

Impact of Immobilization Stress on Erythropoiesis, Alterations in the Antioxidant System, and Potential Correction with *Stevia rebaudiana* Bertoni in Rabbit

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Abstract

This study investigates the impact of immobilization stress as a risk factor for pathological processes, with a focus on erythropoiesis and oxidative stress. Additionally, the potential therapeutic effects of *Stevia rebaudiana* Bertoni in mitigating these stress-induced alterations are explored. Immobilization stress was induced for 30 days, and its effects on erythropoiesis and antioxidant systems were assessed. *Stevia rebaudiana* Bertoni was administered to evaluate its potential for preventing and correcting the observed negative processes.

Prolonged immobilization stress led to disruptions in erythropoiesis, manifested by a delay in proliferative and maturation processes and a moderate decrease in erythrocyte equilibrium. Concurrently, increased stress duration stimulated lipid peroxidation processes and inhibited antioxidant enzyme activity. The use of *Stevia rebaudiana* Bertoni demonstrated significant positive effects on lipid peroxidation processes and restored the activity of antioxidant enzymes, particularly peroxidase and catalase.

Immobilization stress adversely affects erythropoiesis and oxidative balance, contributing to pathological processes. *Stevia rebaudiana* Bertoni emerges as a promising intervention, displaying the potential to attenuate the negative effects of immobilization stress through its positive influence on erythropoiesis and antioxidant mechanisms.

1. Introduction

Treatment of organism resistance effective measures to different stress factors is one of the actual problems of medico-biological sciences since stress is a basis for the development of numerous pathological processes. In the beginning state of the effect of stress factors, the hypothalamus-hypophysis-adrenal system is stimulated, the amount of biologically active compounds in the organism is enhanced, and the level of red-ox processes changes that play an important role in organism adaptation to environment-altered conditions and viability providing [1]. To ensure the normal functioning of the body, physical activity is necessary, which increases the energy supply and oxygen supply of the body and favorably affects the course of the biochemical and physiological functions of organs and systems. Restriction of mobility causes a stress reaction, reduces the body's reserve capacity, and negatively affects the nervous and humoral mechanisms regulating the body's physiological and reproduction functions [2, 3]. During stress reactions, the body quickly mobilizes its compensatory-protective mechanisms to counteract the violation of physiological balance, however, prolonged exposure to stress causes morphological and functional changes in organ systems, and causes the development of pathological processes. The study of biochemical and physiological functions under the influence of various stress factors on the body is of great importance for the development of stress-limiting measures to prevent emerging deviations. It is especially important to study the effect of psycho-emotional stress on the several functions of the body, which include immobilization stress.

Immobilization stress is one of the risk factors for developing pathological processes in the body, which may disrupt adaptation processes. As a stress factor, it causes tension in regulatory mechanisms,

disruption of vegetative functions reduces the reserve capacity of the body, and inhibits energy processes [3, 4]. Short-term exposure to immobilization stress causes erythropenia, a decrease in hemoglobin content and osmotic stability of erythrocytes, and an increase in the number of destroyed erythrocytes in the spleen [5–7] against the background of changes in various organs and systems of the body. When exposed to stress factors, the body quickly mobilizes its adaptive, compensatory, and protective mechanisms to counteract the violation of physiological balance, however, prolonged exposure causes anatomical and functional disorders of organ systems, and causes the development of pathological processes[8]. Therefore, it is important to minimize the disturbances caused by stressors and apply measures that will increase the body's resistance to the stress factor and contribute to preventing and correcting emerging disorders. Currently, medicinal plants and herbal preparations prepared from them are used in medical practice for the prevention and treatment of various diseases [9]. Biologically active substances contained in plants are similar to the natural metabolites of the body, most of them are necessary for the normal functioning of the body. Herbal plants containing flavonoids and glycosides, which include *Stevia rebaudiana*, are distinguished by their medicinal properties and the composition of physiologically active components [10].

It is known that stevia contains natural antioxidants, increases the body's resistance to adverse environmental factors, has anti-stress [11], antibacterial [12, 13], anticarcinogenic, and anti-inflammatory effects [13], normalizes the activity of the secreting glands [14] and exhibits a hypoglycemic effect [11, 15]. Besides its health benefits, industrial applications of stevia particularly as food and food ingredients, as sucrose replacers, as fertilizers and animal feed, and as solubilizing or foaming agents have been discussed [16].

The research aimed to study the effect of immobilization stress on several vital processes, in particular, on the morphological and functional parameters of erythropoiesis, changes in the oxidant and antioxidant systems of the body, and the possibility of their correction using the herb *Stevia rebaudiana*.

2. Materials and methods

2.1. Chemicals and reagents. All chemicals were purchased from Sigma-Aldrich (USA).

2.2. Bioethics. The experimental procedures involving animals were conducted following the guidelines outlined in Directive 2010/63/EU [17]. Ethical approval was obtained from the Armenian National Center of Bioethics.

