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STUDY OF S100 PROTEIN LEVELS IN RATS SUBJECTED TO ENERGY DRINK ADMINISTRATION

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Abstract: In recent years, the increased consumption of energy drinks has become a pressing public health issue. The use of energy drinks, classified as non-alcoholic beverages, is rising significantly among young people each year. At the same time, it is well known that there are enthusiasts who consume these drinks regularly and may even develop dependence on them. These beverages provide temporary energy and strength, reduce sleepiness, improve mood, and enhance activity. In our study, chronic consumption of energy drinks was found to have a negative impact on the development of the nervous system and was associated with an increase in S100 protein levels in the blood. This may indicate a potential risk for the subsequent development of oncological and chronic neurological disorders.

Introduction: Energy drinks are becoming increasingly widespread and popular among young people and adolescents [2,4]. Worldwide, the sales of these beverages are rapidly increasing, even surpassing the consumption of alcoholic products. One of the main risks associated with regular consumption of these drinks is that their caffeine content has been found to be three times higher than that of coffee. Currently, more than 500 different types of energy drinks are consumed in various countries [1].

Consumption of energy drinks provides temporary stimulation of the body, but this is an artificial form of activation. Energy drinks do not provide additional energy; they only deplete the body's reserve resources [4,5,6].

Chronic consumption of energy drinks has been shown to cause significant changes in the histological structure of human organs, potentially leading to pathological processes. In particular, literature reports indicate that this can result in impaired kidney function, liver diseases, respiratory system changes, nervous system disorders, elevated blood pressure, and disruption of cardiac muscle function [3].

Given the current increase in energy drink consumption, the need for preventive strategies to mitigate associated adverse effects is repeatedly emphasized. In scientific studies, in addition to histological examinations, biochemical analyses of blood—particularly the determination of S100 protein levels—can be an important indicator.

S100 belongs to a family of proteins responsible for binding calcium ions. S100 proteins are essential for processes such as cell growth and division, protein synthesis, muscle contraction, and many other functions. They are produced in small amounts by the liver, kidneys, heart, skin, and nerve cells, with the highest concentrations found in brain cells [4,8]. The primary objective of this study was to determine and analyze the levels of S100 protein in the blood.

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Materials and Methods: For the experiment, 9-month-old male white rats weighing 220 ± 30 g were selected, and all procedures were conducted in compliance with bioethical principles. The experimental animals received the widely available energy drink **Iguana** orally via a gastric gavage for 1 and 3 months.

Rats in the control group were administered 7.5 ml of physiological saline once daily via gavage. The dose of Iguana energy drink was calculated at 10 mg/kg of body weight, and it was administered once daily at 7.5 ml for 1 and 3 months. Blood samples from the rats receiving the energy drink were collected at the end of 1 and 3 months for laboratory analysis.

Each of the experimental and control groups included 7 rats, totaling 21 animals. Blood samples were obtained from all animals in each group, and the levels of S100 protein were measured. The collected data were subsequently analyzed.

For enzyme-linked immunosorbent assay (ELISA), blood serum was prepared, and S100 protein levels were determined using specialized ELISA kits designed for this purpose. Laboratory analyses were conducted at the final stages of each experimental period.

Results and Analysis: In the control group, the S100 protein levels in the 7 rats averaged **6.126 ng/ml**. In the experimental group, after 1 month of daily administration of Iguana energy drink, the S100 protein levels in 7 rats averaged **99.125 ng/ml**. After 3 months of continuous daily administration of Iguana energy drink, the S100 protein levels in 7 rats increased dramatically, averaging **1603.57 ng/ml** (Table 1).

As shown in the table, S100 protein levels in both experimental groups were significantly higher compared to the control group. This increase was particularly pronounced in the chronic exposure group, i.e., rats that received the energy drink for 3 months.

According to literature data, S100 protein levels can increase in tumor diseases, especially in malignant neoplasms [4,8]. Russian researchers L.I. Larentsova and co-authors have recommended the measurement of S100 protein in brain tissue as a diagnostic marker in dental procedures, based on clinical observations and animal studies. G.R. Khakimova and colleagues consider the increase in S100 protein as a response to antigen stimulation and emphasize that detecting antibodies against it and administering therapeutic agents can serve as an indicator of treatment efficacy.

Literature also reports elevated S100 protein levels in brain tumors, leukemias, and cancers of the breast, ovaries, stomach, pancreas, and bladder. Based on these data, the significant increase of S100 protein observed in our experimental rats that chronically consumed the Iguana energy drink can be considered a highly important and potentially hazardous indicator. The pronounced difference in S100 levels between experimental and control animals further confirms that the observed increase is likely attributable to the long-term consumption of the Iguana energy drink.

S100 Protein Levels in 9-Month-Old Control and Experimental Rats

Control Group	**Experimental Group**	
	Rats Receiving Energy Drink for 1 Month	Rats Receiving Energy Drink for 3 Months
6,126 **ng/ml**.	99,125 **ng/ml**.	1603,57 **ng/ml**.

Conclusions: The experiments conducted and the data obtained indicate that the widely consumed energy product Iguana, popular among certain individuals today, causes a significant increase in S100 protein levels in the blood of white rats. Compared to animals receiving the

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drink for 30 days, rats that were administered Iguana daily for 90 days showed a dramatic rise in S100 protein levels. This pronounced increase may serve as a warning indicator of the considerable toxic potential of this energy drink.

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