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MORPHOLOGICAL CHANGES OF CORTICOSTEROCYTES AND CHROMAFFIN CELLS AFTER 1 MONTH OF CHEMICAL CASTRATION UNDER CONDITIONS OF CORRECTION WITH QUERCETIN

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It is well known that endocrine pathology is a leading disease in the morbidity structure of the Ukrainian population. It is important to study the metabolic effects of hormones of the structural components of the hypothalamic-pituitary-gonadal axis on other peripheral endocrine glands and body organs. Modern research on testosterone proves its effect on the biochemical processes of nervous tissue, liver, organs of the male and female reproductive systems, and others. The paper describes the main morphological changes of corticosterocytes in the adrenal cortex area and endocrinocytes of the adrenal medulla of white rats during the correction of central deprivation of testosterone synthesis during 1 month of the experiment. It was found that the inhibition of testosterone synthesis caused reactive changes in both - corticosterocytes and chromaffin cells, manifested at the microscopic level by the variability of morphometric parameters.

Keywords: chemical castration, corticosterocytes, chromaffin cells, adrenal gland, correction.

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МОРФОЛОГІЧНІ ЗМІНИ КОРТИКОСТЕРОЦИТІВ ТА ХРОМАФІНОЦИТІВ НА 1-Й МІСЯЦЬ ХІМІЧНОЇ КАСТРАЦІЇ ЗА УМОВ КОРЕКЦІЇ КВЕРЦЕТИНОМ

Відомо, що ендокринна патологія посідає провідне місце у структурі захворюваності населення України. Актуальним є дослідження метаболічного впливу гормонів структурних компонентів гіпоталамо-гіпофізарно-гонадної вісі на інші периферичні ендокринні залози та органи тіла. Сучасні дослідження, що вивчають тестостерон, доводять його вплив на біохімічні процеси нервової тканини, печінки, органи чоловічої і жіночої статевих систем та інші. У роботі описані основні морфологічні зміни кортикостероцитів зон кіркової речовини та ендокриноцитів мозкової речовини надниркових залоз білих шурів під час корекції центральної депривації синтезу тестостерону на 1 місяць експерименту. Встановлено, що пригнічення синтезу тестостерону викликало реактивні зміни кортикостероцитів кіркової речовини та ендокриноцитів мозкової речовини, що на мікроскопічному рівні виявлялось варіабельністю морфометричних показників.

Ключові слова: хімічна кастрація, кортикостероцити, хромафіноцити, надниркова залоза, корекція.

The study is a fragment of the research project "Experimental and morphological study of the effect of cryopreserved preparations of cord blood and embryofetoplacental complex (EFPC), diphereline, ethanol and 1 % methacrylic acid ether on the morphological and functional state of a number of internal organs", state registration No. 0119U102925.

The metabolic relationship between corticosteroids and androgens synthesised by the adrenal cortex, the feedback of the hypothalamus-pituitary-gonadal axis and their modulation caused by the biochemical environment, gender, age and health status remain relevant topics of research [5, 7]. It has been proven that age-related hypogonadism is one of the key causes of biologically planned early ageing of men and the rapid onset of functional disorders in women [5, 10].

The use of testosterone and its regulators, such as gonadotropin-releasing hormone, is of significant clinical importance and is used by scientists and physicians to develop and improve methods of treating diseases of the endocrine glands, reproductive system and other organs and tissues of the human body [6, 9, 14].

With the development of new technologies, the molecular and pathophysiological mechanisms of oxidative stress and the impact of thyroid hormone imbalance, renin-angiotensin system and other endocrine pathology on the structure of the adrenal glands are being studied in depth [1, 3, 11]. Alternative ways of exposure to dihydrotestosterone, which is formed from testosterone in the skin and genitals and is the cause of hyperandrogenic disorders, such as congenital adrenal hyperplasia and polycystic ovary syndrome, are being widely studied. Deficiency of steroid 21-hydroxylase, oxidoreductase P450 and other enzymes have been shown to be a genetic component of the aetiology of these diseases [2, 8]. Thus, studying morphological changes in the adrenal glands against the background of oxidative stress caused by testosterone deficiency is promising.