2.3. Animals and experimental design. The experimental animals had the same gender (male) and weight ((weighing 1400–1500 g.) as *Oryctolagus cuniculus domestic* rabbits, which were in the same conditions of nutrition and care during experiments. Studies have been carried out in the same feeding and caring conditions by two variants. In the first variant the peripheral red blood parameters, changes in lipid superoxide oxidation processes, and activity of enzymes (catalase and peroxidase) were studied in the immobilization stress effect (Control group). For the immobilization of animals, they were fixed 5 hours a day for 30 days on the experimental table [18]. In the second variant, the studies were carried out in

combined stress dynamics of stevia and immobilization stress. The number of animals for blood sampling is 5, and for organ studies, 3 at each time point (0, 10, 20, 30 days). Dynamic changes in the composition of blood were studied at 5-day intervals for 30 days, and as for organs of animals, the interval was 10 days taking into account the exploratory nature of the research. These intervals may change in future research. Stevia crushed leaves with 0.5 g/kg weight were added to animal feed each day then animals were exposed to stress effect.

2.4. Determination of peripheral red blood parameters. Erythrocytes were counted using the Goryaev counting grid [19], and the hemoglobin content was determined with a Sally hemoglobinometer. The relative percentage of hemoglobin was divided by the double number of the first two numbers of erythrocytes to obtain a color index. The relative percentage of reticulocytes was determined by the Egorov method [20]. To determine the absolute number of reticulocytes, the number of erythrocytes in 1 mm³ of blood was multiplied by the relative percentage of reticulocytes on the same day and divided by 1000.

2.5. Quantitative determination of malondialdehyde in the blood and different organs (liver, kidneys, and brain) was performed by spectroscopic measurement of the optical density of products reacting with 2-thiobarbituric acid [21, 22].

2.6. Determination of the activity of enzymes catalase and peroxidase. The catalase activity was measured according to the method of titration [23]. Catalase activity was determined by the μmol of hydrogen peroxide broken down in 1 mg of protein. Peroxidase activity was determined according to the spectroscopic method [24], and the activity of peroxidase is expressed in μmol of hydrogen peroxide/min/mg protein.

2.7. Statistics. All results are presented as means $\pm$ SD. The significance of the results was assessed using one-way and two-way ANOVA and Tukey's multiple comparisons tests. Statistical analyses were performed using GraphPad Prism 8 software (San Diego, CA, USA), and a significance level of $p < 0.05$ was deemed statistically significant.

3. Results

3.1. Effect of prolonged immobilization stress on morpho-functional criteria of erythropoiesis. Analysis of the results of the study showed that 5-day immobilization caused a moderate normochromic increase in erythrocytes and hemoglobin and reticulocytosis. The number of erythrocytes increased by 13% compared with the initial level, and the content of hemoglobin by 9%, resulting in a color index within the normal range (0.73). The relative number of reticulocytes increased by 47%, the absolute number - by 66%, and the rate of maturation of reticulocytes by 33% (Table 1). These changes in the peripheral parameters of red blood result from the mobilization of the body's compensatory mechanisms against the background of stress and have a protective value, providing the need for organ systems in oxygen.

Table 1

Effect of prolonged immobilization stress on peripheral red blood parameters of rabbits without and with treated by *Stevia rebaudiana* Bertoni (The number of erythrocytes in 1 mm³ of blood (thousand), amount of hemoglobin (g%), Color index, the relative number of reticulocytes (%), the absolute number of reticulocytes in 1 mm³ of blood, reticulocyte maturation rate per hour) (n = 5, * p < 0.05, ** p < 0.01, *** p < 0.001).

Peripheral red blood parameters	Groups	Initial data	The days of investigation					
			5	10	15	20	25	30
The number of erythrocytes in 1 mm ³ of blood (thousand)	Control group	5123 ± 169	5790 ± 189*	5896 ± 181*	4927 ± 179**	4605 ± 170**	4422 ± 168**	4400 ± 169**
	Treated	4870 ± 162	5990 ± 169*	5880 ± 171*	5430 ± 165*	5269 ± 158*	5169 ± 156**	5150 ± 153*
Amount of hemoglobin (g%)	Control group	12.8 ± 0.28	14.0 ± 0.32*	12 ± 0.26**	11.4 ± 0.21**	10.8 ± 0.19***	10.8 ± 0.19***	10,6 ± 0.19***
	Treated	12,8 ± 0,23	15,8 ± 0,24**	13,6 ± 0,25**	13,2 ± 0,25**	13,2 ± 0,24**	13,2 ± 0,24**	13,2 ± 0,23**
Color index	Control group	0.75	0,73	0.62	0.75	0.72	0.73	0.71
	Treated	0.80	0.80	0.71	0.73	0.76	0.77	0.77
The relative number of reticulocytes (%)	Control group	17 ± 0.41	25 ± 0.51***	27 ± 0.55***	20 ± 0.48**	16 ± 0.40**	14 ± 0.38**	14 ± 0.38**
	Treated	18 ± 0,35	24 ± 0,41**	24 ± 0,41**	23 ± 0,38*	22 ± 0,36**	21 ± 0,35**	21 ± 0,35**
The absolute number of reticulocytes in 1 mm ³ of blood	Control group	87091 ± 3442	144750 ± 3819***	159152 ± 3849***	98540 ± 3415*	79680 ± 3321**	61908 ± 2645***	61600 ± 2615***
	Treated	87660 ± 2627	143760 ± 2998**	140640 ± 2996**	124890 ± 2885**	115918 ± 2868*	108549 ± 2725**	108150 ± 2721**
Reticulocyte maturation rate per hour	Control group	1.5	2.0	2.0	1.5	1.25	1.25	1.25
	Treated	1	2,75	2.5	2.0	1.75	1.55	1.55

On the 10th day of the study, a hypochromic change in the number of erythrocytes and hemoglobin content was observed. The number of erythrocytes increased by 15%, and the hemoglobin content decreased by 7% of the initial level, as a result of which the color index decreased from 0.75 to 0.62 of the norms. The increase in the number of reticulocytes continued during this period, while the relative number increased by 58% compared with baseline, and the absolute number by 82%. The observed movements of peripheral erythrocytes under the influence of 10-day immobilization stress are due to the activation of

sympathetic mechanisms of blood redistribution, as well as the acceleration of the processes of the release of young cells from the bone marrow to the periphery, as evidenced by reticulocytosis.

