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Morphological and Functional Changes in Seminal Glands of Albino Rats Exposed to Lead Acetate

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Abstract: Using histological, morphometric and statistical research techniques the impact of lead acetate on the seminal glands of male albino rats was explored during postnatal ontogeny. Investigations were carried out using a digital microscope Axio Imager.M2 with the image analysis software AxioVision SE64 Rel. 4.8.3 and ZEN 2011. Statistical processing of digital data was performed using the FStat and Excel program codes. Testing statistical hypothesis was carried out by Student's t-test. The obtained data suggest that the mode of spermatids formation is the most vulnerable link in the process of gametogenesis. A decrease in both the spermatogenesis and the relaxation indices (spermatogenesis intensity) was noted, indicating decreased functional activity of the seminal glands.

Key words: Seminal glands (testis) · Convoluted seminiferous tubules · Seminiferous epithelium · Interstitial tissue · Lead acetate.

INTRODUCTION

Chemical pollution of environment has reached unprecedented scale in recent years. There are about 1000 reprotoxicants, i.e. pollutants that have toxic effects on the reproductive system [1]. This also applies to the salts of certain heavy metals, including lead, having both direct and mediated cytotoxic action [2, 3, 4]. In a number of works the conclusion is made that lead is a reproductive toxin [5, 6, 7] though the experimental data on the effect of lead acetate on the seminal glands is extremely small and the available data is controversial [8, 9].

The aim of the present study was to investigate the morphological and functional changes in seminal glands of albino male rats exposed to lead acetate.

MATERIALS AND METHODS

The pubescent outbread albino male rats weighing 200-250 g were used as a biological test object. Altogether 50 animals were used.

In line with the research objectives, the animals were divided into two groups. The control group consisted of 25 males kept in the vivarium under the common mode. Experimental group consisted of 25 males treated orally by lead acetate Pb $(CH_3COO)_2 \times 3H_2O$ during 7 days at a dose of 45 mg/kg/day.

The animals were killed by decapitation under ether anesthesia with chloroform (1:1) in compliance with the principles of humanity as set out in the directives of the European Community (86/609/EES) and the Declaration of Helsinki and in accordance with the rules of carrying out the works using experimental animals.

Seminal glands of male rats were used as a trial material for study. For histological examination, tissue samples were preserved in 10% solution of neutral formalin. Preserved samples after rinsing in running water were dehydrated by placing in alcohols of increasing concentration and embedded into paraffin according to the conventional methodology. Histological cross-sections of seminal glands were prepared 10-15 microns thick, stained with haematoxylin-eosin and examined by a digital microscope Axio Imager.M2 with the image analysis software AxioVision SE64 Rel. 4.8.3 and ZEN 2011.

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The morphological features of the seminal glands structure was studied by survey microscopy with subsequant determination of the following morphometric parameters:

- The thickness of the seminal gland tunica albouginea;
- The number of convoluted seminiferous tubules in the same visual field, the cross-section of the convoluted seminiferous tubule and its lumen, as well as the area of seminiferous epithelium and its thickness;
- The number of interstitial sites between the convoluted seminiferous tubules in the same visual field as well as their area;
- The number of myoid cells in the wall of the convoluted seminiferous tubule and the area of myoid cells and their nuclei;
- The number of Sertoli cells in the spermatogenetic epithelia of the convoluted seminiferous tubule, basal width and length of the apical parts of the Sertoli cells, as well as the area of the cells and their nuclei;
- The number of spermatogenic cells (spermatogonia, spermatocytes and spermatids) in the seminiferous epithelium of the convoluted seminiferous tubule, the area of the spermatogenic cells and their nuclei, the length and the thickness of the flagellum of late spermatids;
- The number of spermatozoons in the lumen of the convoluted seminiferous tubule, the area of the head and the nucleus, cervix width and tail length;
- The number of Leydig cells in the interstitial site; and the area of Leydig cells and their nuclei.

Morphometric measurements were performed with a zooming of 10×10, 40×10 and 100×10. Resolution of the resulting images was 1300×1030 pixels.

Spermatogenesis index was calculated by the formula: $Is=\Sigma a/N$, where a – is the number of layers selected in each tubule (first layer is spermatogonia, the second layer is spermatocytes, the third layer is spermatids and the fourth layer is spermatozoons); N – is the number of counted tubules [10]. The relaxation index (spermatogenesis intensity) – is the ratio between the sum of all counted germinal cells and the sum of Sertoli cells.

