10. Shahbazi-Gahrouei D, Setayandeh S, Aminolroayaei F, Shahbazi-Gahrouei S. Biological Effects of Non-ionizing Electromagnetic Fields on Human Body and Biological System: A Systematic Literature Review. Journal of Medical Sciences. 2018; 18: 149-156. DOI: 10.3923/jms.2018.149.156.
11. Solhjou KA, Hosseini SE, Vahdati A, Edalatmanesh MA. Changes in the Hypothalamic-Pituitary-Gonadal Axis in Adult Male Rats Poisoned with Proteus and Biscaya Insecticides. Iran J Med Sci. 2019 Mar;44(2):155-162.
12. Walker DM, Gore AC. Epigenetic impacts of endocrine disruptors in the brain. Front Neuroendocrinol. 2017 Jan; 44:1-26. doi: 10.1016/j.yfrne.2016.09.002.
13. Wallace SJ, de Solla SR, Head JA, Hodson PV, Parrott JL, Thomas PJ, et al. Polycyclic aromatic compounds (PACs) in the Canadian environment: Exposure and effects on wildlife. Environ Pollut. 2020 Oct;265(Pt B):114863. doi: 10.1016/j.envpol.2020.114863.

Стаття надійшла 7.12.2024 р.

DOI 10.26724/2079-8334-2025-4-94-178-182
UDC 617.735:616.1:614.31:599.323.4

G.A. Yeroshenko, K.V. Shevchenko, V.A. Synenko, L.E. Vesnina, I.M. Zviaholska, Yu. V. Tymoshenko, V. I. Ischenko[1]
Poltava State Medical University, Poltava
[1]National University "Yuri Kondratyuk Poltava Polytechnic", Poltava

INFLUENCE OF A COMPLEX OF FOOD SUPPLEMENTS ON THE CONDITION OF RETINAL NEURONS IN RATS

e-mail: gala_umsa@ukr.net

The paper presents data from a morphometric study of the diameter of neurocyte nuclei in the outer nuclear, inner nuclear, and ganglion layers of the retina under the combined action of food additives. It has been established that the effect of a complex of dietary supplements – sodium glutamate, sodium nitrite, and Ponceau 4R – causes a progressive decrease in the average diameter of retinal nerve cell nuclei due to a decrease in euchromatin volume, which indicates a decrease in cell activity. The combined effect of food additives leads to the development of oxidative stress, to which proteins, lipids, and DNA respond, the development of nonspecific inflammation, structural degradation, and the development of gliosis in response to damage. Thus, monosodium glutamate, sodium nitrite, and Ponceau 4R affect the function of the visual analyzer, primarily causing deterioration and once again confirming the need for strict quality control of domestic and foreign products.

Key words: food additives, monosodium glutamate, sodium nitrite, Ponceau 4R, retina, eye, oxidative stress, rats.

Г.А. Єрошенко, К.В. Шевченко, В.А. Синенко, Л.Е. Весніна, І.М. Звягольська, Ю.В. Тимошенко, В.І. Іщенко

ВПЛИВ КОМПЛЕКСУ ХАРЧОВИХ ДОБАВОК НА СТАН НЕЙРОЦИТІВ СІТКІВКИ ОКА ЩУРІВ

В роботі представлені дані морфометричного дослідження діаметру ядер нейроцитів зовнішнього ядерного, внутрішнього ядерного та гангліонарного шарів сітківки при комплексній дії харчових добавок. Встановлено, що вплив комплексу харчових добавок – глутамату натрію, нітриту натрію та Понсо 4R, викликає прогресивне зменшення середніх значень діаметру ядер нервових клітин сітківки, внаслідок зменшенням об'єму еухроматину, що свідчить про зменшення активності клітин. Комплексна дія харчових добавок призводить до розвитку оксидативного стресу, на що реагують білки, ліпіди та ДНК, розвитку неспецифічного запалення, структурної деградації та розвитку гліозу у відповідь на пошкодження. Отже, глутамат натрію, нітрит натрію та Понсо 4R впливає на функцію зорового аналізатору, насамперед, викликаючі погіршення та в черговий раз підтверджує необхідність суворого контролю за якістю вітчизняної та закордонної продукції.

Ключові слова: харчові добавки, глутамат натрію, нітрит натрію, Понсо 4R, сітківка, око, окислювальний стрес, щури.

