Morphology of carotid sinus wall under the influence of monosodium glutamate and following its withdrawal: an experimental study

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SUMMARY

The aim of this experimental study was to evaluate micro- and ultrastructural changes of the carotid sinus wall during oral consumption of low doses of monosodium glutamate (MG) and following its withdrawal. Adult male albino rats (n=39) were enrolled into the study. Carotid sinus wall morphology was assessed by light and electron microscopy at the end of week 4 and week 8 of MG oral consumption, as well as 2 weeks after its withdrawal; the results were compared with the control group. After 8 weeks of MG consumption, the wall of the carotid sinus was disorganized, endothelial layer of intima deformed, often without clear margins, the media edematous and dissected with thickened elastic membranes, and the cells of the vascular wall were showing signs of apoptosis while extra fat was present in the adventitia. Upon discontinuation of MG after 4 weeks of its consumption, the structural organization of carotid sinus wall was partially preserved, whereas no compensatory processes were registered after 8 weeks of MG administration followed by 2 week-long withdrawal. Therefore, 8-week-long low-dose MG consumption resulted in pronounced changes of the micro- and ultra-structure of the carotid sinus wall of albino rats. Discontinuation of MG following 4 weeks of its administration partially improved the morphologic characteristics of the carotid sinus wall within 2 weeks. Withdrawal of MG after 8 weeks of its administration did not result in any improvement of the micro- and ultra-structure of the carotid sinus wall within 2 weeks.

Key words: Carotid sinus – Structural changes – Monosodium glutamate – Experimental study

INTRODUCTION

Widespread use of food additives, particularly MG, for the purpose of taste enhancement requires thorough morphological investigation of their possible effects on the structure of organs.

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According to EFSA European Food Safety Authority Panel on Food Additives and Nutrient Sources added to Food, an acceptable daily intake (ADI) for MG was established at 30 mg/kg of body weight per day, expressed as glutamic acid, for glutamic acid and glutamates (E 620-625). The Panel noted that the exposure to glutamic acid and glutamates (E 620-625) exceeded not only the proposed ADI for all population groups at the high level (95th percentile) and at the mean level (except for the elderly), but also that these doses are associated with adverse effects in humans for some population groups. In the past, the Scientific Committee on Food established a group ADI for glutamic acid and its salts as 'not specified' in 1990; and the Joint FAO/WHO Expert Committee on Food Additives in 2006 maintained the previously established group ADI as 'not specified'. The European Commission considers revising the maximum permitted levels (Mortensen et al., 2017).

Despite the prevalence of the additives in food worldwide, scientific works dedicated to their effects on vascular morphology are scarce. Given the role of vascular pathology in the disability and mortality rates among patients of different ages, it continues to be an important medical and social problem in Ukraine and worldwide (Kyyak et al., 2019). Stroke is the fifth leading cause of death and is a leading cause of disability (Virani et al., 2020). Approximately 85% of strokes are ischaemic, they are predominantly caused by large artery disease (atherosclerosis), cardioembolism and cerebral small vessel disease. Extracranial internal carotid artery stenosis is a major cause of ischaemic stroke, as it is estimated to cause about 20% of all ischaemic strokes (Murphy et al., 2020). Atherosclerotic plaque occurs frequently at the carotid artery bifurcation and especially in the area of the carotid sinus – a slightly dilated zone in the internal carotid artery usually located cranially to carotid bifurcation and involved in hemodynamics regulation. Changes in carotid sinus size and position result in blood flow disturbances and can facilitate atherosclerotic plaque formation (Nagargoje, Gupta 2020), which is recognized as a leading cause of stroke in patients without other known cardiovascular risk factors (Cautinho et all., 2017).

MG consumption has been previously linked to obesity and arterial hypertension (Thongsepee et al., 2022), thus contributing to elevated risk of stroke, Alzheimer's disease (Fuchsberger et al., 2019; Saski-Hamada et al., 2021), developmental anomalies of the nervous system (Abdou at al., 2020), erosions of stomach wall (Chakraborty, 2019), as well as body weight increase (Konopelniuk at al., 2016). At the same time, data on the level of endogenous intoxication during long-standing MG consumption is not definitive (Bevzo, 2016). It was established that MG has toxic effects on the tissues of lymphatic nodes (Harapko et al., 2021), colon (Kolenchenko et al., 2017), and liver (Banergee et al., 2021); and it can also cause damage to the reproductive system (Mondal et al., 2018). Previous data show the ability of MG to induce oxidative stress in myocardial cells with the increase of marker enzymes, particularly lactate dehydrogenase, aspartate transaminase and alanine aminotransferase (Banergee et al., 2021). Additionally, in case of MG-induced obesity, extra fat is accumulated in the fatty tissue due to cholesterol level increase that leads to cardiovascular pathology (Airaodion et al., 2019). In recent studies, it was shown that low and moderate doses of MG increased serum levels of cholesterol, triglycerides and low density lipoproteins, as well as atherogenic index within one month of consumption (Hazzaa et al., 2020). Nevertheless, further studies are required to clarify the mechanisms of MG consumption effects on the morphology and function of blood vessels, particularly carotid arteries, when it comes to its role in development and progress of atherosclerosis as well as its direct effects on vascular walls.

MATERIALS AND METHODS

The materials constituted histologic and ultramicroscopic slides of the carotid sinus wall samples of 39 adult male albino rats, 3.5-5 months old, with initial body weight of 180-200 g, which were divided into experimental and control groups. The experimental group was further divided into three subgroups. Subgroup 1 (10 animals) received oral MG (10 mg/kg) daily for the duration of 8 weeks and had unrestricted access to standard vivarium food; subgroup 2 (10 animals) had the same

dose of MG discontinued after 4 weeks of the experiment, switching the animals to the standard vivarium diet for the next 2 weeks; subgroup 3 (10 animals) had MG withdrawn following 8 weeks of the experiment and their carotid sinuses studied 2 weeks later. The animals from the control group (9 animals) were on the standard vivarium diet for the entire duration of the experiment. MG was diluted in distilled water and 1 ml of the solution was administered orally by a syringe once daily (at 9 am) with subsequent unrestricted access to food and water. The dose of MG applied was selected on the basis of the utility patent for MG-induced obesity experimental model (Harapko et al., 2020), with the main focus on the dose to be low enough to create the possibility of results extrapolation on human population with regularly mild to moderate MG consumption. The animals from the control group were administered 1 ml of distilled water orally by a syringe once daily at approximately the same time.

All experimental animals were kept at the vivarium of Danylo Halitskiy Lviv National Medical University. The research was conducted in strict accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986), the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Behavior" (2006), the General Ethical Principles on Animal Experimentation, approved by the First National Congress of Ukraine on Bioethics (2001).

The carotid sinuses of the experimental animals were harvested at the level of carotid bifurcation with cuts made 5 mm below and above it, with subsequent preparation of histological slides or paraffin blocks. While cutting the material, care was taken for cuts to be horizontal, i.e., perpendicular to the vascular axis.