When exposed to 15-day immobilization stress, a normochromic decrease in the number of erythrocytes and hemoglobin content was observed. Compared with the 10th day, the number of erythrocytes decreased by 19%, and the hemoglobin content decreased by 4% and amounted to 11.4 ± 0.21 . The relative and absolute reticulocyte counts decreased from day 5 but were increased compared to baseline by 17% and 13%, respectively. The rate of maturation of reticulocytes was equal to the norm (100%).

On the 20th day of the study, the normochromic decrease in peripheral erythrocytes continued, the number of erythrocytes was 89%, and the hemoglobin content was 84%. The relative number of reticulocytes was within the initial level, the absolute number decreased by 84%, and the rate of maturation of reticulocytes slowed down by 83%. During this period, a significant decrease in the number of erythroid stem cells was observed in the myelogram: 27 ± 1.3 compared with the norm of 33 ± 1.6 . The number of polychromatophilic and oxyphilic normocytes is especially reduced, which is a consequence of the maturation of erythroid stem cells and a slowdown in hemoglobin synthesis. The proof of the latter is the decrease in the maturation index of erythroblast protoplasm – 0.59 compared with the norm of 0.73 (Table 2).

Table 2

The nature of changes in erythroid sprout cells (Total number of erythroid sprout cells, Erythroblasts, and pronormocytes, Basophilic normocytes, Polychromatophilic normocytes, Oxyphilic normocytes) in the dynamics of exposure to immobilization stress (n = 5, * p < 0.05, ** p < 0.01, *** p < 0.001).

Parameters	Groups	Initial data	The days of investigation		
			10	20	30
Total number of erythroid sprout cells	Control group	34 ± 1.6	32 ± 1.2**	27 ± 1.3**	27 ± 1.3**
	Treated	36 ± 1,2	35 ± 1,3**	35 ± 1,5*	34 ± 1,2*
Erythroblasts and pronormocytes	Control group	4	4	5	5
	Treated	5	4	4	3
Basophilic normocytes	Control group	6	6	6	6
	Treated	6	5	5	6
Polychromatophilic normocytes	Control group	11 ± 0.17**	11 ± 0.16*	8 ± 0.09**	8 ± 0.09**
	Treated	11 ± 0.16**	11 ± 0.17*	9 ± 0.09*	8 ± 0.09*
Oxyphilic normocytes	Control group	13 ± 0.21**	11 ± 0.18*	8 ± 0.11***	8 ± 0.11***
	Treated	14 ± 0.21*	15 ± 0.21**	17 ± 0,91**	17 ± 0,85**
An indicator of maturation of erythroblast protoplasm	Control group	0.73	0.68	0.59	0.59
	Treated	0,72	0,74	0,74	0,73

In the following days of the study (25–30 days), the morphofunctional parameters of erythropoiesis did not undergo significant changes compared to the 20th day, and stabilization of erythropoiesis indicators at a moderately low level was observed. The number of erythrocytes was 85%, the hemoglobin content was 83%, the relative reticulocyte count was 82%, the absolute count was 70%, and the maturation rate was 83%. The total number of erythroid stem cells was at a low level – 27 ± 1.3, due to a decrease in the number of polychromatophilic and oxyphilic erythrokaryocytes. The erythroblast protoplasm maturation index was 0.5.

In our opinion, in the initial period of exposure to immobilization stress (5–10 days), an increase in peripheral erythrocytes is an adequate response of the body to stress and is due to the activation of the sympathetic nervous system and an increase in the content of catecholamines - a reduction in the functions of the spleen, the release of erythrocytes stored in the bone marrow and spleen, into the

peripheral blood, which is important to meet the needs of organ systems for oxygen. In the dynamics of the 30-day exposure to immobilization stress, these changes in erythropoiesis parameters are adaptive by the changing conditions of the body.

3.2. Effect of Stevia on morpho-functional criteria of erythropoiesis during prolonged immobilization stress. To mitigate the negative impact of immobilization stress and correct morphological and functional shifts in erythropoiesis, at the next stage of the research, a certain amount of crushed stevia leaves was added to the standard diet of rabbits for 30 days until the end of the experiment. In the initial stage of the experiment, changes in the peripheral parameters of red blood were recorded (Table 1), but against the background of the medicinal plant, immobilization stress did not cause pronounced changes in the parameters of erythropoiesis. On the 5th day of exposure to immobilization stress, a moderate normochromic increase in erythrocytes and hemoglobin was observed. The number of erythrocytes increased by 10%, and hemoglobin content by 12%. The relative number of reticulocytes increased by 14%, absolute by 25%, and maturation rate by 83%.

