding food conversion into energy, while minerals and amino acids contribute to overall physiological function. Some energy drinks incorporate herbal extracts, such as ginseng and guarana, which are believed to enhance energy levels and cognitive function. It's crucial to note

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that the specific formulation of energy drinks can vary significantly between brands, leading to differences in taste, effectiveness, and potential health implications (Alsunni, 2015).

Since the early 90s of the last centuries, when this product appeared on the global market, the consumption of energy drinks has increased rapidly worldwide. With it, the variety of available products has increased. The global ED market size was estimated at 68.1 billion USD in 2021 and is expected to grow at a CAGR (compound annual growth rate) of 7.0% from 2022 to 2032 (Energy Drinks Market…, 2024). Energy drinks are a popular product among all age groups. However, most segments of society that consume large doses of these energy drinks are athletes, teenagers, and students hoping to boost energy levels or compensate for lack of sleep (Jouda *et al.*, 2019). During the COVID-19 pandemic, the overall consumption of ED experienced a slight decline due to widespread closures and restrictions affecting social activities, including schools, gyms, and sports arenas (Koh, 2020). However, the demand for EDs containing caffeine or enriched with vitamins and minerals remained relatively high as consumers sought these beverages for their perceived energy-boosting or nutritional benefits amidst the challenging circumstances of isolation and limited access to traditional sources of stimulation and nutrition (Bakaloudi *et al.*, 2022).

The energy drink market in Ukraine is already showing signs of maturity with moderate growth in retail sales (Jagtap *et al.*, 2022). This is due to the potential shift of consumers from other categories, such as low-alcohol beer, coffee, and carbonated drinks, to energy drinks, attracted by their energy properties. However, economic pressures, including the impact of Russia's invasion of Ukraine and the ongoing pandemic, have led to increased price competition (Zhou *et al.*, 2023). This has resulted in consumers switching from premium brands to cheaper mass products to save money. The market size of Energy Drinks in Ukraine has been impacted by various factors such as health considerations, changing social attitudes, legislation (e.g., sugar tax), demographics, and the changing retail landscape. The COVID-19 pandemic and social restrictions have harmed the consumption of Energy Drinks in Ukraine. Additionally, energy drinks are used in Ukraine, particularly in the military, as a vital asset for forces fighting an exhaustive war. Caffeine in energy drinks is a short-term solution for exhaustion (Leal Filho *et al.*, 2023).

In general, the safety of energy drink consumption remains debatable and has been discussed in several studies (Ehlers *et al.*, 2019; Sikalidis *et al.*, 2020; Soós *et al.*, 2021; Nadeem *et al.*, 2021; Svikis *et al.*, 2022). Limited complex literature reviews illustrate the suitability and risks of energy drink consumption, especially among young people (Ghozayel *et al.*, 2020; Oberhoffer *et al.*, 2022; Khouja *et al.*, 2022). Potential benefits of ED consumption include improved performance and mood due to the content of caffeine, ginseng, taurine, or natural products with high caffeine content, such as guarana (Sikalidis *et al.*, 2020). On the other hand, energy drinks have many side effects on health, including increased heart rate and levels of dopamine and adrenaline, which can lead to hypertension, dehydration, increased urination, and gastrointestinal disorders (Mohammed, 2018). Palpitations, seizures, headaches, strokes, type 2 diabetes, and kidney dysfunction have also been associated with ED use (Edrees *et al.*, 2022). In the study of electrocardiographic and hemodynamic parameters, it was found that the use of energy drinks increases platelet aggregation, blood pressure, and QTc prolongation, which puts patients at increased risk of developing torsades de pointes, which can lead to fatal ventricular arrhythmias (Shah *et al.*, 2019). Frequent energy drink consumption affects blood pressure, heart rate, and blood glucose levels in healthy adults exercising in the gym (Verma *et al.*, 2021).