For histologic studies, the specimens were stained with hematoxylin and eosin. The slides were studied using 400x and 1000x magnification of optic microscope, and computer system "Aver Media" was used for photographing the specimens.

Electron microscopy was also used for assessment of the specimens. Ultrathin slices of the ca-

rotid sinus wall were prepared with ultra-microtome using glass knives. Slices of silver or lemon color were selected for the study. The specimens were first contrasted in 2% solution of uranyl acetate, and then in lead citrate. The material was studied and photographed using electron microscope YEMV-100 K with acceleration voltage of 75 kV and magnification of 2000x-6000x.

RESULTS

After 8 weeks of continuous MG oral consumption (subgroup 1), the carotid sinus wall was noted to be disorganized, endothelial layer deformed, often without clear margins, sometimes interrupted; endothelial protrusions as well as endothelial proliferation were seen, and some endotheliocytes were dislodged into the carotid sinus lumen (Fig. 1a), while the endothelial layer of the carotid sinus wall in the control group did not show the aforementioned abnormalities (Fig. 1b). Subgroup 1 was remarkable for muscular bundles in the media layer that were separated with wide bands – thickened and sometimes deformed elastic membranes; some perivascular edema was present; occasional thrombi were noted in the vascular lumen (Fig. 1a).

Electron microscopy of the carotid sinus wall samples confirmed the presence of micro- and macroangiopathy following MG consumption. After 8 weeks of the experiment, considerable structural changes of the hemomicrocirculatory vessels of the carotid sinus wall were seen on the ultramicroscopic level (Fig .2a) compared to the control group (Fig. 2b). The endotheliocytes of the microcirculatory vessels were edematous with numerous mitochondria and free ribosomes in the cytoplasm. Chromatin blebs were seen on the periphery of the nuclei of endotheliocytes and pericytes, and their nucleoli were atrophied or disappearing. Apical plasmolemma was protruding in occasional places into the vessel's lumen and formed microvilli. The granular endoplasmic reticulum was fragmented; the Golgi complex contained vesicles that often proceeded to become microcysts, and mitochondria were noted to have clear matrix and scarce cristae, dissection and perforations.
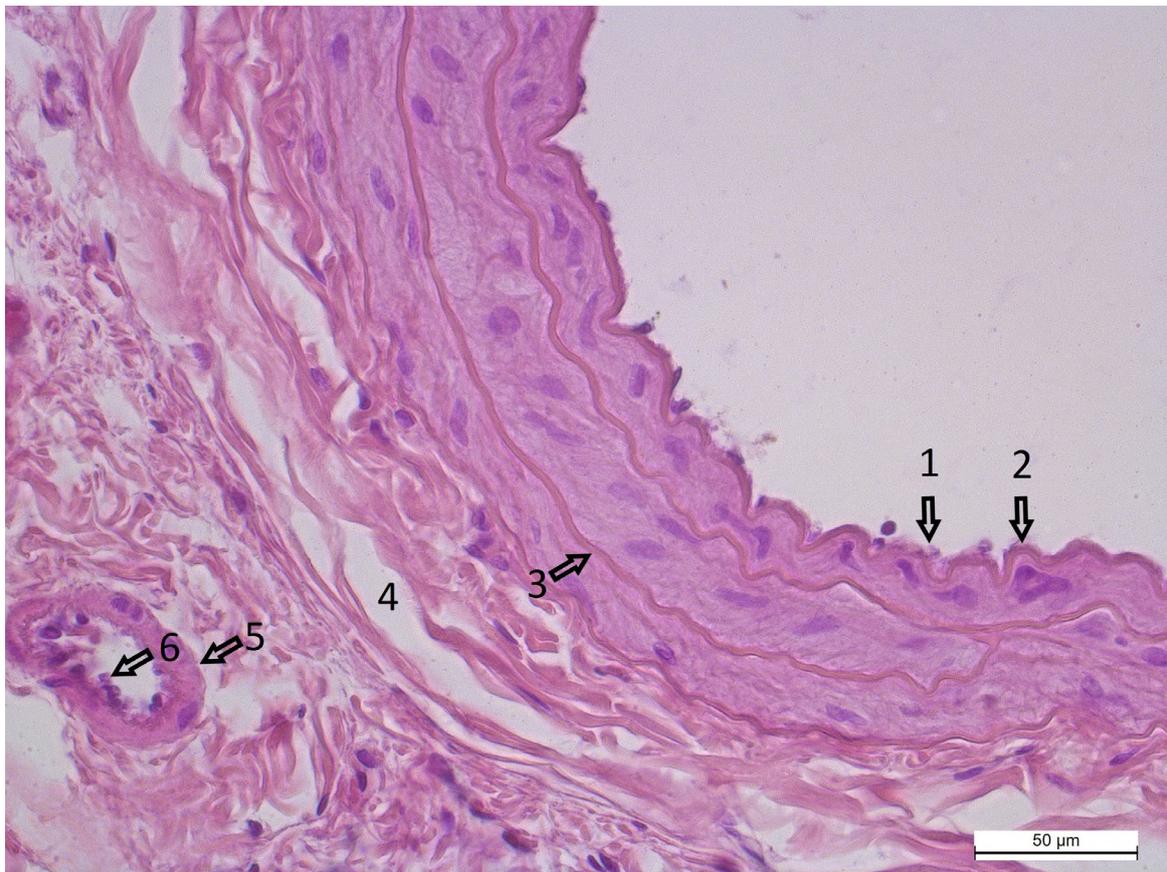
Fig. 1a.- A fragment of carotid sinus wall of an albino rat after 8 weeks of MG oral consumption. Staining with hematoxylin and eosin. Microphotograph. Magnification 400x. 1 – deformed layer of intima endotheliocytes; 2 – endothelial protrusion; 3 – thickened elastic membrane; 4 – perivascular edema; 5 – thickened arteriole wall; 6 – thrombus being formed next to the vascular wall.

Fig. 1b.- A fragment of carotid sinus wall of an albino of the control group. Staining with hematoxylin and eosin. Microphotograph. Magnification 1000x. 1 – intact layer of intima endotheliocytes; 2 – smooth intact elastic membrane.