The study is a fragment of the research project "Restructuring of the organs of the immune, respiratory, and excretory systems under the effect of various exogenous factors (monosodium glutamate, sodium nitrite, ethanol, methacrylate)", state registration No. 0121U108234

Recently, the use of food additives has spread to almost all stages of food production. In order to conceal poor-quality raw materials and make products look attractive, manufacturers add them, sometimes without complying with the standards established by Ukrainian legislation. The available literature often reports on the link between certain food additives and the development of cancerous tumors, allergies, and other adverse effects. However, it is important to recognize that the effect of any chemical substance on the human body depends on several factors, including personal characteristics, the amount of the substance, and the duration of exposure [5].

Previous studies have revealed the effect of food additives on organs and tissues separately from each other. However, an analysis of the content of domestic and foreign products revealed the addition of food additives in combination [12]. Therefore, the impact of food additives, especially when they act in combination, is currently very relevant and requires further research [14].

A study of food additives in food products found that the most common are monosodium glutamate, sodium nitrite, and Ponceau 4R.

The neurotoxic effect of monosodium glutamate from the consumption of monosodium glutamate is that the main neurotransmitter for brain excitation is glutamate, which activates the corresponding receptors, affecting both physiological and pathological processes. Excessive stimulation of these receptors can lead to excitotoxicity. Once glutamate enters Müller cells via the GLAST glutamate transporter, it is metabolized into non-toxic glutamine by glutamine synthetase [1]. Synaptic transmission between bipolar, photoreceptor, and ganglion cells in the retina is facilitated by glutamic acid. However, its presence in excessive amounts can cause neuronal death [5], including retinal ganglion cells [8, 9].

Sodium nitrite is often used as a preservative and color fixative in the manufacture of meat products. It should be noted that the concentration and duration of exposure to sodium nitrite can play a significant role in determining its effect on the retina. Controlled and regulated use of sodium nitrite for food preservation is generally considered safe, as it is used in low concentrations and is carefully monitored by regulatory authorities. However, excessive consumption or exposure to high levels of nitrite may pose a potential risk. On the other hand, high levels of nitrites may be potentially harmful to the retina. Nitrites can react with other compounds in the body to form nitrosamines, which are known to have carcinogenic properties. Nitrosamines can have harmful effects on cells and tissues, including the retina, although the specific effects on retinal cells have not been studied in detail [15].

Regarding the effect of Ponceau 4R on the retina, specific studies on the effect of E124 on this tissue are limited. However, some studies have examined the effect of other food colorings on eye health. For example, studies have shown that some food colorings, such as tartrazine (E102) and sunset yellow (E110), can cause oxidative stress and inflammation in the retinas of animals, potentially leading to retinal damage [6].

The purpose of the study was to determine the dynamics of changes in the diameter of retinal neurocyte nuclei in rats under normal conditions and under the influence of a complex of food additives – monosodium glutamate, sodium nitrite, and Ponceau 4R.

Materials and methods. The study was conducted on 84 sexually mature male Wistar rats. The control group, consisting of 10 rats, received drinking water and orally administered physiological saline. Rats in the experimental group, with free access to water, were given 0.6 mg/kg of sodium nitrite (Sodium nitrite E250, Uralchem, China), 20 mg/kg of monosodium glutamate (Monosodium glutamate E621, Multichem, China), and 5 mg/kg of Ponceau 4R (Ponceau 4R E124, Multichem, China) in 0.5 ml of distilled water once a day orally. The doses of food additives were half of the permissible limit in food products. The adaptive behavior of the rats was assessed using the open field test [13].

The animals were euthanized at 1, 4, 8, 12 and 16 weeks through an overdose of thiopental anesthesia. After euthanasia, fragments of the posterior eye wall were fixed in 10% formalin solution. The pieces of the posterior eye wall were then embedded in Epon-812 and paraffin using conventional technique [11].

Sections with a thickness of 5–10 μm were obtained using the ARM 3600 microtome. Semi-thin sections were obtained using the UNTP-7 ultramicrotome. After staining with hematoxylin and eosin, as well as methylene blue, the sections were placed in polystyrene and examined under a light microscope. Using a digital microscope with the Levenhuk D740T digital microphotography attachment and specialized software adapted for this research, microphotography and morphometric analysis were conducted. The diameter of the nuclei of neurocytes in the outer nuclear, inner nuclear, and ganglion layers of the rat retina was measured.

Statistical processing of the morphometric data was performed using Excel with the built-in “VGA analysis package” add-in, specifically the “descriptive statistics” tool. To test the normality of variances, the Shapiro-Wilk test was applied, and when the data followed a normal distribution, comparisons were made using the Student’s t-test for independent samples. A difference was considered statistically significant at $p < 0.05$ [2, 7].

