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The Impact of Dust and Salt Aerosols of the Aral Sea on the Tissues of Rat Testes in the Experiment

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Abstract

BACKGROUND: The Aral Sea crisis is recognized as one of the global environmental problems of our time. The extreme environmental situation in the region is caused by massive chemical pollution of the territory for several decades by high doses of pesticides, herbicides, dumping of industrial waste into the rivers that feed the Aral Sea. As a result of the Aral Sea desiccation, aridization of the territory, climate change, and salinization of soil and water occurred. This led to increased mineralization of precipitation, climate change, the spread and deposition of dust on an area of about 25 million hectares. One of the factors in reducing fertile activity in humans is a decrease in spermatogenesis activity. Based on identified trends, WHO is forced to review the main indicators of spermograms in the direction of their reduction.

AIM: To study the effect of dust and salt aerosols of the Aral Sea on reproductive function, rat testes were studied after 7 and 24 days of inhalation administration.

MATERIAL AND METHODS: Some morphometric parameters of the testes were evaluated: The diameter of the convoluted seminiferous tubules and the thickness of the germinal epithelium. The obtained comparative morphological and histological characteristics of the testes of the control and experimental groups indicate the negative effect of the components of dust and salt aerosols of the Aral Sea on the reproductive function of male rats.

RESULTS: The thickness of the epithelial spermatogenic layer in the second group of rats is on average 64.52 μm, which is significantly less compared to the control group (81.3 μm). The thickness of the epithelial spermatogenous layer in the third group is on average within 73.36 μm, which is significantly less than in the control group (81.3 μm), but more than in the group exposed to dust and salt aerosols for 7 days (64, 52 μm).

CONCLUSION: The study revealed that the impact of dust and salt aerosols of the Aral Sea leads to a change in the morphological and histological characteristics of the testes of animals. In experimental groups, a decrease in the diameter of the convoluted seminiferous tubule and a decrease in the thickness of the epithelial spermatogenic layer were observed. This indicates the negative effect of the components of dust and salt aerosols of the Aral Sea on the reproductive function of male rats exposed to dust for 7 and 24 days.

Introduction

The Aral Sea crisis is recognized as one of the global environmental problems of our time. The extreme environmental situation in the region is caused by massive chemical pollution of the territory for several decades by high doses of pesticides, herbicides, and dumping of industrial waste into the rivers that feed the Aral Sea. As a result of the Aral Sea desiccation, aridization of the territory, climate change, and salinization of soil and water occurred. This led to increased mineralization of precipitation, climate change, the spread, and deposition of dust on an area of about 25 million hectares.

The negative impact of the environment under the conditions of a massive technogenic load is reflected in the deterioration of demographic indicators, a decrease in the functional capabilities and body defenses, and an increase in the morbidity and mortality of the population of the region [1], [2], [3], [4]. A significant increase in the content of extracellular nucleic acids in the blood and ejaculate of men [5] and DNA oxidation products in the blood of women [6] living in the Aral region was revealed.

In the context of the aggravation of unfavorable trends in medical and demographic processes, the relevance of studying the problem of reproductive health of the population as one of the aspects of the demographic policy of the state significantly increases.

One of the factors in reducing fertile activity in humans is a decrease in spermatogenesis activity. Based on identified trends, the WHO is forced to review the main indicators of spermograms in the direction of their reduction [7], [8].

An analysis of the literature data showed that there is practically no study of the effect of dust and salt aerosols on reproductive function. In this regard, the purpose of this experimental study was to study the impact of dust and salt aerosols of the Aral Sea on the histological characteristics of testes under experimental conditions.

Materials and Methods

The objects of the experimental study were 21 outbred white rats (males) with an initial weight of 180–220 g, 14 rats were experimental groups and 7 rats – the control group. The animals of the experimental group were divided into two groups: I – with inhalation administration for 7 days and II – with administration for 24 days. Animals were kept in an equipped vivarium under standard conditions and on a standard diet in compliance with international ethical standards of the European Convention for the Protection of Animals Used for Scientific Purposes [9].

Inhalation exposure on white laboratory male rats was carried out in a special cylindrical chamber with a volume of 150 L with animals placed for 4 h a day according to the technique of Borisova *et al*. [10]. For administration, a dust and salt mixture from the bottom of the Aral Sea was used, previously crushed into a fine aerosol with particle sizes up to 5 μg.