Fig. 2a.- An arteriole of the carotid sinus wall of an albino rat after 8 weeks of MG oral consumption. Magnification 6000x. 1 – vacuolated mitochondria in an endotheliocyte; 2 – chromatin accumulation at the periphery of the nucleus of an endotheliocyte (a) and pericyte (b); 3 – atrophied nucleolus; 4 – microcyst; 5 – protrusions inside the vascular lumen (microvilli) of apical plasmalemma; 6 – thickened, swollen, deformed basal membrane.
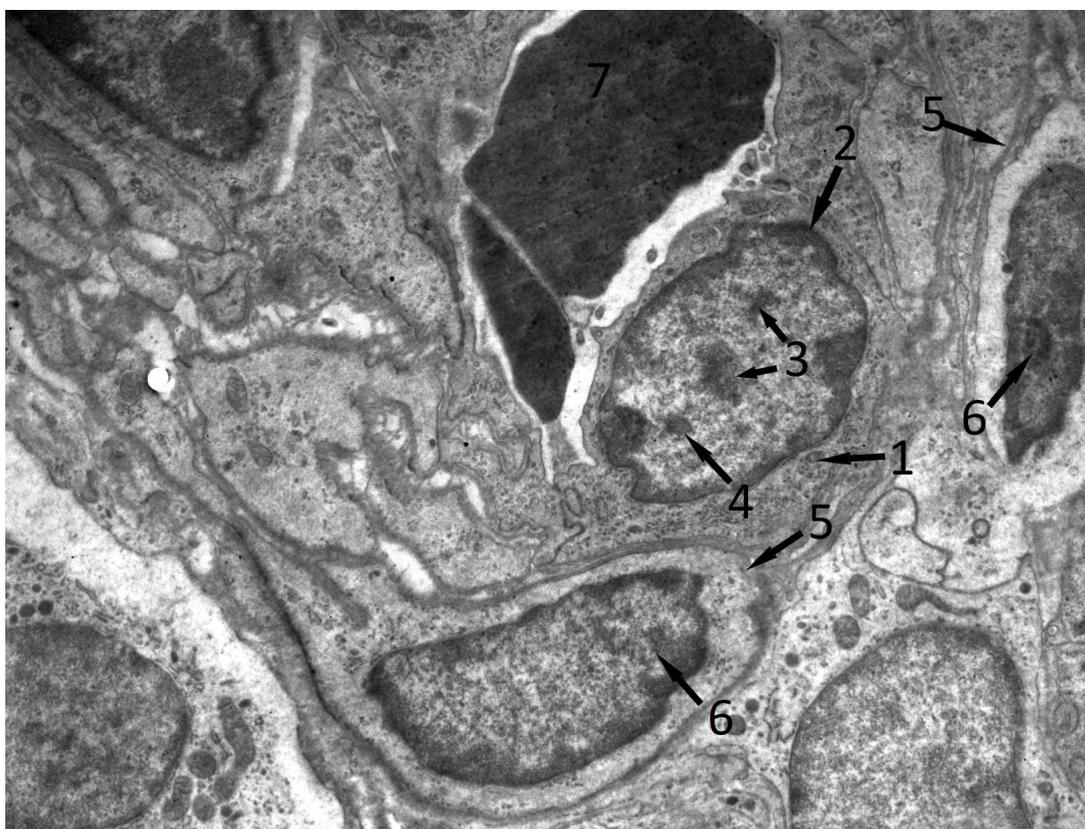
Fig. 2b.- A capillary of the carotid sinus wall of an albino rat of the control group. Magnification 4000x. 1 – intact mitochondria of an endotheliocyte; 2 – intact nucleus of a regular shape of an endotheliocyte; 3 – condensed chromatin within the nucleus; 4 – structured nucleolus within the nucleus; 5 – pericytes; 6 – structured nucleoli within the pericytes nuclei; 7 – erythrocyte in the microvascular lumen.

Regarding the structure of the carotid sinus intima after 8 weeks of the experiment, its endotheliocytes were considerably damaged, with their contours distorted and their shape bizarrely changed. The cytoplasm was edematous, with vacuoles and fused pinocytic vesicles seen within the endothelial cells, their mitochondria swollen with impaired cristae; the granular endoplasmic reticulum was damaged, and some nuclei contained condensed chromatin located peripherally, while other ones were translucent, their nuclear membrane featuring deep invaginations. Many nuclei showed signs of apoptosis and karyopycnosis. The media layer was also disorganized. Most nuclei of the smooth myocytes had irregular shape and their chromatin was represented by heterochromatin mostly located peripherally, perinuclear and pericellular spaces enlarged, mitochondria swollen, their cristae homogenized for the most part. Structureless areas of the cytoplasm were observed in the majority of the myocytes. Elastic membranes were thickened, fuzzy

and without clear margins (Fig. 3a). The control group, however, showed no pathological changes in the morphology of intima or media structures (Fig. 3b). Hence, in the control group, micro- and ultra-structural organization of the carotid sinus wall was preserved and within the species norm if compared to subgroup 1, which that was subjected to oral consumption of MG and unrestricted access to food for the duration of 8 weeks, resulting in some morphological changes as described above.

In case of MG withdrawal and switching of the animals to the standard vivarium diet with evaluation of carotid sinus wall morphology 2 weeks upon MG discontinuation following the preceding period of 4 weeks (subgroup 2) and 8 weeks (subgroup 3) of MG oral consumption and unrestricted access to food, partial preservation of the carotid sinus wall structure was observed on assessment with light microscopy in subgroup 2 (Fig. 4a), while almost no improvement was noted in sub-

Fig. 3a.- A fragment of carotid sinus wall of an albino rat after 8 weeks of MG oral consumption. Magnification 2000x. 1 – translucent cytoplasm of an endotheliocyte of the carotid sinus wall intima; 2 – vacuole; 3 – swollen mitochondria with damaged cristae; 4 – translucent nucleus of an endotheliocyte with condensed chromatin located on the periphery of the nucleus; 5 – a smooth myocyte nucleus of irregular shape; 6 – dilated pericellular space; 7 – swollen mitochondria with homogenized cristae; 8 – thickened, disorganized elastic membrane lacking clear margins.
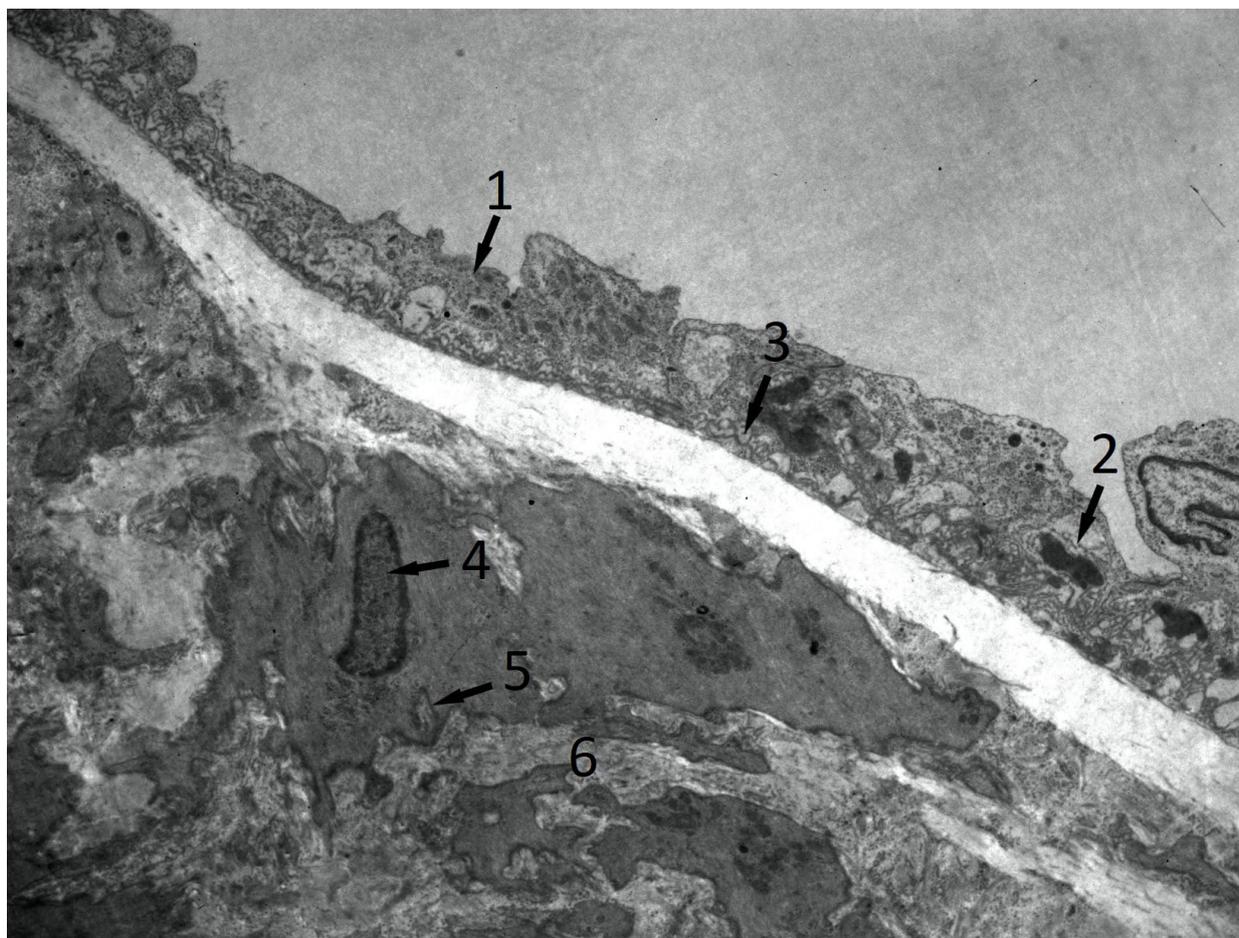
Fig. 3b.- A fragment of carotid sinus wall of an albino rat of the control group. Magnification 2000x. 1- intact endothelial layer; 2 – intact nucleus of an endotheliocyte featuring condensed chromatin; 3 – intact mitochondria of an endotheliocyte; 4 – intact nucleus of a myocyte; - intact mitochondria of a myocyte; 6 – intercellular space.