Almost all works presented in the literature related to the effects of ED on the cardiovascular system, gastrointestinal tract, or neurophysiological parameters, and data on the impact on hematological blood indices are very limited (Khayyat *et al.*, 2014; Posokhov *et al.*, 2019; Bertolone *et al.*, 2020). Erythrocyte indices are important indicators as they provide information on red blood cell size and haemoglobin concentration and characterize erythropoiesis. They are digital characteristics of morphological changes in cells. The results obtained from the study (Khayyat *et al.*, 2014) showed a significant decrease in the number of red blood cells, hemoglobin content, hematocrit, platelets, and neutrophils in animals consuming certain brands of ED. Ultrastructural

changes were also observed, including peripheral blood cells' nucleus and cytoplasm. Prolonged oral administration of caffeinated energy drinks to rats caused an increase in erythrocyte membrane viscosity (Posokhov *et al.*, 2019). Energy drink consumption increased plasma and erythrocyte taurine levels and increased glycolysis and glutathione metabolism in erythrocytes (Bertolone *et al.*, 2020). However, the presented works lack data on the effect of ED on the oxidative modification of proteins (OMP) and the state of antioxidant defence of the body.

Although existing research extensively explores the impact of ED consumption on cardiovascular, gastrointestinal, and neurophysiological parameters, limited attention has been given to erythrocyte indices, which are crucial for understanding erythropoiesis and morphological changes in cells. Therefore, this work aimed to study the changes in erythrocyte indices of rats after using ED to assess the state of endogenous intoxication and the development of oxidative stress. The main questions for this study could be formulated as:

1. How does the consumption of energy drinks affect erythrocyte indices in experimental rats, and what insights does this provide into the development of endogenous intoxication and oxidative stress?
2. What are the specific changes observed in the antioxidant system of experimental rats following the consumption of energy drinks, and how do these changes contribute to the development of oxidative stress?

Methods

Experimental design

The research was conducted by directive of the European Parliament and of the Council No. 2010/63/Eu "On the Protection of Animals Used for Scientific Purposes" (2010), based on the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1991). The experiments were conducted according to the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006), as well as the recommendations of the First National Congress of Ukraine on Bioethics (Reznikov *et al.*, 2006).

The experiments were conducted on adult male Wistar rats weighing 180-250 g, kept in a vivarium under standard conditions on a standard diet and free access to water at 20 ml per day per rat. All experimental animals showed weight gain. According to the experimental conditions, the animals were divided into two groups (each group consisted of 7 animals): the intact (control) group received only drinking water; the experimental group received an energy drink for a month. The required amount of the drink was calculated based on the conversion per kg of adolescent body weight. The ED contained water, caffeine, taurine, carbon dioxide, citric acid, and sodium citrate as acidity regulators, anthocyanin and sugar color IV as natural colorants, sugar, natural and identical to natural flavors, preservative potassium sorbate, glucuronolactone, inositol, ascorbic acid, vitamins B3, B5, B6 and B12. The duration of ED use was 1 month. Material for analysis was collected on the 10th day after the end of the experiment. For this purpose, rats were anesthetized by intramuscular injection of sodium thiopental (60 mg/kg), and blood samples were taken and centrifuged. Plasma and erythrocyte masses were selected separately, from which the hemolysate necessary for analysis was prepared.

Ethical approval

The Scientific Committee of Ethics of the Ivano-Frankivsk National Medical University, Ukraine, revised and approved all animal procedures.

Hematological and biochemical analysis

To assess the state of endogenous intoxication, two leading indicators were determined: erythrocyte intoxication index (EII) and the content of middle mass molecules (MMM). The basis of the method used to determine the EII is the idea of the erythrocyte as an adsorbent; that is, the

erythrocyte membrane can absorb and pass-coloured substances, namely methylene blue (Kazimirko and Maltsev, 2007). The content of MMM in hemolysate was determined at wavelengths of 254 nm (incomplete protein breakdown products) and 280 nm (aromatic amino acids), according to the method of Habryielian *et al.* (1985). The intensity of oxidative modification of proteins in erythrocyte hemolysate was estimated according to the process of Dubinina and Shuhalei (1993).