At the end of the experiment, the rats of the control and experimental groups were withdrawn from the experiment by the method of incomplete decapitation under light ether anesthesia in compliance with the rules for working with laboratory animals of the "International recommendation for medical and biological research using animals" guide [11].

The testes were fixed in the Bouin's fixative (saturated picric acid – 75 ml, formalin – 25 ml, and glacial acetic acid – 5 ml). To exclude the influence of the anatomical features of blood supply on the result, the right testis of rats was chosen for morphological studies.

In a tissue processor, tissue samples were dehydrated in five shifts of isopropanol, clarified and compacted in two portions of a mixture of mineral oil and isopropanol, then impregnated with paraffin (three shifts) in pure mineral oil at 58°C. Blocks were made at the Sakura fill station (Japan) Tissue-Tek TEC-5. Sections were made on a Sakura Accu-Cut® SRM™ 200 Rotary Microtome, section thickness 4–6 μ. The preparations were stained with hematoxylin-eosin; control tests were performed for histochemical reactions. The stained preparations were enclosed in a histomount mounting medium under a coverslip.

To study the microstructure, stained testis preparations were examined using a Nikon Eclipse Ci light microscope (Japan) at a total magnification of ×40, ×100, ×200, and ×400. Sections were photographed using a DS-Fi2 camera.

The following morphometric parameters of the testes were evaluated: The diameter of the convoluted seminiferous tubules (CST) in cross-section and the thickness of the germinal epithelium in μm [12].

Statistical processing of the results was carried out using the software package "Statistica," 8.0. Character attributes distribution was determined by the Shapiro–Wilk test (W). Due to the fact that the studied indicators obeyed the law of normal distribution, for paired comparisons, the Student's test (t) was used. The data in the text and the table are given in the form M ± m, where M is the arithmetic mean and m is the error of the arithmetic mean [13].

Results

When studying the histological preparations of the testes of animals of the control group stained with hematoxylin and eosin, the transverse sections of the CSTs are well distinguished. The testes are coated externally with a fibrous capsule consisting of fibrillary structures of the connective tissue.

The capsule basically retains its integrity, is uniform in thickness and consistency, in some places winding. In some cases, there is a slight separation of the fibrillary structures. As part of the connective tissue, empty blood vessels, a few cells with basophilic nuclei of a flattened shape are distinguishable, and slight infiltration by small basophilic cells is distinguishable in places (Figure 1).

When studying with a microscope, the histological preparations of the testes of rats exposed to dust and salt aerosols from the bottom of the Aral

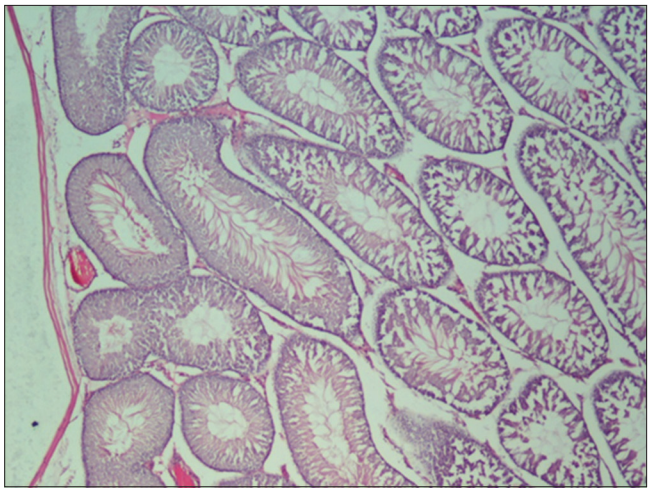

Figure 1: Cross-section of the testes of animals from the control group, x40

Sea, it was found that the organs outside are also covered with a connective tissue capsule. The capsule itself consists of adjacent fibrillary structures, which in places has a more loose arrangement of collagen fibers in the first experimental group, and in the second group of animals, fibrillary structures are separated for a considerable length. Connective tissue also contains blood vessels, in some places, small cells with basophilic stained nuclei are determined, often in small quantities. The vessels of the capsule in some cases have hypervolemia, and the walls of the vessels themselves look thickened. Around the vessels with hypervolemia, the connective tissue is more pronounced, and also slight infiltration by small basophilic cells is noted in the tissue. The own wall of the CSTs in animals of the control group is thin, uniform in color, cell infiltration and tears are not observed. The diameter of the CST on average is 290.09 μm (Table 1). The CSTs are covered with an epithelial spermatogenic layer from the inside. The histological picture in various sections of the CST is different in the cellular composition of the spermatogenic row. However, in general, in this layer, the usual two populations of cells are clearly distinguishable: Supporting epithelial cells (Sertoli cells or sustentocytes) and spermatogenic cells at various stages of differentiation. The thickness of the spermatogenic epithelium averages 81.30 μm.

