

## ANTIOXIDANT PROPERTIES OF STEVIA (*STEVIA REDAUDIANA BERTONI*) DURING IMMOBILIZATION STRESS

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The changes of antioxidant enzymes – catalase and peroxidase activity and lipid superoxide oxidation processes in rabbit blood have been determined in conditions of immobilization stress long duration. It was also studied stevia (*Stevia rebaudiana Bertoni*) effect on oxidant and antioxidant systems. It was revealed that with stress effect duration lengthening fat superoxide oxidation processes is activated and malonic dialdehyde amount is increased. It was shown that antioxidants contained in stevia suppress oxidation processes providing preservation of activity of antioxidant enzymes in blood. It can be suggested that stevia leaves antioxidant properties are a measure of correction of immobilization stress results.

*Immobilization stress – stevia-malonic dialdehyde-catalase*

Ուսումնասիրվել է ճագարի արյան մեջ լիպիդների գերօքսիդային օքսիդացման գործընթացների և հակաօքսիդանտային ֆերմենտների կատալազի և պերօքսիդազի ակտիվության փոփոխությունների օրինաչափությունները անշարժացնող սթրեսի երկարատև ազդեցության պայմաններում, ինչպես նաև հետազոտվել է մեղրախոտի (*Stevia rebaudiana Bertoni*) ազդեցությունն այդ գործընթացների վրա: Ցուց է տրվել, որ սթրեսի ազդեցության տևողության երկարացմանը զուգընթաց ճագարի արյան մեջ խթանվում են ճարպերի գերօքսիդային օքսիդացման գործընթացները, ինչի հետևանքով բարձրանում է մալոնային երկալդեհիդի քանակը, իսկ մեղրախոտում պարունակվող հակաօքսիդանտային հատկություն ունեցող նյութերը ճնշում են օքսիդացման գործընթացները՝ օժանդակելով արյան մեջ հակաօքսիդանտային ֆերմենտների ակտիվության պահպանմանը: Առաջարկվում է մեղրախոտի տերևների հակաօքսիդանտային հատկությունները կիրառել որպես անշարժացնող սթրեսի հետևանքները շտկող միջոց:

*Անշարժացնող սթրես-մեղրախոտ-մալոնային երկալդեհիդ-կատալազ*

Изучены закономерности изменения процессов перекисного окисления липидов и активности антиоксидантных ферментов каталазы и пероксидазы в крови кроликов в условиях длительного воздействия иммобилизационного стресса. Также было исследовано влияние антиоксидантных соединений листьев стевии (*Stevia rebaudiana Bertoni*) на эти процессы. Показано, что после длительного воздействия стресса в крови у кроликов активируются процессы перекисного окисления липидов и повышается количество малонового диальдегида. Антиоксидантные вещества, содержащиеся в листьях хстеви, подавляли окислительные процессы, тем самым содействуя сохранению активности антиоксидантных ферментов. Предлагается, антиоксидантные свойства листьев стевии использовать как меру коррекции последствий иммобилизационного стресса.

*Иммобилизационный стресс-стевия – малоновый диальдегид-каталаза*

Treatment of organism resistance effective measures to different stress factors is one of actual problems of medico-biological sciences, since the stress is a basis for development of numerous pathological processes. In the beginning state of the effect of stress factors, hypothalamus-hypophysis-adrenal system is stimulated, the amount of biologically active compounds in organism enhances, the level of red-ox processes changes that play an important role in organism adaptation to environment altered conditions and viability providing. Nowadays sharp change of human lifestyle results in motor activity confinement, and organism natural viability is possible only at sufficient motor background which makes a favorable impact on natural processing of organism physiological functions;

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especially the investigation of physico-emotional stresses, including immobility becomes relevant.

