

Thyroid and Extrathyroid Effects of Methimazole in Modeling Hypothyroidism with Different Doses of antithyroid drug: morfofunctional study

.ABSTRACT

Aims: Methimazole (MMI) is widely used in experimental thyroidology. However, the effect on thyroid function can vary widely depending on the route of administration and doses of the antithyroid drug. In some cases, it is difficult to determine whether the results obtained are a manifestation of thyroid dysfunction or a direct toxic effect of xenobiotic. The aim of the study was to establish the optimal dose of methimazole for the induction of hypothyroidism with minimal toxic effects on tissues.

Study design: The effect of various doses of methimazole administered intragastrically to rats on thyroid status, histological structure of thyroid, liver and kidney was studied.

Place and Duration of Study: Bashkir institute of Technology and Management (branch) of Moscow State University of Technology and Management n.a. K. G. Razumovsky, city of Meleuz, Bashkir State Medical University, Ufa, Russia, 2018-2019.

Methodology: Studies were conducted on 60 nonlinear white male rats administered different doses of MMI intragastrically. To assess the thyroid status, the concentration of circulating thyroid hormones in blood plasma and body temperature rhythms were determined. Along with the indicators of thyroid status, morphological changes in the thyroid gland, liver and kidneys were compared in rats that were administered different doses of MMI.

Results. Hormonal profile of rats received MMI in the dose 2,5 mg/100 g of body weight. for 3 weeks revealed decrease in mean free T_4 ($10,8 \pm 2,14$ against $16,2 \pm 0,57$ in control, $P < 0,001$), total T_3 ($3,12 \pm 0,57$ against $2,36 \pm 0,19$, $P < 0,001$ with the increase of TSH ($0,12 \pm 0,01$ against $1,96 \pm 0,18$, $P < 0,001$). The hypothyroid state in this group of animals is also confirmed by decrease in the arithmetic mean values of body temperature.

Conclusion. The results obtained indicate that a dose of 2.5 mg/100 g of b. w. for 3 w. leads to the development of symptoms characteristic of endemic thyroid dysfunction, and is accompanied by minimal toxic effects on tissues.

Keywords: methimazole, hypothyroidism, experimental model, rat, thyroid

1. INTRODUCTION

Analysis of the dynamics of the primary incidence of subclinical and other forms of hypothyroidism in the Russian population indicates a steady increase in the rate in all age groups, which varies in different regions from 25 to 99 cases per 100000 populations per year [1]. The main causes of the development of primary hypothyroidism are iodine-deficiency in areas endemic for iodine content in soil and water, genetic predisposition to autoimmune diseases, congenital or hereditary defects in enzymes and other proteins involved in hormone genesis, transport and reception of thyroid hormones, and also

destruction of thyroid tissue after surgical interventions and treatment of thyrotoxicosis with radioactive iodine.

Iodine deficiency is one of the most common alimentary-dependent disorders, causing a wide range of negative consequences, the most common of which are goiter and its complications, hypothyroidism and cognitive disorders. About 2 billion people in 130 countries live in conditions of iodine deficiency, more than 740 million have goiter, and 43 million people on the planet have mental retardation [2,3]. Iodine deficiency increases infant mortality and is a major preventable cause of brain damage in fetuses and infants, as well as delayed psychomotor development in young children. The total number of miscarriages, stillbirths and neonatal deaths associated with severe iodine deficiency in early pregnancy is at least 600000 per year. [4].

Iodine deficiency is one of the large-scale microelementoses in Russia. The relevance of thyroid dysfunction of the endemic type is determined by the low content of iodine in the biosphere in most regions of Russia, occupying almost 2/3 of the country's territory. Analysis of data from epidemiological studies in 2003-2012 on the provision of iodine to the population showed that the prevalence of endemic goiter in children and adolescents in the regions of the central part of Russia is 5-20%, the median ioduria ranges from 25.9 [18.8; 32.2] $\mu\text{g} / \text{l}$ to 152.4 [69.8; 209.0] $\mu\text{g} / \text{l}$, averaging only 82.2 $\mu\text{g} / \text{l}$ [5].

Insufficient iodine intake creates serious health problems for tens of millions of Russians, threatens the physical and mental development of 33.7 million children. Every year, more than 200,000 children are born in Russia with impaired brain function due to iodine deficiency [6,7]. In this regard, the problem of experimental modeling of hypothyroidism by endemic type seems to be an urgent task.

The objectives of experimental research are to obtain data that most fully and objectively reflect the essence of the pathophysiological changes taking place in the body, which determines the importance and priority of choosing an adequate experimental model [8,9,10]. Due to the great similarity in many aspects of the physiology of the thyroid system of different mammalian species, the results of experimental studies are quickly translated into human thyroidology. The proximity of the main mechanisms of biosynthesis, transport, intracellular signaling, physiological effects and metabolism of thyroid hormones in humans and rodents makes it possible to quickly introduce the achievements of experimental biology into clinical medicine. It is experimental models using laboratory animals and cell cultures that make it possible to reveal the subtle mechanisms of functioning and regulation of the thyroid system, the structure and expression of hormone receptors in vivo, the differentiation and growth of cells in the prenatal and postnatal periods and associated organ pathology, which, in turn, serves as a fundamental basis for the development of new methods for the diagnosis and treatment of thyroid diseases.

Modern experimental thyroidology has a wide range of methods for modeling thyroid dysfunction. To model hypothyroid conditions, surgical methods are used, including complete or partial removal of the thyroid gland [11,12,13,14]. It is reported that with partial (about half the volume) removal of the thyroid gland, a persistent increase in TSH is observed and after 20 days [14]: the detected level of TSH in rats in the postoperative period was $4.2 \pm 1.0 \text{ ng} / \text{ml}$ (in control animals - $2.4 \pm 0.9 \text{ ng} / \text{ml}$), and the level of triiodothyronine in both groups did not differ, amounting to $1.8 \pm 0.3 \text{ nmol} / \text{l}$. Such changes in the thyroid system are characteristic of subclinical hypothyroidism. Secondary hypothyroidism can be caused by hypophysectomy. In specially equipped laboratories, it is possible to perform radioisotope destruction (ablation) of the thyroid gland with radioactive I^{131} preparations.

Morphologic and functional changes in thyroid follicles with the development of hypothyroidism are detected by the introduction of certain trace elements [15], after exposure to laser radiation [16].

