

# Features of Morpho-Functional States of the Adrenal Glands in Rats with Nephrolithiasis Against the Background of Androgen Deficiency

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**Abstract** The features of morpho-functional states of the adrenal glands of rats with nephrolithiasis against the background of androgen deficiency. Objective: To study the dynamics of structural and functional changes in the adrenal glands of rats with nephrolithiasis against the background of androgen deficiency. The experiments were carried out on 30 white outbred male rats of a mixed population with an initial weight of 180-200 g, kept in a laboratory diet in vivarium conditions. For morphological examination, pieces of kidneys of the control and experimental groups of experimental animals were selected, from which pieces of 1.0 cm in size were cut out and placed in a fixing solution of 15% formalin for 24 hours, then the pieces were fixed in wire alcohols and chloroform. As a result of the morphological study of the adrenal gland of the intact group, no significant pathological changes were revealed; only weakly expressed vacuolization of focal cells was noted.

**Keywords** Experiment, Adrenal gland, Rats, Nephrolithiasis, Cortisol

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## 1. Introduction

Urolithiasis is a polyetiological disease that occupies an important place in the practice of a urologist. It is widespread in the world and can occur in all age groups. The main approach to the treatment of urolithiasis is to find the cause of the disease and competent correction of disorders, which will help to avoid recurrence of stone formation. In the prevention and treatment of this disease, an interdisciplinary approach should be used approach [2]. Urolithiasis (nephrolithiasis) is a common renal disease characterized by the deposition of stones in the renal cavity and urinary tract and can occur at any age. The incidence of nephrolithiasis currently accounts for 1-2% of the total disease structure [4]. In the structure of the terminal stage of chronic kidney disease, nephrolithiasis accounts for up to 3% [1,3]. The incidence of androgen deficiency and metabolic syndrome increases with age, which is explained mainly by endogenous mechanisms that lead to impaired testosterone production and increased insulin resistance. The influence of environmental factors on the development of these diseases is known, but they receive much

less attention than they deserve. However, we should not forget that the body of a modern person is constantly affected not only by endogenous, but also paraecological pathogenic factors, which, in addition to those associated with the individual's activities - stress, bad habits, eating disorders - also include anthropogenic environmental factors - chemical compounds, medications, etc. [6,7].

## 2. Purpose of the Study

To study the dynamics of structural and functional changes in the adrenal glands of rats with nephrolithiasis against the background of androgen deficiency.

## 3. Materials and Methods

The experiments were conducted on 30 white mongrel male rats of a mixed population with an initial weight of 180-200 g, kept on a laboratory diet in vivarium conditions. After receipt from the nursery, the experimental rats underwent a 14-day quarantine period in the quarantine block of the vivarium in order to exclude animals with somatic and / or infectious pathology from the experiment. The study was carried out in accordance with the ethical

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principles of handling animals, observed in accordance with the "European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes. CETS No. 123". All animals were divided into 3 groups: 1) intact group (10 rats), 2) control group (10 rats without castrated males), 3) experimental group (10 castrated rats, which were castrated before the 5th day of the experiment). The animals of the control and experimental groups, against the background of the general viviparous diet, received 1% aqueous solution of ethylene glycol instead of drinking water for 21 days with free access, which induced the development of experimental oxalate nephrolithiasis. The condition of the animals was observed daily for 21 days.

Rats were given standard species-appropriate food and drinking water. On the 21st, 27th and 37th days, blood was collected from the animals (3-4 ml) from the cardiac region under ether anesthesia (inhalation) for laboratory research.

For morphological study, kidney pieces of the control and experimental groups of experimental animals were selected, from which pieces measuring 1.0 cm were cut out and placed in a fixing solution of 15% formalin for 24 hours, then the pieces were fixed in conduction alcohols and chloroform. Then they were loaded into a thermostat at 37 degrees for 1-2 hours, and 57 degrees for impregnation for 1 hour, after hardening, paraffin blocks were cut out. Serial sections were prepared from the finished blocks. Glasses were smeared with protein and pierced with an alcohol lamp, the cut materials were fixed on the glass and stained with hematoxylin and eosin. The finished preparations were viewed under a BIO BLUE binocular microscope with an adapter and a Euromex Microscopen BV camera.

