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# PROOXIDANT-ANTIOXIDANT STATUS OF THE HEART OF RATS UNDER THE ACTION OF MONOSODIUM GLUTAMAT AND CORRECTION WITH MELATONIN

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*Monosodium glutamate is the most common food additive used as a taste and aroma enhancer of other product components and as a salt substitute. Consuming food products containing monosodium glutamate on a permanent basis leads to exceeding the maximum permissible consumption norms and the occurrence of metabolic changes, in particular, oxidative stress in the heart. Drugs with antioxidant properties are used to correct disorders that arise as a result of oxidative stress. One of the antioxidants that has a cardioprotective effect is melatonin. The purpose of the study was to determine the changes in the pro-oxidant-antioxidant system of the heart of rats under the influence of monosodium glutamate and the correction of pathological changes with melatonin.*

*The experimental animals were divided into three groups: a control group and two experimental groups. The animals of the first (control) group were kept under normal vivarium conditions without the influence of additional factors. The animals of the second group per os received a 3% aqueous solution of monosodium glutamate at the rate of 20 mg/kg. Rats of the third group received a 3% aqueous solution of glutamate (20 mg/kg), and for the purpose of correction, melatonin was administered intraperitoneally to the animals at a dose of 1.0 mg/kg of body weight once a day. Metabolic changes in the heart muscle of rats were studied on the 7th, 14th, 21st, 28th day of the study.*

*It was established that the daily consumption of monosodium glutamate at a dose of 20 mg/kg of body weight led to the activation of lipid peroxidation processes throughout the study period, an increase in total antioxidant activity on the 7th and 14th days of the study, and its subsequent gradual decrease on the 21st and 28th days of the experiment. The introduction of exogenous melatonin contributed to a decrease in the intensity of peroxidation of lipids and activation of the enzymatic link of the antioxidant system, which is accompanied by an increase in the activity of superoxide dismutase and catalase and a decrease in the level of primary and secondary products of peroxidation. Therefore, melatonin is a potentially useful drug for reducing the manifestations of oxidative stress in the myocardium.*

***Key words:*** *monosodium glutamate, rats, heart, catalase; superoxide dismutase.*

**Connection of the publication with planned research works.**

The present work is part of the research topic of the Department of Anatomy, Clinical Anatomy, Operative Surgery, Pathomorphology and Forensic Medicine of the Petro Mohyla Black Sea National University of the Ministry of Education and Culture of Ukraine «The influence of ecologically dangerous factors on the mechanisms of development of diseases of civilization and their correction with physiologically active substances», state registration number 0122U002033.

**Introduction.**

Modern society is characterized by a significant acceleration of the pace of life and its global changes, which are accompanied by a violation, first of all, of the nutritional regime, a change in the quality of the diet, as a result of giving preference not a rational and balanced diet, but to fast food, which has led to the consumption by people of an uncontrolled amount of food additives. Monosodium glutamate is the most common food additive in the whole world, used as a taste and aroma enhancer of other components of the product, and as a substitute for salt (E621) [1, 2]. Consuming food products containing monosodium glutamate on a permanent basis leads to the entry into the body of its increased amount, exceeding the maximum permitted consumption norms, which causes morphological and functional changes in almost all organs and systems and the occurrence of metabolic and toxic effects, in particular oxidative stress [3]. It is known that monosodium glutamate consumption has a negative effect on the morpho-functional state of the heart, which consists in heart rhythm disturbances, the occurrence of heart attacks and the development of oxidative stress. The negative consequences of oxidative stress are the strengthening of the processes of free radical peroxidation of lipids and the destabilization of antioxidant protection, which is one of the factors in the occurrence of heart pathologies at the cellular level and the development of diseases of the cardiovascular system of various etiologies [4].

Despite the comprehensive study of the effect of food additives on the body, the issue of pharmacological correction of pathological changes induced by the influence of food additives remains the most relevant [5]. Drugs with antioxidant properties are used to correct disorders that arise as a result of oxidative stress [6]. One of the antioxidants that has a cardioprotective effect is melatonin [4]. At the same time, the study of pathological changes in the pro-oxidant-antioxidant system of the heart under the influence of monosodium glutamate and their correction by melatonin is insufficient, which prompted us to conduct our own research.

**The aim of the study.**

To determine the changes in the pro-oxidant-antioxidant system of the heart of rats under the influence of monosodium glutamate and the correction of pathological changes with melatonin.