The use of lines of laboratory animals with mutations or genetically engineered modifications of key enzymes of the hypothalamic-pituitary-thyroid axis opens up great prospects. Of interest is the method of induction of cell- and tissue-specific hypothyroidism by blocking

thyronine deiodinase type 2 (D2), or thyronine deiodinase type 3 (D3) [17,18]. Modulation of the activity of different types of deiodinases makes it possible to change the intracellular concentration of triiodothyronine and other forms of iodothyronines in certain types of cells and tissues without changing the level of circulating thyroid hormones.

The most widely used in the modeling of thyroid dysfunction are chemical models - pharmacological and chemical agents that affect the function of the thyroid gland and the thyroid status. Substances that suppress the hormonal activity of the thyroid gland include potassium or sodium perchlorate, potassium thiocyanate, thiourea derivatives (thioamides) - methylthiouracil, propylthiouracil, methimazole (mercazolyl, thiamazole), mercaptoimidazole, methylmercaptoimidazole. When deciding on the design of a chemical model of thyroid dysfunction, the mechanism of action of the chemical agent is important, as well as the determination of single and course doses of thyrostatics. The mechanism of action of thioamides is associated with inhibition of thyroid peroxidase activity. The detailed mechanism of inhibition is not completely clear. It is assumed that due to the thioamide group, they form a stable electron-donor-acceptor complex with iodide, and also enter into a coordination interaction with the heme iron atom in the active site of the enzyme [19]. There are some differences in the action of various thioamides. In particular, this concerns their activity against various types of thyroperoxidases: thyroid peroxidase-1, which is more active in iodide oxidation, is most sensitive to methimazole, and thyroid peroxidase-2, which catalyzes the condensation of mono- and diiodotyrosines into thyronines, is more sensitive to the action of propylthiouracil. Propylthiouracil is also able to inhibit the activity of thioredoxin-containing selenoprotein - type 1 deiodinase (D1), responsible for the intrathyroid and peripheral deiodination of T_4 into more active T_3 . In this case, propylthiouracil interacts with an intermediate product in the active site of the enzyme - selenocysteine, blocking the conversion of T_4 to T_3 [20]. Methimazole also accelerates the excretion of iodides from the thyroid gland.

The antithyroid effect of perchlorates and thiocyanate is based on competition with iodide for transmembrane transport in the thyroid gland with the help of NIS (sodium-iodide symporter), localized on the basolateral surface of thyrocytes [21].

There are large variations in the modes and methods of administration of thyrostatics. They can be administered intraperitoneally, orally, by adding to food or drinking water. The dose is critical. On the one hand, the dose must be sufficient for the development of hypothyroidism, on the other hand, it is maximally secure from the point of view of minimizing a toxic effect on the body of an experimental animal. Thiouracil derivatives are well absorbed, food intake does not affect the amount and rate of absorption. Their bioavailability is more than 90%. They practically do not bind to plasma proteins, reach the maximum level in the plasma after 30-60 minutes, accumulate in the thyroid tissue, and a single dose of the drug acts within 24 hours [22,23].

To verify hypothyroidism in laboratory animals, physical and laboratory studies are used. Diagnostic criteria can be anemia, leukopenia, hypercholesterolemia, changes in the intensity of metabolic processes, but the leading criterion for the diagnosis of hypothyroidism is a significant and steady decrease (by 40-80%) in the content of thyroxine and triiodothyronine against the background of an increase in thyroid-stimulating hormone in the blood serum [23]. Dynamic study of these indicators, as well as assessment of the development of clinical symptoms (decrease in body temperature, weight gain, decreased motor activity, etc.) allow with a high degree of confidence to diagnose this pathology. It should be considered that a single change in the rectal temperature of experimental animals (rats, mice) does not allow judging the development of hypothyroidism, since it is subject to significant daily fluctuations, so multiple thermometry is required during the day. [24].

The most widely used for scientific research is a chemo-induced model of hypothyroidism using thiourea derivatives. In this case, the necessary reduction in the levels of thyroid hormones is achieved by the introduction of relatively high doses of thyrostatics (50.0 and 20.0 mg / 100 g of body weight). However, when using high doses, pronounced

morphofunctional changes in the organs and tissues of experimental animals can be observed, which clearly depend on the regimens for the introduction of a thyrostatic [24]. As the most optimal, the following modes of administration of thyrostatics are recommended: 1-2 mg of propylthiouracil per 100 g of body weight, 1-5 mg of mercazolil per 100 g of body weight intraperitoneally for 2-3 weeks; three-week intragastric administration of mercazolil at a dose of 5 mg per 100 g of body weight; addition to the feed of 0.02% -0.15% propylthiouracil solution or 1.25% potassium perchlorate solution [23]. A methodological method for modeling "short-term" and "long-term" hypothyroidism is also proposed. [24]. This method suggests the administration of mercazolil to immature rats per os for 14 days at a dose of 0.5 mg / 100 g of body weight, followed by the continuation of the administration of a maintenance dose for a month - 0.25 mg / 100 g of body weight (short-term hypothyroidism). In modeling the prolonged hypothyroidism, animals further receive a thyrostatics until puberty.

However, an ideal experimental model of hypothyroidism probably does not exist. It should not be ignored that the pathochemical shifts observed during the chemical induction of hypothyroidism may be partly caused by the extrathyroid effects of the injected xenobiotic. When studying the developing changes in oxidative metabolism, it is impossible to exclude the possibility of an inhibitory effect of thioamides in addition to thyroperoxidase and on the activity of other oxidative enzymes, in particular, members of the myeloperoxidase family.

In this regard, we have conducted targeted studies to determine the single and course doses of methimazole (mercazolil), which provide modeling of hypothyroidism while minimizing the side effects of a thyrostatics. [22,25].

2. MATERIAL AND METHODS

2.1 Animals

Studies were conducted on 60 nonlinear white male rats weighing 180-220 g in vivarium conditions: temperature in the range of 18-20°C, relative humidity - 60-70%, free access to water, complete dry compound feed for laboratory animals («Chara», produced by «Assortiment-Agro» LLC, Russia) in compliance with ethical standards and recommendations for humane treatment of laboratory animals (order of the Ministry of Healthcare of Russia N 199 dated 01.04.2016 "On Approval of the Rules of Good Laboratory Practice").

2.2 Experimental design

Animals were divided into 5 groups (n=12 for each group).