group 3 (Fig. 4b). For subgroup 2, signs of carotid sinus wall edema, fibrosis, hyperemia of adventitial capillaries, many of them with damaged walls, and additionally some diapedesis bleedings were noted (Fig. 4a), while in subgroup 3 destructive changes of the endothelium, as well as signs of connective tissue and perivascular edema, were seen along with microcirculatory vessels hyperemia (Fig. 4b).

While assessing the aforementioned experimental material with electron microscopy, carotid sinus morphology as well as vasa vasorum were found to be in satisfactory state in subgroup 2. However, some endotheliocytes were remarkable for karyopyknosis. Additionally, fibrosis of vascular walls was noted, as well as occasional cytoplasmic protrusions of endothelial cells into the lumen of micro- and macro-vessels. (Figs. 5, 6, 7).

In case of MG withdrawal after 8 weeks of oral consumption combined with unrestricted access to food, no compensatory processes were seen in the wall of carotid sinus of the experimental animals of subgroup 3 on electron microscopy (Fig. 8a) compared to the control group (Fig. 8b). Carotid sinus wall was notably thickened, intima showed signs of exudative-proliferative inflammatory processes, media was remarkable for the presence of vacuole-type dystrophy of myocytes, fibrotic and sclerotic changes and elastic membranes deformation. Adventitia showed structural changes of all hemomicrocirculatory vessels: hyperemia of capillaries and arterioles, formation of thrombi adjacent to vascular walls, dilation and thinning of venules' walls, perivascular edema as well as marked poikilocytosis of erythrocytes – fragmented cells, basket cells, degmacytes were seen in the microvascular lumen (Fig. 9a), while no significant changes in vascular or cellular morphology were observed in the control group (Fig. 9b, 9c), suggesting that structural impairment was less likely to regress over time in case of more prolonged exposure to MG and excessive food intake.
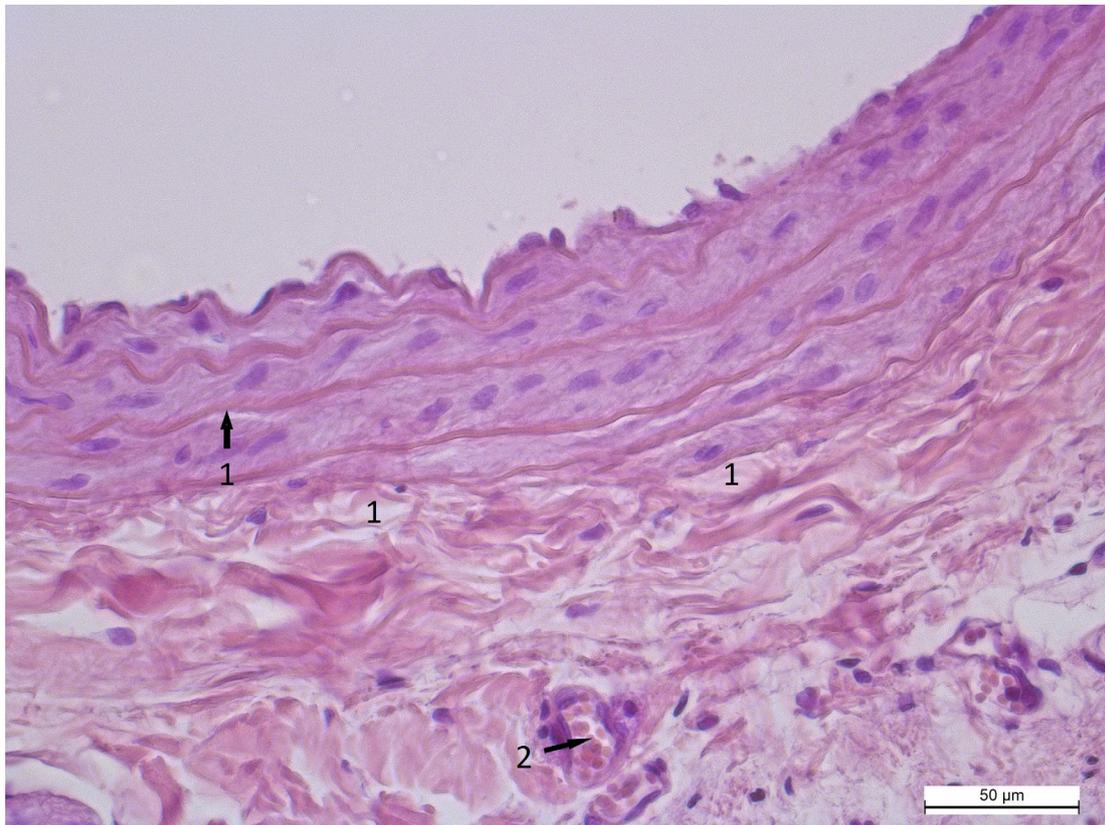
Fig. 4a.- A fragment of carotid sinus wall of an albino rat 2 weeks after withdrawal of MG following 4-week-long oral consumption. Hematoxylin & Eosin staining. Magnification 400x. 1 – signs of swelling in the carotid sinus wall; 2 – hyperemia of an adventitia capillary, damaged walls of the capillary and diapedesis bleeding.
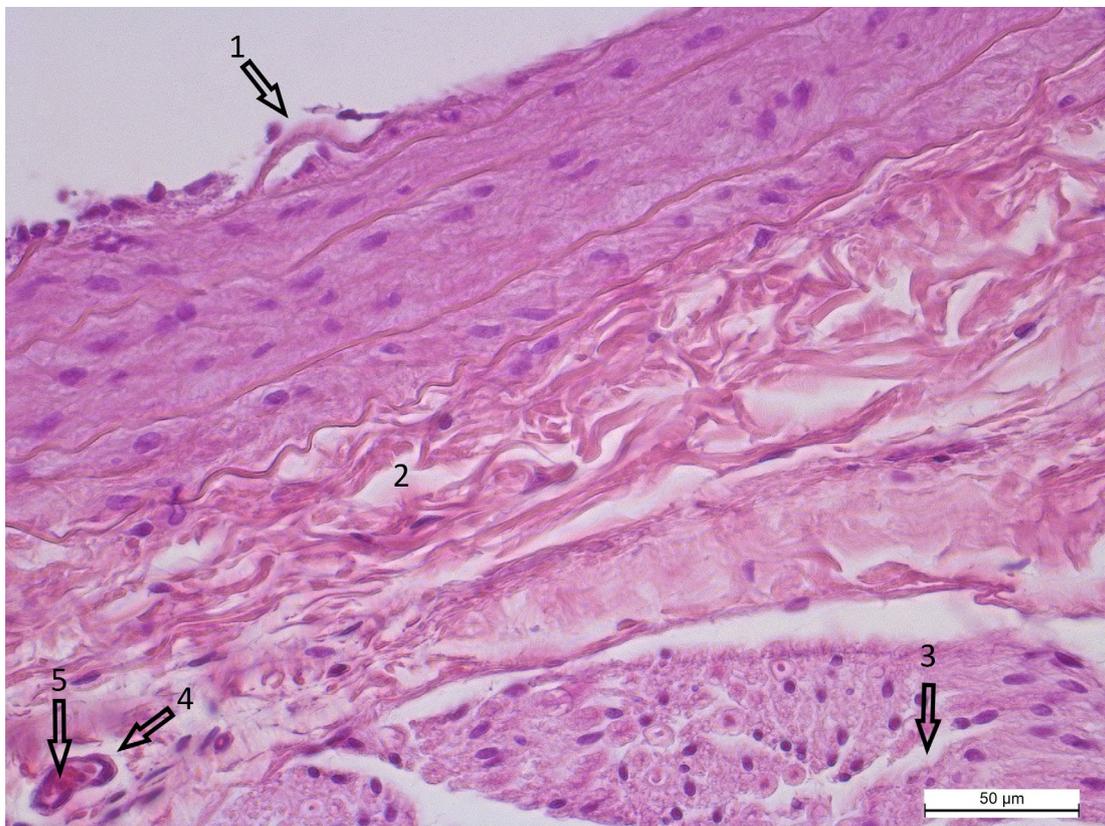
Fig. 4b.- A fragment of carotid sinus wall of an albino rat after 8 weeks of MG oral consumption and 2 week-long withdrawal. Hematoxylin & Eosin staining. Magnification 400x. 1 – de-structured endothelial layer; 2 – edematous connective tissue layers in the carotid sinus wall; 3 – widened connective tissue area in the carotid glomus; 4 – perivascular edema; 5 – hyperemia in a capillary of the carotid sinus wall.