The study of the impact of central testosterone deprivation on not only zona reticularis (ZR) but also zona glomerulosa (ZG) and zona fasciculata (ZF) of the adrenal cortex and adrenal medulla cells (AMC) remains relevant since corticosterocytes synthesise both cortisol and substances that are precursors of cortisol and testosterone. The adrenal medulla (AM) produces hormones that are responsible for the implementation of stress reactions [13].

Modelling of the pathological condition during the study was carried out by administering triptorelin acetate, a synthetic analogue of gonadotropin-releasing hormone used in the treatment of tumour diseases of the male and female reproductive systems. The main pharmacological effect during long-term use is the development of chemical castration due to a decrease in the production of luteinizing (LH) and follicle-stimulating hormones [4, 12, 15].

Correction of changes that occurred in the tissues of the studied organs as a result of the experiment was carried out by applying the active substance quercetin. It has been proven that it has an antioxidant effect and can be used for the purpose of pathogenic therapy and prevention of changes in the body caused by a decrease in testosterone levels [11, 12, 15].

Thus, our study is aimed at identifying the relationship between central testosterone deprivation and correlated reactive changes in corticosterocytes adrenal cortex zones (AC) and endocrinocytes of the adrenal medulla (AM). Particular attention should be paid to the corrective antioxidant effect of quercetin.

The purpose of the study was to investigate the morphological changes of corticosterocytes in the adrenal cortex zones and the adrenal medulla endocrine cells of white rats during the correction of central testosterone deprivation after the first month of the experiment.

Materials and methods. Adrenal glands of 25 male white rats were studied and divided into three groups: group 1 consisted of 5 intact rats, group 2 consisted of 10 rats that were subcutaneously injected with triptorelin acetate at a dose of 0.3 mg of active substance per kg body weight, and group 3 consisted of 10 rats that were injected with triptorelin acetate and also received quercetin at a dose of 100 mg per kg body weight three times a week for a month. The control group was injected with a 0.9 % sodium chloride solution. The collection of the investigated material was carried out at the end of the first month of the experiment [4, 12, 13].

During the animal experiments, the national regulations "General ethical principles of experiments on animals" (Ukraine, 2001), which were coordinated with the requirements of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1985), the Law of Ukraine No. 3447-IV dated 21.02.2006 "On the Protection of Animals from Cruelty", and the Helsinki Declaration on the humane treatment of animals were followed.

The histological method was used to study the morphological features of corticosterocytes in the zona reticularis (CZR), zona glomerulosa (CZG) and zona fasciculata (CZF) of the adrenal cortex and chromaffin cells (CC) of the adrenal medulla.

Measurements and calculations were made of the average size and nucleus volume (NV) of corticosterocytes in all adrenal cortex zones (AC), the average size and nucleus volume of CC, and the density of AC cords was determined by counting the number of ZG, ZF and ZR corticosterocytes, while the density of medulla cords was determined by counting the number of CC. The average space of the investigated areas was 36422.511±2082.09 μm². After preliminary photographing of the sections at high magnification, measurements were made using the computer program AimImageExaminer.

Statistical method – to determine the objectivity and reliability of the results obtained, the morphometric analysis was carried out in accordance with generally accepted statistical methods using Microsoft Office Excel 2007.

Results of the study and their discussion. On histological preparations of intact rat adrenal glands, the gland's structure does not differ from normal. The adrenal gland is surrounded by a connective tissue capsule and consists of cortical and medullary substances. Zona glomerulosa is formed by small corticosterocytes with basophilic cytoplasm and rounded nuclei, with an average size (AS) of 13.78±0.341 μm and a mean nucleus volume (NV) of 336.23±1.05 μm^3. Dark and light spongy corticosterocytes of the zona fasciculata form straight cords. Light cells have vacuolated cytoplasm, while dark cells have basophilic cytoplasm. The AS of CZF is 19.93±0.631 μm, and the NV is 427.37±1.07 μm^3.

The cells of the zona reticularis have smaller sizes compared to spongy corticosterocytes, with an average size of 13.63±0.472 μm and a NV of 174.57±1.27 μm^3. The cells form cords that go in different directions and anastomose with each other. The medullary substance has an average size of CC 18.42±0.497 μm and a nucleus volume of CC 420.82±1.52 μm^3.