Group 1: control animals; *Group 2:* rats were administered methimazole (MMI) solution intragastrically at the daily single dose 20 mg per 100 of body weight for two weeks; *Group 3:* rats received MMI solution intragastrically at the daily single dose 10 mg per 100 of body weight for two weeks; *Group 4:* rats received MMI solution intragastrically at the daily single dose 2,5 mg per 100 of body weight for three weeks; *Group 5:* rats received MMI solution intragastrically at the daily single dose 1 mg per 100 of body weight for three weeks.

On the second day after of the last dose rats were anesthetized by ether, blood samples were obtained and organs (thyroid gland, liver, kidney) dissected out.

2.3 Light microscopy

Histological examination of the tissues according to generally accepted methods were carried out: tissues were fixed with neutral 10% formalin solution, embedded in paraffin, sectioned with a microtome to obtain 7 µm-thick sections. Dewaxed sections are then stained with hematoxylin and eosin. Ocular. 10x, objective 40x.

2.4 Assessment of thyroid status

2.4.1 Assessment of serum thyroid hormones

Content of free T_4 , total T_3 and thyroid stimulating hormone (TSH) was determined in the blood serum using the ELISA method using the standard test systems of AlkorBio CJSC (Russia).

2.4.2 Body temperature

Daily biorhythms of body (rectal) temperature on the 1st and 2nd days after the last dose of MMI with an interval of 4 hours - at 7.00, 11.00, 15.00, 19.00 and 23.00 were determined.

2.5 Statistical analysis

Statistical analysis was carried out using Student's t-test, the data obtained are presented as Means $\pm \sigma$.

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Histological analysis

3.1.1.1 Thyroid gland

Light microscopy examination revealed morphological alterations in organs of MMI treated animals. In animals of the control group (Fig 1.A), the thyroid gland is covered with a connective tissue capsule, from which thin partitions depart deep into the gland, dividing the gland into lobules. Follicles are of round or angular in the form of closed vesicles lined with single-layered epithelium. Between the follicles there are poorly differentiated cells that form interfollicular islets. Follicle thyrocytes, which have a cubic shape, are stained oxyphilically, tightly adjacent to each other, the nucleus is located in the basal half of the cell. Follicles contain a colloid that fills the entire space.

In the thyroid gland of animals treated with MMI at a dose of 20 mg / 100 g of body weight for 2 weeks (Fig. 1.B), both in the central and in the marginal part of the follicles of the gland, there is no colloid. Separate destructively altered thyrocytes located on the basement membrane have cytoplasmic outgrowths of various configurations directed into the cavity of the follicle. Thyrocytes are deformed, the nuclei undergo karyopycnosis and are reduced in size, chromatin is dense, stained in dark blue, a narrow rim of the cytoplasm is preserved around the nucleus. In the walls of the follicles, areas devoid of glandular cells are determined, where fibroblastic cells of loose connective tissue are adjacent to them. In the central part of the gland, follicles with a deformed cavity are observed, and in the peripheral part, thyrocytes completely line the walls of follicles devoid of intrafollicular colloid. At the same time, the follicles seem to be compressed, which gives the wall a reticulate structure. Part of the deformed thyrocytes is devoid of connections with the basement membrane and is freely located in the intrafollicular cavity in the form of clusters, and in the subcapsular zones of the gland, a well-preserved wall and cavity containing traces of colloid are determined. In the central part of the gland there are clusters of densely spaced cells of cubic or polygonal shape, containing a uniformly colored cytoplasm and a centrally located nucleus of a rounded shape: these structures probably represent interfollicular islets. Small and large blood vessels are altered, in most cases they are free of blood and gaping, especially in the central areas, in the peripheral areas they are characterized by moderate blood filling.

In the thyroids of animals treated with MMI at a dose 10 mg/100 g of b.w. for two weeks (Fig. 1.C) single follicles contain a small amount of colloid. In the central part, deformed thyrocytes were determined, connected to the basement membrane by a narrow band of cytolemma. The apical ends of glandular cells are represented by cytoplasmic outgrowths turned into a colloid-free intrafollicular cavity, where fragments of nuclei and thyroid epithelium are found. In the overwhelming number of follicles, the main structural elements are preserved: the wall is lined with a single-layered cubic epithelium; the nucleus occupies a central position and has a rounded shape, chromatin is evenly distributed and stained basophilically. Based on the described histological picture, it can be concluded that the

processes of synthesis and accumulation of intrafollicular colloid in this mode of thyrostatic administration are blocked while preserving the main structural elements of the parenchyma and blood supply to the thyroid gland.