The optical density of the formed dinitrophenylhydrazones, expressed in conventional units, was recorded using a SPECORD M 40 spectrophotometer (Germany) at wavelengths 356 nm and 370 nm (aldehyde and ketone derivatives of neutral character) and 430 nm and 530 nm (aldehyde and ketone derivatives of essential character). Catalase activity (CAT) [KF 1.11.1.6] was determined as the ratio of catalase number to the number of red blood cells in 1 ml of blood.

CAT was expressed in units according to the method, which is based on determining the amount of hydrogen peroxide split in the catalase reaction. Quantitative determination of catalase was performed using methods A. Bakh and S. Zubkova (Babenko, 1999). The activity of superoxide dismutase (SOD) [KF 1.15.1.1] was calculated based on the level of inhibition of the reduction process of nitro blue tetrazolium in the presence of NADH (nicotinamide adenine dinucleotide, H for hydrogen) and phenazine methosulfate (Chevary *et al.*, 1991).

Statistical analysis

Statistical calculations were performed using STATISTICA 7 software with Student's t-test. Given the small sample size and the assumption of normal distribution, the t-test was chosen as a suitable statistical method to analyze the observed differences in EII, content of MMM, OMP, CAT, and SOD. The p-values ($p<0.001$) indicate statistically significant differences in EII and MMM between the two groups, confirming the appropriateness of using the t-test for comparing means in this experimental context.

Results

The results of determining the content of middle mass molecules and erythrocyte intoxication index in the erythrocytes of experimental animals under conditions of energy drink consumption are presented in [Table 1].

Table 1: Indicators of endogenous erythrocyte intoxication in rats of intact and experimental groups.

Indicators	Intact group (n=7)	Experimental group (n=7)
MCM_{254}, unit.	0.108 ± 0.005	0.132 ± 0.005*
MCM_{280}, unit.	0.017 ± 0.002	0.051 ± 0.015*
EII, %	29.16 ± 2.34*	51.75 ± 2.35*

***Note:** * – the reliability of the experimental group compared to the intact group of animals <0.001; MMM_{254} and MMM_{280} – the content of middle mass molecules at wavelengths of 254 and 280 nm, respectively; EII – erythrocytes intoxication index*

The obtained values for the intact and experimental groups are expressed as ± the mean value of standard deviation (M±m). As can be seen from the results of Table 1, an increase in endogenous intoxication products was observed during the study. Indicators of MMM_{254} and MMM_{280}, which reflected, respectively, the content of incomplete breakdown products of proteins and aromatic amino acids, increased in experimental animals that consumed energy drinks during the month, compared to the control group. Thus, the content of MMM_{254} in the blood of rats of the experimental group significantly increased from 0.108±0.005 units to 0.132±0.005 units ($p<0.001$). This is 1.2 times more compared to the intact group. As for the content of MMM_{280}, this indicator increased much more – 3 times ($p<0.001$), from 0.017±0.002 to 0.051±0.015 units. At the same time, a significant increase of 1.8 times ($p<0.001$) in the level of EII in the experimental group relative to the intact group was noted. The erythrocyte intoxication index increased from 29.16 2.34% to 51.75 2.35%.

The functioning of red blood cells is primarily determined by how well-balanced the oxidation processes of lipids and membrane proteins are and how active the antioxidant system is. Proteins are essential in metabolic processes in living organisms. They are essential ferments for various metabolic and regulatory processes in the body. However, they react rapidly with oxidants, and their abundance in cells, extracellular tissues, and body fluids makes them the primary target for oxidation reactions. Due to the influence of various exo- and endogenous factors, reactive oxygen species (ROS) are constantly formed in the body, the functions of which can be both positive (mobilization of the immune system) and negative (oxidation processes in proteins and lipids). In the body, under normal conditions, ROS formation and neutralisation processes are in a balanced state. Under the influence of stress or other factors, the existing balance is disturbed, oxidized products accumulate, and the body does not have time to neutralize them. As a result, the work of almost all organs is disrupted. With such an imbalance between the generation and disposal of ROS, oxidative stress develops, which is the cause of many pathologies. Due to the development of oxidative stress caused by the excessive generation of ROS, the processes of uncontrolled modification of proteins are actively developing. The negative consequences of this process are not only the denaturation and fragmentation of proteins but also the formation of primary amino acid radicals, which can enter secondary reactions with adjacent amino acid residues. In other words, proteins lose their biological activity.

