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PATHOMORPHOLOGICAL SIGNS OF EXPERIMENTAL THYROTOXICOSIS OF THE ADRENAL AND TESTIS GLANDS

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ABSTRACT

The adrenal glands and testis of intact rats and animals that received a single dose of levothyroxine through a probe do not differ significantly in weight. With a 14-day administration of levothyroxine, the morphometric parameters of the adrenal glands and testis of rats are significantly different from those of intact animals. Thus, in the control group, the proliferative index in the cortical layer of the adrenal glands was above 10%, and in the cerebral layer 7-9%. When exposed to levothyroxine sodium in the experimental group, the proliferative index in the cortical layer decreased to 5%, and in the cerebral layer below 1%. Significant changes were also observed in testicular tissue, notably in the experimental group the seminiferous tubes were found to be smaller and smaller to medium in size compared to the control group and the basal membrane thinned. In addition, there is a significant disorganization of the spermatogenic epithelium, and 60% of the ducts are emptied. In small quantities, only spermatogonium and sperm are visible. There are calcinates in the epithelial layer of some twisted tubes, intermediate sex cells were not observed in these tubules.

Key words: adrenal glands, thyrotoxicosis, immunomarkers (Ki-67; BCL-2), testicles, sexual cells, apoptosis, proliferation.

INTRODUCTION

Thyroid gland hormones help regulate lipid and carbohydrate metabolism, normalize and contribute to the development of diabetes. Elevated levels of thyroid hormones (TH) in the blood (hyperthyroidism or hypothyroidism) may be

associated, for example, with diseases of the thyroid gland and Graves, as well as with impaired thyroid function. [9]. In this regard, according to a study conducted, thyroid glance has adrenal dysfunction, an increase in the level of sex hormones, impaired ureteral function and, possibly, infertility [4.2]. Sperm quality depends on several factors, including sperm size, sperm count and sperm morphology. In particular, the semantic signs of hyperthyroidism include hypospermia, oligozoospermia, asthenozoospermia and teratozoospermia, and hypothyroidism also includes teratozoospermia [8].

In thyrotoxic rats, sperm mitochondrial activity is reduced, and the proliferative period of neonatal Sertoli cells is shortened in hyperthyroid conditions [7]. In contrast [6], observed that thyroid hormone deficiency reduces sperm viability and delays sperm passage through the epididymis.

Histological examination of the adrenal glands in rats treated with a mixture of benzene and chromium showed that increased secretory activity of corticotropocytes was not only not accompanied by an adequate response of the adrenal cortex [10].

The glomerular structure of the outer cortex is typical for the adrenal gland. The bundle zone contains typical radially oriented strands of cells, the distribution of sudanophilic lipids in them is almost uniform. The mesh area is relatively thin. Giant cells have been identified in the bundle zone of the cortex. The medulla occupies 1/5 of the area of the sections of the adrenal gland of the evening red and is formed by strands of cells in the form of glomeruli of various shapes [1].

After a single injection of the pesticide, there is a decrease in the synthetic processes of the secretory cycle in the cells of the cortical substance zones, indicating an increase in the phase of hormone release from cortical cells. There is a sharp increase in the morphofunctional activity of the adrenal glands along with the development of reactive and destructive changes in it against the background of a violation of synthetic processes in cells [5,3].

But despite the presence of morphological works dedicated to the study of the structures of the adrenal gland and testicles, in immunogystochemistry of the sphere of pathomorphology (Ki-67; BCL2), has many questions in the extensions are unresolved or require clarification.

The purpose of the work: to study the pathomorphological indicators of the cellular structures of the rat in the adrenal gland and testicles.

Materials and research methods: the experiment was carried out in 30 white male rats of the reproductive period with a 90-day weight of 200-250 G.

The animals were taken from the laboratory vivarium of the Bukhara Medical Institute. They were divided into two groups: rats in the first control group were sent distilled water in the stomach in a size of 0.5 ml through a metallic probe. In the second experimental groups, forced levothyroxine sodium suitability for 14 days at 50mkg/100 gr micdor [11] applied.