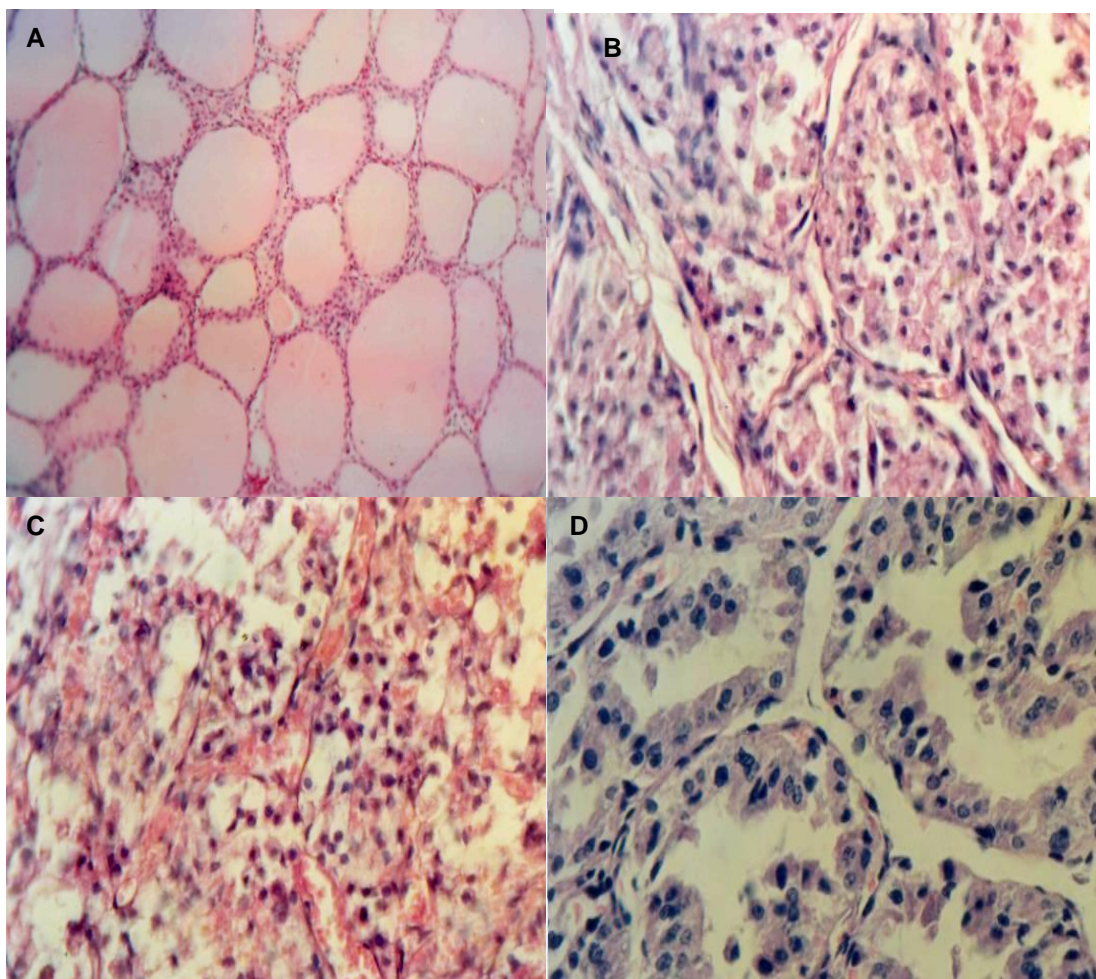


Figure 1. A. Follicles with colloid in thyroid gland of rats of the control group.
B. Destructive processes in the thyroid gland: MMI at a dose of 20 mg / 100 g of b.w. for 2 w.
C. Deformation of thyrocytes in the thyroid follicles: MMI at a dose of 10 mg / 100 g of b. w.
D. Absence of colloid in the thyroid gland: MMI at a dose of 2.5 mg / 100 g of b. w. for 3 w.

Significant changes were also detected in the thyroid gland of rats treated with MMI at a dose of 2.5 mg / 100 g of body weight for three weeks (Fig1.D). In the central part of the gland, a reduced amount of colloid is observed. At the same time, the colloid, decreasing in volume, gradually moves away from the thyrocytes, a free space is formed between the follicular cells and the colloid. In some cases, the mass of the follicular colloid shifts to one side and is adjacent to the apical end of the thyrocytes. In some follicles, the colloid is completely absent, which is due to the cessation or sharp inhibition of the synthesis of thyroglobulin against the background of its active absorption. In the cells of the follicular epithelium, signs of destruction are noted. Often the height of thyrocytes decreases, which

acquire an oval shape and are characterized by dense chromatin. Some thyrocytes, which have a flattened shape and an uneven apical part, shift into the follicle cavity. Around the destructively altered follicles with deformed walls in the form of various forms of folds and protrusions, individual lymphocytes and macrophages are observed, sometimes they can be located in the follicle cavity. Interfollicular thyrocytes are also in a state of destruction and gradually wedge themselves into the total mass of altered cells. Thyrocytes having a foamy, vacuolized pale-colored cytoplasm are increased in volume due to severe hydropic and vacuole dystrophy; the nuclear-cell ratio decreases.

The administration of the lower dose of MMI (1mg/100 g of b.w.) led to less pronounced destructive processes in the thyroid gland (Fig 2). In the follicles of the central part of the gland, colloid is absent, thyrocytes are mainly cubic in shape, the phenomena of karyopycnosis and caryorexis are observed, some of the thyrocytes are exfoliated and freely located in the follicle cavity. Areas with lymphoid infiltration are detected.

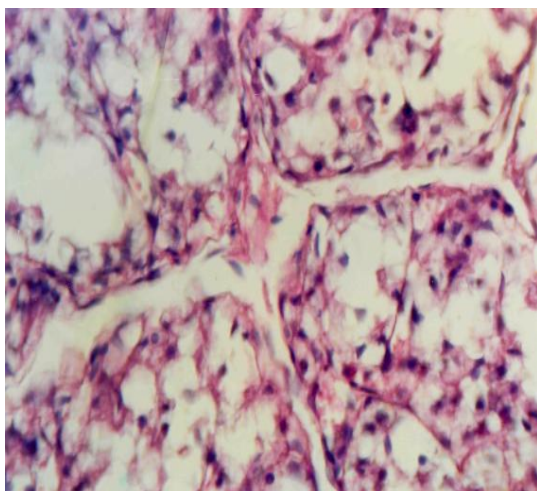


Figure 2. Various forms of thyrocytes after the use of MMI at a dose of 1.0 mg / 100 g of b. w. for three weeks

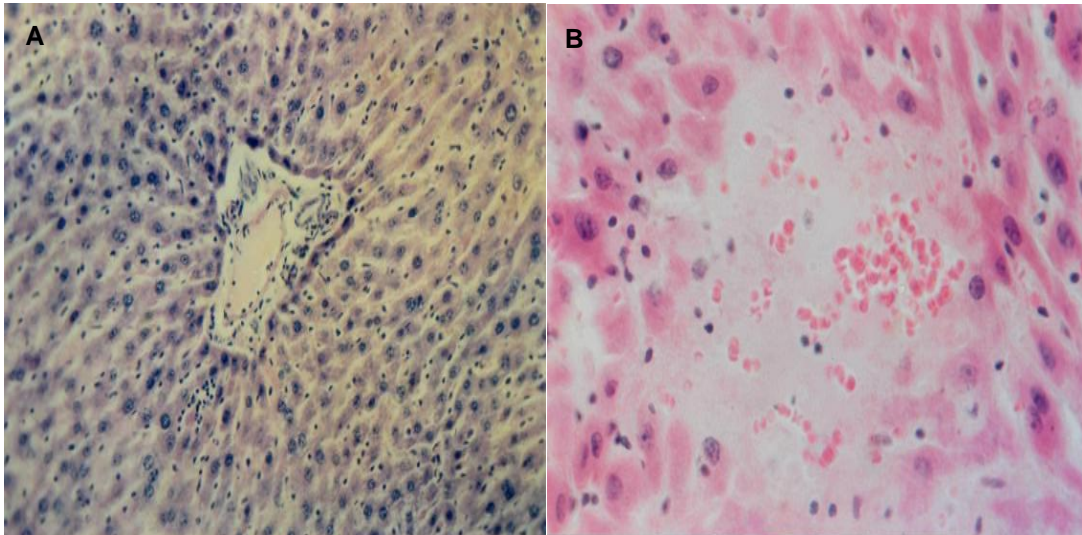
3.1.1.2 Liver

In control animals (Fig.3.A) the liver lobules are separated by underdeveloped connective tissue, in the center of the lobule is the central vein, from which the hepatic plates begin, formed by two rows of hepatocytes that have a cubic or polygonal shape. In the center of the hepatocyte is a rounded nucleus. Intralobular sinusoid capillaries of moderate blood filling. Lymphoid tissue is represented in the form of small foci diffusely located along the course of hemocapillaries.