Table 1: Morphometric indicators of rat testes

Groups	CST, μm	SE, μm
Control	290.09 ± 3.90	81.30 ± 1.33
Experimental group 1 (7 days)	194.51 ± 3.20*	64.52 ± 1.43*
Experimental group 2 (24 days)	218.66 ± 3.23*	73.36 ± 1.38*

*The reliability of differences between the experimental groups and analogous indicators of the control group, p<0.001. The number of replicates for each of the control indicators and experimental groups, n=36

In histological preparations of the sex glands of rats exposed to dust and salt aerosols from the bottom of the Aral Sea for 7 and 24 days, sections of CSTs are seen in both transverse and longitudinal directions (Figure 2).

On the preparations, the wall of the CSTs of the testis is clearly distinguishable and is internally covered with a layer of spermatogenic epithelium. The wall of the convoluted tubule has mainly smooth contours; in some cases, in small areas, they are winding and slightly densified. In individual CSTs, the shape of the tubules is changed, it has uneven contours, more often these phenomena are observed in the group of animals exposed to dust for 24 days (Figure 3).

The diameter of the CST in the second group of animals averages 194.51 microns, which is significantly smaller than the corresponding parameter of the control group (290.09 μm). The diameter of the CST in the third group averages 218.66 μm, which is significantly less than the same indicator in the control group (290.09 μm) and slightly larger than in the group exposed to dust for 7 days (197.51 μm).

The inner layer of different portions of the tubules in the sections consists of spermatogenic epithelium at various stages of development and supporting Sertoli cells. Sustentocytes, or Sertoli cells, larger in size, are located on the basal membrane, the boundaries are hardly distinguishable. The cytoplasm of the cells is evenly colored pink, the nuclei are in the basal part of the cells, basophilic. Cells of spermatogenous epithelium at different stages of development are determined in sections of different portions of CST. The arrangement of cells corresponds to the general idea of the arrangement of cells at the stages of the development of spermatogenic cells. In some tubules, small basophil cells are detected. In other tubules, segregating spermatozoa in a small amount are visible.

In groups of animals exposed to dust-salt aerosols of the Aral Sea for 7 days, the layer of spermatogenic epithelium is heterogeneous in thickness, it is formed by several layers of gametes that are in the process of differentiation, the development and maturation of which occurs in the direction from the basement membrane to the tubule lumen. The basal part of the tubule is occupied by smaller cells with small basophilic nuclei. Relatively large cells with uneven edges, sometimes elongated, whose nuclei are also basophilic and somewhat larger, are located above. In other neighboring tubules, basophilic cells with small sizes are determined, which

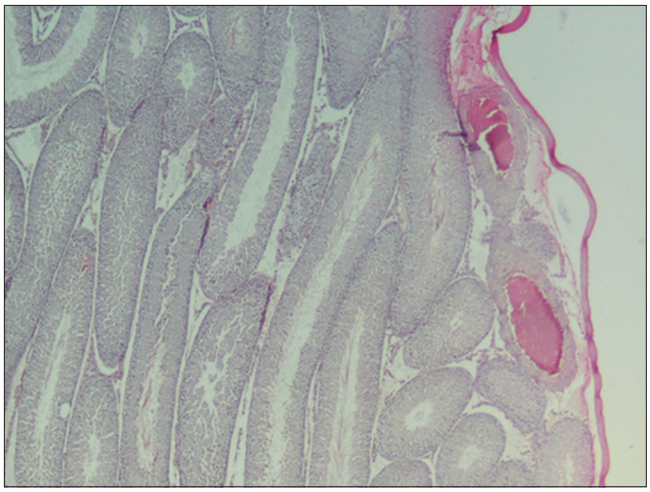

Figure 2: Cross-section of the testes of animals from the experimental Group 1, ×40