All animal experiments were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986), in accordance with the rules for keeping experimental animals established by European Parliament and Council Directive (2010/63/EU) and the Order №134 of the Ministry of Education and Science, Youth and Sports of Ukraine as of 01.03.2012, No. 249 “On approval of the procedure for conducting tests, experiments on animals by research institutions”, as well as the recommendations of the First National Congress of Ukraine on Bioethics (2001).

Results of the study and their discussion. Morphometric analysis of the mean diameters of neuronal nuclei in the outer nuclear layer of the posterior wall of the rat eye revealed that the average value was 3.93±0.13 μm (Table 1).

Table 1

Morphometric parameters of the mean diameters of nuclei of retinal nerve cells in rats

Parameters	Outer nuclear layer	Inner nuclear layer	Ganglion cells
Control group	3.93±0.13	6.56±0.12	9.16±0.41
Week 1	3.87±0.10	5.73±0.22	8.64±0.21
Week 4	3.7±0.04 *.**	5.47±0.05 *.**	8.3±0.12 *.**
Week 8	3.48±0.04 *.**	4.73±0.07 *.**	7.43±0.13 *.**
Week 12	3.23±0.06 *.**	4.18±0.06 *.**	6.61±0.16 *.**
Week 16	3.06±0.04 *.**	4.08±0.07 *	6.06±0.08 *.**

Notes: * – $p < 0.05$ compared with the control group; ** $p<0.05$ compared with the previous observation period.

Consumption of the complex of food additives on week 1 of the experiment resulted in a non-significant decrease in the mean values (3.87±0.10 μm ($p<0.05$)).

Consumption of the complex of monosodium glutamate, sodium nitrite and Ponceau 4R on week 4 of the experiment led to a decrease in the mean diameter of neurons in the outer nuclear layer to 3.70±0.04 μm, that was significantly lower by 4.39% compared with the results of the previous observation period and significantly lower by 5.85% compared with the control group ($p<0.05$).

By week 8 of the experiment, the effect of the food additives led to a significant decrease in the mean nuclear diameter values by 5.95% compared with those on week 4 of the experiment and amounted to 3.48±0.04 μm, that was also significantly lower by 11.45% compared with those of the control group of animals ($p<0.05$).

By week 12 of the consumption of monosodium glutamate, sodium nitrite and Ponceau 4R, the mean diameter of neuronal nuclei in the outer nuclear layer of the rat retina was 3.23±0.06 μm, which was significantly lower by 7.18% compared with the previous observation period and significantly lower by 17.81% compared with the control group ($p<0.05$).

By week 16 of the experiment, the mean diameter of neuronal nuclei in the outer nuclear layer was 3.06 ± 0.04 μm, which was significantly lower by 5.26% compared with the values on week 12 of consuming monosodium glutamate, sodium nitrite and Ponceau 4R, and significantly lower by 22.14% compared with the control group ($p<0.05$).

Morphometric analysis of the posterior wall of the eye revealed that the mean diameter of neuronal nuclei in the inner nuclear layer of the retina in the control group of rats was 6.56±0.12 μm.

On week 1 of the consumption of food additives, the mean nuclear diameter in the inner nuclear layer was 5.73±0.22 μm, which did not differ significantly from the control group ($p<0.05$).

The impact of the chemicals by week 4 resulted in a decrease in the mean nuclear diameter of the inner nuclear layer by 4.54% compared with the previous observation period, reaching 5.47±0.05 μm, which was also significantly lower by 16.62% compared to the control group ($p<0.05$).

By week 8 of the experiment, the mean nuclear diameter of the inner nuclear layer was 4.73±0.07 μm, which was significantly lower by 13.53% compared with the week 4 and significantly lower by 27.90% compared with the control group ($p<0.05$).

Consumption of monosodium glutamate, sodium nitrite and Ponceau 4R by week 12 led to a decrease in the mean diameter of nuclei in the inner nuclear layer of the rat retina by 11.63% compared with the previous observation period, reaching 4.18±0.06 μm, and this value was also significantly lower by 36.28% compared with the control group ($p<0.05$).

By week 16 of the experiment, the mean nuclear diameter of the inner nuclear layer of the posterior wall of the rat eye was 4.08±0.07 μm, which did not differ significantly from the previous observation period but was significantly lower by 37.81% compared with the control group ($p<0.05$).

Morphometric analysis of the posterior wall of the eye revealed that the mean diameter of nuclei in the ganglion cell layer of the retina was 9.16±0.41 μm in the control group of rats.

On week 1 of the consumption of monosodium glutamate, sodium nitrite and Ponceau 4R, the mean nuclear diameter in the ganglion cell layer was 8.64±0.21 μm, which did not differ significantly from the control group ($p<0.05$).