Fig. 5.- An arteriole of carotid sinus wall of an albino rat following 4 weeks of MG oral consumption and 2 week-long withdrawal. Magnification 4000x. 1 – karyopyknosis in an endotheliosyte; 2 – intact perycite nucleus; 3 – endothelium; 4 – neurocyte nucleus, 5 – vascular lumen.
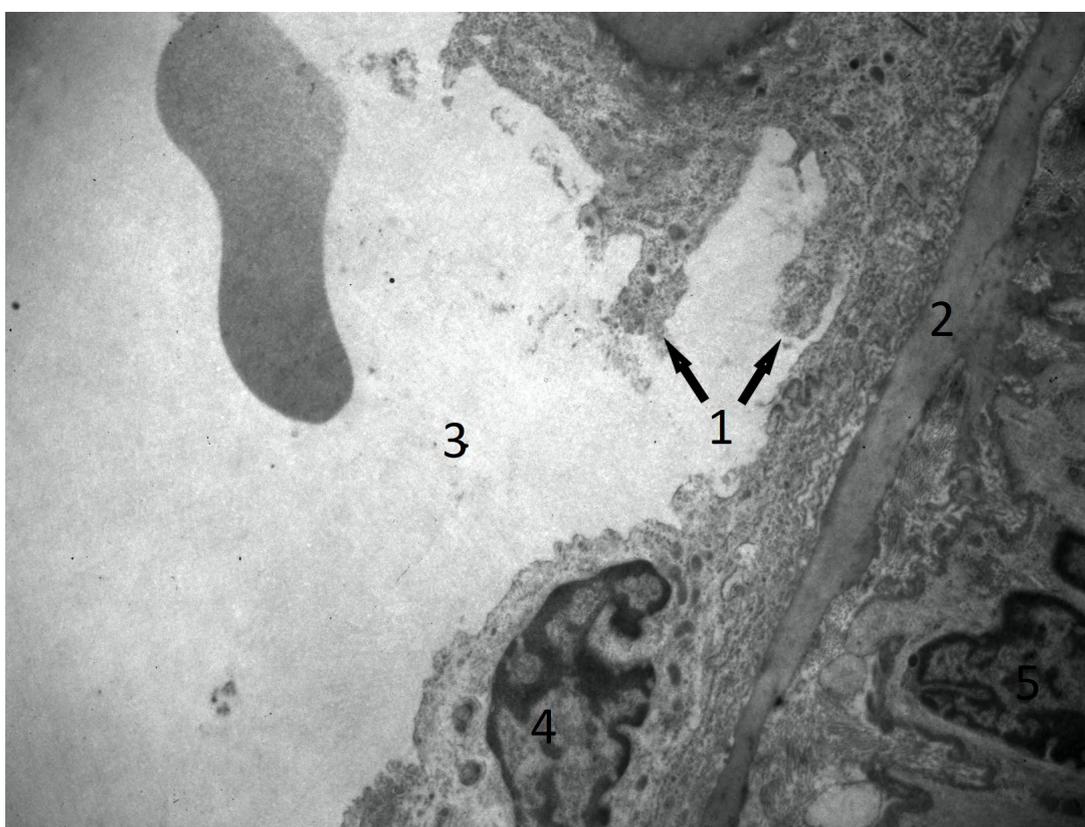
Fig. 6.- A fragment of carotid sinus wall of an albino rat following 4 weeks of MG oral consumption and 2 week-long withdrawal. Magnification 4000x. 1 – cytoplasmic protrusion of an endothelial cell into the macrovascular lumen; 2 – elastic membrane of media; 3 – macrovascular lumen; 4 – intact endotheliocyte nucleus; 5 – intact myocyte nucleus.
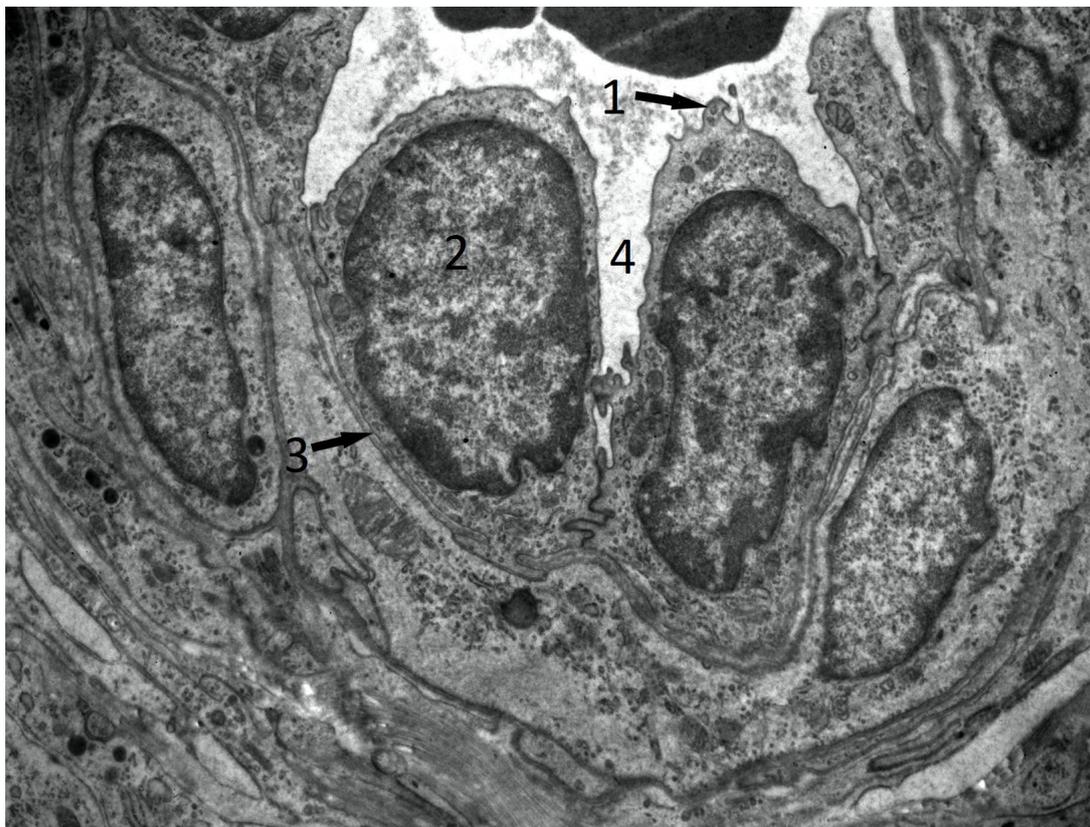