To perform morphological analysis, the resulting tissues are fastened with 10% buffered formaldehyde for 24 hours. The connection of regular tissues was carried out in the STP 120 carousel processor, Thermofisher, Germany. Successive sections on slides 3 μm thick were subjected to deparaphinization and dehydration to stain the sections with hematoxylin-eosin. Then they are kept in a solution of Erlix hematoxylin for 2-5 minutes. Sections are washed in distilled water. Color control was carried out under a microscope. The staining is considered satisfactory if the nuclei are intensely red-purple, the nuclei and chromatin fragments are visible within the nucleus, but the cytoplasm is not stained. Parts painted with hematoxylin and washed with water were transferred to distilled water for 3-5 minutes. To stain the cytoplasm of cells, the fragments were placed in an eosine solution for 0.5-2 minutes. The building is considered successful if the cross section is uniformly yellowish-pink, with the Blue cores clearly visible. After staining in eosin solution, the lumps were washed in distilled water, dehydrated with alcohol, purified in xylene and installed in a preservative environment.

In Van-gizone staining, the Flakes were paraffinized in xylene and brought in through alcohols with a reducing force to distilled water. The cuts are stained in Weigert iron hematoxylin for 3-10 minutes, then washed in two pieces of running water for 1-2 minutes, and in picrofoxin for 2-3 minutes, quickly rinsed and dehydrated in distilled water (5-15 C). two parts 96% are ethanol, part of absolute ethanol, identified in two parts of xylene. The Living time of the sections in each section was 1-2 minutes, after which they were wrapped in a preservative. As a result of staining, the cell nuclei acquired a black color, Collagen - Red and other tissue elements (including muscle fibers and red blood cells) - yellow.

Immunogystochemical studies (IHS) were conducted in 2 samples ("control", "experiment"). The 3 μm thick sequential incisions are used using a special Ventana automated system for deparaphinization, dehydration, unmasking, and antigen staining . Benchmark XT, Roche, Switzerland. The study was conducted with immunomarkers (Ki-67; BCL2).

PERSONAL CHECKING RESULTS:

Control group, staining with hematoxylin-eosin

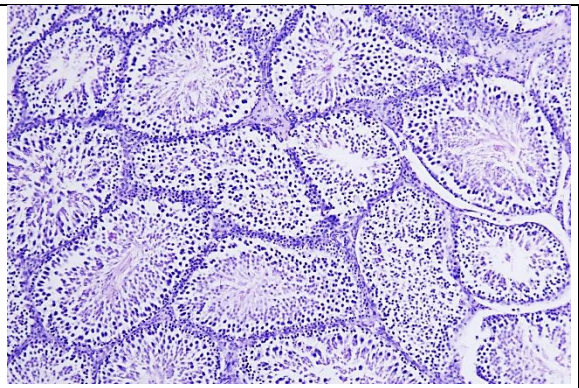
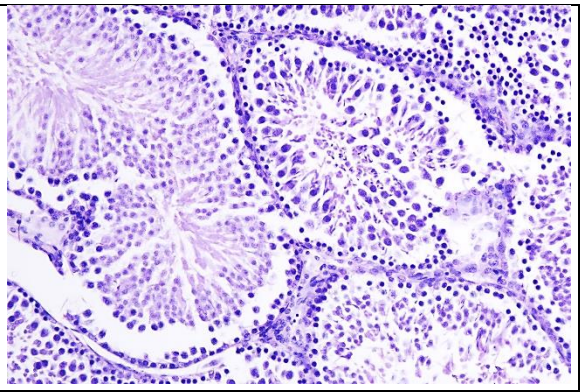
	

Figure 1. It is stained with hematoxylin-eosin. Ob.10 x oc.20	Volume 2. It is stained with hematoxylin-eosin. Ob.10 x oc.40
Seminiferous tubes are usually medium to large in size and have an oval shape. spermatogenesis was intact, with an average sperm count of 25 in 10 visual fields of 50 µm. Figure 1-2.	

Control group, immunohistochemical research

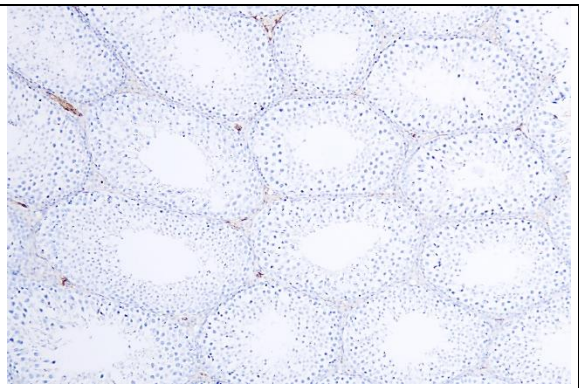
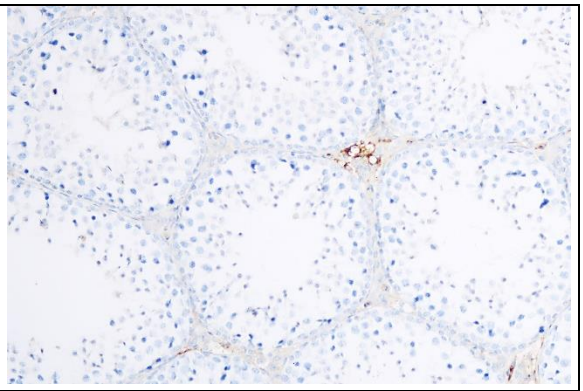
	