There are pronounced destructive processes in the liver tissue of rats treated with MMI at a dose of 20 mg/g of b.w. (Fig. 3.B,C). A characteristic feature of the destructive process of the liver is the presence of foci of destruction, as a result, the boundaries between hepatocytes disappear. Violation of the integrity of the liver plates is accompanied by the destruction of sinusoidal hemocapillaries without pronounced signs of hemorrhage. In the blood vessels of the lobules of the liver, stagnation phenomena are observed with the expansion of sinusoidal capillaries, creating conditions for the release of blood plasma into the perivascular zone.

Morphological alterations in the group received MMI at a dose of 10 mg/100 g of b.w. (Fig.3.D) were characterized by the destruction of the hepatic lobules and the hepatocytes.

The nuclei decrease in size, the chromatin is compacted, the boundaries of the liver cells are not clearly defined. Between the destructively altered hepatocytes are the shaped elements of the blood. In sinusoidal hemocapillaries, blood stasis phenomena are observed. Interlobular connective tissue does not undergo morphological transformations.



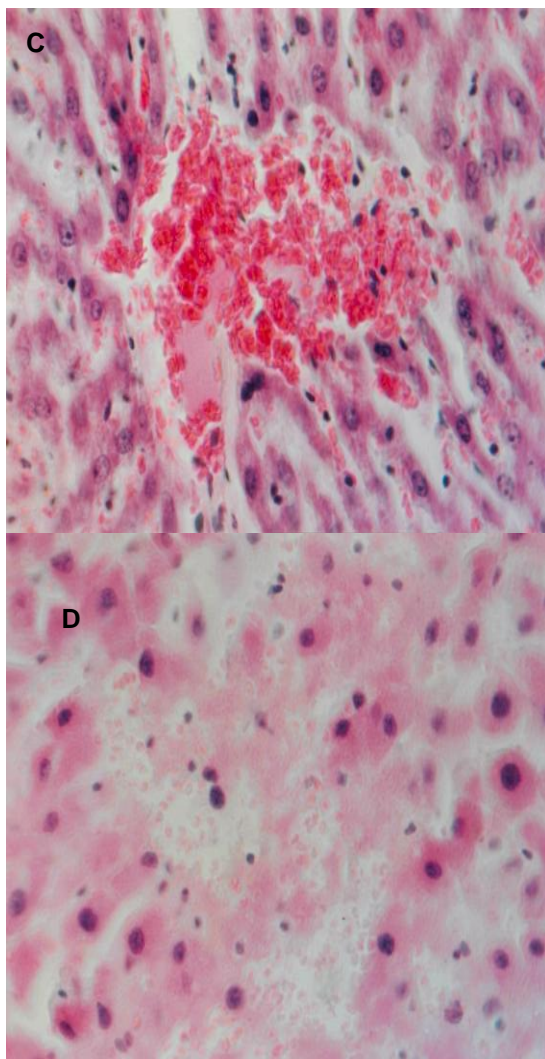


Figure 3.

A. Central vein of the liver lobule of control rats.

B. Destruction of the liver after the use of MMI at a dose of 20 mg / 100 g of b. w. for 2 weeks.

C. Fullness of the central vein of the liver lobule of rats after the use of MMI at a dose of 20 mg / 100 g of body weight.

D. Destruction of the liver when using MMI at a dose of 10 mg / 100 g of b. W

When using MMI at a dose of 2.5 mg / 100 g of b. w. (Fig.4) in all parts of the lobules of the liver, hepatocytes are uniformly stained with a basophilic tint of the cytoplasm, which indicates a high functional activity of cells . In hepatocytes, mitoses and polyploid nuclei are determined. However, in the blood capillaries, stagnant phenomena are detected to a certain extent, whereas in most cases fullness is not determined. In various parts of the liver, lymphoid cells are observed, their number, location features and size vary greatly

MMI at a daily dose of 1 mg / 100 g of body weight for three weeks caused minor changes in tissue structures: moderate phenomena of blood stagnation in the sinusoidal hemocapillaries of individual lobules and small foci of lymphocyte and macrophage clusters

along large blood vessels were determined. At the same time, destructive processes were not detected both in hepatocytes and in the interlobular connective tissue

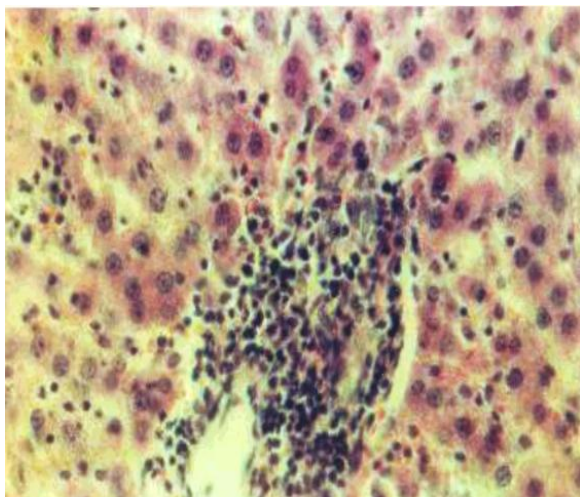


Figure 4. Lymphoid accumulations in the region of the central vein of the liver lobule of rats received at 2.5 mg of MMI / 100 g of b.w.

3.1.1.1 Kidney

In the kidney of the control group of animals, renal corpuscles are distinguished among the tubules of the nephron, represented by a two-layer capsule and a vascular glomerulus (Fig. 5.A). The proximal section of the nephron is formed from cubic-shaped nephrocytes. The narrow part of the nephron is built of flat cells, the distal section is made of cubic cells, and the collecting tubules are made of cylindrical cells. In the course of the tubules, blood capillaries are determined, and at the border of the cortical and cerebral matter - the arc artery. The administration of MMI at doses of 20 and 10 mg / 100 g of b.w. accompanied by changes in the structure of the nephron and blood circulation (Fig. 5. B,C,D). In the cortical substance, non-functioning renal corpuscles formed by intensively colored densely spaced cells are determined.

