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Original article

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Electron microscopic features of thyroid parafollicular cells in rats after a 60-day administration of Tartrazine and Mexidol ®

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Abstract:
Objective: to identify the effect of a 60-day isolated tartrazine administration, as well as in combination with the Mexidol ®, on the structural features of parafollicular cells in the thyroid of rats at the electron microscope level.
Materials and Methods. We distributed 30 white male rats weighing 200–210 g among five groups of equal sizes. Group I served as a control. Groups II and III included rats receiving tartrazine at concentrations of 750 and 1,500 mg/kg, respectively, for 60 days. Groups IV and V comprised animals under similar conditions, but with Mexidol ® administered at a dose of 50 mg/kg. Qualitative changes in parafollicular cells were examined using electron microscopy, while quantitative changes were assessed via morphometry.
Results. After exposure to tartrazine, fine-grained or fibrous contents were detected in cisternae of the rough endoplasmic reticulum, and in some mitochondria, there were areas of destroyed matrix. The euchromatin to heterochromatin areas ratio decreased in groups II and III by 5.7% and 56.9%, respectively, and the diameter of secretory granules did so by 12.3% and 19%, correspondingly, vs. the control (Group I). However, the above ratio in Group V increased by 79.6%, and the diameter of secretory granules did so by 8.2% and 6.5% in Groups IV and V, respectively, compared with animals of Groups II and III.
Conclusion. Administration of tartrazine in different doses for 60 days triggered dose-dependent qualitative and quantitative changes in the ultrastructure of parafollicular cells, while administration of the Mexidol ® against this background caused a reduction in the severity of changes.
Keywords: thyroid, parafollicular cells, ultrastructure, morphometry, tartrazine, Mexidol ®.

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Introduction

Tartrazine is a representative of azo dyes with a bright yellow or orange color and good solubility in water. This food additive is frequently used in the production of food products, cosmetics, and various medicines [1]. Possessing a rich yellow or orange color, tartrazine improves the attractiveness of the product's appearance, which is accompanied by a proportional rise in consumer interest in it, thereby increasing profits for manufacturers.

Despite the fact that the content of tartrazine in products is regulated by law, questions remain about the safety of its use for human health. There is information about the genotoxic and teratogenic effects of tartrazine [2, 3], its ability to cause allergic reactions (asthma, rash) [4] and behavioral changes in children (hyperactivity combined with attention deficit disorder), as well as about the morphology and biochemical markers of the kidneys, liver, and brain [5, 6].

It was established that tartrazine also affects the metabolism of calcium in the human body, increasing its interstitial content and decreasing its concentration in the blood [7]. However, the available literature does not contain data on morphological changes in the thyroid parafollicular cells involved in the regulation of calcium and phosphorus metabolism. There is also no information about possible pharmacological ways to correct changes in the structural organization of these cells during the long-term administration of tartrazine. Considering that one of the mechanisms of tartrazine adverse effects is its ability to act as a pro-oxidant, we chose Mexidol ®, which, among other things, has antioxidant properties, as a corrector of the above-mentioned changes [8].

Objective –– to identify the effect of a 60-day isolated tartrazine administration, as well as in combination with the Mexidol ®, on the structural features of parafollicular cells in the thyroid of rats at the electron microscope level.

Materials and Methods

For the study, we used 30 mature white male rats weighing 200–210 g. The animals were distributed among five groups of equal sizes (four experimental and one control). Group I (C) served as a control; it included animals that were intragastrically injected with saline solution. In Group II (T1), laboratory animals were exposed to tartrazine administered by gavage at a concentration of 750 mg/kg for 60 days. Group III (T2) differed from Group II by the

