ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Effect of Lead on the Process of Formation of the Testes of Wistar Rats.

Olga Sergeevna Shubina* and Natalia Anatolievna Dudenkova.

Federal State Budgetary Educational Institution of Higher Professional Education "M. E. EvsevievMordovian State Pedagogical Institute", Mordovia, 430007, Saransk, Studencheskaya Street, 11A.

ABSTRACT

Using histological, morphometric methods research studied the effect of lead acetate on the formation of the testes of Wistar rats. Investigations were carried out using a digital microscope Axio Imager.M2. It is shown that when exposed to lead acetate a decrease in the production of all populations of spermatogenic cells, decreased spermatogenesis index and an index of relaxation (tension spermatogenesis), the increase in the index of ripening, index meiotic activity and germinative index, which indicates a decrease in the functional activity of the testes. On preparations it is possible to see that after influence of acetate of lead the head of spermatozoa becomes more roundish, breaks of tails observed. The results of the studies suggest a negative impact of lead acetate in the course of the process of spermatogenesis in the testes of male white rats.

Keywords: Seminal glands, epididymis, spermatogenesis, spermatozoa,spermatogenic cells, lead acetate.

*Corresponding author

ISSN: 0975-8585

INTRODUCTION

With the onset of puberty in male sex glands (testes) begins the process of maturation of male germ cells - spermatogenesis, which is extremely sensitive to the damaging effects, including the effects of heavy metals, which is lead [1-5]. However, experimental data on the influence of heavy metals on the testes very little, and the available data are rather contradictory. Not enough experimental data on the effect of lead on the process of spermatogenesis, and also not clear what level of gametogenesis in quantity suffers most [6, 7, 8].

Aim of this study was to investigate the morphological and functional features of the process of spermatogenesis in the testes of male albino rats when exposed to lead acetate.

TECHNIQUE

The pubescent out bread albino male rats weighing 200-250 g were used as a biological test object. Seminal glands were used as a trial material for study.

The experiment was conducted during the year in the premises with air temperature 22-25°C, and a relative humidity 67-70%. In line with the research objectives, the animals were divided into two groups. The control group of animals was rats contained on the common regime of the vivarium. Experimental group included animals that received within 7 days of oral acetate lead Pb(CH3COO)2×3H2O in intermediate toxicity dose of 45 mg/kg/day (in terms of lead). 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.

For histological study tissue samples seminal glands were fixed in 10% solution of the 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.

Morphometric measurements were performed with a zooming of 40×10. The preparations were photographed with a digital camera AxioCam MRc5 (ZEISS, Japan) and then the images were processed in the Abode Photoshop Elements 11. Resolution of the resulting images was 1300×1030 pixels.

Using histological research methods and morphometric analysis studied the structural and quantitative changes of various kinds of the spermatogenic cells in normal conditions and after 7 days of exposure of lead acetate $Pb(CH_3COO)_2 \times 3H_2O$.

On the basis of morphometric data of the testes were counting the number of informative parameters, characterizing the state of spermatogenesis:

1) Spermiogramma – percentage distribution of spermatogenic epithelium cells [9].

2) Index of spermatogenesis – ratio of the sum of all the layers of cells counted in one tubule to the number of counted tubules.

Spermatogenesis index was calculated by the formula: $Is=\sum a/N$, 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].

3) Index of relaxation (tension of spermatogenesis) – the ratio of the sum of all the spermatogenic cells to the amount of Sertoli cells [9].

4) Index of ripening – the ratio of young (spermatogonia, spermatocyte) and mature forms of spermatogenic epithelium (spermatids, spermatozoa).

5) Index meiotic activity – ratio of meiotic cells (spermatocytes) to a sum the remaining germ cells.

6) Germinative index – the ratio of spermatogonia to a sum the Sertoli cells [11].

ISSN: 0975-8585

To determine the index of relaxation and germinative index counted the number of Sertoli cells in the spermatogenic epithelium convoluted seminiferous tubule testes using a digital microscope Axio Imager.M2 (ZEISS, Japan) with software for image analysis AxioVision SE64 Rel. 4.8.3 and ZEN 2011 with an increase of 40×10 [12].

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 $p \leq 0.05$.

MAIN PART

Histological examination of the testes white rats showed that in the first outer layer spermatogenic epithelium in the tortuous seminiferous tubules are lying on the basal membrane of spermatogonia with dark optically dense core and narrow bezel cytoplasm.

Closer to the center of the tubule located spermatocytes. These large cells with a large nucleus and cytoplasm of a wide rim having a rounded shape.