The intensity of oxidative modification of proteins in erythrocyte hemolysate was studied since this value, that is, the indicator of oxygen-dependent oxidation of proteins directly indicates damage to organs and tissues of the body. The results are shown in [Figure 1]. In the animals of the experimental group on the 10th day after the completion of ED administration, the value of optical density at a wavelength of 356 nm increased from 0.23 to 0.26 units, i.e., a significant increase in the level of OMP by 1.1 times ($p<0.001$) was observed. A significant increase in the level of OMP by 1.2 times ($p<0.001$) compared to the intact group was noted at a wavelength of 530 nm. The value of optical density increased from 0.14 to 0.17 units. A similar pattern of increase in the level of OMP is characteristic of the wavelengths of 370 nm and 430 nm. The analyzed indicators increased by 1.3 times ($p<0.05$) and 1.2 times ($p<0.05$), respectively. Such a significant increase in oxidative modification indicates a pronounced activation of free radical oxidation of proteins in experimental animals that consumed energy drinks.

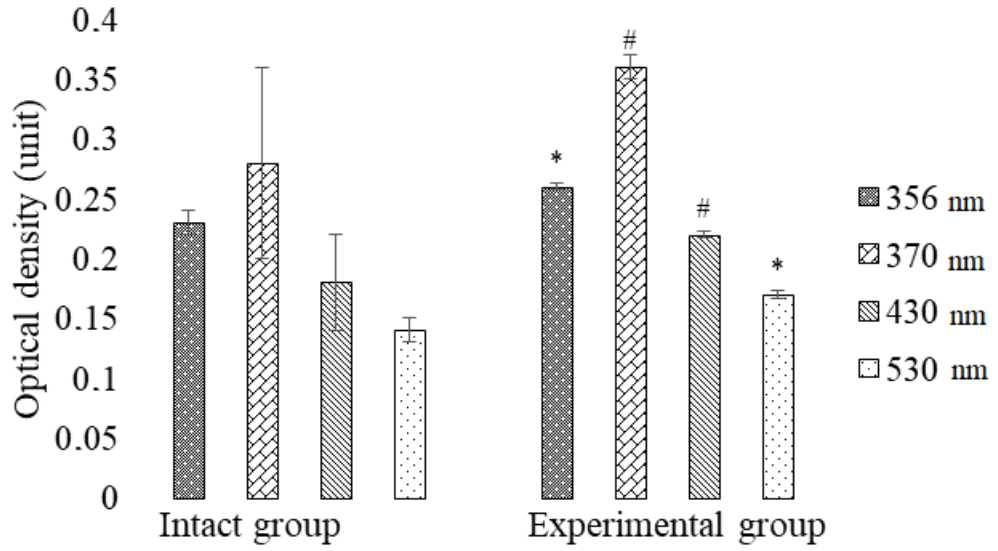

Figure 1: The level of oxidative modification of proteins in the hemolysate of erythrocytes of rats of intact and experimental groups, units (M±m; n=7)

Notes: *symbols in the experimental group mean: * – reliability of the experimental group compared to the intact group of animals <0.001; # – reliability compared to the intact group of animals <0.05.*

The next stage of research was to study the state of the antioxidant system of red blood cells, which are among the first to respond to various influences. The antioxidant system plays a protective role against the harmful effects of various factors, including ROS. The antioxidant system includes both antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase, and non-enzymatic antioxidants, such as vitamin E, vitamin A, vitamin C, glutathione, flavonoids, carotenoids, and uric acid. Antioxidant enzymes neutralize the action of hydrogen peroxide and superoxide radicals, which are the leading cause of harmful oxidative damage. By reducing their concentration to a minimum, they thereby reduce the likelihood of the formation of reactive OH• radical.