tartrazine concentration (1,500 mg/kg). Groups IV and V were formed to establish the efficacy of using Mexidol ® to correct the adverse effects of the food additive. With this goal in mind, rats of Group IV (T1+M) were intramuscularly injected with 1 mL of a 5% solution of Mexidol ® at a dose of 50 mg/kg during a 60-day exposure to tartrazine at a dose of 750 mg/kg, while Group V (T2 +M) differed from Group IV by a dosage of tartrazine (1,500 mg/kg of body weight). We used tartrazine manufactured by Roha Dyechem Pvt. Ltd., India, and Mexidol ® by Ellara Medical Center LLC, commissioned by the Pharmasoft research and production company, Moscow, Russia. Laboratory animals were handled during the experiment and cared about in the vivarium in accordance with the rules established by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [9]. The study design was approved at a meeting of the Bioethics Committee of Saint Luke Lugansk State Medical University, protocol #2 of 25 March 2022. After the completion of the experiment, the animals were removed from the study by decapitation. Processing of the thyroid pieces was conducted in accordance with the standard protocol for electron microscopy [10], after which sections of the gland were photographed and analyzed. The area of the nuclei of parafollicular cells and the areas of euchromatin and heterochromatin were measured using the NIS-Elements BR 4.60.00 software (Nikon Corporation, Japan), and their ratio was calculated. The diameters of secretory granules and the mean area of mitochondria were measured in the cytoplasm.

Data processing was carried out using Statistica 10.0 software (StatSoft, Inc., USA) and Microsoft Office Excel 2017 (Microsoft, USA) (mean, standard error of the mean, and median were calculated). To assess the type of distribution of each parameter, the Shapiro–Wilk criterion was employed. In cases of normal distribution, as well as equality of variances, we used Student's t-test to compare means. Equality of variances was assessed using Fisher's F-test. In the case of distributions other than normal, as well as in case of inequality of variances, the nonparametric Mann–Whitney U test was applied. Differences were considered statistically significant at $p<0.05$.

Results

Parafollicular cells of the thyroid in sexually mature rats of the control group have a typical structure. After exposure to the first concentration of tartrazine (750 mg/kg of body weight), the parafollicular cells of the thyroid were large in size and elongated in shape. Their nuclei contained accumulations of heterochromatin mainly under the karyolemma. Areas of rough endoplasmic reticulum were unevenly expanded with fine-grained contents of moderate electron density. Mitochondria were oval in shape with moderately electron-dense content. Secretory granules were numerous, mostly small, of varying electron density, and unevenly distributed in the cell cytoplasm (*Figure 1*).

When rats were exposed to tartrazine at double the concentration (1,500 mg/kg of body weight), parafollicular cells were large in size and elongated in shape. The nucleus contained accumulations of heterochromatin, which was located at the periphery of the nucleus, as well as in the form of clumps in the central regions of the karyoplasm. In the cytoplasm, the cisternae of the rough endoplasmic reticulum were unevenly expanded. In some areas, in the lumens of the tubules, there were single fibrous structures of different orientations located loosely in relation to each other. Mitochondria had an oval shape and moderate electron density. Some of them contained electron-bright areas of irregular shape with destroyed contents. Secretory granules were numerous, mostly small, of varying electron density, unevenly distributed in the cytoplasm of the cell. Some of them were located very close to each other, thereby looking like electron-dense clusters.

When measuring the structural components of parafollicular cells in Group II, we established that the area of the nucleus was smaller than that in the control group by 20.5%, areas occupied by euchromatin and heterochromatin were smaller by 22.3% and 17.5%, respectively, and the euchromatin to heterochromatin areas ratio was reduced by 5.7%. In Group III, the nucleus area decreased by 28.7%, the area occupied by euchromatin was reduced by 52.1%, the ratio of euchromatin to heterochromatin areas was smaller by 56.9%, while the heterochromatin area was, on the contrary, larger by 11.1%. The diameters of secretory granules in the cytoplasm of parafollicular cells in Groups II and III were smaller vs. rats of the control group by 12.3% and 19%, respectively, and the mean area of mitochondria in the cytoplasm was smaller by 15.8% and 24.8%, correspondingly (*Table*).

After exposure to Mexidol ®, in animals receiving tartrazine at a concentration of 750 mg/kg of body weight, the morphological features of the thyroid parafollicular cells were close to those of the control group. The cells were large and oval in shape. The elongated or round nucleus had small clumps of chromatin in the karyoplasm. In the cytoplasm, rough endoplasmic reticulum formed flattened cisternae with contents of medium electron density, and a moderate number of predominantly oval mitochondria was detected. Numerous secretory granules of different sizes and electron density occupied the perinuclear and basal parts of the cell.

After exposure to Mexidol ®, in animals receiving tartrazine at a concentration of 1,500 mg/kg of body weight, a partial leveling of morphological changes in thyroid parafollicular cells was observed as well. Areas of rough endoplasmic reticulum with dilated cisternae were preserved, and secretory granules of varying sizes were detected in the cytoplasm, either single or located in groups (*Figure 2*).