Fig. 7.- An arteriole of the carotid sinus wall of an albino rat following 4 weeks of MG consumption and 2 week-long withdrawal. Magnification 4000x. 1 – cytoplasmic protrusion; 2 – intact endotheliocyte nucleus; 3 – structured basal membrane; 4 – microvascular lumen.
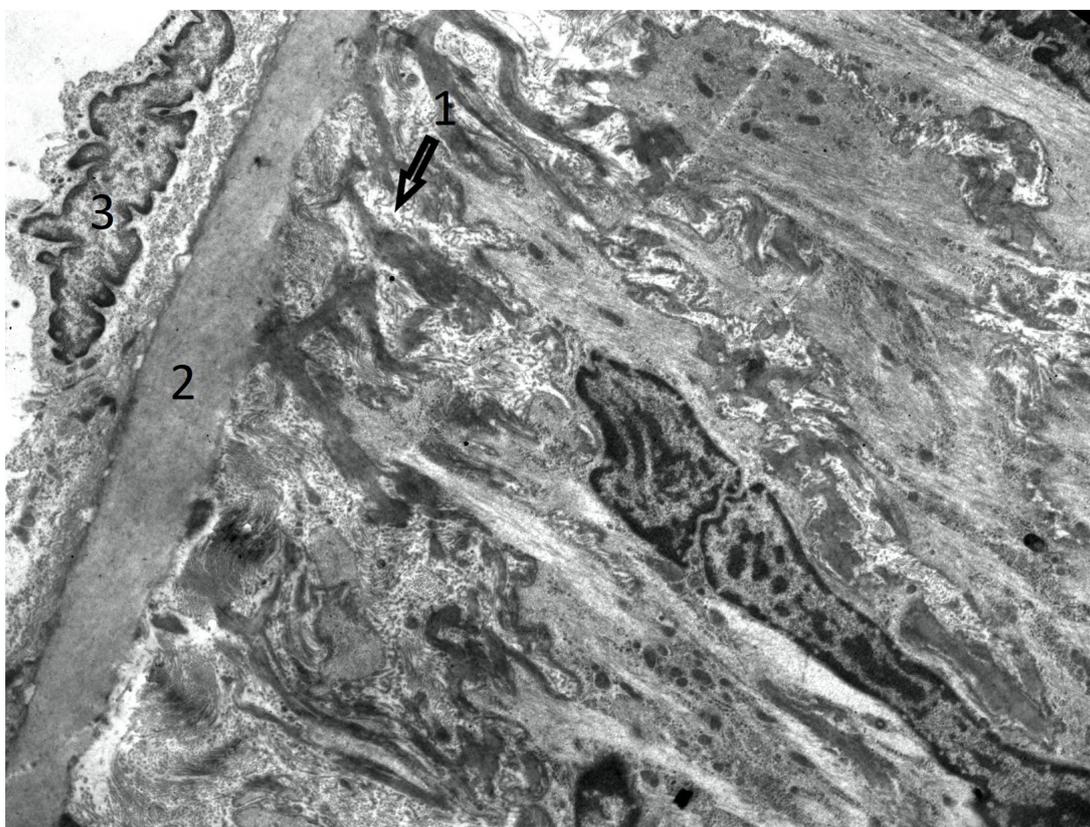
Fig. 8a.- A fragment of the carotid sinus wall of an albino rat after 8 weeks of MG oral consumption and 2 week-long withdrawal. Magnification 2000x. 1 – vacuolated dystrophy of a myocyte; 2 – deformed elastic membrane; 3– fragmented nucleus of a de-structured endotheliocyte.
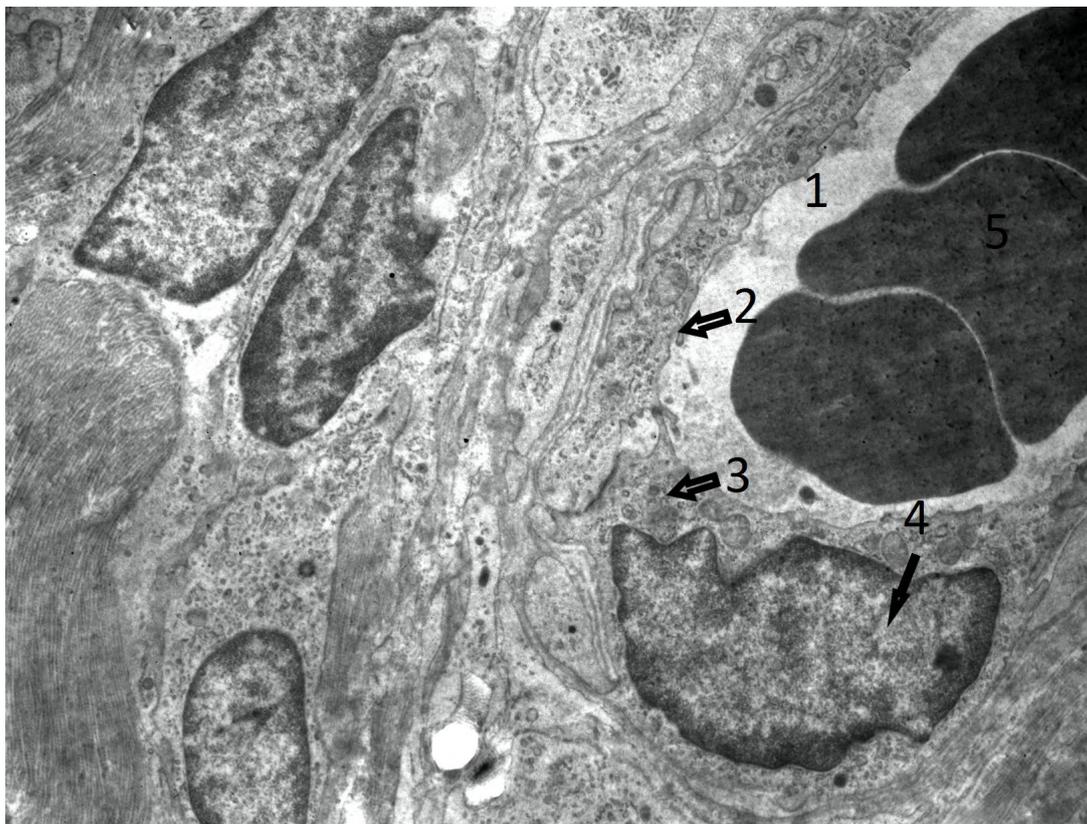
Fig. 8b.- A capillary of the carotid sinus wall of an albino rat of the control group. Magnification 3800x. 1 – lumen of the vessel; 2 – vascular wall; 3 – endotheliocyte; 4 – intact nucleus of the endotheliocyte; 5 – erythrocyte in the vascular lumen.