**Object and methods of research.**

The research was conducted on 54 sexually mature white male Wistar rats weighing 200-240 g. The choice of

**Table – Indicators of the prooxidant-antioxidant status in the heart muscle of rats under the conditions of exposure to monosodium glutamate and correction by melatonin (M±m)**

| Group | Index | | | |
|---|---|---|---|---|
| | Diene conjugates (mmol/kg) n=6 | MDA (mmol/kg) n=6 | SOD (RU/g) n=6 | Catalase (mcat/kg) n=6 |
| **Indicators of the pro-oxidant-antioxidant status of the control group of animals** | | | | |
| Intact | 5,62±0,71 | 53,30±3,29 | 73,47±1,61 | 4,89±0,12 |
| **Indicators of prooxidant-antioxidant status under conditions of exposure to monosodium glutamate** | | | | |
| 7 days | 7,31 ±0,28* | 69,87±6,11* | 87,25±1,94* | 6,95±0,18* |
| 14 days | 8,75±0,42* | 76,23±6,48* | 95,53±2,03* | 8,35±0,07* |
| 21 days | 7,19±0,65 | 71,34±5,81* | 71,13±1,54 | 4,02±0,06* |
| 28 days | 6,05±0,44 | 64,38±6,27 | 59,42±1,75* | 3,84±0,05* |
| **Indicators of prooxidant-antioxidant status under the conditions of sodium glutamate exposure and melatonin correction** | | | | |
| 7 days | 7,03±0,56 | 65,70±5,38 | 99,18±1,45# | 7,01±0,13 |
| 14 days | 6,84±0,37# | 61,45±6,01 | 104,22±1,51# | 8,96±0,09 |
| 21 days | 6,25±0,48 | 58,16±5,67 | 110,41±1,60# | 9,65 ±0,11# |
| 28 days | 5,79±0,59 | 55,27±6,23 | 114,95±1,82# | 10,58 ±0,09# |

**Notes:** *significantly with the control (p<0.05), #significantly with the group of animals exposed to monosodium glutamate for the corresponding period (p<0.05)

males for the study is due to the absence of fluctuations in the level of melatonin in the blood plasma compared to females, in which the concentration of melatonin and the morphofunctional state of the pineal gland depend on the phase of the sexual cycle. The animals were kept in standard vivarium conditions under natural lighting and had free access to food and drinking water. The experiments were conducted in the spring period.

The experimental animals were divided into three groups: a control group and two experimental groups. The control group included 6 animals, and each of the experimental groups included 24 animals. For the purpose of comparative analysis of biochemical indicators, rats of two experimental groups were removed from the experiment at different times of the study, namely on 7, 14, 21, 28 days. At each stage of the study, 6 rats were selected from each group, which is the minimum acceptable standard for the number of animals required for statistical research. The animals of the first (control) group were kept under normal vivarium conditions without the influence of additional factors. The animals of the second group per os received a 3% aqueous solution of monosodium glutamate at the rate of 20 mg/kg. The dose of monosodium glutamate was were twice lower the allowable normal rate in food products and according to the literature, does not show a negative impact on health [7, 8]. The rats of the third group received a 3% aqueous solution of glutamate (20 mg/kg) and, for the purpose of correction, the animals were injected intraperitoneally with melatonin (Vita-Melatonin JSC «Kyiv Vitamin Plant», Ukraine) at a dose of 1.0 mg/kg of body weight in 1.0 ml of solvent once a day at 19.00 [4].

Animals were decapitated under thiopentane anesthesia (25 mg/kg, intraperitoneally). For research, the heart was removed from the animals. A 10% homogenate was prepared from the exsanguinated heart in an isotonic solution. Metabolic processes in the myocardium were analyzed by the concentration of primary (conjugated dienes) and secondary (malondialdehyde) products of free radical peroxidation, as well as the activity of antioxidant enzymes (superoxide dismutase, catalase) in the homogenate of the heart [9].

All stages of the research were carried in accordance with the general principles of work with experimental animals in accordance with the following standards: Convention on Bioethics of the Council of Europe (1997); European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, General Ethical Principles of Animal Experiments, approved by the First National Congress of Ukraine on Bioethics (2001); Law of Ukraine «On Protection of Animals from Cruelty» (2006) and other international agreements and current national legislation in the field of medical and biological research.