The activity of antioxidant defence ferments largely determines how intense the processes of free radical oxidation are. Enzyme's superoxide dismutase and catalase play prominent roles as antioxidant defenders. Superoxide dismutase has a high catalytic rate of the superoxide radical dismutation reaction with subsequent hydrogen peroxide and oxygen formation. As a critical cellular antioxidant, SOD is mainly responsible for the removal of O_2^-, which damages cells when it is in excessive concentration. Therefore, SOD maintains and controls not only free radical levels but also the oxygen homeostasis of the body. The regulation of SOD activity is based on the principle of reversible feedback, i.e., an excessive amount of H_2O_2, which is formed, significantly reduces the activity of superoxide dismutase. SOD is a metalloprotein found not only inside cells and intercellular space of tissues but also in biological fluids such as plasma or lymph. In blood cells, SOD successfully deactivates reactive oxygen species. As mentioned above, after the breakdown of ROS, hydrogen peroxide is formed, which can destroy SOD molecules, which is why superoxide dismutase always functions together with catalase. Catalase (CAT) is an enzyme that belongs to the class of oxidoreductases. Its peculiarity is that it has both catalase and peroxidase activity. CAT reduces hydrogen peroxide to water and molecular oxygen quite rapidly, i.e., its main function is the accelerated decomposition of hydrogen peroxide, which is formed because of various oxidative processes in the body, in red blood cells. Since this enzyme has a key responsibility for neutralizing hydrogen peroxide through decomposition, it, therefore, maintains the optimal level of the molecule in the cell, which is also important in the processes of cell signalling.

[Figure 2] shows the change in the activity of SOD and CAT in the erythrocyte hemolysate of rats after the use of energy drinks compared to the intact group. It should be noted that on the 10th day after the end of the experiment, there was a decrease in SOD activity from 58.19% to 52.47%. That is, in animals that received the energy drink for a month, compared to the intact (control) group, SOD decreased by 1.1 times ($p<0.05$). When analyzing CAT activity, it was noted that there was a significant increase in this indicator by 1.3 times ($p<0.001$) in rats of the experimental group relative to animals of the control group. CAT value increased from 13.96 mg H_2O_2/ml to 18.38 mg H_2O_2/ml. Thus, according to the results obtained, multidirectional changes occur in the antioxidant system of erythrocytes.

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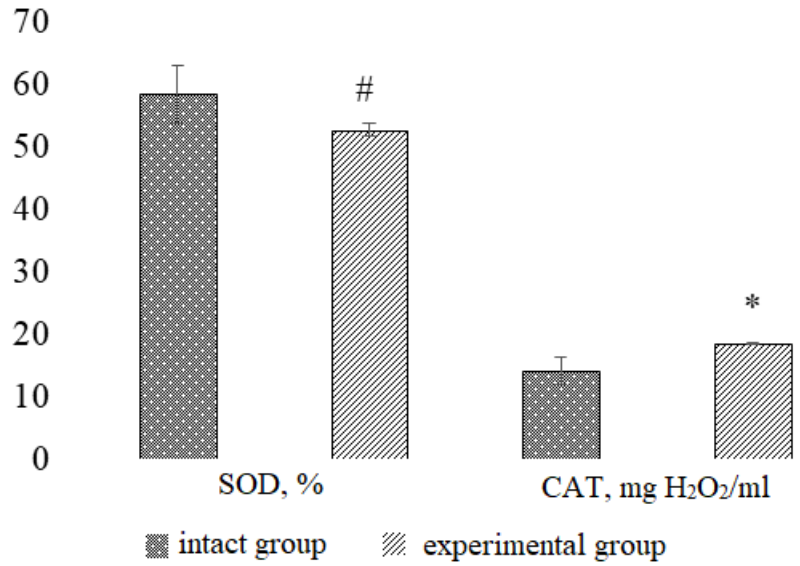

Figure 2: Changes in the activity of antioxidant enzymes SOD and CAT in erythrocyte hemolysate of rats of intact and experimental groups (M±m; n=7)
Notes: *symbols in the experimental group mean: * – reliability of the experimental group compared to the intact (k) group of animals 0.001; # – reliability compared to the intact (k) group of animals 0.05.*

Analyzing on the 10th day the dynamics of changes in the activity of the studied enzymes in the hemolysate of rats when they consumed the energy drink during the experimental period, significantly higher ($p<0.001$) values of SOD against catalase were found [Figure 3].