Fig. 9a.- A venule of the carotid sinus wall of an albino rat after 8 weeks of MG oral consumption and 2 week-long withdrawal. Magnification 3000x. 1 – dilated lumen of a venule in the carotid sinus wall; 2 – basket cells in the lumen of a venule; 3 – degmacyte in the lumen of a venule; 4 – perivascular edema; 5 – de-structured wall of a venule.
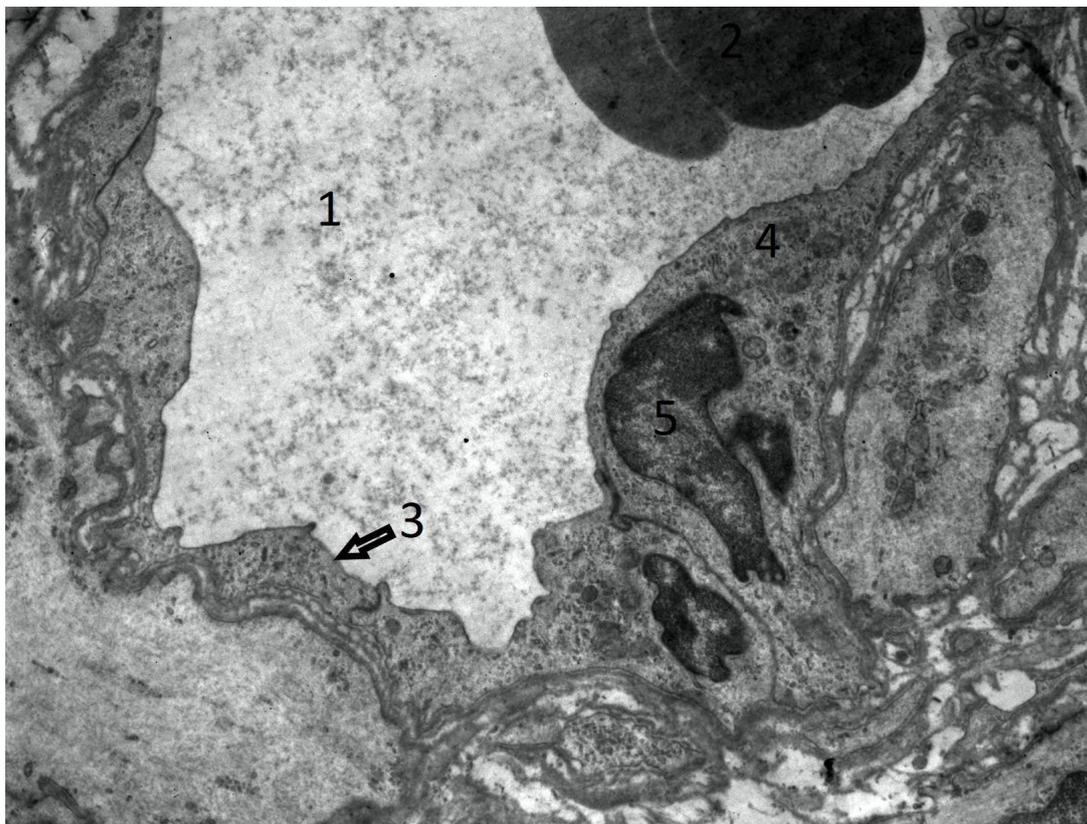
Fig. 9b.- A venule of the carotid sinus wall of an albino rat from the control group. Magnification 3800x. 1 – lumen of the venule; 2 – erythrocyte in the lumen; 3 – wall of the venule; 4 – endotheliocyte; 5 – intact nucleus of the endotheliocyte.
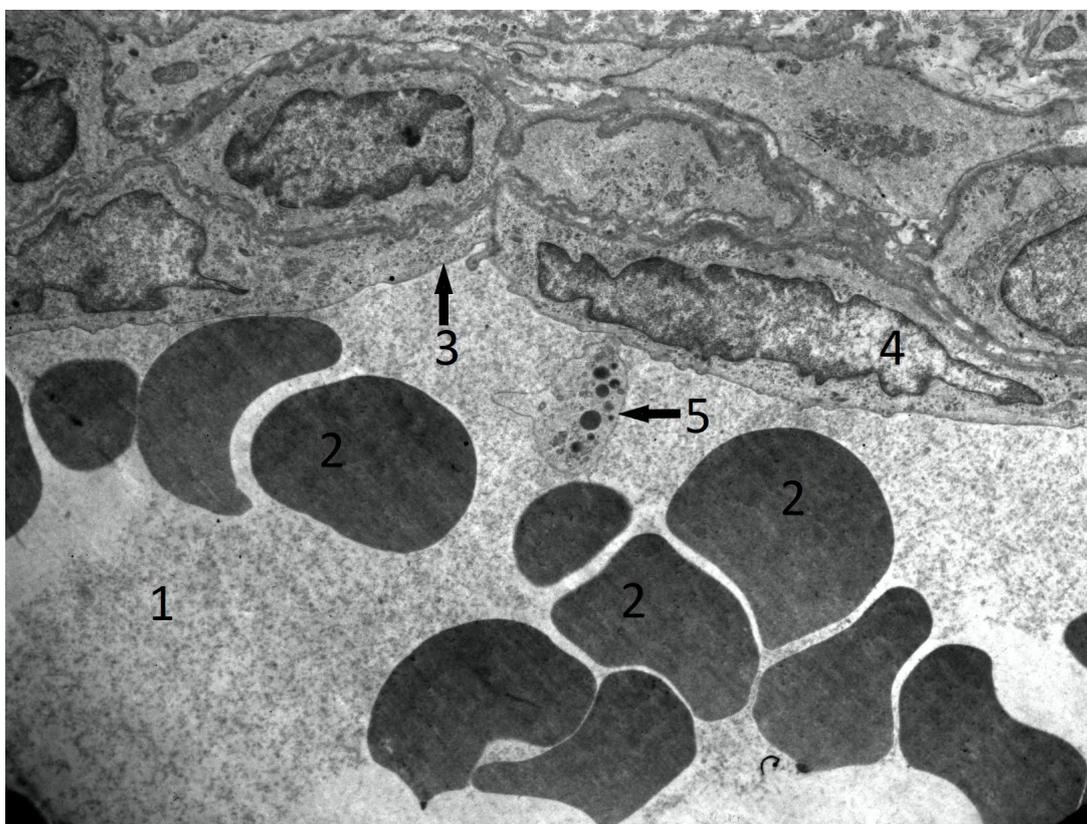
Fig. 9c.- A venule of the carotid sinus wall of an albino rat from the control group. Magnification 4000x. 1 – lumen of the venule; 2 – erythrocytes in the lumen; 3 – wall of the venule; 4 – endotheliocyte; 5 – platelet in the lumen.