In the case of influence of different stressful irritants superoxide oxidation processes of lipids strengthen, amount of intermediate and final products of these reactions increases in blood plasma which cause structural and functional distortions of different organs and tissues [3,4]. To confine these pathologies the important role belongs to antioxidant enzymes of organs and tissues (catalase, superoxide-dismutase, glutathione-peroxidase) which are the first ring of intracellular defensive mechanisms against free radicals. The effect of stressful irritants is accompanied by deviation of the equilibrium between free radical processes and organism antioxidant system [2]. From this point of view the studies directed to the revelation of different preparations possessing antioxidant property are considered to be quite actual topic. Among plants containing anti oxidant compounds it should be mentioned the stevia which contains flavonoids and glycosides (*Stevia rebaudiana Bertoni*). This herb is known due to its anti-inflammation, anti-sclerotic, anti-allergic, bactericide and anti-histamine effects and is applied as medico-preventive measure [10, 12].

Taking into account the role of antioxidant system enzymes in regulation of free radical processes, we have had a task to study regularities of lipid superoxide oxidation and antioxidant system activity changes in combined effect dynamics of stevia and immobilization stress in rabbit blood.

### **Materials and methods**

Studies have been carried out on 10 rabbits with the same sex, weight in the same feeding and caring conditions by two variants. In the first variant the changes of lipid superoxide oxidation processes and activity of enzymes (catalase and peroxidase) were studied in immobilization stress effect (control group). For immobilizing of animals they were fixed 5 hours a day during 30 days on the experimental table. In the second variant the studies were carried out in combined stress dynamics of stevia and immobilization stress. Stevia crushed leaves with 0.5 g/kg weight were added to animal feed each day then animals were exposed to stress effect. Studies in rabbit blood were realized in norm, after immediate stress direct effect, then one time per 5 days during 30 days.

Catalase activity was determined by manganese-metric method, peroxidase activity – by spectrophotometric method [5].

### **Results and discussion**

In the first state of studies the changes of malonic dialdehyde amount as well as catalase and peroxidase activities in rabbit blood were carried out in immobilization stress effect dynamics. It follows from analysis of the results that in early stress state for animals exposed to immobilization stress effect the amount increasing of malonic dialdehyde being final product of lipid superoxide oxidation is observed (Fig. 1). After 5 hours of stress effect the amount of malonic dialdehyde increased by 160%, after 5 days – 106%, in 20<sup>th</sup> day – 119%, then it begins to decrease then in 30<sup>th</sup> day the amount of malonic dialdehyde was higher by 41% as compared to norm.

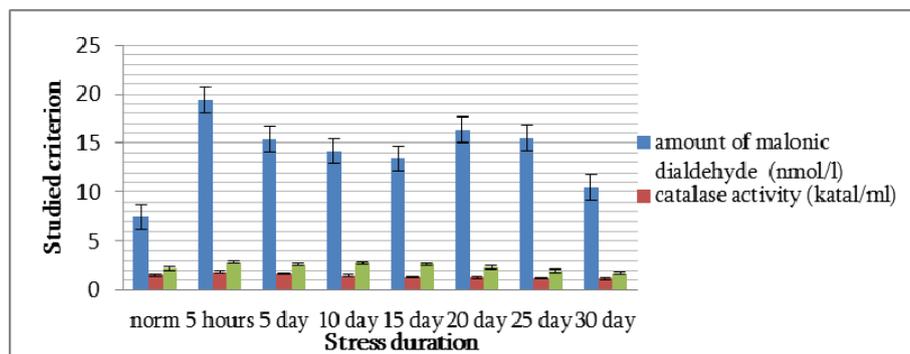


Figure 1. Immobilization stress effect on malonic dialdehyde amount, catalase and peroxidase activities in rabbit blood.

As far as the activity of antioxidant enzymes is concerned after 5 hours from the stress effect, the catalase activity increases by 21 % as compared to the norm, and after 5 days – by 10%. Catalase activity increasing at the early stages of different stress factor effect was mentioned by other authors as well [6, 11]. With stress effect influence enhancement in chronic stage the catalase activity gradually decreases. In 15<sup>th</sup> day its activity decreases by 11% compared to the norm, in 20<sup>th</sup> day – by 16%, 25<sup>th</sup> day – 20%, 30<sup>th</sup> day – 22%.