*Enzyme immunoassays* were performed on serum obtained after centrifugation of blood samples at 3000 rpm for 8 minutes. The serum was stored at  $-20^{\circ}\text{C}$  until analysis for enzyme immunoassay parameters such as Luteinizing Hormone (LH), Cortisol (Cor) and Testosterone (Tes). For the analysis of enzyme immunoassay parameters, a semi-automatic Elisa microplate reader "Scitek EMLR-112" and enzyme immunoassay kits were used.

The results were processed using the ANOVA variation statistics at a significance level of  $p=0.05$ , using the GraphPad Prism version 8.0.0 program for Windows, GraphPad Software, San Diego, California, USA, [www.graphpad.com](http://www.graphpad.com) [5].

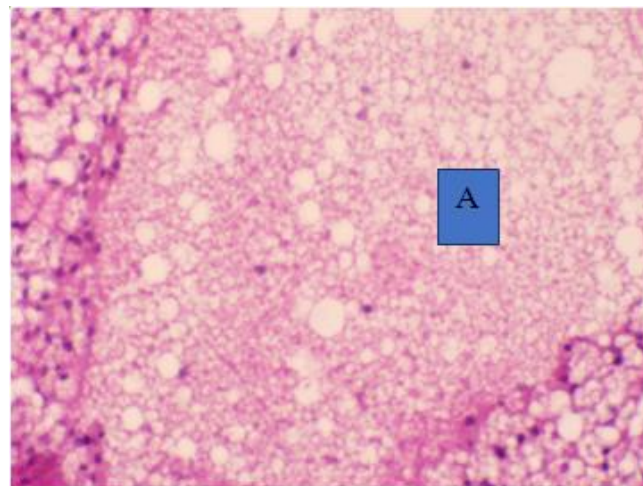
## 4. Results and Discussion

For morphological study, pieces of adrenal glands were selected from intact, control and experimental groups of experimental animals, from which pieces measuring 1.0 cm were cut out and placed in a fixing solution of 15% formalin for 24 hours, then the pieces were fixed in conductive alcohols and chloroform. Then they were loaded into a thermostat at 37 degrees for 1-2 hours, and 57 degrees for impregnation for 1 hour, after hardening, paraffin blocks were cut out. Serial sections were prepared from the finished blocks. Glasses were smeared with protein and pierced with

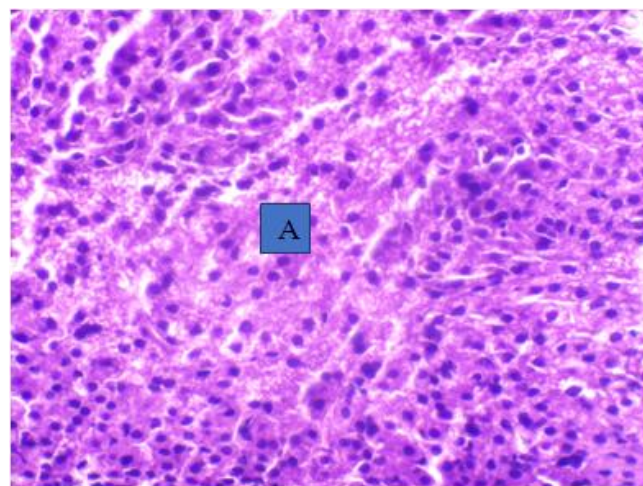
an alcohol lamp, the cut materials were fixed on the glass and stained with hematoxylin and eosin. The finished preparations were viewed under a BIO BLUE binocular microscope with an adapter and a Euromex Microscopen BV camera.

For an experimental study of the androgenic effect on the development of nephrolithiasis, we selected outbred mice, which were divided into 3 groups by the terms of the 22nd day. The first group is an intact group, the 2nd group is a control group (castrated rats) and the 3rd experimental group (males with nephrolithiasis).

As a result of the morphological study of the adrenal gland of the intact group, no significant pathological changes were revealed; only weakly expressed vacuolization of focal cells was noted (Fig. 1).