DISCUSSION

Mechanisms of MG toxic action on humans and animals have been triggering interest in researches worldwide for quite some time (Umbuzeiro et al., 2017; Bevzo, 2017). During decades, MG has been allowed for consumption, considered relatively safe and widely used in many countries. At the same time, it was shown in numerous scientific studies that prolonged and systematic MG consumption may be associated with a number of pathological conditions, such as metabolic syndrome (Banerjee et al., 2021a, b), diabetes mellitus, dyslipidemia and obesity (Bautista et al., 2019), hypertension and other cardiovascular disorders (Malik, Sabahelkhier, 2019), neuroendocrine disturbances, depression and anxiety (Kumar et al., 2021; Kraal at al., 2020), reproductive and urinary disorders (Pongking at al., 2020), liver disease (Albrahim et al., 2018) and others. MG was also reported to cause increase in food intake (Onaolapo, 2019), which we also observed in our study. This fact might have contributed to the accumulation of excessive fat in the tissues surrounding the carotid sinus and within its wall in our study. A link between extracranial carotid pathology and covert brain infarctions has previously been established (Baradaran et al., 2022). Notably, large artery atherosclerosis has been also associated with cerebral small vessel disease (Ding et al., 2017). These considerations point towards possible pathogenetic implications of morphological changes of the carotid arteries, particularly the carotid sinus wall, in the context of cerebral circulation impairment with subsequent cerebrovascular disturbances and events. Moreover, in our study, impairment of endotheliocytes' nuclei was noted, which indicates MG's ability to cause nuclear damage, potentially leading to genotoxicity. Gene mutations can lead to development of different pathologies, including neurologic defects, metabolic disturbances and neoplasia that may manifest in the next generations (Syed Imam at al., 2019). Therefore, further research is needed to clarify the role of MG in cells apoptosis induction, as well as its possible ability to interfere with structural integrity of cellular genetic material. MG was shown to have a role in metabolic disturbances, namely increase in pros-

tanoid synthesis, together with hypersensitivity to thromboxane A2 combined with potassium channels inhibition, and decrease in nitric oxide levels were shown to have the ability to lead to arterial hypertension in the setting of MG-induced obesity (Majewski at al., 2018). MG consumption combined with high-fat diet causes rise in nitric oxide levels and oxidative stress, hence increasing the affected area in myocardial infarction in experimental setting (Aghajani at al., 2017). Structural changes of myocytes of carotid sinus media observed by us were similar to the damage of cardiomyocytes described in the setting of ischemia and diabetes mellitus (Nadraga at al., 2020; Vlasiuk at al., 2018). Besides changes in the intima and media layers of the carotid sinus wall seen in our study, adventitia was also remarkable for structural changes, with microvasculature impairment and fatty deposits being the most notable ones. Our results are supported by a previous study of MG consumption effects on the aortic wall (Heil et al., 2020). Our research suggests that MG consumption in the experimental setting affects each layer of the vascular wall. This can potentially result in cerebral perfusion compromise due to impairment of the morphology of the carotid arteries.

In our study, some of the structural changes of the carotid sinus that occurred as a result of MG consumption and high food intake were reversible, following MG discontinuation and switching to standard diet, provided that this occurred early enough, suggesting that diet composition and food intake modifications can alter the unfavorable effects of MG consumption, especially at early stages.

The original results of our study are related to identification of specific morphological changes of the carotid sinus wall of albino rats under the influence of MG, and after its withdrawal at different stages of the experiment using light and electron microscopy.

Prospects for further development

Further investigation of possible ways of correction of micro- and ultra-structural changes of the carotid sinus wall in the setting of MG influence is a promising direction for research, opening pos-

sibilities of minimization of its unfavorable action and prevention of vascular disorders associated with structural damage of the carotid sinus wall and adjacent structures.

CONCLUSIONS

Eight week-long MG consumption causes pronounced micro- and ultrastructural changes of the carotid sinus wall in experimental albino rats.

Withdrawal of MG within 4 weeks of its consumption and switching the experimental animals to standard diet considerably improves the morphology of the carotid sinus wall.

Withdrawal of MG within 8 weeks of consumption does not result in restoration of micro- or ultra-structure of the carotid sinus wall.

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