Processing of the obtained research results was carried out using the methods of variational statistics, namely the Student's t-test. The difference was considered reliable when the difference in numerical parameters between the studied groups was at least p<0.05. Statistical calculations were performed on a personal computer using the standard software «STATISTICA 6» for computer equipment with the Windows operating system.

**Research results and their discussion.**

As a result of the study of the biochemical parameters of the pro-oxidant-antioxidant system of the heart of rats exposed to monosodium glutamate, a probable increase in the content of primary peroxidation products (conjugated dienes) was established on the 7th and 14th day of the experiment compared to similar indicators in the group of control animals **(table)**. It was established that on the 7th day of monosodium glutamate use, the content of diene conjugates in the homogenate increased by 30.07% (p<0.05) and reached its maximum value on the 14th day, increasing by 55.69% (p<0.05) relative to the control which indicates the activation of lipid peroxidation. Starting from the 21st day, the content of diene conjugates gradually decreases and by the 28th day approaches the control indicators, which can be considered as manifestations of adaptation to relatively long-term use of monosodium glutamate.

Similar changes are observed when studying changes in the level of malondialdehyde. It was established that in the group of animals exposed to monosodium glutamate, the concentration of malondialdehyde on the 7th day of the study increased by 31.09% (p<0.05) compared to the normal value, which may indicate damage to cardiomyocytes by free radicals. It should be noted that the maximum concentration of malondialdehyde was detected on the 14th day of the study and exceeded the value of the control group by 43.02%. On the 21st day, the studied indicator decreased, but significantly exceeded the control value. On the 28th day of the experiment, the level of MDA decreased, but the indicator was higher than the intact group by 20.79%.

Thus, the introduction of monosodium glutamate into the body leads to the accumulation of primary and secondary products of lipid peroxidation in the heart

homogenate, which indicates the development of oxidative stress. The most important link of protection against stress is the antioxidant system, which is a set of protective mechanisms aimed at preserving and maintaining the body's homeostasis. Catalase and superoxide dismutase are the main markers of the enzymatic antioxidant system that slow down the processes of formation of free radicals and inactivate excess content of reactive oxygen species.

According to the results of our research, in animals injected with monosodium glutamate, a probable increase in catalase activity was observed by 42.13% ($p<0.05$) and 70.76% ($p<0.05$), respectively, on the 7th and 14th day of the study, which may indicate a compensatory increase in the activity of the studied enzyme due to the intensification of lipoperoxidation processes. Starting from the 21st day of the study, catalase activity decreased sharply. The results of our biochemical studies showed that on the 21st day of the experiment, the concentration of catalase decreased by 17.79% ($p<0.05$), and on the 28th day it decreased by 21.47% ($p<0.05$) relative to the indicators of the control group of rats.

The dynamics of changes in the activity of superoxide dismutase when monosodium glutamate was administered was characterized by a similar increase in concentration levels on the 7th and 14th day of the study by 18.76% ($p<0.05$) and 30.03% ($p<0.05$) compared to the control. On the 21st and 28th day of the experiment, a decrease in the level of superoxide dismutase was found relative to the control. Thus, the activity of the studied enzyme on day 21 decreased by 3.18% compared to the control group of animals. The maximum decrease in superoxide dismutase activity relative to the intact group was found on the 28th day of the experiment by 19.12% ($p<0.05$).

Since both enzymes play a key role in protecting cells from damage induced by the development of oxidative stress, a decrease in their activity indicates the depletion of key components of the enzymatic part of the antioxidant system on the 28th day of the study. Thus, the results of our research indicate an imbalance in the balance between the processes of lipid peroxidation and the antioxidant defense system, which leads to a change in the structural and functional properties of cardiomyocytes membranes, which is an unfavorable condition for the functioning of the cardiovascular system [10]. Changes of a similar nature are indicated by the results of studies by other researchers who studied the influence of monosodium glutamate on the state of the prooxidant-antioxidant system of other tissues and organs [11, 12]

Biochemical analysis of the heart homogenate of a group of experimental animals that were under the influence of monosodium glutamate and corrective therapy with the drug «Vita-melatonin» showed a decrease in the intensity of lipid peroxidation processes, which is confirmed by a gradual decrease in the content of diene conjugates and malondialdehyde compared to a group of animals that were not effect of the corrector. It was established that the level of diene conjugates decreases at all stages of the study and is maximally close to control values on day 28, exceeding the control indicator by 3.02%.