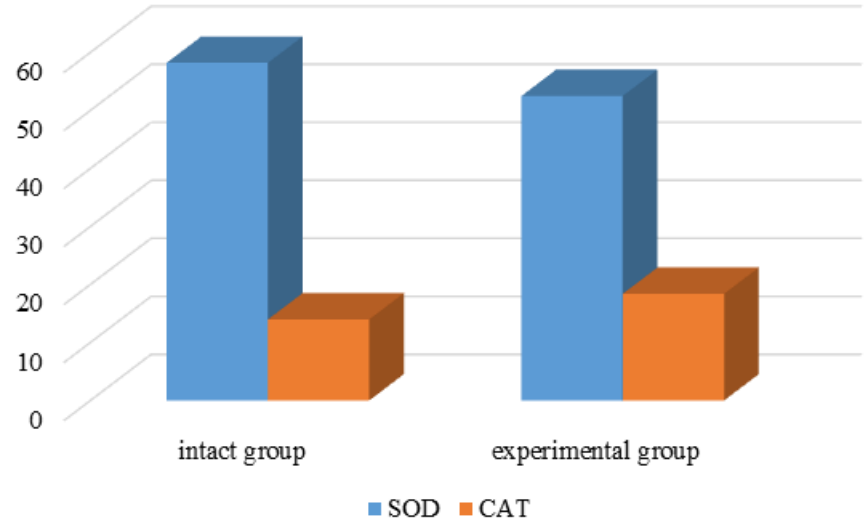

Figure 3: Dynamics of changes in the activity of antioxidant enzymes in erythrocyte hemolysate of rats of intact and experimental groups (M±m; n=7)

The ratio of SOD/CAT in rats of the experimental group decreased approximately 1.5 times (from 4.17 to 2.85) compared to this value in rats of the intact group.

Discussion

Since MMM have high biological activity, they can block cell receptors by binding to the active centers of albumin molecules and thus disrupt the process of humoral regulation. The increase in MMM content indicates the development and spread of endogenous intoxication. A significant

increase in MMM_{254} and MMM_{280}, revealed during the study, indicates a substantial increase in endogenous intoxication of experimental animals during the experimental period. Moreover, the increase in the content of MMM_{280} was more intense than that of MMM_{254}, indicating a higher content of aromatic amino acids in the composition of medium molecules. This may be due to the loss of aromatic amino acids by proteins because of oxidative modification and fragmentation of molecules. A similar trend in the relationship between MMM content and the development of endotoxemia has been noted in the study of the effect of drugs on indicators of endogenous intoxication (Lys and Regeda, 2019; Demkovych *et al.*, 2021) or in the assessment of cardiovascular risks (Strilchuk and Kondratyuk, 2021). In these works, it is noted that with increasing levels of endogenous intoxication, the permeability of erythrocyte membranes increases, and the content of middle mass molecules increases. Such changes are associated with direct injury, destructive changes, inflammation, activation of hypoxia and peroxia, and lipid oxidation. The study of the data presented in the literature allows us to state the lack of special studies on the effect of ED consumption on the development of endotoxemia in the body of experimental animals and the development of oxidative stress.

Oxidative stress triggers the mechanisms of free radical oxidation, which leads to the accumulation of reactive oxygen species. The latter activates the processes of oxidative modification of proteins and lipid peroxidation. Both exogenous and endogenous toxins accumulate in the body. This deepens the endogenous intoxication of the body, which is accompanied by the accumulation of aldehyde and keto derivatives of a neutral and basic nature in erythrocytes (Demasi *et al.*, 2021). The obtained research results confirm this conclusion. The increase in OMP values determined in the work indicates the activation of protein modification. The likely consequence of such modification may be losing their biological activity, fragmentation, and denaturation (Hawkins and Davies, 2019). In turn, intoxication causes changes in protective systems, particularly antioxidant and immune systems (due to the formation of many compounds with antigenic properties). It is known from the literature (Al Yacoub *et al.*, 2020) that the level of MMM depends on both the intensity of biopolymer breakdown and the decrease in the rate of their excretion through the detoxification organs, which are the kidneys and liver. Given the experimental data obtained in this work, it can be assumed that both process components are affected.