On the 10th day of the study, a high level of the number of erythrocytes and reticulocytes remained, and the hemoglobin content was within the normal range. On the 15th day, there were no significant changes in the peripheral parameters of red blood compared to the 10th day. On the 20th day of exposure to immobilization stress in the bone marrow, an increase in the number of erythrocytes containing hemoglobin was observed. The number of oxyphilic normocytes increased by 21% compared with the baseline (Table 2). During this period, the indicators of peripheral red blood were within the physiological norm, which persisted until the 30th day. A high level of oxyphilic normocytes remained in the bone marrow, which indicates the activation of maturation processes. Throughout the study, the indicator of maturation of erythroblast protoplasm was at the initial level. Apparently, under the influence of biologically active substances contained in stevia, many adaptive changes occur in the blood system aimed at strengthening reparative processes, increasing the oxygen capacity of the blood, expanding the limits of compensatory-protective mechanisms, and increasing the number of regulatory structures involved in erythropoiesis.

3.3. Effect of prolonged immobilization stress on the lipid peroxidation processes and the activity of antioxidant enzymes. The effect of immobilization stress on lipid peroxidation processes and the activity of antioxidant enzymes (catalase, peroxidase) was also studied in the blood of animals (Fig. 1). An analysis of the research results revealed that immobilization stress was accompanied by an increase in the processes of lipid peroxidation, as a result of which the content of malondialdehyde increased in the blood plasma and the organs under study. After the first 5 hours of exposure to stress, the content of malonaldehyde in the blood plasma increased by 85.6% compared with the norm, and after 5 days by 74%, then, as the duration of stress increased, it continued to increase by 98% until the 20th day, and by 30th day - although the amount of MDA decreased, the absolute values were above the norm (Fig. 1, A). The activity of antioxidant enzymes in the blood plasma of animals subjected to stress changed as follows as far as the activity of antioxidant enzymes is concerned after 5 hours from the stress effect, the catalase activity (Fig. 1, B) increases by 21% as compared to the norm, and after 5 days – by 14%. Catalase activity

increasing at the early stages of different stress factor effects was mentioned by other authors as well [5, 7]. With stress effect influence enhancement in the chronic stage, the catalase activity gradually decreases. On the 15th day, its activity decreases by 11% compared to the norm, on the 20th day – it by 16%, 25th day – it is 20%, 30th day – 22%.

As it follows from Fig. 1 (C) regularities of the peroxidase activity change depending on stress effect duration are preserved as in the case of catalase; if only catalase activity begins to decrease in the 15th day after stress exposure, the peroxidase activity decreases in the 25th day compared to the norm. Concluding the results of this stage it is obvious that with stress duration enhancement the intensity of lipid superoxide oxidation remains higher than the norm even on the 30th day of stress effect, but the activity of antioxidant enzymes in stress early stages rises, in chronic stage – decreases from the norm.

Changes in the processes of lipid peroxidation and the activity of antioxidant enzymes under conditions of exposure to immobilization stress were also studied in the liver, kidneys, and brain of the rabbit. The processes of lipid peroxidation in the studied organs were most intense in the kidneys (Fig. 2). On the 10th day of the study, the content of malondialdehyde increased by 12% compared to the norm, on the 20th day by 32%, and on the 30th day, it exceeded the norm by 86%.

In the liver on the 10th day of exposure to stress, the content of malonic aldehyde increased by 15%, and on the 30th day, the norm was exceeded by 70%. The same pattern was observed in brain tissue. An increase in the content of malondialdehyde in the bone marrow, spleen, and thymus after a 30-day exposure to immobilization stress was also noted by other authors [6, 25].

As the duration of stress exposure increased, peroxidase activity in the studied organs gradually decreased and on the 30th day, compared with the control group, it was 46% in the liver, 42.2% in the kidneys, and 61.25% in the brain (Fig. 2, C). An increase in catalase activity was observed in the liver and kidneys: on the 20th day of stress exposure, it was 16% and 13%, respectively. An increase in catalase activity in the early stages of exposure to various types of stress was also noted by other authors [5–7].

Thus, with a 30-day exposure to immobilization stress, probably under the influence of catecholamines and glucocorticoids, the processes of lipid peroxidation are activated, resulting in an increase in the density of intermediate and final products of peroxidation, which affect the activity of antioxidant enzymes.

3.4. Combined effects of stevia and immobilization stress on the lipid peroxidation processes and antioxidant enzyme activity. According to our investigations, in the blood of animals fed with stevia and exposed to stress for 5 hours, the malonic dialdehyde amount increased by 148%, after 5 days of stevia receiving and stress exposure – by 49%, on the 10th day – 13%, and with stress duration lengthening it starts gradually decreasing in such way that in 30th day it is lower by 26% than the norm (Fig. 1, A). What concerns antioxidant enzymes in dynamics of stevia and immobilization stress combined to affect the catalase activity does not decrease, and vice versa the activity gradual increase is observed and on the 30th day, it is higher by 13% than the norm. A higher criterion is observed on the 20th day – by 18%.