Correction with melatonin contributed to a gradual decrease in the level of malondialdehyde concentration throughout the experiment. On the 28th day of the experiment, the level of malondialdehyde practically reached physiological values and fluctuated within the range of 55.27±6.23 mmol/kg, while in the first experimental group it was 64.38±6.27 mmol/kg. Along with the reduction of primary and secondary products of peroxidation, a gradual increase in the enzymatic activity of the antioxidant system was established. The use of melatonin increased the activity of superoxide dismutase throughout the experiment. Thus, on the 7th day of the experiment, the activity of the enzyme in the heart homogenate increased by 13.67% ($p<0.05$) compared to the group of animals that did not receive correction. On the 14th and 21st days of the experiment, the activity of superoxide dismutase continued to increase relative to the first experimental group by 9.10% ($p<0.05$) and 55.22% ($p<0.05$), respectively. The highest activity of the studied enzyme was found on the 28th day of the experiment, where it increased by 93.45% ($p<0.05$) compared to the group of rats that were not exposed to melatonin.

The introduction of melatonin contributed to the increase in the activity of catalase. However, it should be noted that the lowest activity of catalase was on the 7th day of the experiment, where, accordingly, it fluctuated within the range of 7.01±0.13 μkat/kg, which exceeded the indicator of the first experimental group by only 0.86%. On the 14th and 21st days of the experiment, a sharp increase in catalase activity by 7.31% and 140.04% ($p<0.05$) was recorded relative to rats that were exposed to monosodium glutamate and in which the studied indicator decreased. On the 28th day of the study, the concentration of catalase in the heart homogenate reached maximum values and was 10.58±0.09 μkat/kg.

The results of our research show that under the influence of melatonin, the processes of lipid peroxidation are inhibited with the simultaneous activation of the enzyme link of the antioxidant system of the rat heart. Other researchers also point to the normalizing effect of melatonin on the antioxidant system of other organs under the influence of adverse factors [4, 9, 12, 13].

**Conclusions.**

The influence of monosodium glutamate is accompanied by the intensification of lipid peroxidation processes throughout the study period, an increase in total antioxidant activity on the 7th and 14th days of the study compared to the control, and a gradual decrease in antioxidant activity on the 21st and 28th days of the experiment. The identified biochemical changes are manifested by a significant increase in the content of diene conjugates and malondialdehyde in the heart homogenate and a gradual decrease in the activity of catalase and superoxide dismutase, which indicates the exhaustion of the enzymatic link of the antioxidant system. Exogenous melatonin helps to reduce the intensity of peroxidation of lipids and activation of the antioxidant system, which is accompanied by an increase in the activity of superoxide dismutase and catalase and a decrease in the level of primary and secondary products of peroxidation. Melatonin has a normalizing effect on the antioxidant system and is a potentially useful tool for reducing the manifestations of oxidative stress in the myocardium.

**Prospects for further research.**

In the future, it is planned to investigate the state of the pro-oxidant-antioxidant system of the lungs of rats under the conditions of exposure to monosodium glutamate.

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### ПРООКСИДАНТНО-АНТИОКСИДАНТНИЙ СТАТУС СЕРДЯ ЩУРІВ ПРИ ДІЇ ГЛУТАМАТУ НАТРІЮ ТА КОРЕКЦІЇ МЕЛАТОНІНОМ

**Пшиченко В. В., Черно В. С., Чеботар Л. Д., Юлевич О. І., Петрова О. І.**

**Резюме.** Порушення режиму та зміна якості раціону харчування, обумовлені пришвидшенням темпів життя та наданням переваги не раціональному і збалансованому раціону, а продуктам швидкого приготування, призвели до споживання неконтрольованої кількості харчових добавок. Постійне вживання продуктів, що містять глутамат натрію, супроводжується перевищенням його гранично допустимих норм споживання та виникненням метаболічних змін, зокрема окисного стресу в серці. Для корекції порушень, які виникають внаслідок окиснювального стресу використовують препарати з антиоксидантними властивостями. Одним з антиоксидантів, який має кардіопротекторну дію, є мелатонін. Метою роботи було визначити зміни прооксидантно-антиоксидантної системи серця щурів під впливом глутамату натрію та корекцію патологічних зрушень мелатоніном.