During morphometric study AC and AM the small and large diameters of the nuclei of endocrine cells and the number of cells in the studied areas with an average area of 36422.511±2082.09 μm² were determined. By counting the number of cells in areas of a given size, we determined the density of the cortical and medullary zones, i.e., the preference of the cellular component over the stromal component or vice versa. The small and large diameters were measured to calculate the nucleus volume of the cells. The nucleus volume of the zona glomerulosa, zona fasciculata, zona reticularis, and chromaffin cells nucleus volume (NVCC) were calculated using the formula for the volume of an ellipsoid: $V = \pi/6 \times D \cdot d^2$, where

V is the volume of the nucleus, d is the length of the minor axis of the nucleus, and D is the length of the major axis of the nucleus.

Changes in the values of average size of the zona glomerulosa, zona fasciculata, zona reticularis corticosterocytes and the nucleus volume of the ZG, ZR, ZF are reflected in Table 1.

Table 1

Change in ASCZG, ASCZF and ASCZR, and mean values of NVCZG, NVCZF, NVCZR of the adrenal gland against the background of central testosterone deprivation

Investigated morphological indices	Groups and study periods		
	Group 1 (Intact rats)	Group 2 (Injection of triptorelin acetate, sample collection at the end of 1 month)	Group 3 (Injection of triptorelin acetate + quercetin, sample collection at the end of 1 month)
ASCZG (μm)	13.78±0.341	19.82±1.28*	25.12±3.03*×
NVCZG (μm^3)	336.23±1.05	529.87±1.09*	723.36±0.503*×
ASCZF (μm)	19.93±0.631	21.86±1.60*	28.43±4.29*×
NVCZF (μm^3)	427.37±1.07	554.94±0.197*	620.74±0.654*×
ASCZR (μm)	13.63±0.472	17.29±0.432*	22.72±3.12*×
NVCZR (μm^3)	174.57±1.27	136.75±1.14*	583.97±0.268*×

Notes: * p<0.05 compared to the intact group; × p<0.05 compared to the previous observation term.

In group 2, a significant and reliable increase in the average size of corticosterocytes and an increase and decrease in the nucleus volume were observed in the adrenal cortex compared to the intact group after 1 month of the experiment. The average size of ZG corticosterocytes increased by 44 %, and their nucleus volume increased by 58 %. The average size of ZF corticosterocytes increased by 9%, while their nucleus volume increased by 28 %. The average size of ZR corticosterocytes increased by 27 %, while their nucleus volume decreased by 22 %.

Thus, a significant and reliable increase in the average size of ZG and ZR corticosterocytes, accumulation of lipid inclusions in their cytoplasm, which gave them the appearance of ZF corticosterocytes, the absence of signs of cellular atypia, and the appearance of corticosterocytes with large lipid vacuoles pushing the nucleus to the periphery ("ring-shaped cells") indicated the phenomenon of fatty degeneration and proved significant reactive changes in the zona glomerulosa and zona reticularis of the adrenal cortex after the administration of triptorelin acetate. [13] (fig. 1).

In the third group, after 1 month of correction of central testosterone deprivation by quercetin administration, a significant and reliable increase in average size of corticosterocytes and nucleus volume was observed compared to intact and second groups. Average size of CZG increased by 27 %, and nucleus volume increased by 36 % compared to the second group. Average size of CZF increased by 31%, and nucleus volume increased by 12 % compared to the second group. It should be noted that in the third experimental group, changes in CZF were more pronounced than in the second group. Average size of CZR increased by 35 %, and nucleus volume increased four times compared to the second group. The restoration of cytoplasmic basophilia and the absence of vacuolization in CZG and CZF indicate the activation of protein-synthesizing activity of cells (fig. 2).

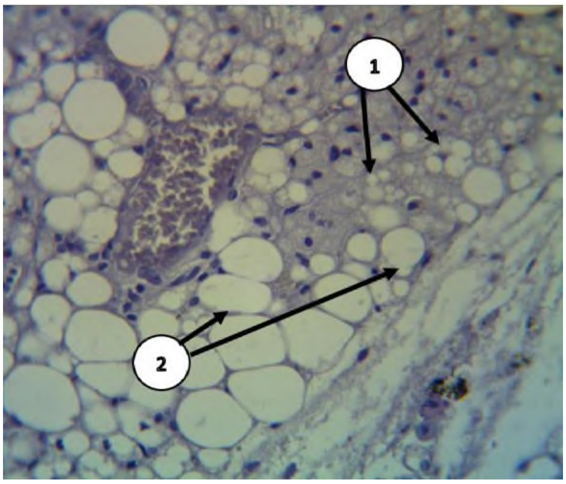

Fig. 1. Reactive changes of CZG after 1 month of central testosterone deprivation. 1 – lipid inclusions in the cytoplasm of corticosterocytes, 2 – "ring-shaped cells". Staining: hematoxylin-eosin. Magnification: ×400.