Consumption of the food additives by week 4 led to a decrease in the mean nuclear diameter of the ganglion cell layer of the posterior wall of the rat eye to 8.30±0.12 μm, which was significantly lower by 3.94% compared with the previous observation period and significantly lower by 9.39% compared with the control group ($p<0.05$).

Consumption of the complex of monosodium glutamate, sodium nitrite and Ponceau 4R by week 8 led to a decrease in the mean nuclear diameter of the ganglion cell layer of the rat retina by 10.48% compared with the previous observation period, reaching 7.43±0.13 μm. These values were also significantly lower by 18.89% compared with the control group ($p<0.05$).

Exposure to the same chemical complex by week 12 resulted in a further decrease in the mean nuclear diameter of neurons in the ganglion cell layer of the posterior wall of the rat eye by 11.04% compared with the previous observation period, reaching 6.61±0.16 μm, which was also significantly lower by 27.84% compared with the control group ($p<0.05$).

By week 16 of the experiment, the mean diameter of nuclei in the ganglion cell layer of the rat retina was 6.06±0.08 μm, which was significantly lower by 8.32% compared with the previous observation period and significantly lower by 33.84% compared with the control group ($p<0.05$).

Table 2

Mean number of cell rows in nuclei of retinal nerve cells in rats

Parameters	Outer nuclear layer, cell rows	Inner nuclear layer, cell rows
Control group	11.8±0.49	4.8±0.21
Week 1	11.62±0.48	4.19±0.18 *
Week 4	11.11±0.46 *. †	4.00±0.18 * .†
Week 8	10.45±0.43 **. ††	3.46±0.15 **. ††
Week 12	9.70±0.40 **. †	3.06±0.13 **. †
Week 16	9.19±0.38 **	2.99±0.13 **

Notes: * — $p < 0.05$ compared with the control group; ** — $p < 0.01$ compared with the control group; † — $p < 0.05$ compared with the previous observation period; †† — $p < 0.01$ compared with the previous observation period.

Thus, the effect of the complex of food additive, namely, monosodium glutamate, sodium nitrite and Ponceau 4R, on the posterior wall of the eye led to a progressive reduction in morphometric parameters, specifically the mean diameters of neuronal nuclei in the outer nuclear, inner nuclear and ganglion cell layers of the retina, that, at early stages, manifested as a significant decrease in the mean values starting from week 4 of the experiment compared with the control group ($p<0.05$). These changes are associated with the primary response of neuronal cells to the components of the complex of food additives, resulting in reduced cellular activity due to a decrease in euchromatin content, as a reaction to oxidative stress. This finding is supported by previous studies on the effects of monosodium glutamate and sodium nitrite [4, 10]. Subsequently, it was the inner nuclear layer that responded most strongly to the complex of chemicals, as evidenced by a significant decrease in mean nuclear diameter by 37.81% on week 16, compared with the control group ($p<0.05$), primarily indicating the degenerative effects of the complex's components. Consumption of the food additives increases extracellular glutamate levels, leading to excessive activation of AMPA-receptors, which causes rapid Na^+ influx and membrane depolarization. This depolarization displaces Mg^{2+} ions that normally block NMDA-receptor, allowing Ca^{2+} entry. Ca^{2+} influx through NMDA-receptors triggers a signaling cascade that can potentiate synapses, increasing the activity of both AMPA- and NMDA-receptors, ultimately resulting in excitotoxic damage to bipolar and amacrine cells located in this layer. This phenomenon is confirmed by a significant reduction in the number of nuclear rows in the inner nuclear layer, with marked changes (in $p<0.01$) starting from week 8 of the experiment, compared with both control values and the results by week 4. This represented a turning point in the study, reflected in structural degradation and gliosis in response to cellular damage (Table 2). Cells of the ganglion layer were less affected by the components of the complex, but still showed a significant reduction in mean nuclear diameter by 33.84% compared with the control group of animals ($p<0.05$). This was associated with their high sensitivity to oxidative stress caused by sodium nitrite and the azo dye Ponceau 4R, leading to an imbalance between free radical production, specifically reactive oxygen and nitrogen species, and the body's antioxidant capacity. As a result, cellular structures, particularly proteins, lipids and DNA, are damaged, which contributes to premature aging and the development of nonspecific inflammation. In the presence of superoxide radicals, nitric oxide (NO) is converted into peroxynitrite ($ONOO^-$), a potent oxidant that induces severe cellular and mitochondrial damage, disproportionately affecting neurons.