Similar regularities are observed in studying peroxidase activities. A decrease in the content of malonic aldehyde was observed in the liver on all days of the study, by 30% on day 10, by 46% on day 20, and by 34% on day 30 (Fig. 2, A) compared to the control group. A decrease in the content of malondialdehyde in the kidneys was observed on the 20th day of the study by 51%, on the remaining days, it was within the initial level. On the 10th and 20th days, lipid peroxidation was activated in the brain, as a result of which the content of malonic aldehyde compared to the control group exceeded by 61% and 44%, respectively, then gradually decreased and on the 30th day was decreased by 24% (Fig. 2, A). As for the effect of the herbal on peroxidase and catalase activity under immobilization stress, the picture is as follows (Fig. 2, B, and C). Under these conditions, there was an increase in catalase activity in the kidneys and liver by an average of 14 and 17%, respectively. The action of stevia is more effective concerning peroxidase, here the activity of the latter is stimulated by 31,4% in the brain and by 28.5% in the liver. Thus, the antioxidant compounds contained in stevia leaves inhibit the processes of lipid peroxidation during immobilization stress, most likely, as a result of which the number of free radicals decreases, and the optimal level of antioxidant system activity is provided.

4. Discussion

An analysis of the regularities and nature of the changes found in the systems we study allows us to make the following generalizations. In the case of prolonged exposure to immobilization stress, erythropoiesis is inhibited, the number of erythrocytes and the hemoglobin content in the peripheral blood decrease to a normochromic level, and moderate erythropenia develops. In addition to quantitative changes, qualitative changes are also observed, the processes of maturation of erythroblast germ cells slowdown in the bone marrow, as a result, the bone marrow index of erythronormoblast protoplasm maturation drops from 0.73 to 0.59 and a moderately low level of erythrocyte balance develops. Inhibition of erythropoiesis, slowing down of hemoglobin synthesis, and an increase in the content of catecholamines can lead to oxygen starvation of tissues and cause stress damage to internal organs. As is known, the pathogenesis of stress lesions is closely related to the activation of fat peroxidation processes [4, 6, 26], structural changes in cell membranes, including erythrocytes, and, as a result, the development of hemolytic situations in the blood, the degree of which depends both on the strength of the stimulus and on initial cell resistance [27]. Being the first link in the defense mechanisms of living organisms, the antioxidant system quickly responds to both physiological and biochemical changes, while simultaneously participating and facilitating the activity of adaptation mechanisms.

Summarizing the results of studies in all the presented areas, the fact of an increase in the activity of the adaptive-compensatory mechanisms of the body in the initial period and a clear leveling of the consequences of the negative impact of immobilization stress with the help of the herbal stevia draws attention. The leaves of *Stevia rebaudiana*, a natural sweetener, contain more than 30 different steviol glycosides, of which stevioside and rebaudioside A are found in major proportion. The main chemistry structures of glycosides found in stevia are diterpenes with strong antioxidant and anti-inflammatory properties [28]. The stevioside contained in stevia leaves plays an important role in the synthesis of hemoglobin proteins. Those processes contribute to the iron, copper, and amino acid lysine contained in

leaves. The organic acids contained in the leaves have antioxidant properties, convert inactive folic acid into an active form, which is involved in the synthesis of globin, and take part in the formation of nucleic acids in erythroblasts. Stevia, rich in natural antioxidants, increases the body's resistance to unfavorable environmental factors.

5. Conclusion

In the process of studying the questions posed, it was clear that an increase in peripheral erythrocytes is an adequate response of the body to stress and is due to the release of erythrocytes stored in the bone marrow and spleen into the peripheral blood. Changes in the parameters of erythropoiesis have become adaptive following the body's changing conditions. Under the influence of biologically active substances of stevia, there was an increase in reparative processes and an expansion of the limits of compensatory-protective mechanisms involved in erythropoiesis. The obtained data suggest that the saturation of the body with exogenous antioxidants under stress prevents the processes of lipids peroxidation due to the activation of stress-limiting systems, which also include antioxidant enzymes that act as membrane protectors, prevent stress damage to the cell membrane, and compensate for the impact of dangerous factors.

The data obtained allow us to state that the use of stevia is a non-drug method for correcting immobilization stress and can subsequently be used in the prevention and treatment of stress conditions.

Declarations

CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

ETHICAL APPROVAL

Animal research was approved by the National Center of Bioethics (Armenia) and according to regulations outlined in the 2010/63/EU.

Author Contribution

All authors contributed to the study's conception and design. In the process of carrying out scientific research, the author's group made a certain contribution to the implementation of this project. Work on the preparation of experimental animals and the implementation of immobilization stress was carried out by N. Avtandilyan and H. Karapetyan. All questions on the use of the medicinal plant stevia at all stages of the work were carried out by E. Baresgyan and H. Karapetyan. The determination of the parameters of peripheral red blood was carried out by Ts. Adamyan and A. Karapetyan. Quantitative determination of malonic dialdehyde and determination of enzyme activity was carried out by H. Karapetyan and E.

Barseghyan. N. Avtandilyan and H. Karapetyan corrected and edited the manuscript. All authors revised and accepted the final version of the manuscript.

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DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