Figure 3. IHC study using the BCL2 mark. Zoom x20.	Figure 4. IHC study using the BCL2 mark. Zoom x40.
No pathomorphological expression was observed.	

Cells	Result: (number of cells, segment - 50 µm)
Diameter of convoluted seminiferous |tubule (µm) 150-200	150-200
Spermatogonium	6
Spermatocyte I	5
Spermatocyte II	5
Spermatids	25
Spermatozoa	12
Sertoli cells (nuclei)	1
Myoid cells	1
Leydig cells (150 micron Segment)	6

Experimental group, staining with hematoxylin-eosin

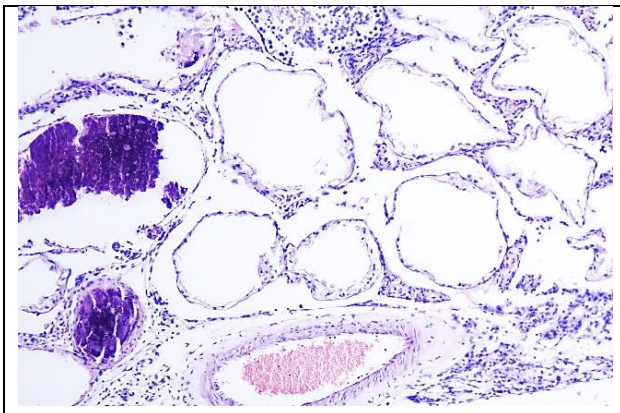
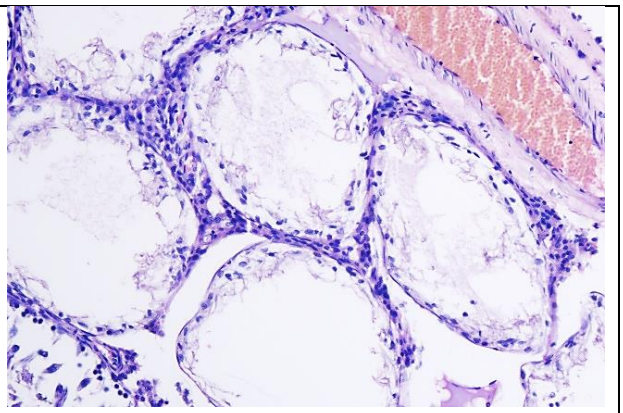
	

Figure 5. It is stained with hematoxylin-eosin. Ob.10 x oc.20	Volume 6. It is stained with hematoxylin-eosin. Ob.10 x oc.40
The seminiferous tubes are small to medium in size, the basal membrane thinned. There is a significant disorganization of the spermatogenic epithelium, and 60% of the ducts are emptied.in small quantities, only spermatogonium and sperm are visible. There are calcinates in the epithelial layer of some twisted tubes, intermediate sex cells are not observed in these tubules (figure 5-6). The average number of sperm in 10 visual fields of 50 µm was 3.	