Піддослідних тварин розподілили на три групи: контрольну і дві дослідні. Тварини I (контрольної) групи перебували за звичайних умов віварію без впливу додаткових факторів. Тварини II групи per os отримували 3% водний розчин глутамату натрію з розрахунку 20 мг/кг. Щури III групи отримували 3% водний розчин глутамату (20 мг/кг) та з метою корекції тваринам вводили внутрішньоочеревинно мелатонін в дозі 1,0 мг/кг маси тіла 1 раз на добу. Метаболічні зміни у серцевому м'язі щурів вивчали на 7, 14, 21, 28 добу дослідження.

Отримані дані свідчать про те, що щоденне споживання глутамату натрію в дозі 20 мг/кг маси тіла призводить до активації процесів перекисного окиснення ліпідів, що починається вже на ранніх термінах експерименту і триває до його закінчення і свідчить про розвиток окислювального стресу. Виявлено і підвищення загальної антиоксидантної активності на 7-му та 14-ту доби дослідження, з подальшим її поступовим зниженням на 21 і 28 добу досліду, що вказує на виснаження ключових компонентів ферментативної складової антиоксидантної системи. Таким чином, результати наших досліджень свідчать про дисбаланс у рівновазі між процесами перекисного окиснення ліпідів і системою антиоксидантного захисту, що призводить до зміни структурно-функціональних властивостей мембран кардіоміоцитів, що є несприятливою умовою для функціонування серцево-судинної системи. Введення екзогенного мелатоніну сприяло зменшенню інтенсивності перикисного окиснення ліпідів та активації ферментативною ланки антиоксидантної системи що супроводжується підвищенням активності супероксиддисмутази і каталази та зменшенням рівня первинних і вторинних продуктів перекисного окиснення. Тож, мелатонін є потенційно корисним засобом для зменшення проявів окислювального стресу у міокарді.

**Ключові слова:** глутамат натрію, щури, серце, каталаза; супероксиддисмутаза

### PROOXIDANT-ANTIOXIDANT STATUS OF THE HEART OF RATS UNDER THE ACTION OF MONOSODIUM GLUTAMAT AND CORRECTION WITH MELATONIN

**Pshychenko V. V., Cherno V. S., Chebotar L. D., Yulevich O. I., Petrova O. I.**

**Abstract.** The violation of the regime and the change in the quality of the diet, due to the acceleration of the pace of life and giving preference to fast food rather than a rational and balanced diet, have led to the consumption of an uncontrolled amount of food additives. The constant use of products containing monosodium glutamate leads

to exceeding the maximum permissible consumption rates and the occurrence of metabolic changes, in particular, oxidative stress in the heart. Drugs with antioxidant properties are used to correct disorders caused by oxidative stress. One of the antioxidants that has a cardioprotective effect is melatonin. The purpose of the study was to determine the changes in the pro-oxidant-antioxidant system of the heart of rats under the influence of monosodium glutamate and the correction of pathological changes by melatonin.

The experimental animals were divided into three groups: a control group and two experimental groups. The animals of the first (control) group were kept under normal vivarium conditions without the influence of additional factors. The animals of the second group per os received a 3% aqueous solution of monosodium glutamate at the rate of 20 mg/kg. Rats of the third group received a 3% aqueous solution of glutamate (20 mg/kg), and for the purpose of correction, melatonin was administered intraperitoneally to the animals at a dose of 1.0 mg/kg of body weight once a day. Metabolic changes in the heart muscle of rats were studied on the 7th, 14th, 21st, 28th day of the study.

The obtained data indicate that the daily consumption of monosodium glutamate in a dose of 20 mg/kg of body weight leads to the activation of the processes of lipid peroxidation, which begins already in the early stages of the experiment and continues until its end and indicates the development of oxidative stress. An increase in total antioxidant activity was also detected on the 7th and 14th days of the study, followed by its gradual decrease on the 21st and 28th days of the study, which indicates the exhaustion of the key components of the enzymatic component of the antioxidant system. Thus, the results of our research indicate an imbalance in the balance between the processes of lipid peroxidation and the antioxidant defense system, which leads to a change in the structural and functional properties of cardiomyocyte membranes, which is an unfavorable condition for the functioning of the cardiovascular system. The introduction of exogenous melatonin contributed to a decrease in the intensity of peroxidation of lipids and activation of the enzymatic link of the antioxidant system, which is accompanied by an increase in the activity of superoxide dismutase and catalase and a decrease in the level of primary and secondary products of peroxidation. Therefore, melatonin is a potentially useful drug for reducing the manifestations of oxidative stress in the myocardium.

**Key words:** monosodium glutamate, rats, heart, catalase; superoxide dismutase

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