The results of the analysis of the obtained values of the erythrocyte intoxication index show an increase in this value, which indicates the sensitivity of red blood cells to the action of energy drinks. Changes in the level of destruction of erythrocyte membranes (the level of dye absorption) are caused by the fact that after sufficiently long use of ED in erythrocytes, energy metabolism is affected, transport of substances is impaired, and the permeability and sorption capacity of membranes increases. These conclusions are consistent with the results of previous studies of the authors on the effect of ED on the state of erythrocyte membranes and hemolytic parameters (Partsei *et al.*, 2017). Other authors have also reported changes in the state of erythrocyte membranes due to the use of energy drinks (Posokhov *et al.*, 2019). Oxidative stress, which reduces the antioxidant capacity, irreversibly damages red blood cells, particularly susceptible to its effects due to the high polyunsaturated fatty acids in the membrane and the auto-oxidation of hemoglobin inside the cell. This leads to the final damage of red blood cells by hemolysis and their removal by circulation (Maurya and Namdeo, 2021). Red blood cells are equipped with an antioxidant defence system in the form of enzymatic and non-enzymatic antioxidants to prevent damage.

The experimental results obtained indicate an unclear effect of ED on the state of antioxidant defence in the body of experimental animals. On the one hand, the enzymatic activity of SOD, an enzyme that is the main obstacle to the formation of ROS, decreased. Such a decrease is a characteristic feature of damage to the antioxidant system and reduced protection of red blood cells from the toxic effects of superoxide radicals. The decline in SOD activity is likely associated with an increase in the generation of superoxide radicals, resulting in the formation of

hydrogen peroxide and oxygen. Hydrogen peroxide can be easily converted into hydroxyl radical, which is highly reactive and damages the metal-protein complex of the enzyme (Islam *et al.*, 2021). On the other hand, catalase activity in rats of the experimental group increased. It cannot be excluded that the consumption of energy drinks initiates the formation of hydrogen peroxide, increasing CAT enzymatic activity, which can be considered an adaptive response.

A review of the reactions of catalases with their primary substrate, hydrogen peroxide, and with various oxidants, such as hydroxyl radical, superoxide, nitric oxide, peroxynitrite, hypochlorous acid, and singlet oxygen, was conducted by Gebicka and Krych-Madej (2019). Another possible reason for the increase in catalase activity may be the course of glycosylation reactions by glucose oxidation products. When consuming ED, blood glucose levels increase significantly, which is confirmed by the results of studies by various authors (Nowak *et al.*, 2018; Graneri *et al.*, 2021), which activates CAT activity. Since both superoxide dismutase and catalase are the main components of the enzymatic link of antioxidant protection, their ratio indicates changes in oxidative protection. A decrease in the ratio of SOD and CAT enzymes, which in this case is due to an increase in the activity of catalase, is possible evidence of a disorder in the coordination of enzymatic antioxidants and confirms a decrease in the level of antioxidant protection (Aliiev, 2023).

According to the authors, the presence of taurine as a component in ED can, to some extent, compensate for the decrease in the activity of antioxidant enzymes. This assumption is confirmed by the data presented in the literature (Bertolone et al., 2020) on the antioxidant activity of taurine in erythrocytes. Taurine is an amino acid that is not synthesized in the human body. It promotes weight loss, helps muscle recovery, and improves oxygen transfer in the body. The authors of this work suggest using taurine to counteract oxidative stress. Non-enzymatic low molecular mass antioxidants may also contribute, one of which is ascorbic acid, also present in the ED taken for the study. Ascorbic acid has antioxidant effects, protecting cellular structures from free radical damage (Vora Axita *et al.*, 2022). In general, several studies in the literature review the impact of individual ED ingredients such as caffeine, taurine, guarana, and/or their combinations.