References

1. Sies, H., Berndt, C., & Jones, D. P. (2017). Oxidative Stress. <https://doi.org/10.1146/annurev-biochem>
2. Karna, K. K., Soni, K. K., You, J. H., Choi, N. Y., Kim, H. K., Kim, C. Y., ... Park, J. K. (2020). Motiliperm ameliorates immobilization stress-induced testicular dysfunction via inhibition of oxidative stress and modulation of the NRF2/HO-1 pathway in SD rats. *International Journal of Molecular Sciences*, 21(13), 1–17. <https://doi.org/10.3390/ijms21134750>
3. Bagheri, Y., Keshtmand, Z., Rahbarghazi, R., Gharamaleki, M. N., Barati, A., Bagheri, S., ... Delashoub, M. (2020). Salvia officinalis hydroalcoholic extract improved reproduction capacity and behavioral activity in rats exposed to immobilization stress. *Animal Science Journal*, 91(1). <https://doi.org/10.1111/asj.13382>
4. SEMERCİ, N., BİLİCİ, G., YILMAZ, F., ÇAVDAR, Z., SAKIK, U., KAYIŞLI, Ü., & ERBİL, G. (2021). THE EFFECTS OF IMMOBILIZATION STRESS ON PLACENTA AND FETUS IN PREGNANT MICE. *SDÜ Tıp Fakültesi Dergisi*, 28(1), 31–39. <https://doi.org/10.17343/sdutfd.651091>
5. Solin, A. V., Lyshev, Y. D., & Tsygan, N. V. (2019). Hepatoprotective effect of opioid peptides in stress. *Research Results in Pharmacology*, 55(1), 77–96. <https://doi.org/10.3897/rpharmacology.5.34472>
6. Solin, A. V., & Lyshev, Y. D. (2015). Effects of Dynorphin A (1-13) on Lipid Peroxidation and the Activity of Antioxidant Enzymes in Immobilization Stress of Different Durations. *Neuroscience and Behavioral Physiology*, 45(7), 776–779. <https://doi.org/10.1007/s11055-015-0143-3>
7. Solin, A. V., & Lyshev, Y. D. (2014). Stress-Induced Changes in the Liver of Rats with Different Resistance to Stress. *Bulletin of Experimental Biology and Medicine*, 157(5), 571–573. <https://doi.org/10.1007/s10517-014-2617-7>
8. Doering, T. M., Thompson, J. L. M., Budiono, B. P., MacKenzie-Shalders, K. L., Zaw, T., Ashton, K. J., & Coffey, V. G. (2022). The muscle proteome reflects changes in mitochondrial function, cellular stress and proteolysis after 14 days of unilateral lower limb immobilization in active young men. *PLoS ONE*, 17(9 September). <https://doi.org/10.1371/journal.pone.0273925>

9. Ginovyan, M., Javrushyan, H., Petrosyan, G., Kusznierevich, B., Koss-Mikołajczyk, I., Koziara, Z., ... Avtandilyan, N. (2023). Anti-cancer effect of *Rumex obtusifolius* in combination with arginase/nitric oxide synthase inhibitors via downregulation of oxidative stress, inflammation, and polyamine synthesis. *The International Journal of Biochemistry & Cell Biology*, 158, 106396. <https://doi.org/10.1016/j.biocel.2023.106396>
10. Leszczyńska, T., Piekło, B., Kopeć, A., & Zimmermann, B. F. (2021). Comparative assessment of the basic chemical composition and antioxidant activity of *stevia rebaudiana bertonii* dried leaves, grown in Poland, Paraguay and Brazil—preliminary results. *Applied Sciences (Switzerland)*, 11(8). <https://doi.org/10.3390/app11083634>
11. Shivanna, N., Naika, M., Khanum, F., & Kaul, V. K. (2013). Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana*. *Journal of Diabetes and its Complications*, 27(2), 103–113. <https://doi.org/10.1016/j.jdiacomp.2012.10.001>
12. Forouzi, A., Ghasemnezhad, A., & Nasrabad, R. G. (2020). Phytochemical response of *Stevia* plant to growth promoting microorganisms under salinity stress. *South African Journal of Botany*, 134, 109–118. <https://doi.org/10.1016/j.sajb.2020.04.001>
13. Ruiz-Ruiz, J. C., Moguel-Ordoñez, Y. B., & Segura-Campos, M. R. (2017). Biological activity of *Stevia rebaudiana* Bertoni and their relationship to health. *Critical Reviews in Food Science and Nutrition*, 57(12), 2680–2690. <https://doi.org/10.1080/10408398.2015.1072083>
14. Jeppesen, P. B., Gregersen, S., Poulsen, C. R., & Hermansen, K. (2000). *Stevioside Acts Directly on Pancreatic β Cells to Secrete Insulin: Actions Independent of Cyclic Adenosine Monophosphate and Adenosine Triphosphate-Sensitive K^+ -Channel Activity*.
15. Chowdhury, A. I., Rahanur Alam, M., Raihan, M. M., Rahman, T., Islam, S., & Halima, O. (2022). Effect of *stevia* leaves (*Stevia rebaudiana* Bertoni) on diabetes: A systematic review and meta-analysis of preclinical studies. *Food Science and Nutrition*. John Wiley and Sons Inc. <https://doi.org/10.1002/fsn3.2904>
16. Ahmad, J., Khan, I., Blundell, R., Azzopardi, J., & Mahomoodally, M. F. (2020, June 1). *Stevia rebaudiana* Bertoni.: an updated review of its health benefits, industrial applications and safety. *Trends in Food Science and Technology*. Elsevier Ltd. <https://doi.org/10.1016/j.tifs.2020.04.030>
17. /63/EU. (2010). Directive 2010/63/EU of the European parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Official Journal of the European Union*, (June), 1–61. Retrieved from <http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32010L0063&from=EN>
18. Aghajanyan, A., Movsisyan, Z., & Trchounian, A. (2017). Antihyperglycemic and Antihyperlipidemic Activity of Hydroponic *Stevia rebaudiana* Aqueous Extract in Hyperglycemia Induced by Immobilization Stress in Rabbits. *BioMed Research International*, 2017. <https://doi.org/10.1155/2017/9251358>
19. Reichenwallner, A. K., Vurmaz, E., Battis, K., Handl, L., Üstün, H., Mach, T., ... Richter, L. (2021). Optical investigation of individual red blood cells for determining cell count and cellular hemoglobin concentration in a microfluidic channel. *Micromachines*, 12(4). <https://doi.org/10.3390/mi12040358>