Thus, high daily doses of MMI (20 and 10 mg / 100 g) contributed to the development of destructive processes in both the renal corpuscles and the tubules of the nephrons. Lower doses of the antithyroid drug – 2,5 and 1.0 mg / 100 g - introduced a less pronounced nephrotoxic effect: histological transformations were found in single renal corpuscles (Fig 5.E,F).

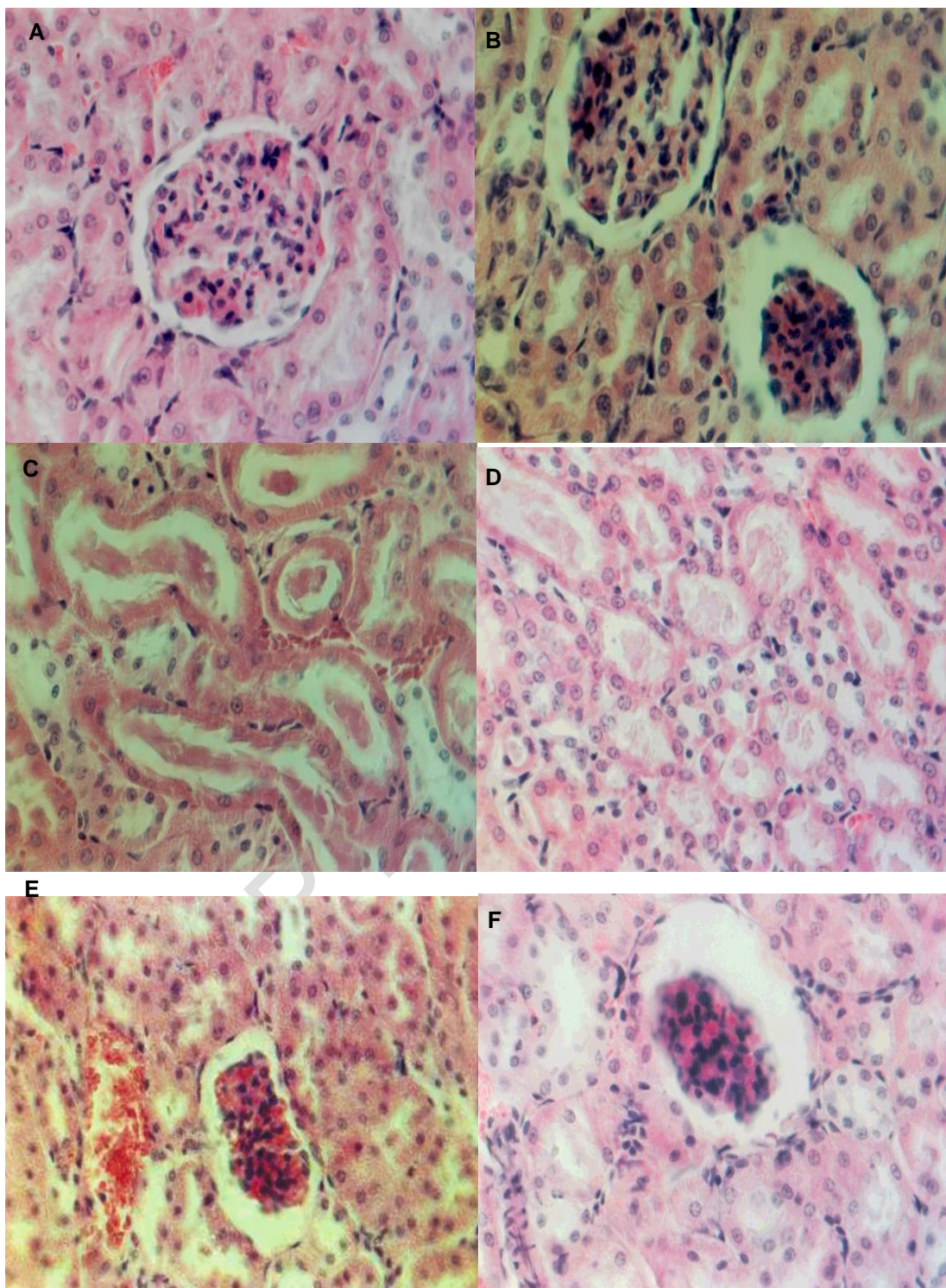


Figure 5.A Renal corpuscle and tubules of the nephron of control rats.

B. Normal and altered renal corpuscle: MMI at a dose of 20 mg / 100 g of b.w.

C. Cylinders in the tubules of the nephron: MMI at a dose of 10 mg / 100 g of b.w.

D. Cylinders in the tubules of the nephron: MMI at a dose of 10 mg / 100 g of b.w.

E. Dystrophic changes in the kidneys: MMI at a dose of 2.5 mg / 100 g of b.w.

F. Destructively altered renal corpuscle: MMI at a dose of 1 mg / 100 g of b.w.

3.2.1.1 Thyroid status

3.2.1.1 Serum concentration of thyroid hormones

Study of the level of circulating thyroid hormones (table 1, fig 6) showed that with all modes of administration of MMI, the concentration of the free T_4 fraction in the blood serum decreases, and almost dose-dependent (up to 36, 26, 67 and 88% of the control for groups 2, 3, 4 and 5, respectively).

The concentration of total T_3 also decreased in the groups receiving 20, 10, and 2.5 mg of MMI per 100 g of b. w. (up to 77, 87 and 76% of the control, respectively), while in the group receiving a low dose of antithyroid drug (1 mg / 100 g of b. w.), the concentration of the most active of iodine-containing hormones T_3 slightly increased (up to 123% of the control level).

The decrease in iodine-containing hormones was accompanied by the logical increase in the concentration of TSH (with the exception of group 3), especially pronounced in the group receiving a daily single dose of 2.5 mg / 100 g of body weight.