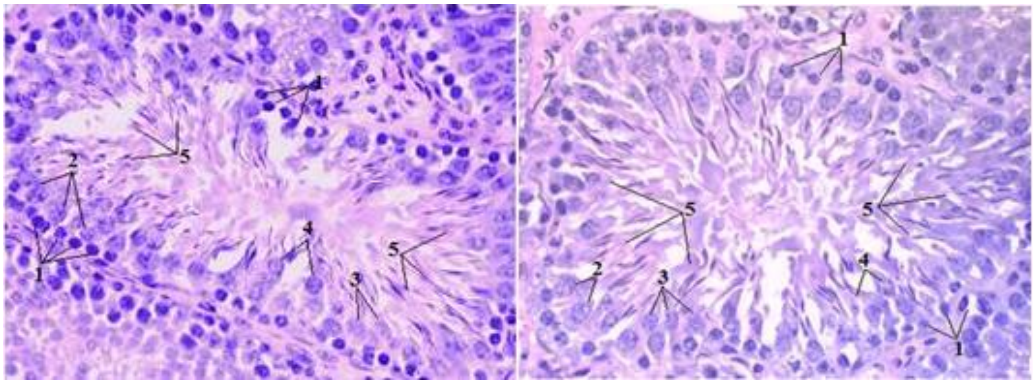
The innermost layer of convoluted tubules are spermacide, small with a light nucleus of the cell, lying in rows. Early spermatids rounded shape with a spherical nucleus is in the middle layers of ofspermatogenic epithelium. Late spermatids are in the layer adjacent to the lumen of the tubule, have an elongated shape. Some late spermatids detected flagellum.

In some tubules are seen formed spermatozoa. Their dark elongated head focused on the periphery of the tubule and tails hanging in the lumen of the tubule. Spermatozoa in the lumen of convoluted tubules groups are located in the amount of 6-8 around the contour of the lumen (Fig. 1A).

However, a closer examination of spermatozoa using a digital microscope Axio Imager.M2 at increase of 40×10 established that the head has the shape of a hook. After research on the impact of lead acetate in the spermatozoa suspension revealed that in the control group of animals is cloudy or milky white in color, has a thick consistency. The observed high concentration of spermatozoa in the mix (Fig. 2A).

Histological research of drugs testes white rats after 7 days of exposure to lead acetate showed that spermatogonia, compared to control, are smaller. Spermatocytes become oval, rarely spherical. Early and late Spermatid practically does not differ. They mainly oval. Their nuclei are displaced in the center of the cell (Fig. 1B). Noted a single location of spermatozoa in the lumen of the tubule. Found convoluted tubules, in the lumen of which were absent spermatozoa (Fig. 3).

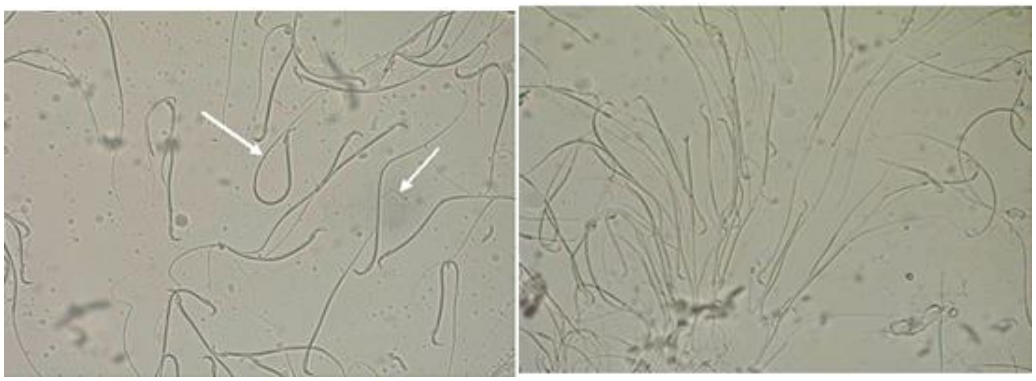
Observed chaotic arrangement of spermatozoa in the lumen of the tubule. Change in the shape of the spermatozoa head. She has a more rounded form. On historypath observed on cliffs tails and of spermatozoa agglutination (Fig. 2B).



A **B**

Fig 1: Convoluted seminiferous tubule. Stained with haematoxylin-eosin. Zooming 40×10: A – control, B – experiment, 1 – spermatogonia, 2 – spermatocytes, 3 – early spermatids, 4 – late spermatids, 5 – spermatozoa.