As it follows from the figure 1, 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 15<sup>th</sup> day after stress exposure, the peroxidase activity decreases in 25<sup>th</sup> 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 from the norm even in the 30<sup>th</sup> day of stress effect, but the activity of antioxidant enzymes in stress early stages rises, in chronic stage – decreases from the norm.

Literature review showed that according to the some authors at immobilization stress long duration, lipase, phospholipase are activated by catecholamines and glucocorticoids that strengthen fat superoxide oxidation processes. The result of this is that the enhancement of superoxide oxidation product density suppresses the activity of antioxidant enzyme activity [4,6]. It is also known that in conditions of oxidative stress or oxygen active form formation a distortion of antioxidant system enzyme functions can occur [9].

As it was mentioned, antioxidant enzymes are the first area of intracellular defense against free radicals and belong to peripheral stress confining system. Hence the strengthening of organism antioxidant power should contribute to increasing of activity of the mentioned enzymes. Taking all these into consideration in the next stage of experiments the changes of antioxidant enzyme activities and lipid superoxide oxidation in blood of animals exposed to stress and fed by food enriched by stevia leaves that in turn are enriched by antioxidant have been studied.

As it is obvious from Fig. 2, in blood of animals fed by stevia and exposed to stress during 5 hours the malonic dialdehyde amount increases by 148%, after 5 days of stevia receiving and stress exposure – by 49%, in 10<sup>th</sup> day – 13%, and with stress duration lengthening it starts gradually decreasing in such way that in 30<sup>th</sup> day it is lower by 26% than the norm. What concerns to antioxidant enzymes in dynamics of stevia and immobilization stress combined effect the catalase activity does not decrease, vice versa the activity gradual increase is observed and in 30<sup>th</sup> day it is higher by 13% than the norm. Higher criterion is observed in 20<sup>th</sup> day – by 118%. Similar regularities are observed at studying of peroxidase activities.

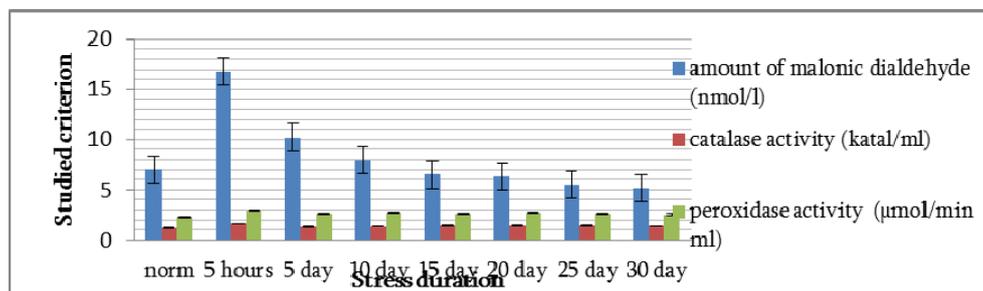


Figure 2. Combined effect of immobilization stress and stevia (*Stevia rebaudiana Bertoni*) on malonic dialdehyde amount, catalase and peroxidase activities in rabbit blood.

The obtained results can be explained by suppressing effect of natural antioxidant compounds contained in stevia leaves on fat superoxide oxidation processes preventing the performance of inhibiting effect of their products on antioxidant enzyme activities [1].

It was shown the antioxidant effect of tea plant *Echinodorus grandiflorus* on rabbit by other authors. It was revealed that tea preparations prevent stimulated damaging of lipids and proteins by sodium aside as well as contribute to restoration of antioxidant enzymes – catalase and superoxide-dismutase [7]. It was also shown that flax seedling polyphenols and omega-3 fat-acids decrease chronic stress effect in rabbits [8].

Therefore, it might be concluded that in stevia (*Stevia rebaudiana Bertoni*) leaves antioxidant compounds can be a measure of correction of immobilization stress results.