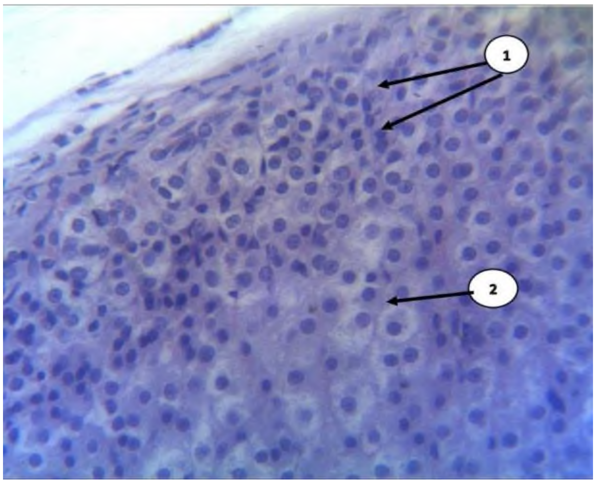

Fig. 2. Reactive changes in the CZG and CZF after 1 month of central testosterone deprivation correction, 1 – zona glomerulosa, 2 – zona fasciculata. Staining: hematoxylin-eosin. Magnification: ×400.

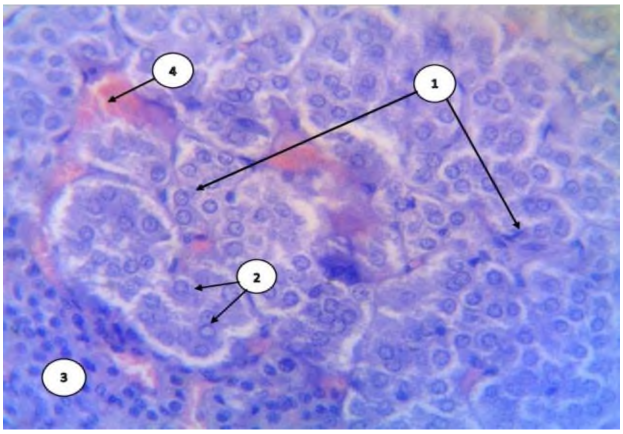

Fig. 3. Reactive changes of CC after 1 month of correction of central testosterone deprivation, 1 – adrenal medulla, 2 – chromaffin cells of the adrenal medulla, 3 – zona reticularis, 4 – blood capillaries. Staining: hematoxylin-eosin. Magnification: ×400.

During the AM study in group 2, a significant increase in the average size of CC – 23.02±3.64 µm and a non-significant increase in the nucleus volume of CC – 461.71±3.09 µm^3 were detected compared to the intact group. In group 3, the average size of CC was 32.94±5.12 µm and the nucleus volume of CC was 978.43±1.03 µm^3.

Thus, the average size of CC increased by 43 % and the nucleus volume of CC increased by 112 % in group 3 compared to group 2. These changes indicate a significant reactivity of the CC during correction (fig. 3).

The average density values of the AC and AM zones according to the study terms are presented in Table 2.

Table 2

The indicators of the average density values of the zona glomerulosa (DZG), zona fasciculata (DZF), zona reticularis (DZR) and adrenal medulla (DAM)

The investigated morphological indices	Groups and study terms		
	1st group (intact rats)	2nd group (administration of triptorelin acetate, material collection at the end of 1 month)	3rd group (administration of triptorelin acetate + quercetin, material collection at the end of 1 month)
DZG	57.7±7.19	47.35±4.08*	66.42±7.30*×
DZF	33.9±3.55	45.33±2.81*	69.93±6.23*×
DZR	47.2±4.49	48.76±2.63	77.53±5.59*×
DAM	14.7±2.89	33.8±5.93*	44.46±4.37*×

Notes: * $p<0.05$ compared to the intact group; × $p<0.05$ compared to the previous observation period.