Experimental group, immunohistochemical research

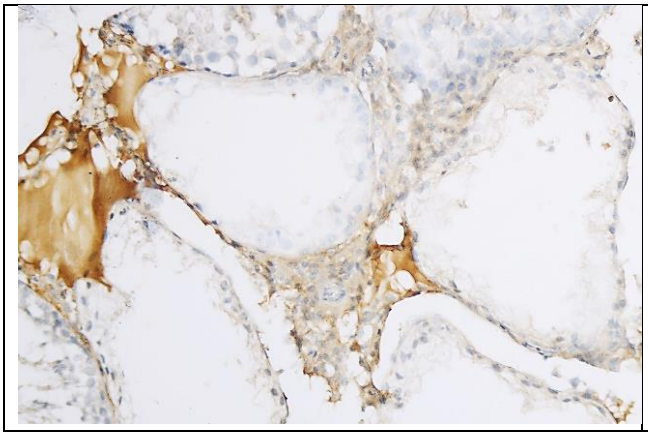
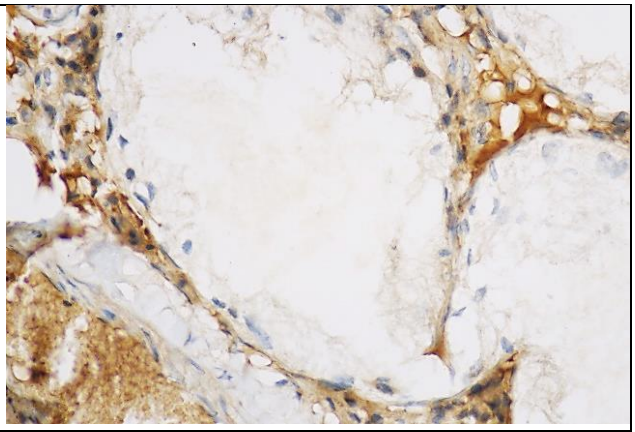
	

Figure 7. IHC study using the BCL2 mark (124). Zoom x20.	Figure 8. IHC study using the BCL2 mark (124). Zoom x40.
"Ki -67 expression.. X 400. Positive cytoplasmic staining was observed in single spermatozoa .	

Cells	Result: (number of cells,

	segment - 50 µm)
Diameter of convoluted seminiferous tubule (µm) 150-200	140-160
Spermatogonium	2
Spermatocyte I	1
Spermatocyte II	1
Spermatids	0
Spermatozoa	1
Sertoli cells (nuclei)	1
Myoid cells	1
Leydig cells (150 micron Segment)	7

Histological architectonics of the adrenal gland structure without features. The slice shows a normal pattern of cortical zonality (clusters of cells with weakly basophilic cytoplasm in the glomerular zone, columns of cells with transparent cytoplasm in the bundle zone and cells with acidophilic cytoplasm in the reticular zone). The glomerular zone consists of dense clusters and short trabecular cells under the adrenal capsule. The nuclei of the glomerular zone are oval; the nuclei of the bundle zone are round. There is a clear boundary between the zona reticularis and the medulla (clusters of cells with basophilic cytoplasm).

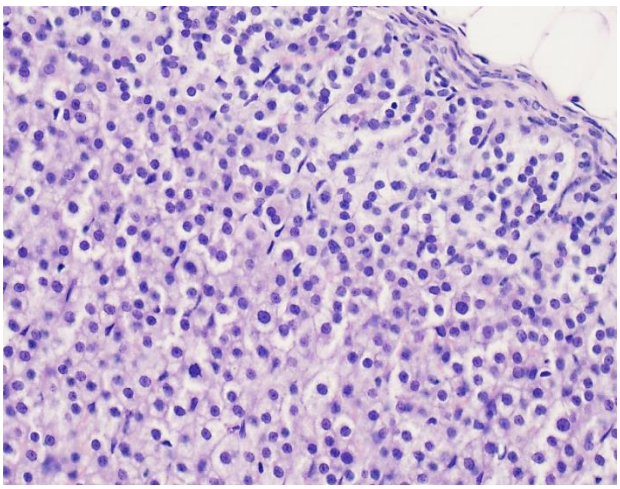
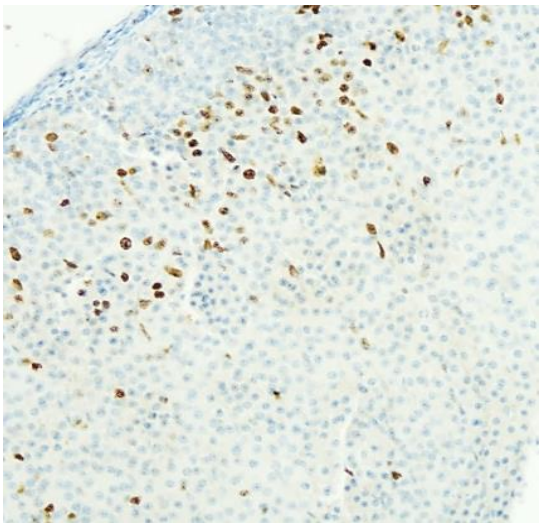


Fig. 9. GE coloring. Zoom x20.