In Groups IV and V, the use of Mexidol ® as a corrector made it possible to reduce the severity of changes in the morphometric parameters of the thyroid parafollicular cells in rats. In Group IV, the nucleus area was greater than the same parameter in the group without Mexidol ® administration by 7.8%, the area occupied by euchromatin was larger by 8.5%, while the area occupied by heterochromatin and the euchromatin to heterochromatin areas ratio did not change statistically significantly. In Group V, the nucleus area increased by 9%, the area occupied by euchromatin augmented by 46.5%, the area occupied by heterochromatin decreased by 18.7%, and the euchromatin to heterochromatin areas ratio was higher by 79.6%. The diameter of secretory granules and the mean area of mitochondria in the cytoplasm of parafollicular cells increased by 8.2% and 6.5%, and by 8.1 and 8.8%, vs. animals in groups without administration of the Mexidol ®, i.e., vs. Groups IV and V, respectively (*Figures 3, 4, and 5*).

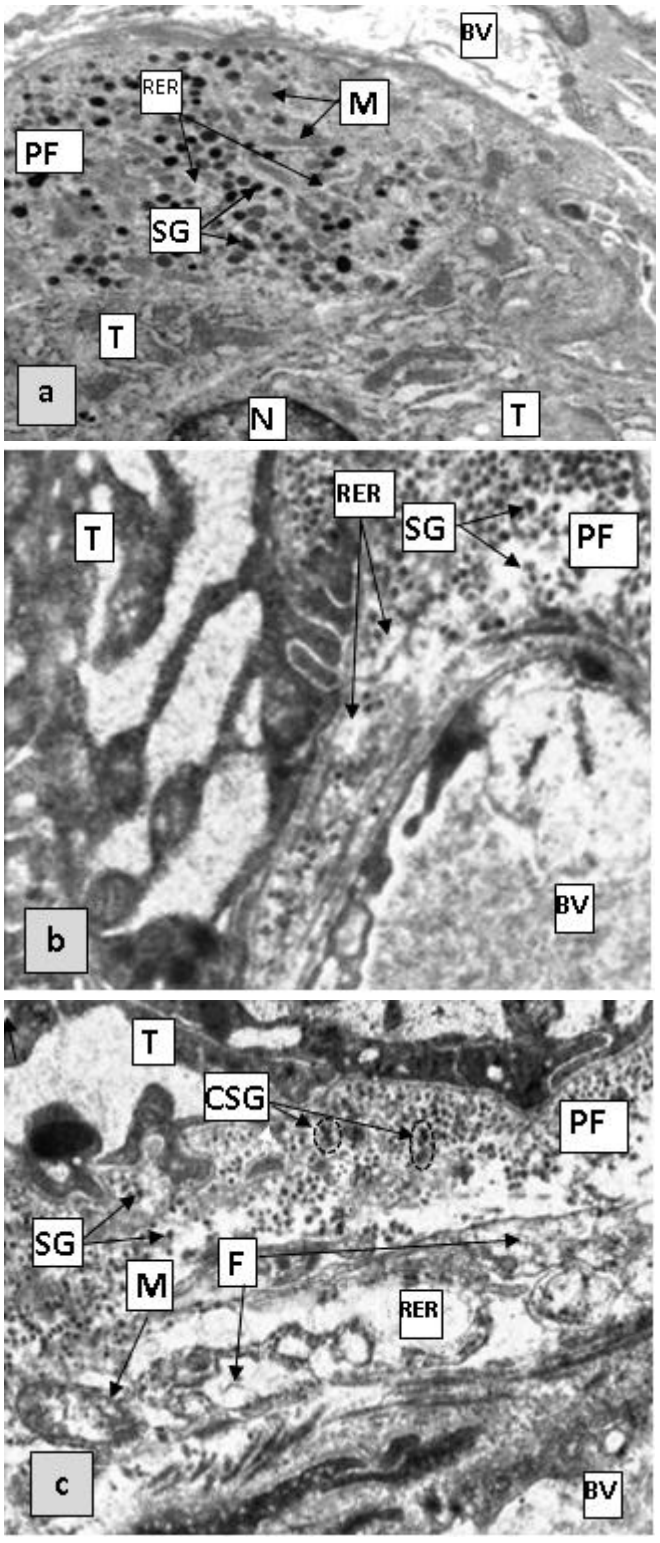

Figure 1. Areas of the thyroid in sexually mature rats: a, control group; b, experimental group with tartrazine administration at a concentration of 750 mg/kg of body weight; c, experimental group with tartrazine administration at a concentration of 1,500 mg/kg of body weight; T, thyrocyte; PF, parafollicular cell; N, nucleus; RER, rough endoplasmic reticulum; M, mitochondria; SG, secretory granules; CSG, clusters of electron-dense secretory granules; F, single fibrous structures; C, blood vessel. Magnification: ×12,000