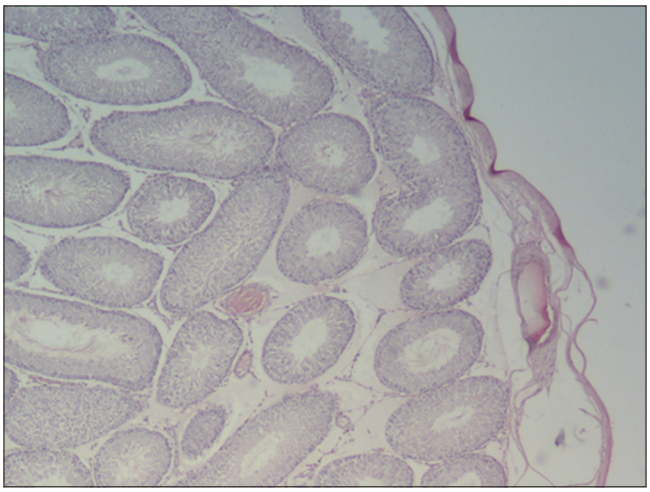

Figure 3: Cross-section of the testes of animals from the experimental Group 2, ×40

are located closer to the lumen of the CSTs of the testis. Moreover, in some tubules, forming spermatozoa are visible in a small amount, more often they are closer to the lumen or are already in the lumen.

Sertoli cells are larger, the cytoplasm is weakly basophilic, the edges of the cell are invaginated, in which mature gametes of varying degrees of maturity have settled. Cell nuclei are basophilic, with uneven edges. There are no visible features in the cytoplasm.

In some tubules, changes in the state of the epithelial spermatogenous layer are noticeable in the form of gaps between cells. In some CSTs, desquamation of the spermatogenic epithelium from the basal membrane is seen with the formation of a free space between them.

In animals with a duration of exposure to dust for 24 days in the epithelial spermatogenous layer, disorganization phenomena are determined, which is expressed in the loss of communication between individual cells, rarefaction of the cell population, vacuolization of their cytoplasm, and fluid accumulation between individual maturing spermiums.

The thickness of the epithelial spermatogenic layer in the second group of rats is on average 64.52 μm, which is significantly less compared to the control group (81.3 μm). The thickness of the epithelial spermatogenous layer in the third group is on average within 73.36 μm, which is significantly less than in the control group (81.3 μm), but more than in the group exposed to dust and salt aerosols for 7 days (64, 52 μm).

Discussion

Our experiment confirms that the impact of dust and salt aerosols of the Aral Sea leads to a change in the morphological and histological characteristics of the testes of animals.

In the second group of animals (exposure to dust and salt aerosols of the Aral Sea for 7 days), between the CSTs, there is an interstitial connective tissue, in the structure of which dilated and sharply hypervolemia blood vessels are visible. The wall of blood vessels is more often than the usual layered structure, in isolated cases, there is impregnation with small basophilic cells. In the third group of animals (exposure to aerosols for 24 days), in some areas, the CSTs are located at a considerable distance from each other, and the interstitial tissue of the organ is not visually detected, there are free spaces between the tubules that appear empty (Figure 3).

When exposed to dust and salt aerosols of the Aral Sea in the body of experimental animals, a decrease in the diameter of the CST and a decrease in the thickness of the epithelial spermatogenic layer in the first experimental group were revealed. This indicates the negative effect of the components of dust and salt aerosols of the Aral Sea on the reproductive function of male rats. In this case, the inclusion of compensatory and recovery resources of the animal organism is also determined, which is illustrated by a slight increase in the values of the studied parameters in the second experimental group. Despite this, the diameter of the CSTs and the thickness of the epithelial spermatogenous layer in the testes of rats exposed to dust for 24 days remain significantly smaller than those in the control group.

Conclusion

The study revealed that the impact of dust and salt aerosols of the Aral Sea leads to a change in the morphological and histological characteristics of the testes of animals. In experimental groups, a decrease in the diameter of the convoluted seminiferous tubule and a decrease in the thickness of the epithelial spermatogenic layer were observed. This indicates the negative effect of the components of dust and salt aerosols of the Aral Sea on the reproductive function of male rats exposed to dust for 7 and 24 days.

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