Statistical processing of digital data was performed using the FStat and Excel program codes. Testing of statistical hypothesis was carried out by Student's t-test. When testing statistical hypotheses, the accepted significance points were $p \leq 0.05$. Mathematical treatment of morphometric studies was performed using the correlation analysis method.

The Main Part: At external inspection, the seminal glands of rats are pinkish- white, with soft-elastic consistency, having elliptical shape.

After conducted studies it was revealed that in the control group, the tunica albouginea consisting of dense fibrous connective tissue, covers seminal glands from outside in form of strips with uniform thickness. Convoluted seminiferous tubules of round or elliptical shapes are firmly against each other. Outline of their lumen is clearly visible. Interstitial sites are uniformly arranged, predominantly in a triangular shape.

After 7-day oral exposure to lead acetate it was revealed that tunica albouginea surrounding the seminal glands has a nonhomogenious thickness. Convoluted seminiferous tubules are arranged freely, not fitting tightly to each other. Loose connective tissue layer 12-17 microns thick is situated between them. They are of irregular polyhedral shape. Convoluted seminiferous tubules of ellipsoidal shape occur just occasionally. The proper tunic of convoluted seminiferous tubules is characterized by separation of fibers and disorganization of the basal membranes.

The boundary between the seminiferous epithelium and the lumen of the tubule is not clearly visible and has a fuzzy outlines. Interstitial areas between the convoluted seminiferous tubules are situated unevenly. They are of triangular or polygonal shape (Fig. 1).

According to morphometric studies, in the experimental group of animals as compared to the control group, the thickness of the testis tunica albuginea, the number of convoluted seminiferous tubules and the interstitial sites in the same visual field were reduced by 42.24%, 25.14% and 10.34%, respectively (Table 1).

As a result of histological studies it was revealed that in the control group the myoid cells are spaced evenly over the entire boundary of the convoluted seminiferous tubule. They have scaly, crescent or elongated shape.

After 7-day exposure to lead acetate, the shape of myoid cells changes as compared to control shape, becoming semicircular or oval. The cells are unevenly arranged over the boundary of the convoluted seminiferous tubule, lying either in groups or solitary.

When studying the Sertoli cells it was revealed that in the control cells their bases are lying on the basal membrane between the spermatogonia. The apical portion of the cell is faced to the lumen of the seminiferous tubule and has a triangular or pyramidal shape.

Table 1: Macroscopic indicators of the seminal glands of male albino rats

Indicators	Control	Experiment
The thickness of the testis tunica albuginea, μ	35.23±3.42	20.35±4.73*
The number of convoluted seminiferous tubules in the same visual field	34.68±0.94	25.96±0.69*
The number of interstitial sites between the convoluted seminiferous tubules in the same visual field	42.56±2.26	38.16±1.77*

Note: * – P ≤ 0.05 as compared with the control animals.

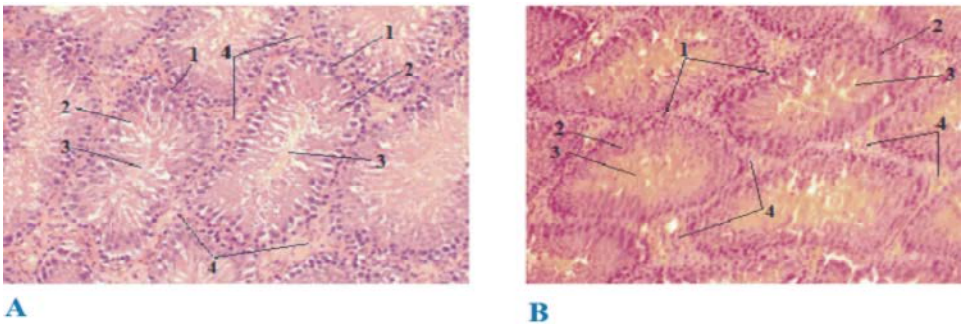


Fig. 1: A cross section of seminal glands. Stained with haematoxylin-eosin; Zooming 10×10: A - control, B - experiment, 1 - convoluted seminiferous tubule, 2 - seminiferous epithelium, 3 - tubule lumen, 4 - interstitial tissue