Division rates are rare, proliferative activity is observed near the periphery of the cortex, is <10%, mainly in the bundle zone.



IHC study of Ki-67 markers (30-9). Zoom x20.

The sections show a hypertrophied cortical layer of the adrenal gland with a predominance of its weak blood supply. Subtotal marked delipidization of the cytoplasm of adrenocorticocytes. In the glomerular zone, cytoplasm wrinkling, densification and fragmentation of nuclei and swelling of membranes are observed.

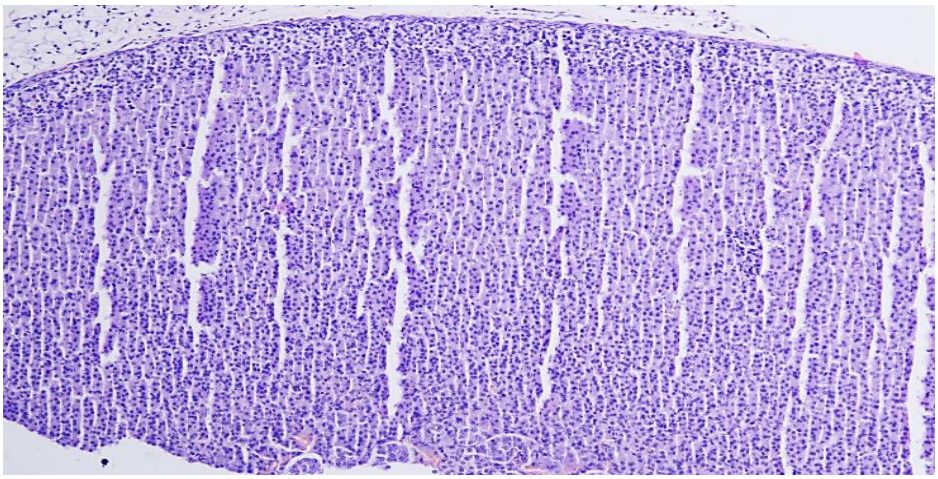


Fig. 10. GE coloring. Zoom x10.

The proliferative index in the cortical layer is <5%, mainly in the bundle zone.

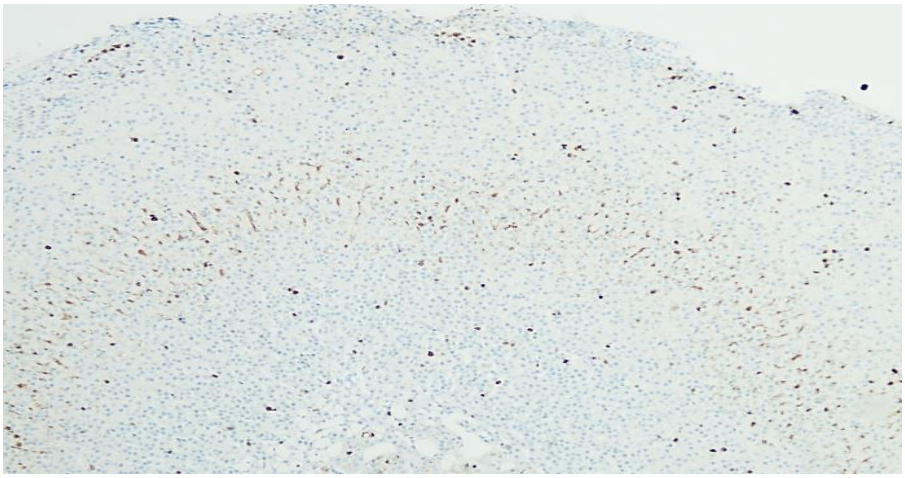


Fig. 11. IHC study of Ki-67 markers (30-9). Zoom x20.

CONCLUSION:

Thus, in the control group, the proliferative index in the cortical layer of the adrenal glands was above 10%, and in the cerebral layer 7-9%.

When exposed to levothyroxine sodium in the experimental group, the proliferative index in the cortical layer decreased to 5%, and in the cerebral layer below 1%.

Significant changes were also observed in testicular tissue, notably in the experimental group the seminiferous tubes were found to be smaller and smaller to medium in size compared to the control group and the basal membrane thinned. In addition, there is a significant disorganization of the spermatogenic epithelium, and 60% of the ducts are emptied. In small quantities, only spermatogonium and sperm are visible. There are calcinates in the epithelial layer of some twisted tubes, intermediate sex cells were not observed in these tubules.

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