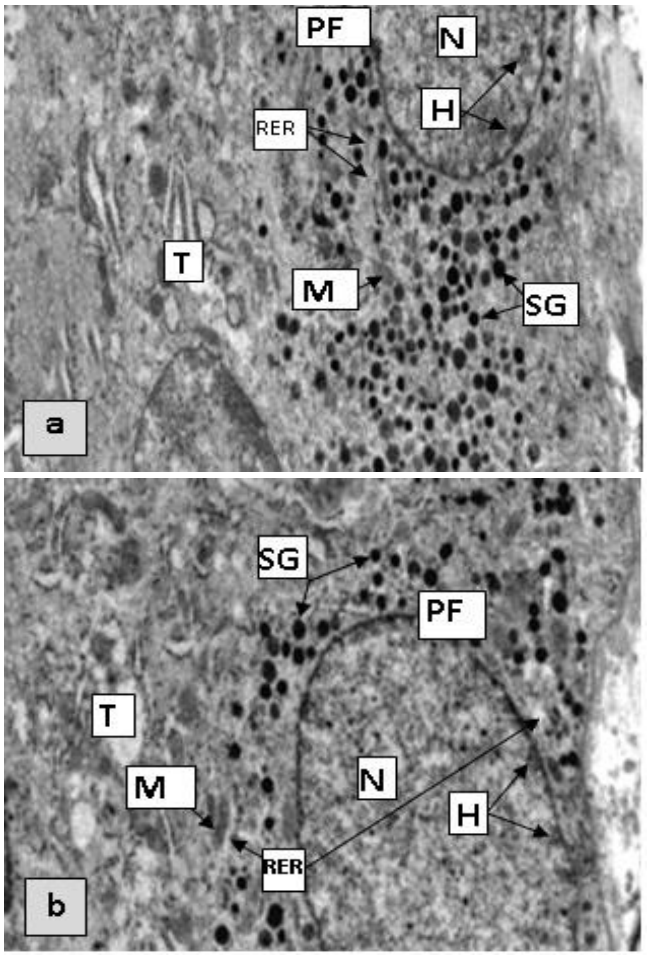

Figure 2. Areas of the thyroid in sexually mature rats: a, experimental group of animals receiving Mexidol ® after exposure to tartrazine at a concentration of 750 mg/kg of body weight; b, experimental group of animals receiving Mexidol ® after exposure to tartrazine at a concentration of 1,500 mg/kg of body weight; T, thyrocyte; PF, parafollicular cell; N, nucleus; H, heterochromatin; RER, rough endoplasmic reticulum; M, mitochondria; SG, secretory granules. Magnification: ×12,000

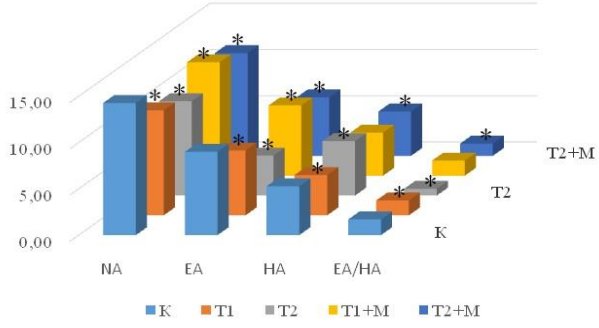

Figure 3. Nuclear parameters of parafollicular cells: NA, nucleus area; EA, area occupied by euchromatin; HA, area occupied by heterochromatin; EA/HA, ratio of euchromatin to heterochromatin areas; C, control group; T1, experimental group with exposure to tartrazine at a concentration of 750 mg/kg of body weight; T2, exposure to tartrazine at a concentration of 1,500 mg/kg of body weight; T1+M, experimental group of animals receiving Mexidol ® after exposure to tartrazine at a concentration of 750 mg/kg of body weight; T2+M, experimental group of animals receiving Mexidol ® after exposure to tartrazine at a concentration of 1,500 mg/kg of body weight