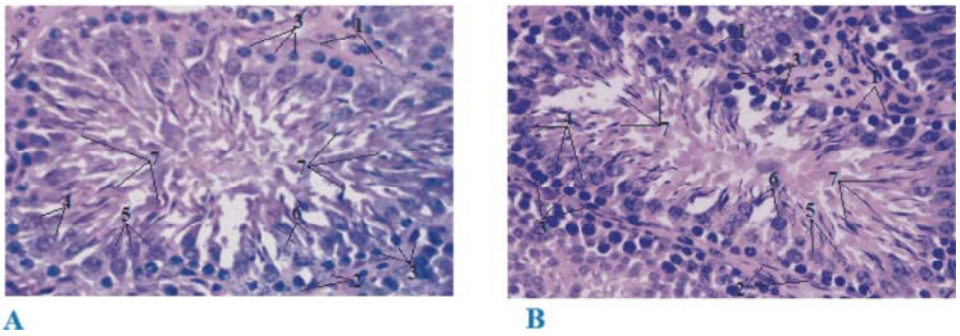


Fig. 2: Convoluted seminiferous tubule. Stained with haematoxylin-eosin; Zooming 10×10: A - control, B - experiment, 1 - myoid cells, 2 - Sertoli cells, 3 - spermatogonia, 4 - spermatocytes, 5 - early spermatids, 6 - late spermatids, 7 - spermatozoons

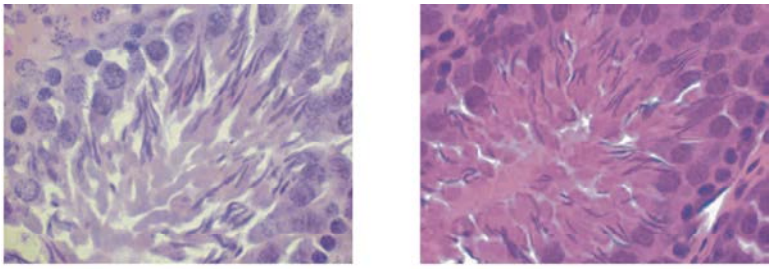
After 7-day exposure to lead acetate, the shape of Sertoli cells is changed as compared with the control cells. The basal part of the cell is significantly reduced in size and has a more rounded shape. The apical part of the cell has more elongated shape.

When examining the spermatogenous cells in control group, distribution of spermatogonia over the entire boundary of the tubule was found to be even. Spermatocytes are located in invaginations of Sertoli cells, are round or oval in shape and somewhat removed from the basal membrane. Early spermatids with rounded shape with a spherical nucleus are located in the middle layers of the seminiferous epithelium. The late spermatids lie in a layer adjacent to the tubule lumen and have an elongated shape. Some late spermatids have flagellum.

After 7-day exposure to lead acetate, the reduction in the size of spermatogonia was noted. Spermatocytes take oval or more rarely spherical shape. Early and late spermatids practically do not differ against each other. They are predominantly oval. Their nuclei are displaced largely from the proximal end (the end facing the wall of the tubule) to the center of the cell (Fig. 2).

When staining the slices of semen glands with hematoxylin-eosin in the control group, the presence of spermatozoons in the lumen of the convoluted seminiferous tubules was revealed. They were arranged in groups in an amount of 6-8 over the entire boundary of the lumen. Head of the spermatozoons has the shape of a hook.

After 7-day exposure to lead acetate, as compared with the control group, it was indicated that the spermatozoons are characterized by disordered arrangement in the tubule lumen. The shape of the spermatozoons head changes, becoming rounded with the decrease in its size compared with the control sample. Tissue samples show ruptures of spermatozoon tails and



A B

Fig. 3: Spermatozoons in the lumen of convoluted seminiferous tubule. Zooming 100×10: A - control, B - experiment

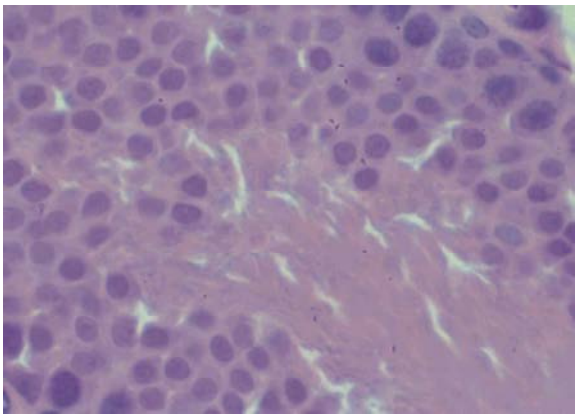


Fig. 4: Convoluted seminiferous tubule lumen (experiment). The lack of spermatozoons is clearly visible. Stained with haematoxylin-eosin; Zooming100×10.

agglutination of the spermatozoons (Fig. 3). Convoluted seminiferous tubules were revealed with the lumens lacking the spermatozoons (Fig. 4).