ISSN: 0975-8585



A B

Fig 2: Spermatozoa of male albino rats. Zooming 40×10: A – control, B – experiment (arrows indicate breaks tails and spermatozoa agglutination).

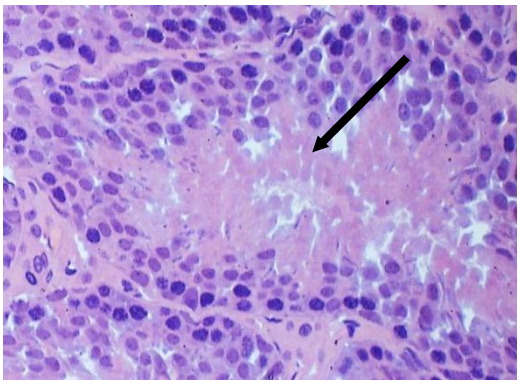


Fig 3: Convoluted seminiferous tubule (experiment). It is clear absence of spermatozoa in the lumen of the tubule. Stained with haematoxylin-eosin. Zooming 40×10.

Morphometric studies have shown that in the experimental group of animals compared to the control, there is a decrease in the number of spermatogonia, spermatocyte, spermatids and spermatozoa respectively on 6.31% ($p \leq 0.05$), 8.43% ($p \leq 0.05$), 17.36% ($p \leq 0.05$) and 26.70% ($p \leq 0.05$) (Table 1).

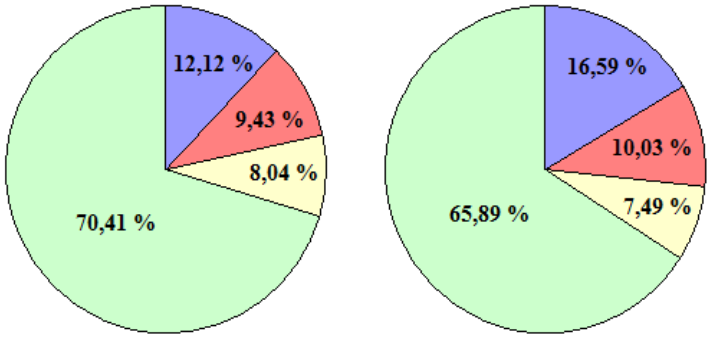
Table 1. Quantitative and percentage change of the different types of spermatogenic cells in the tortuous seed tubules of the testes of male white rats under the influence of lead acetate.

Indicators	Control		Experiment	
	The number of cells in the tortuous seminiferous tubule	% of total number of spermatogenic cells	The number of cells in the tortuous seminiferous tubule	% of total number of spermatogenic cells
Spermatogonia	52.44±1.46	12.12±2.71	49.44±1.30*	16.59±2.56*
Spermatocytes	40.80±1.97	9.43±1.61	37.36±1.71*	10.03±2.37*
Spermatid	34.80±1.52	8.04±1.20	28.76±1.31*	7.49±1.35*
Spermatozoa	304.52±13.14	70.41±4.14	223.20±31.02*	65.89±5.20*

Note: * – $p \leq 0.05$ versus control animals.

In the study spermiogram male albino rats found that when exposed to lead acetate reduced the percentage of more mature forms of the spermatogenic cells - spermatids and spermatozoa and increases the percentage of spermatogonia and spermatocyte (Fig. 4).

ISSN: 0975-8585



Control Experiment

Fig 5: Spermiogramma male white rats: –spermatogonia, – spermatocytes, – spermatid, – spermatozoa.

Для определения индекса релаксации и герминативного индекса подсчитывали количество клеток Сертоли в сперматогенном эпителии извитого семенного канальца семенных желез. Морфометрические исследования показали, что в опытной группе животных, по сравнению с контролем происходит достоверное уменьшение количества клеток Сертоли с 23.84±3,16 до 18.48±2,52, т.е. на 22.48% (p≤0.05).

To determine the index of relaxation and its germinative index calculated the number of Sertoli cells in the spermatogenic epithelium of convoluted tubules seminal glands. Morphometric studies have shown that in the experimental group of animals compared to the control, there was a significant decrease in the number of Sertoli cells with at 23.84±3.16 to 18.48±2.52, i.e. to 22.48% (p≤0.05).

After investigating, the marked decrease of the index of spermatogenesis and index relaxation (tension of spermatogenesis), compared with the control, respectively 10.24% (p≤0.05), 4.46% (p≤0.05), indicating a decrease of functional activity of the seminal glands.