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## **17 $\beta$ -ESTRADIOL ATTENUATES THE LPS-INDUCED INFLAMMATORY RESPONSE IN WHOLE BLOOD CELLS CULTURE**

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Estradiol belongs to a family of steroid hormones that primarily controls the reproductive system. 17 $\beta$ -estradiol (E2) is the predominant and most biologically active estrogen. Growing evidences suggest that E2 may have a regulatory effects on immune system and can directly influence the function if cell-mediated immunity. Nevertheless, the effect of E2 on innate immune cells, particularly monocytes and neutrophils, has been so far poorly investigated. This study was aimed to investigate *in vitro* production of TNF- $\alpha$ , IL-1 $\beta$ , MCP-1 and IL-8 by whole blood cells following short-term exposure (4 hours) to 17 $\beta$ -estradiol (E2) in the presence or absence of LPS. The impact of E2 on  $\beta$ 2 integrin (CD11b/CD18) and L-selectin (CD62L) expression on the surface of human blood monocytes and neutrophils was also evaluated. We demonstrated an inhibitory effect of E2 on LPS-induced TNF- $\alpha$  production, the feature that could play a critical role in the regulation of inflammatory response. Expression of CD62L on neutrophils and monocytes was also decreased in the presence of E2. Thus, the results of our study indicate that E2 may have an immunomodulatory action on innate immune cells and modulate ongoing inflammatory response.

*Neutrophils, monocytes, estradiol, gene expression, cytokines*

Եստրադիոլը պատկանում է ստերոիդային հորմոնների ընտանիքին, որի առանցքային դերը վերարտադրողական համակարգի աշխատանքի կարգավորումն է: Եստրոգեն հորմոնի հիմնական և կենսաբանորեն առավել ակտիվ ձևը հանդիսանում է 17 $\beta$  էստրադիոլը (E2): Մի շարք հետազոտություններ ցույց են տվել, որ E2-ն կարող է ունենալ կարգավորիչ ազդեցություն իմունային համակարգի վրա և անմիջականորեն ազդել բջջային միջնորդավորված իմունային պատասխանի վրա: Այնուամենայնիվ, մինչ օրս, բնածին իմունային բջիջների՝ մասնավորապես մոնոցիտների և նեյտրոֆիլների վրա E2-ի ազդեցությունը բավականաչափ ուսումնասիրված չէ: Տվյալ հետազոտության նպատակն է ուսումնասիրել *in vitro* պայմաններում ամբողջական արյան բջիջների կողմից TNF- $\alpha$ , IL-1 $\beta$ , MCP-1 և IL-8 արտադրելու ունակությունը 17 $\beta$ -էստրադիոլի (E2) կարճատև ազդեցությունից հետո (4 ժ)՝ LPS-ի առկայության կամ բացակայության պայմաններում: Ինչպես նաև ուսումնասիրվել է E2-ի ազդեցությունը մոնոցիտների և նեյտրոֆիլների մակերեսային մոլեկուլներ  $\beta$ 2 ինտեգրինի (CD11b/CD18) և L սելեկտինի (CD62L) էքսպրեսիայի վրա: Մեր կողմից ցույց է տրվել E2-ն ցուցաբերում է ճնշիչ ազդեցություն LPS-ով խթանված TNF- $\alpha$ -ի արտադրության վրա, ինչը, հնարավոր է, կարող է որոշիչ դեր ունենալ բորբոքային պատասխանի կարգավորման ժամանակ: E2-ի ազդեցությունից հետո նեյտրոֆիլներում և մոնոցիտներում նվազում է նաև CD62L-ի էքսպրեսիայի մակարդակը: Այսպիսով, ստացված արդյունքները ցույց են տալիս, որ բնածին իմունային բջիջներում E2-ն կարող է ունենալ իմունոկարգավորիչ ազդեցություն և modulate ընթացիկ իմունային պատասխանը:

*Նեյտրոֆիլներ, մոնոցիտներ, Եստրադիոլ, գենային էքսպրեսիա, ցիտոկիններ*