Table 1. Serum thyroid hormones profile in MMI administered group

MMI dose	Free T_4 , pM/L		Total T_3 , nM/L		TSH, μ IU/ml	
	control	MMI	control	MMI	control	MMI
20mg/100g of b.w.	16,88 \pm 0,5	6,0 \pm 0,23*	1,88 \pm 0,13	1,44 \pm 0,1*	0,12 \pm 0,01	0,2 \pm 0,02*
10mg/100g of b.w.	10,35 \pm 0,67	2,7 \pm 0,24**	1,88 \pm 0,13	1,64 \pm 0,12*	0,12 \pm 0,01	0,118 \pm 0,01
2,5mg/100g of b.w.	16,2 \pm 0,57	10,8 \pm 2,14**	3,12 \pm 0,57	2,36 \pm 0,19**	0,12 \pm 0,01	1,96 \pm 0,18**
1mg/100g of b.w.	16,88 \pm 0,13	14,0 \pm 0,07**	1,88 \pm 0,13	2,32 \pm 0,07**	0,12 \pm 0,01	0,216 \pm 0,01

Data values Mean \pm σ , * - P < 0.05, ** - P < 0.001 vs control group

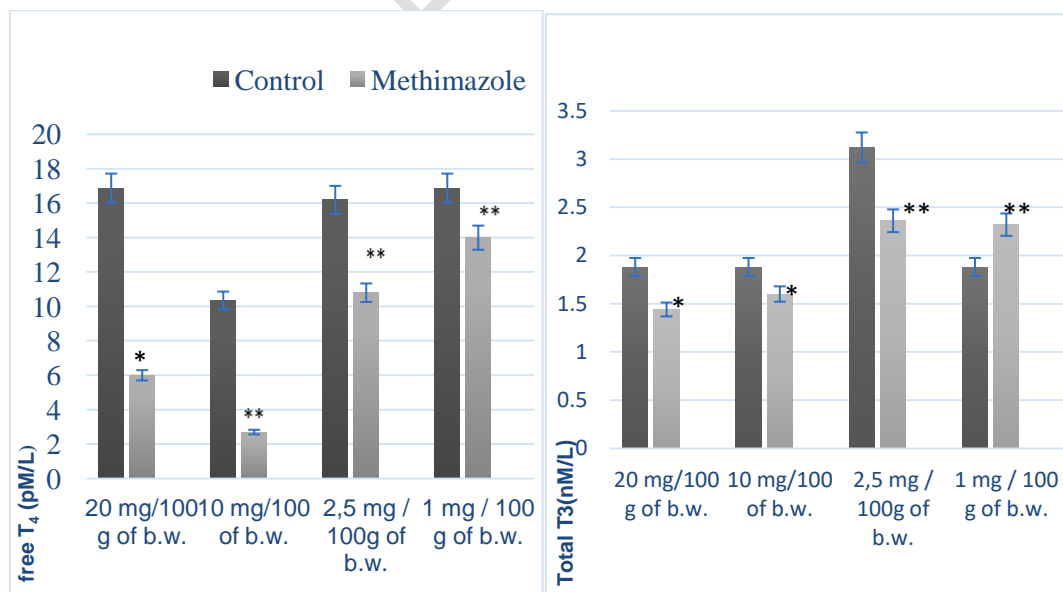


Figure 6. Free T_4 and total T_3 in blood serum in MMI administered groups.

Data values Mean \pm σ , * - P < 0.05, ** - P < 0.001 vs control group

3.2.1.2 Body temperature

Comparison of temperature curves (table2, fig 7) indicates dysregulation of heat production in animals receiving MMI at a dose 20 mg/100 g of b.w. At the same time, instead of the relative hypothermia expected for hypothyroidism, in some phases of the study there were higher values of body temperature compared to the control group. In general, the pattern of the temperature curve, that is, its general structure, is not similar to the structure of the temperature curve of the control group, which indicates profound pathophysiological shifts, including in chemical and physical thermoregulation systems.

Table 2. Body temperature (°C) on the 1st and 2nd day after discontinuation of intragastric administration of MMI at a daily dose of 20 mg / 100 b. w. for 2 weeks

time / group of rats	7.00	11.00	15.00	19.00	23.00	7.00	11.00	15.00	19.00	23.00
Control, n=12	38,4±0,07	36,9±0,11	37,8±0,15	37,1±0,08	38,0±0,09	38,0±0,07	37,0±0,06	36,9±0,15	37,6±0,11	37,2±0,08
MMI 20 mg/100g of b. w.	38,0±0,16**	37,5±0,12**	37,2±0,14**	36,8±0,01*	36,7±0,01**	36,9±0,19**	37,5±0,11*	36,7±0,11**	36,8±0,14**	36,3±0,13**

Data values Mean ± σ, * -P < 0.05, ** -P < 0.001 vs control group

Table 3. Body temperature on the 1st and 2nd day after discontinuation of intragastric administration of MMI at a daily dose of 2,5 mg / 100 b. w. for 3 weeks

time / group of rats	7.00	11.00	15.00	19.00	23.00	7.00	11.00	15.00	19.00
Control, n=12	37,9±0,09	36,9±0,06	37,8±0,08	37,4±0,08	37,2±0,08	37,9±0,13	36,8±0,10	38,1±0,10	38,3±0,05
MMI 2,5 mg/100g of b. w.	35,6±0,10**	35,9±0,08**	37,3±0,08*	37,2±0,05*	36,1±0,07***	36,5±0,11**	35,9±0,11**	37,4±0,09**	37,8±0,07**

Data values Mean ± σ, * -P < 0.05, ** -P < 0.001 vs control group

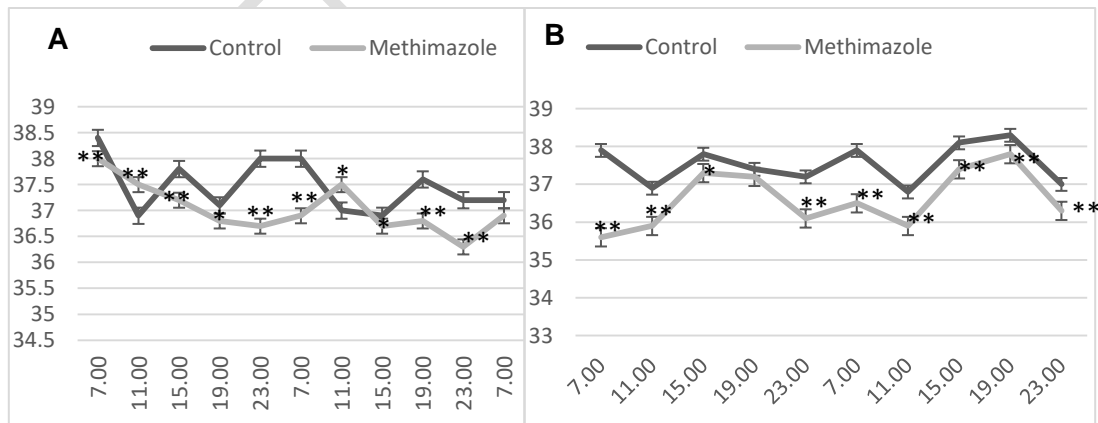


Figure 7. Body temperature on the 1st and 2nd day after discontinuation of intragastric administration of MMI at a daily dose 20 mg /100 g of b.w. (A) and 2,5 mg / 100 b. w. (B)