The morphometric study has shown the following differences between the control and experimental groups of animals.

- The increase in cross-sectional area of convoluted seminiferous tubules and their lumen by 13.72% and 58.01%, respectively.
- Reduction in the seminiferous epithelium area and its thickness by 13.76 % and 21.19 %, respectively.
- Reduction in the amount of myoid cells in the wall of convoluted seminiferous tubule by 35.80%, at that the area of myoid cells and their nuclei increases by 44.26% and 22.45%, respectively.
- Reduction in the area and the width of the basal part of the Sertoli cells by 19.81% and 7.39%, respectively; increase in the height of their apical part by 10.54%. Also, a decrease in Sertoli cells area by 36.35% compared with the control case was noted. The number of Sertoli cells in the seminiferous epithelium of convoluted seminiferous tubule decreased by 22.48%.
- Reduction in the number of spermatogonia in seminiferous epithelium of the convoluted seminiferous tubule, decrease of their area and the area of their nuclei by 6.31%, 29.77% and 33.33%, respectively.
- Reduction in the number of spermatocytes, their area and the area of their nuclei by 8.43%, 19.83% and 12.24%, respectively.
- Reduction in the number of spermatids in the seminiferous epithelium of the convoluted seminiferous tubule, their area and the area of their nuclei by 17.36%, 12.27% and 28.67%, respectively. At the same time the flagellum length of the late spermatids was increased by 20.06%, while reducing simultaneously in its thickness by 12.30%.
- Reduction in the number of spermatozoons in the lumen of the convoluted seminiferous tubule and in the area of the spermatozoon head and its nucleus, as well as the width of cervix by 26.70%, 12.41%, 25.97% and 10.11%, respectively. At the same time the length of spermatozoon tail was increased by 8.05%.

A spermatogenesis index is decreased by 10.24 %, while the relaxation index (spermatogenesis intensity) reduces by 4.46% indicating a decrease in functional activity of the semen glands (Table 2).

According to morphological investigations, in the interstitial tissue of semen glands of the animals from control group there are a few glandulocytes lying in groups of 5-7 cells, predominantly around the vessels. Occasionally there are also solitary cells. They have round, oval, or polygonal shape. Leydig cell nuclei are large and spherical.

After 7-day exposure to lead acetate, Leydig cells in the interstitial site are mostly solitary, but occasionally there are small groups of 2-3 cells (Fig. 5).

Table 2: Morphometric characteristics of the convoluted seminiferous tubules of albino rats.

Indicators	Control	Experiment
The cross sectional area of convoluted seminiferous tubule, μ^2	45469.74±1746.76	52701.15±2703.18**
Tubule lumen area, μ^2	8878.17±832,41	21146.15±1091.75**
Seminiferous epithelium area, μ^2	36591.57±1243.36	31554.72±2526.31**
Seminiferous epithelium thickness, μ	36.62±2.34	28.86±1.77*
The number of myoid cells in the wall of the convoluted seminiferous tubule	19.44±1.42	12.48±1.49**
Myoid cell area, μ^2	10.63±2.55	19.07±4.49**
The area of myoid cell nucleus, μ^2	1.14±0.30	1.47±0.29*
The number of Sertoli cells in the seminiferous epithelium of convoluted seminiferous tubule	23.84±3.16	18.48±2.52**
Sertoli cell area, μ^2	189.73±18.59	152.15±13.96**
The width of the basal part of the Sertoli cell, μ	13.39±1.04	12.40±1.38*
The height of the apical part of the Sertoli cell, μ	15.78±4.14	17.64±2.96*
The area of Sertoli cell nucleus, μ^2	15.82±0.73	10.07±1.72**
The number of spermatogonia in seminiferous epithelium of the convoluted seminiferous tubule	52.44±1.46	49.44±1.30**
Spermatogonium area, μ^2	27.58±2.07	19.37±2.68**
The area of spermatogonium nucleus, μ^2	5.55±1.52	3.70±0.74**
The number of spermatocytes in the seminiferous epithelium of the convoluted seminiferous tubule	40.80±1.97	37.36±1.71**
Spermatocyte area, μ^2	41.19±5.86	33.02±2.07**
The area of spermatocyte nucleus, μ^2	3.35±0.43	2.94±0.34**
The number of spermatids in seminiferous epithelium of the convoluted seminiferous tubule	34.80±1.52	28.76±1.31**
Spermatid area, μ^2	32.69±4.36	28.68±4.26**
The area of spermatid nucleus, μ^2	2.93±0.52	2.09±0.43**
Flagellum length of late spermatids, μ	10.08±2.15	12.61±3.02*
Flagellum thickness of late spermatids, μ	3.17±0.75	2.78±0.56*
The number of spermatozoons in the lumen of the convoluted seminiferous tubule	304.52±13.14	223.20±31.02**
The area of the spermatozoon head, μ^2	17.48±2.12	15.31±0.82**
The width of spermatozoon cervix, μ	2.97±0.23	2.67±1.46*
The length of the spermatozoon tail, μ	20.11±0.96	21.87±1.05*
The area of the spermatozoon nucleus, μ^2	1.81±0.56	1.34±0.81**
Spermatogenesis index	3.32±0.15	2.98±0.12**
Relaxation index	18.14±1.72	17.33±1.02**