If we compare the density indicators of the adrenal cortex zones 1 month after the administration of triptorelin acetate (experimental group 2) with the indicators of the intact group, it can be seen that there was a decrease in the density of the zona glomerulosa (DZG) by 18 %, and an increase in the density of the zona fasciculata (DZF) by 34 %. The density of the zona reticularis (DZR) increased by only 3 %, which is not significant. When comparing the indicators of experimental group 3 (correction of central testosterone blockade) with the indicators of group 2, an increase in the density of the zona glomerulosa (DGZ) by 40 %, zona fasciculata (DZF) by 54 %, and zona reticularis (DZR) by 59 % was observed. The density of the adrenal medulla (DAM) increased by 16 %. Thus, the diagram identifies a significant increase in the density of all adrenal cortex zones and adrenal medulla specifically after 1 month of correction of central testosterone deprivation (group 3) (fig. 4).

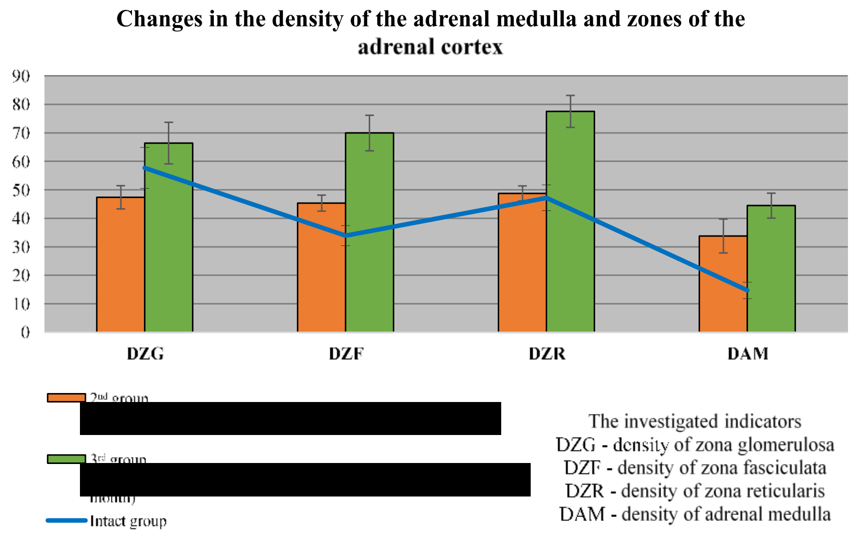

Fig. 4. Comparative characteristics of changes in the density of the adrenal cortex zones and the adrenal medulla.

By conducting morphological and morphometric studies of the indicators of the average size and nucleus volume of the CZG, CZF, CZR, CC of the AM, and the density of the adrenal cortex zones and the adrenal medulla, visualising the results in the form of tables and diagrams, the main features of reactive changes in the adrenal glands against the background of central testosterone deprivation and its correction within 1 month can be identified. It is possible to demonstrate the interrelation of the processes that occur in the male reproductive system not only with the reactivity of the adrenal cortex but also with morphometric changes in the adrenal medulla.

Therefore, the unreliable changes in the density of ZG, ZR, and AM and the significant change in cell size prove the reactivity of the changes in these zones due to the stromal and cellular components during testosterone deprivation after 1 month of the experiment (group 2). Morphological changes in the CZG and CZR were reflected in the accumulation of lipid inclusions in the cytoplasm and the appearance of "ring-shaped cells". The changes were interpreted by us as the primary reaction of the CZG and CZR to the introduction of triptorelin acetate.

The reliable increase in density, average size and nucleus volume of corticosterocytes in the AC and CC of the AM in the 3rd group (administration of quercetin against testosterone deprivation), the restoration of basophilic cytoplasm and the absence of vacuolization in the CZG and CZR indicate the activation of protein-synthetic activity of cells and positive reactive changes in the AC and AM due to the cellular component under the influence of quercetin.

By analyzing scientific papers related to the topic of our study, we found that the data in this article describe pathophysiological reactions and biochemical processes of oxidative stress [11, 15] in the adrenal glands on the background of thyroid hormone imbalance [3], type 2 diabetes [1], and the administration of other chemical substances [12]. Our study of changes in the adrenal glands caused by oxidative stress induced by testosterone deficiency aims to investigate the morphological features and morphometric indicators of corticosterocytes in the AC and CC of the AM.