Most of these studies focus on caffeine, whose mechanism of action is almost entirely studied, and its effects on the body have been examined (Soós *et al.*, 2021). Such studies regarding the impact of other components are rare (Jouda *et al.*, 2019). The effects of guarana have not been sufficiently studied, although it is well known that the beans of this plant contain about twice as much caffeine as coffee beans, which is why the caffeine content in energy drinks increases (Sikalidis *et al.*, 2020). The morphological and biochemical effects in vitro of caffeine, taurine, and guarana, alone or in combination with taurine, have been studied, and their cytotoxicity has been evaluated (Zeidán-Chuliá *et al.*, 2013). Evidence of both positive and negative effects of the main components of energy drinks or their cumulative effect on the state of erythrocytes and the development of oxidative stress was not found in the literature. Therefore, the experimental results indicate that energy drinks can have potential adverse health effects and are accompanied by a pronounced increase in endogenous intoxication and changes in the state of the antioxidant system.

The study's findings advocate for comprehensive policy reforms targeting energy drink regulation. It necessitates the development of stringent guidelines for ingredient disclosure, health risk warnings, and consumption advisories on packaging. The evidence supports advocating for age-restricted sales to protect young consumers from potential adverse effects. Moreover, it underscores the importance of public health campaigns to educate consumers about the risks associated with energy drink consumption and encourages further scientific research to inform policy decisions. These measures aim to mitigate health risks and promote safer consumption practices.

Conclusions

Experimental studies of changes in erythrocyte indices in experimental rats after the use of ED were conducted to evaluate the state of endogenous intoxication and the development of oxidative stress. The data obtained indicate the activation of endogenous intoxication processes and changes in the state of the antioxidant system. Endotoxemia develops in the body of experimental animals, manifested by the accumulation of endotoxins in the blood hemolysate. This fact is confirmed by clearly expressed changes in the leading indicators of endogenous intoxication – EII and MMM. A significant increase in the value of EII by 1.8 times ($p<0.001$) was found, indicating the sensitivity of erythrocytes to ED. The content of MMM_{254} in the hemolysate of the rats of the experimental group significantly increased by 1.2 times ($p<0.001$) compared to the intact group, and the content of MMM_{280} – by 3 times ($p<0.001$).

It should be noted that the higher intensity of the increase in the content of MMM_{280}, compared to MMM_{254}, indicates an apparent rise in the concentration of aromatic amino acids present in the middle molecules. The activation of the processes of oxidative modification of proteins is proved by the increase in the OMP index by 1.1-1.2 and 1.2-1.3 times, determined by aldehyde and keto derivatives of neutral and essential character, respectively. It is shown that the state of the antioxidant system shows multidirectional changes. SOD activity decreases by 1.1 times, and CAT activity increases by 1.3 times. The decrease in the SOD/CAT ratio by almost 1.5 times is caused by disturbances in the consistency of enzymatic antioxidants and a decrease in the level of antioxidant protection. Reduction of antioxidant and superoxide dismutase activities in the blood hemolysate of experimental rats after using energy drinks confirms the development of oxidative stress and the effect of endogenous intoxication. The increase in catalase activity may be due to adaptive synthesis as a response to the increase in ROS or possible glycosylation by glucose oxidation products. The results obtained in this work may help study the potential adverse health effects of ED consumption.

The study was limited by its small sample size of only 14 rats divided into two groups and the short duration of 1 month for ED consumption. To further validate the results, the study should be expanded to include more animals, additional experimental groups with varying durations of ED intake, and more extended follow-up periods after ceasing consumption. There is also a need for research on the effects of individual ingredients like caffeine or taurine, comparison of different types of energy drinks, and tracking changes over extended periods after stopping intake. Studies on actual ED consumption patterns and amounts in human populations are also warranted.

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

Data availability statement: The data supporting this study's findings are available on request from the corresponding.

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