Table. Morphometric parameters of the thyroid parafollicular cells in rats of the control and experimental groups, *M±m*

Indicators	Group				
	I	II	III	IV	V
Nucleus area, μm²	14.28±0.16	11.36±0.21*	10.19±0.11*	12.25±0.14 ‣	11.11±0.20‣
Area occupied by euchromatin, μm²	9.00±0.10	7.00±0.10*	4.32±0.07*	7.59±0.09 ‣	6.32±0.09‣
Area occupied heterochromatin, μm²	5.28±0.08	4.36±0.08*	5.87±0.07*	4.66±0.07‣	4.79±0.09‣
Euchromatin to heterochromatin areas ratio	1.70±0.0098	1.61±0.0078*	0.74±0.0032*	1.63±0.0061	1.32±0.0053‣
Diameter of secretory granules, nm	161.58±1.92	141.71±1.70*	130.85±1.90*	153.38±1.94‣	139.33±1.58‣
Mean area of mitochondria, μm²	10.03±0.14	8.45±0.18*	7.54±0.18*	9.14±0.08‣	8.21±0.10‣

*, statistically significant difference between the indicator values of Groups II and III from the control values; ‣, statistically significant difference between the indicator values of Groups IV and V from the values in Groups II and III, respectively.

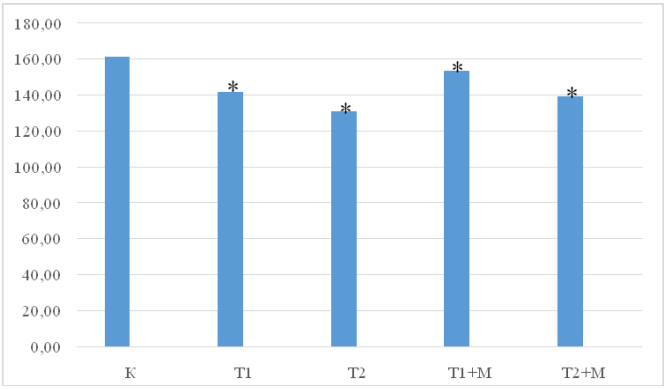

Figure 4. Changes in the diameter of secretory granules in the cytoplasm of parafollicular cells, nm: C, control group; T1, experimental group with exposure to tartrazine at a concentration of 750 mg/kg of body weight; T2, exposure to tartrazine at a concentration of 1,500 mg/kg of body weight; T1+M, experimental group of animals receiving Mexidol ® after exposure to tartrazine at a concentration of 750 mg/kg of body weight; T2+M, experimental group of animals receiving Mexidol ® after exposure to tartrazine at a concentration of 1,500 mg/kg of body weight

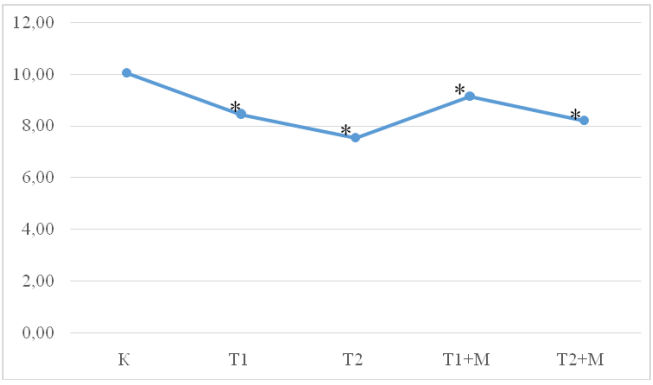

Figure 5. Changes in the mean area of mitochondria in the cytoplasm of parafollicular cells, μm2: C, control group; T1, experimental group with exposure to tartrazine at a concentration of 750 mg/kg of body weight; T2, exposure to tartrazine at a concentration of 1,500 mg/kg of body weight; T1+M, experimental group of animals receiving Mexidol ® after exposure to tartrazine at a concentration of 750 mg/kg of body weight; T2+M, experimental group of animals receiving Mexidol ® after exposure to tartrazine at a concentration of 1,500 mg/kg of body weight

Discussion

Parafollicular cells of the thyroid (also known as C cells) in the control group of mature rats had morphological features that were similar to the data presented by J. Dadan et al. [11].