Conclusions

1. Short-term effect of intramuscular administration of triptorelin acetate leads to reactive changes in the structure of cortisterocytes of zona glomerulosa and zona reticularis of the adrenal cortex and the adrenal medulla of rats.

2. Significant positive effect of quercetin was confirmed by a reliable increase in the average size, nucleus volume, number of cortisterocytes and chromaffin cells of adrenal medulla during the correction of chemical castration after 1 month of the experiment.

3. The direct correlation between changes in the cells of the zona glomerulosa and zona reticularis of the adrenal cortex and adrenal medulla of white rats glands during chemical castration and its correction, which we have discovered, indicates the involvement of not only hypothalamic-pituitary-dependent endocrine organs but also the organs of the sympathetic-adrenal system in the process.

Prospects of further research. A histochemical study of the adrenal cortex and adrenal medulla zones is planned to biochemically prove the effect of testosterone on metabolic processes of the adrenal cortex and medulla.

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PATHOMORPHOLOGICAL CHANGES IN SALIVARY GLANDS OF RATS UNDER THE CONDITION OF DIABETIC NEUROPATHY AND CORRECTION

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The purpose of the study was to evaluate the effect of diabetic polyneuropathy on the development of pathological changes in the salivary glands of rats, as well as the feasibility of their correction by the drug Cocarnit. Immature white nonlinear rats of both sexes were simulated with streptozocin-induced diabetic neuropathy, which was confirmed by the Randall-Selitto tensoalgometric method. For correction, Cocarnit 1 mg/kg was administered intramuscularly for 9 days. The subjects were the submandibular salivary glands of rats. It was found that streptozocin-induced diabetic neuropathy leads to changes in the parenchymatous components in the lobules of submandibular salivary glands of rats, which was manifested by dystrophic and destructive changes in the epithelial cells of the terminal sections and ducts and blood perfusion in the hemomicrocirculatory vessels. Administration of Cocarnit reduces manifestations of periacinaric edema and improves trophism of epitheliocytes. Restoration of blood flow contributes to the normalization of secretory function of the glandular apparatus of submandibular salivary glands in rats.

Key words: diabetic peripheral neuropathy, streptozocin, salivary glands, Cocarnit, nicotinamide, cobalamin, thiamine diphosphate.

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ПАТОМОРФОЛОГІЧНІ ЗМІНИ У СЛИННИХ ЗАЛОЗАХ ЩУРІВ ЗА УМОВ ДІАБЕТИЧНОЇ НЕЙРОПАТІЇ ТА КОРЕКЦІЇ

Метою роботи було вивчити вплив діабетичної полінейропатії на розвиток патологічних змін в слинних залозах щурів, а також доцільність їхньої корекції препаратом Кокарніт. Статевозрілим білим нелінійним щурам моделювали стрептозоцин-індуковану діабетичну нейропатію, розвиток якої підтверджували тензоалгометричним методом Randall-Selitto. Для корекції виявлених змін щурам вводили Кокарніт. Об'єктами дослідження були піднижньощелепні слинні залози щурів. Виявлено, що стрептозоцин-індукована діабетична нейропатія призводить до змін паренхіматозних компонентів у часточках піднижньощелепних слинних залоз щурів, що проявлялось дистрофічними і деструктивними змінами епітеліоцитів кінцевих відділів і проток та перфузії крові у судинах гемомікроциркуляторного русла. Введення Кокарніту зменшує прояви периацинарного набряку і поліпшує трофіку епітеліоцитів. Відновлення кровотоку сприяє нормалізації секреторної функції залозистого апарату піднижньощелепних слинних залоз щурів.

Ключові слова: діабетична периферична полінейропатія, стрептозоцин, слинні залози, Кокарніт, нікотинамід, кобаламін, тіаміндифосфат.

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Diabetes mellitus is one of the top three diseases most often leading to disability and death. According to recent estimates by the International Diabetes Federation, 537 million adults live with diabetes, about 90 % of whom have type 2 diabetes. [6] Currently, there are 1 million 134 thousand registered diabetics in Ukraine. Diabetic peripheral polyneuropathy is the most common complication of diabetes, with high morbidity and reduced quality of life [4, 5, 7] impaired performance in a large number of patients and its development in 50 % of patients with both types of diabetes.

The pathogenesis of diabetic neuropathy is multifactorial and involves multiple interrelated mechanisms. Experimental evidence suggests that hyperglycemia, glucotoxicity and insulin deficiency act