**Figure 1.** Adrenal gland with vacuolization of cells - A, amongchromaffinocytes increase in the number of cells with light cytoplasm-B. Intact, day 22. Hematoxylin and eosin staining. Magnification 100



**Figure 2.** Under high magnification of the objective lens in the adrenal medulla there is mucous edema of the stroma and macrophage-mononuclear infiltration - A. Day 22, nephrolithiasis. Hematoxylin and eosin staining. Magnification 400

The results of the study of the second group of animals with nephrolithiasis in males were characterized by signs of chronic inflammatory cell infiltration of the adrenal glands (Fig. 2).

In the third group, castrated rats showed more pronounced inflammatory processes with lymphohistiocytic infiltration, the lumen was dilated, and necrotic changes in the adrenal gland were observed (Fig. 3).

Morphological characteristics of the adrenal glands of experimental animals with nephrolithiasis under androgenic influence. (37 day)

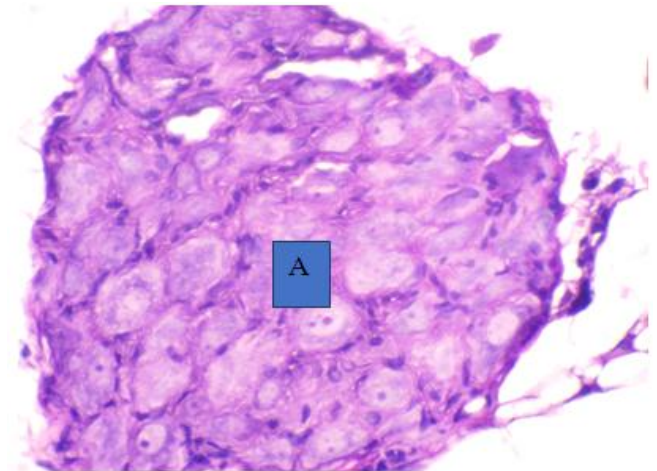
During histological examination of the adrenal glands of the second group of animals with nephrolithiasis in males, massive hemorrhages and atrophic changes in the cortical layer were found in the adrenal glands (Fig. 4).

In the experimental group, hyperplastic changes in the cells of the adrenal cortex were observed in castrated rats (Fig. 5).

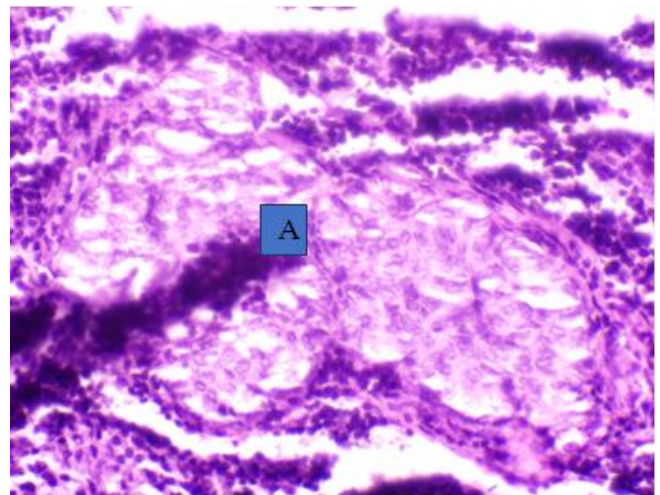
Results immune enzyme Blood parameters after 21 days are presented in Table 1. The average cortisol level in the intact group was 255.71 ng/mL. Cortisol levels were significantly higher in the control group — 812.86 ng/mL. The highest cortisol value was observed in castrated rats — 934.29 ng/mL. This significant increase in cortisol may indicate a strong stress response of the body, especially during castration and in conditions of nephrolithiasis. The average testosterone level in the control group was 5.01 nmol/L. Differences were observed in the groups with nephrolithiasis: in males the level was lower (4.66 nmol/L), in females it was significantly reduced (0.73 nmol/L). In the group with a high emotional background, testosterone was higher compared to the control group (6.04 nmol/L), and in the group with a low emotional background, its level also increased (6.48 nmol/L). In castrated animals, the testosterone level was the lowest (0.35 nmol/L). These data may indicate the effect of nephrolithiasis, emotional state and castration on testosterone levels. Increased testosterone levels in the groups with high and low emotional background may be the result of compensatory processes, while a significant decrease in females and castrated animals confirms the effect of hormonal changes on the regulation of androgen status.