The use of tartrazine caused dose-dependent changes in the thyroid parafollicular cells of sexually mature rats (noteworthy accumulations of heterochromatin under the karyolemma; unevenly expanded cisternae of the rough endoplasmic reticulum, in some cases with fibrous structures of different orientations; small granules of different electron densities, in some cases located in the form of clusters; and the presence of single mitochondria with damaged cristae). According to the results of the study by G.E. El-Desoky et al., tartrazine reduced the levels of glutathione peroxidase and superoxide dismutase, increased the number of markers of oxidative stress, one of the main consequences of which, according to S.P. Gonzalez-Hunt, M. Wadhwa, L.H. Sanders, was damage to the genetic material [12, 13]. Thus, according to a 2019 study by S. Shakoor et al., tartrazine had a genotoxic effect in the cells of nervous, connective and epithelial tissues, which could disrupt protein synthesis and its removal from the tubules and could cause the so-called endoplasmic reticulum stress [14], thereby exerting an effect directly on parafollicular cells.

According to S. Shakoor et al. 2022, the use of food coloring significantly reduced the level of triiodothyronine (T3) and thyroxine (T4) in experimental rats, which indirectly implied hypofunction of thyrocytes [15]. At the same time, the studies by J. Dadan et al. [11] and by A.M. Makhmurova, M.A. Yuldasheva and A.Yu. Yuldashev [16] drew attention to unidirectional ultramicroscopic changes in thyrocytes and parafollicular cells of the thyroid in experimental animals, which indicates a paracrine effect of some cells on others. According to the data by L. Alioui et al. [17], the use of tartrazine reduced the level of free calcium, and also, based on the results of a study by M. Cemek et al. [6], increased the content of tissue calcium in the liver of experimental animals, which could reduce the endogenous synthesis of calcitonin [6]. Analysis of presented published data allowed us suggesting that the identified ultramicroscopic features of parafollicular cells of the thyroid in rats after exposure to tartrazine were associated with a decrease in the functional activity of these cells through the direct mechanism of the drug action on the cells, via the indirect mechanism (the effect on the calcium levels in the blood and tissues), and by

the paracrine pathway (through the influence of thyrocytes on parafollicular cells).

After exposure to the Mexidol ®, positive dose-dependent dynamics was observed (the lower the concentration of tartrazine affecting the body, the more pronounced the positive dynamics of changes in the structure of parafollicular cells) in terms of changes in the morphometric features of parafollicular cells becoming closer to control values. According to A.V. Shchulkin, Mexidol ® is able to reduce the level of oxidative stress markers (in particular, malondialdehyde), neutralize lipid peroxidation products, increase the activity of glutathione peroxidase, as well as penetrate the mitochondrial matrix and bind reactive oxygen species, thereby providing antioxidant and antihypoxic effects [8]. The presented materials suggest that the antioxidant effect of Mexidol ® can reduce the genotoxic effect and neutralize the oxidative stress caused by the use of tartrazine in different concentrations on rat thyroid cells, which, accordingly, allows restoring protein biosynthesis and the synthesis of secretory granules with the hormone partially or entirely.

Conclusion

Isolated administration of tartrazine in different doses for 60 days results in stress of the endoplasmic reticulum (accumulation of fine-grained and fibrous contents in its cisternae), matrix destruction in some mitochondria, and reduced values of the following parameters: areas of the nucleus and euchromatin, euchromatin to heterochromatin ratio, diameter of the secretory granules, and mean area of mitochondria.

Administration of the Mexidol ® against the background of tartrazine intake causes positive dose-dependent dynamics of changes in the morphometric parameters of parafollicular cells towards approaching the control values. Such dynamics includes the increase in nucleus area, euchromatin area, euchromatin to heterochromatin ratio, diameter of secretory granules, and mean area of mitochondria.

Author contributions. Luzin V.I.: scientific supervision of the experiment and manuscript preparation, interpretation of the results. Morozov V.N.: conducting an experiment, writing sections of an article, statistical data processing, reviewing available publications on the topic of the research.

Conflict of interest. None declared.

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