20. Briggs, C., Culp, N., Davis, B., d'Onofrio, G., Zini, G., & Machin, S. J. (2014). ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting. *International Journal of Laboratory Hematology*, 36(6), 613–627. <https://doi.org/10.1111/ijlh.12201>
21. Mejía-Barajas, J. A., Montoya-Pérez, R., Salgado-Garciglia, R., Aguilera-Aguirre, L., Cortés-Rojó, C., Mejía-Zepeda, R., ... Saavedra-Molina, A. (2017). Oxidative stress and antioxidant response in a thermotolerant yeast. *Brazilian Journal of Microbiology*, 48(2), 326–332. <https://doi.org/10.1016/j.bjm.2016.11.005>
22. Avtandilyan, N., Javrushyan, H., Ginovyan, M., Karapetyan, A., & Trchounian, A. (2022). Anti - cancer effect of in vivo inhibition of nitric oxide synthase in a rat model of breast cancer. *Molecular and Cellular Biochemistry*, (0123456789). <https://doi.org/10.1007/s11010-022-04489-y>
23. Chen, Q., Cheng, M., Wang, Y., Yao, M., Chen, Y., Gao, Y., & Ding, W. (2015). A simple method of catalase purification for the undergraduate experimental course. *Molecular Medicine Reports*, 11(2), 1340–1343. <https://doi.org/10.3892/mmr.2014.2806>
24. Ginovyan, M., Javrushyan, H., Karapetyan, H., Koss-Mikołajczyk, I., Kusznierevich, B., Grigoryan, A., ... Avtandilyan, N. (2024). Hypericum alpestre extract exhibits in vitro and in vivo anticancer properties by regulating the cellular antioxidant system and metabolic pathway of L-arginine. *Cell Biochemistry and Function*, 42(1). <https://doi.org/10.1002/cbf.3914>
25. Kuchukashvili, Z., Menabde, K., Chachua, M., Burjanadze, G., Chipashvili, M., & Koshoridze, N. (2011). Functional state of rat cardiomyocytes and blood antioxidant system under psycho-emotional stress. *Acta Biochimica et Biophysica Sinica*, 43(6), 480–486. <https://doi.org/10.1093/abbs/gmr037>
26. Karapetyan, H., Barseghyan, E., & Trchounian, A. (2018). ANTIOXIDANT PROPERTIES OF STEVIA (STEVIA REDAUDIANA BERTONI) DURING IMMOBILIZATION STRESS. *Biolog. Journal of Armenia* (Vol. 1).
27. Alghareeb, S. A., Alfhili, M. A., & Fatima, S. (2023, March 1). Molecular Mechanisms and Pathophysiological Significance of Eryptosis. *International Journal of Molecular Sciences*. Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/ijms24065079>
28. Ramos-Tovar, E., & Muriel, P. (2017). Stevia as a Putative Hepatoprotector. In *Liver Pathophysiology: Therapies and Antioxidants* (pp. 715–727). Elsevier. <https://doi.org/10.1016/B978-0-12-804274-8.00051-5>