Note: * – $P \leq 0.05$ comparing with control animals; ** – $P \leq 0.001$ comparing with control animals.

Table 3: Morphometric indicators of the interstitial tissue of seminal glands of albino rats.

Indicators	Control	Experiment
The area of interstitial tissue, μ^2	1226.14±103.75	1592.66±138.96**
The number of Leydig cells in the interstitial site	9.20±1.20	6.20±1.80**
Leydig cell area, μ^2	40.44±1.30	15.72±2.07**
The area of the Leydig cell nucleus, μ^2	10.82±1.06	3.28±1.33**

Note: ** – $P \leq 0{,}001$ comparing with control animals.

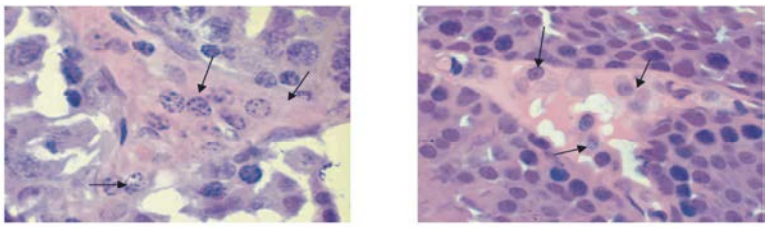


Fig. 5: Interstitial tissue of the seminal glands. The arrows show the Leydig cells. Stained with haematoxylin-eosin; Zooming 100×10. A - control, B - experiment

According to the morphometric study, in the experimental group of animals as compared to the control group, the area of interstitial tissue between the convoluted seminiferous tubules increases by 23.01%, the area of Leydig cells and their nuclei reduces by 61.13% and 69.68%, respectively; the number of Leydig cells in the interstitial site decreases by 32.61% as well (Table 3).

Final Part: The data array obtained during the study suggest that the impact of lead acetate results in the following morphological and morphometric changes in the semen glands of albino male rats:

- Decrease in the thickness of tunica albouginea of semen glands;
- Increase in the cross sectional area of convoluted seminiferous tubules and interstitial area;
- Decrease in area of seminiferous epithelium and its thickness;
- Decrease in the number of myoid cells in the wall of the convoluted seminiferous tubule and an increase in the area of the cells and their nuclei;
- Reduction in the production of all kinds of cell populations of the seminiferous epithelium, especially of spermatids as its mature form;
- Reduction in the number of Leydig and Sertoli cells, change in their shape, as well as reduction in the area of cells and their nuclei.

After being subjected to lead acetate, a spermatozoon head changes the shape, its cervix diameter decreases, while the tail length increases.

When exposed to lead acetate, both the spermatogenesis and the relaxation indices (spermatogenesis intensity) are reduced.

CONCLUSIONS

The conducted study has shown that the lead acetate exposure is associated with numerous adverse effects on the reproductive system of male rats.

Production of all kinds of seminiferous epithelium populations and especially spermatids as the mature forms is reduced, as well as the number of stem cells i.e. spermatogonia, that is an unfavorable prognostic factor.

Reduction in the number of Leydig and Sertoli cells may lead to a decrease in testosterone production and androgen binding protein that slows reduction division of the germinal cells.

Reduction in spermatogenesis and relaxation indices (spermatogenesis intensity) indicates a decrease in functional activity of the semen glands.

Thus, this study has shown a direct impact of lead acetate on the violation of the spermatogenesis regulation.

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