The outer nuclear layer exhibited the least pronounced changes, with a significant reduction in mean nuclear diameter by 22.14% compared with control values ($p<0.05$), since photoreceptor cells lack glutamate receptors capable of mediating excitotoxic cell death as seen in other layers [3].

Conclusion

The effect of the complex of food additives, namely, monosodium glutamate, sodium nitrite and Ponceau 4R, causes a progressive reduction in the mean diameters of retinal neuronal nuclei due to a decrease in euchromatin volume, indicating reduced cellular activity. The combined action of the abovementioned food additives induces oxidative stress, affecting proteins, lipids and DNA, leading to nonspecific inflammation, structural degradation and gliosis in response to damage. Therefore, monosodium glutamate, sodium nitrite and Ponceau 4R adversely affect the function of the visual analyzer, primarily causing impairment, and once again underscore the need for strict control over the quality of both domestic and imported products.

References

1. Bevzo VV. Vplyv tryvaloho vvedennia hlutamatu natriiu na riven deiakykh metabolitiv azotystoho obminu v syrovattsi krovi shchuriv. Visnyk problem biolohii i medytsyny. 2017; 1(135): 83-6. [in Ukrainian]
2. Shpihelkhalter D. Mystetstvo statystyky. Pryiniattia arhumentovanykh rishen na osnovi danykh. Kyiv : KM-Buks. 2023; 384 s. [in Ukrainian]
3. Airaodion AI, Ogbuagu EO, Osemwowa EU, Ogbuagu U, Esonu CE. Toxicological Effect of Monosodium Glutamate in Seasonings on Human Health. Glob. J. Nutri. Food Sci. 2019; 1: 522.
4. Ansari FA, Ali SN, Mahmood R. Sodium nitrite-induced oxidative stress causes membrane damage and alters major metabolic pathways in human erythrocytes. Toxicol In Vitro. 2015; 29(7): 1878-1886.
5. Bera TK, Kar SK, Yadav PK, Mukherjee P, Yadav S, Joshi B. Effects of monosodium glutamate on human health: A systematic review. World Journal of Pharmaceutical Sciences, 2017; 5(5): 139-144. Available from: https://www.wjpsonline.org/admin/uploads/m9d27N.pdf
6. Hammer M, Sandra G, Karl-Heinz D. Retinal pigment epithelium cell damage by A2-E and its photo-derivatives. Molecular vision. 2006; 12: 1348-54.
7. Kay R. Fast Facts: Medical Statistics: Understanding clinical trial results. Karger Publishers Limited. 2020; 108 p.
8. Maluly HDB, Arisseto-Bragotto AP, Reyes FGR. Monosodium glutamate as a tool to reduce sodium in foodstuffs: Technological and safety aspects. Food Sci Nutr. 2017; 5(6): 1039-48.
9. Maeda K, Sawada A, Matsubara M, Nakai Y, Hara A, Yamamoto T. A novel neuroprotectant against retinal ganglion cell damage in a glaucoma model and an optic nerve crush model in the rat. Invest Ophthalmol Vis Sci. 2004 Mar; 45(3):851-6. doi: 10.1167/iovs.03-0365. PMID: 14985301.
10. Ozen T. Acute sodium nitrite induces redox imbalance and oxidative damage in rat tissues. PLoS One. 2017; 12(4): e0175196.
11. Pronina OM, Koptev MM, SM Bilash SM, Yeroshenko GA. Response of hemomicrocirculatory bed of internal organs on various external factors exposure based on the morphological research data. World of Medicine and Biology. 2018; 1(63): 153-57.
12. Wexler P, Editor. Encyclopedia of Toxicology. Reference Work. Third Edition. Academic Press; 2014; 5220 p.
13. Yachmin AI, Kononov BS, Yeroshenko GA, Bilash SM, Bilash VP. Ameasure of the effect of complex food additives on rats'adaptive responses. World of Medicine and Biology. 2020; 1(71): 232-235.
14. Yachmin AI, Yeroshenko GA, Shevchenko KV, Perederii NO, Ryabushko OB. Monosodium glutamate (e621) and its effect on the gastrointestinal organs. Georgian medical news. 2021; 5(319): 147-151.
15. Yamasaki M, Mishima HK, Yamashita H, Kashiwagi K, Murata K, Minamoto A, Inaba T. Neuroprotective effects of erythropoietin on glutamate and nitric oxide toxicity in primary cultured retinal ganglion cells. Brain Res. 2005 Jul 19; 1050(1-2): 15-26. doi: 10.1016/j.brainres.2005.05.037. PMID: 15979589.

Стаття надійшла 9.12.2024 р.