Data values Mean ± σ, * -P < 0.05, ** -P < 0.001 vs control group

In animals treated with MMI at a daily dose of 2.5 mg / 100 g of b. w. for 3 weeks (table 3, fig 7), the arithmetic mean values of body temperature on the first and second days after discontinuation of treatment with thyrostatic agent were lower relative to control in all phases of the circadian rhythm.

In animals treated with MMI at a daily dose of 1 mg / 100 g of b. w. for 3 weeks, the arithmetic mean values of body temperature on the first and second days after discontinuation of thyrostatic treatment did not differ from the control values in all phases of the circadian rhythm.

3.2 DISCUSSION

Numerous models of hypothyroidism induced by MMI have been proposed, differing in both the methods of administration and the recommended doses. Large variations in the regimens and doses of administration make it difficult to compare the results obtained by different authors, and sometimes raise the question of the mechanisms of occurrence of the identified changes. To what extent are these changes caused by a deficiency of thyroid hormones, and to what extent are they a manifestation of the possible toxic effect of the injected xenobiotic? To determine the optimal dose of methimazole, we conducted a comprehensive morphofunctional study, during which we compared morphological changes in the thyroid gland, liver and kidneys with indicators of thyroid status - the concentration of circulating thyroid hormones and basal temperature mesor in animals when administered different doses of MMI.

Histological examination revealed, that the thyroid gland of animals treated with MMI at a daily dose of 20 mg / 100 g of b. w. for 2 w. (group 2) is subject to pronounced destructive and dystrophic changes, which cannot but affect the hormone-producing function of the thyroid gland. Shifts in the concentration of thyroid hormones in the blood serum of animals of this group consisted in a decrease in the level of total T_3 and, especially, free T_4 , (up to 76.6 and 37.5% of the control level, respectively), against the background of an increase in TSH (up to 166% of the control level), which quite corresponds to the picture of hypothyroidism. However, along with these changes, pronounced destructive processes were found in the liver and kidney tissue. The daily temperature curve of animals after a two-week administration of MMI at a daily dose of 20 mg/100 g of b.w. included uncharacteristic for hypothyroidism episodes of an increase in body temperature above control values. Thus, it cannot be concluded that in this case the experimental model fully corresponded to the type of endemic effect, since this dose of the antithyroid drug causes acute intoxication and profound pathological changes in the tissues of experimental animals.

A two-week daily intake of MMI at a dose of 10 mg / 100 g of b. w. also led to functional depletion of the pituitary-thyroid system, which was manifested by the absence of an intrafollicular colloid in the central and peripheral zones of the thyroid gland and a decrease in serum levels of T_3 and T_4 with a simultaneous increase in the concentration of TSH. Pronounced morphological changes of a destructive-dystrophic nature in the liver and kidney tissue, inadequate to the nature of hypothyroidism, also indicate the development of acute intoxication with this mode of administration of MMI. Similar results were obtained by other authors when studying the histological structure of the liver in rats in a state of MMI-induced hypothyroidism, according to the scheme: 10.0 mg / 100 g of b. w. for 8 weeks [26].

Pathomorphological changes in the thyroid gland of animals receiving MMI at a daily dose of 2.5 mg / 100 g of b. w. for three weeks (group 4) were characterized by a decrease in colloid volume until its complete disappearance in individual follicles. The study of the hormonal profile revealed shifts characteristic of hypothyroidism: a pronounced persistent decrease in the levels of free T_4 and total T_3 with a simultaneous sharp increase in the concentration of TSH. The development of the hypothyroidism in animals of this group was also confirmed by a decrease in the arithmetic mean values of body temperature after a three-week administration of MMI. At the same time, no significant changes of toxic genesis were

detected in the histological structure of the liver in this group of animals. Some lymphoid infiltration was observed. In the kidney tissue, the introduction of a thyrostatic at a dose of 2.5 mg / 100 g of body weight causes changes in the blood circulation system.

In order to minimize the side effects of the thyrostatic agent, rats of the 5th group were administered MMI at a dose of 1.0 mg / 100 g of b. w. for 3 w. Rats relatively easily tolerated a small dose of the drug, minor histological alteration were found in the liver and kidney, as it have reported in the following sources [22,25,26]. In the thyroid gland, when using this dose of MMI, pathomorphological changes characteristic of hypothyroidism develop with a decrease in the amount of colloid in the follicles. Likely, these changes were partially compensated, possibly due to activation of peripheral deiodination, as it might be evidenced by the detected slight increase in more active T_3 in the blood serum. The absence of one of the main symptoms of hypothyroidism - hypothermia also indicates compensation for thyroid shifts in animals of this group. Administration of MMI at a dose of 2.5 mg /100 g of b. w. for 3 weeks is optimal and leads to the development of symptoms characteristic of endemic thyroid dysfunction, and is accompanied by minimal toxic effects on tissues.

4. CONCLUSIONS

Thus, our studies showed that MMI in regimes 20 and 10 mg / 100 g of b. w. daily for 2 weeks induces the development of tissue pathology of toxic genesis, which imposes side effects that are not characteristic of hypothyroidism. MMI at a dose of 1 mg / 100 g for 3 weeks does not induce the persistent hypothyroidism, as evidenced by the absence of one of the characteristic symptoms of thyroid insufficiency - hypothermia. It was found that a dose of 2.5 mg per 100 g of body weight for 3 weeks leads to the development of symptoms characteristic of endemic thyroid dysfunction, and is accompanied by minimal toxic effects on tissues

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985), as well as the order of the Ministry of Healthcare of Russia N 199 dated 01.04.2016 "On Approval of the rules of good laboratory practice" were followed. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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