Figures

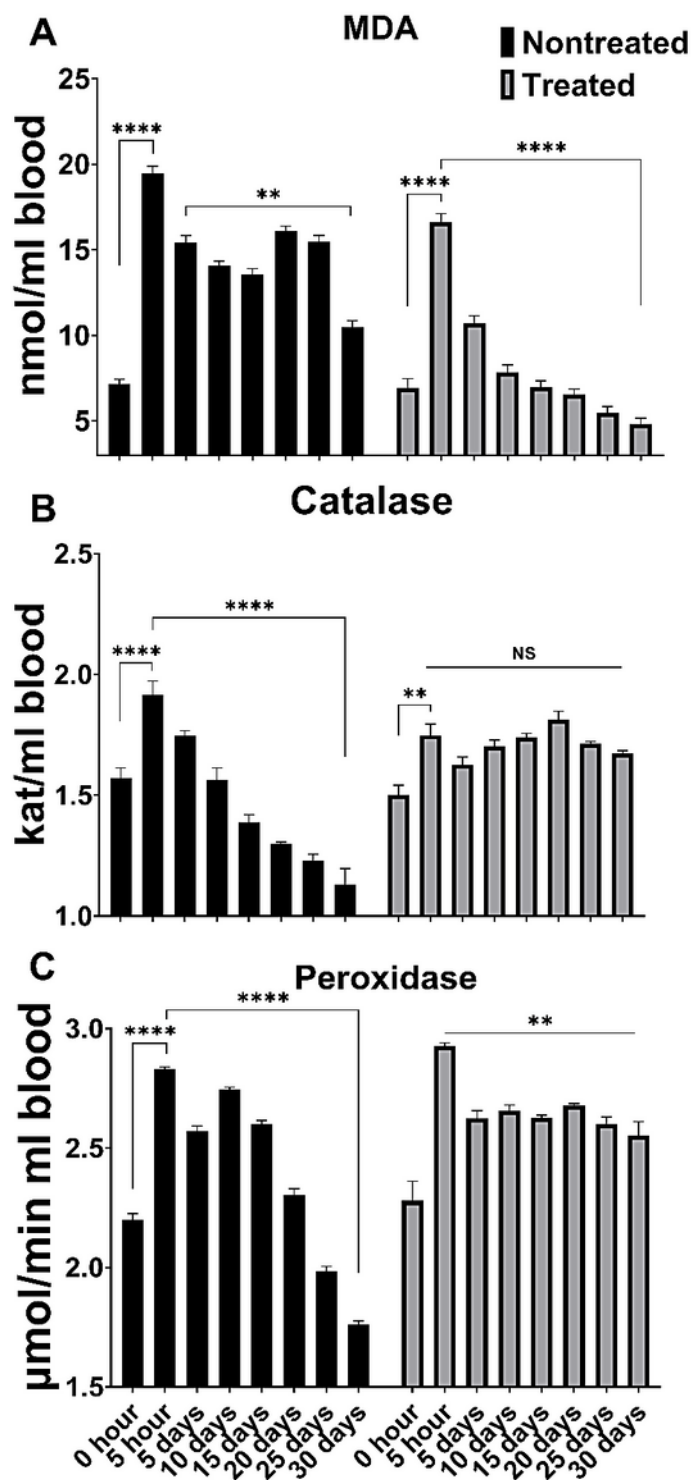


Figure 1

Effect of prolonged immobilization stress and treatment with stevia on the content of malondialdehyde (A) and antioxidant enzymes (Catalase (B) and peroxidase (C)) activities in rabbit blood plasma (n=5, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001).

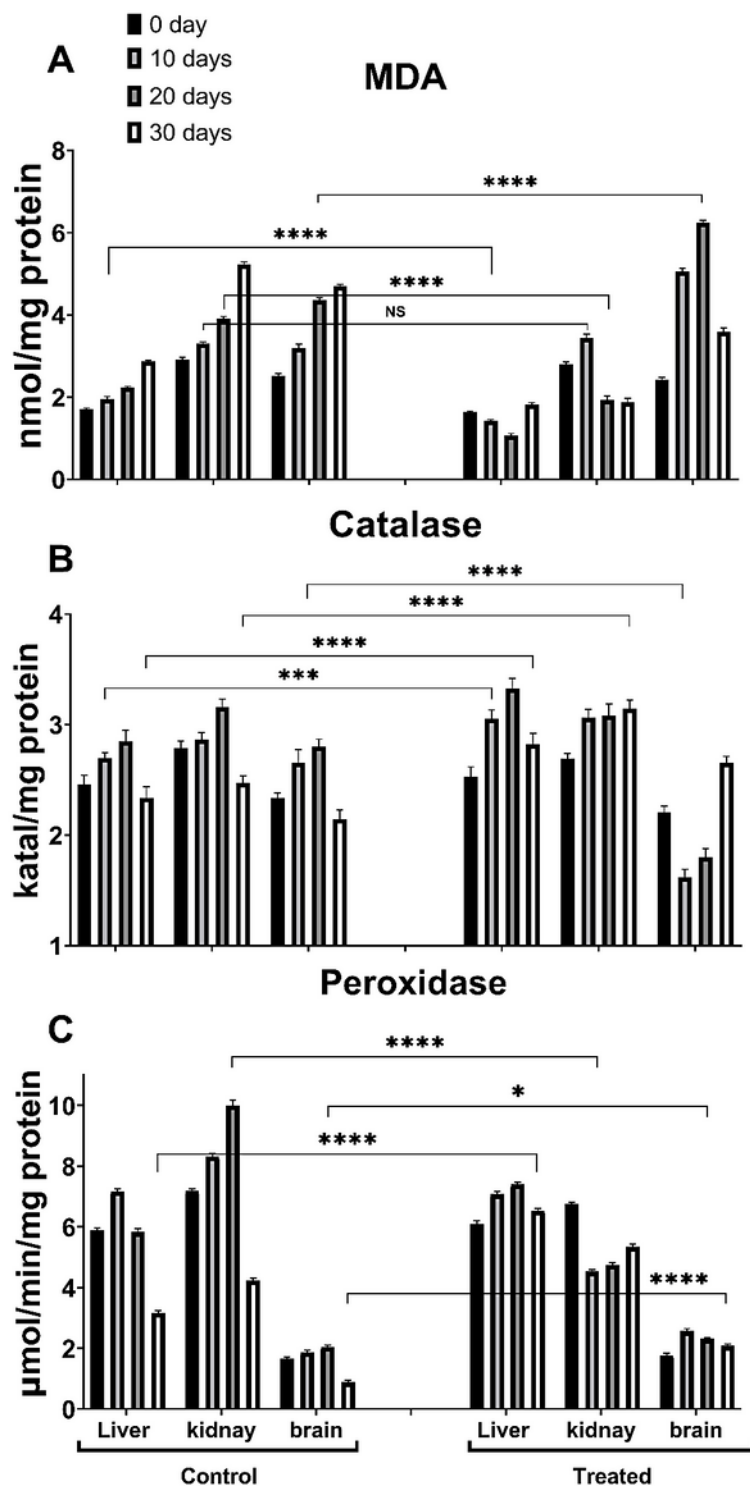


Figure 2

The effect of prolonged immobilization stress and treatment with stevia on the content of malondialdehyde, peroxidase, and catalase activities in the different organs of rabbits ($n=3$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).