Results immune enzyme Blood parameters after 27 days are presented in Table 1. The average cortisol level in the intact group was 298.57 ng/mL. Cortisol levels were significantly higher in the control group — 898.57 ng/mL. In the experimental group, cortisol levels were also elevated — 577.14 ng/mL. Elevated cortisol levels in all groups with nephrolithiasis indicate a stress response of the body. The mean testosterone level in the intact group was 4.11 nmol/L. In the groups with nephrolithiasis, 4.29 nmol/L was observed. In castrated rats, the testosterone level was the lowest (0.41 nmol/L).

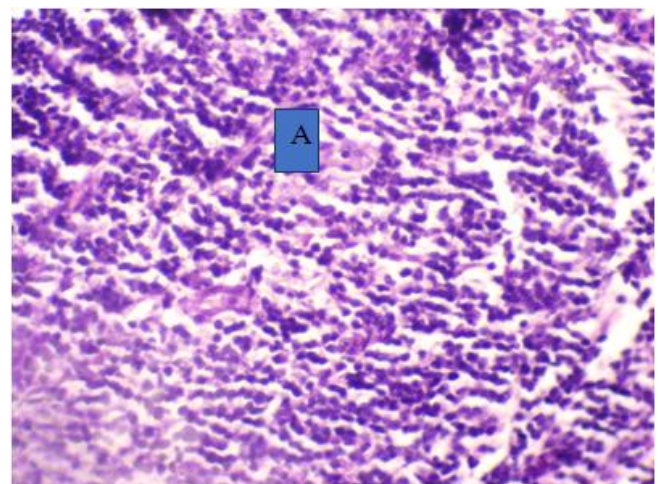
Results immune enzyme Blood parameters after 37 days are presented in Table 1. The average cortisol level in the intact group was 241.43 ng/mL. Cortisol levels in the control group were 734.29 ng/mL. In the experimental group, the cortisol level was also elevated — 498.57 ng/mL. The average testosterone level in the intact group was 4.11 nmol/L. In the control group, testosterone was slightly lower (4.18 nmol/L). In the experimental group, the testosterone level was the lowest (0.43 nmol/L).



**Figure 3.** Dystrophy of parenchyma cells, necrotic changes in the adrenal gland. Day 22. Castrated rat. Hematoxylin and eosin staining. Magnification 400



**Figure 4.** Under high magnification of the objective lens, adrenocorticocytes in small quantities, in the adrenal medulla, mucous edema of the stroma - A. 37 days, nephrolithiasis. Hematoxylin and eosin staining. Magnification 400



**Figure 5.** Adrenal adrenocorticocytes are closely located, their nuclei are darkly stained and rounded -A of the adrenal gland. Day 37. Castrated rat. Hematoxylin and eosin staining. Magnification 400

**Table 1.** The results of the study of enzyme immunoassays of blood plasma in the dynamics of the experiment (M±SD; p=0,05; n=8)

Experiment timing	Cortisol (COR), ng/mL			Testosterone (TES), nmol/L		
	Intact group	Control group	Experimental group	Intact group	Control group	Experimental group
Day 21	255.71 ± 30.30	812.86 ± 151.52	934.29 ± 20.20	5.01 ± 1.19	4.66 ± 0.53	0.35 ± 0.19
Day 27	298.57 ± 30.30	898.57 ± 50.51	577.14 ± 40.41	4.11 ± 0.94	4.29 ± 0.27	0.41 ± 0.29
Day 37	241.43 ± 30.30	734.29 ± 60.61	498.57 ± 70.71	4.11 ± 0.94	4.29 ± 0.27	0.41 ± 0.29

## 5. Conclusions

1. In experimental nephrolithiasis against the background of androgen deficiency, morphological changes in the adrenal glands worsen with the duration of the pathological process as dystrophy of parenchymal cells,
2. In experimental nephrolithiasis, cortisol concentrations in experimental groups (castrated rats) increase slightly compared to control groups. This is probably due to a decline in the compensatory capacity of the adrenal gland against the background of androgen deficiency.

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