Simultaneously, the index is increased maturation index meiotic activity and germinative index, compared with the control, respectively at 20.00% (p≤0.05), 23.08% (p≤0.05) and 31.79% (p≤0.05), suggesting the predominance young cells of more mature, and delay maturation of male germ cells (Table 2).

Table 2: The change of the functional activity of the testes of male white rats under the influence of lead acetate.

Indicators	Control	Experiment
Index of spermatogenesis	3.32±0.15	2.98±0.12*
Index of relaxation (tension of spermatogenesis)	18.14±1.72	17.33±1.02*
Index of ripening	0.28±0.01	0.35±0.04*
Index meiotic activity	0.10±0.01	0.13±0.01*
Germinative index	2.21±0.17	3.24±0.36*

Note: * – p≤0.001 versus control animals.

CONCLUSION

Its research findings highlight the negative impact of lead acetate on the formation of the testes of Wistar rats and the occurrence in them of the process of spermatogenesis:

1. Found that when exposed to lead acetate reduced production of all populations of spermatogenic cells and especially their mature forms – spermatids and spermatozoa.

2. Reduces the number of stem cells – spermatogonia, which is an adverse prognostic factor of the process of spermatogenesis.

3. After exposure to lead acetate in preparations there is a lack spermatozoa heads in the majority and change their shape.

4. Reduced index of spermatogenesis and the index of relaxation (tension of spermatogenesis), in comparison with control, which indicates a reduction of the functional activity of the testes. Simultaneously with this increase, in comparison with the control index ripening, index meiotic activity and germinative index that indicates the prevalence of young cells of a mature and delayed ripening of male germ cells.

ACKNOWLEDGEMENT

The study was carried out with the financial support of the Ministry of Education and Science of the Russian Federation within the state programme of FSBEI HPE "Mordovian State Pedagogical Institute named after M.E. Evsevyev" (project "The influence of anthropogenic factors on the morphofunctional state of the organism").

REFERENCES

[1] Lutsk, D.L., S.V. Vibornov, A.M. Lutsk, L.A. Goncharova and R.M.2009. The influence of chemical factors on the condition of the male reproductive system (review of the literature). Problems of reproduction, 6: 48-64.

[2] Paranko, N.M., N.I. Rublevskaya, E.N. Belitskaya, T.A. Golovkova, T.D. Zemlyakova, L.E. Chub and G.G. Shmatkov, 2002. Role of heavy metals occurs reproductive disorders. Hygiene and sanitation, 1: 28-30.

[3] Dawson, E.B., D.R. Evans, W.A. Harris and L.C. Powell, 2000. Seminal plasma trace metal levels in industrial work-ers. Biol Trace Elem Res, 74: 97-105.

[4] Sallmen, M., M.L. Lindbohm and A. Anttila, 2000. Time to pregnancy among the wives of men occupationally exposed to lead. Epidemiology, 11: 141-147.

[5] Sallmen, M., 2001. Exposure to lead and male fertility. Int J Occup Med Environ Health, 14: 219-222.

[6] Dudenkova N.A., 2014. Peculiarities of the process of spermatogenesis in the seminal glands male white rats under the influence of lead acetate. XXI International Conference of Students, Postgraduates and Young Scientists "LOMONOSOV-2014": Section "Biology". Publishing house of the Moscow University, P. 5.

[7] Shubina, O.S. and N.A. Dudenkova, 2013. Morphological and functional changes in seminal glands of albino rats exposed to lead acetate. World Applied Sciences Journal, 25 (6): 886-891.

[8] Hess, R.A. and R.L. Franca, 2008. Spermatogenesis and cycle of the seminiferous epithelium. Molecular mechanisms in spermatogenesis. Austin. TX: Landes Bioscience / Springer Science, pp. 1-15.

[9] Sheiko, D.D., 1998. The influence of small doses of hexavalent chromium on the reproductive function of small mammals: Model experiment. Ural research Institute of maternity and infancy, Ekaterinburg.

[10] Narbutova, T.E., 2011.The dynamics of structural and functional changes in the testes of the second generation mice at the cumulation of lead in the body and the introduction of alpha-tocopherol. Biomedical and Biosocial Anthropology, 16: 27-31.

[11] Shevantaeva, O.N., 2012. Spermatogenesis after the extreme hypoxic and ischemic effects and the possibility of medical correction in the experiment. Russian University of Peoples' Friendship, Moscow.