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Оригинальная статья

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Assessment of the safety of short-term exposure of peat thermal degradation products in sublethal concentrations on rats

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ABSTRACT

Introduction. The lack of information about the health safety of firefighters and those taking part in extinguishing landscape fires from short-term exposure to sublethal concentrations of carbon monoxide determines the relevance of this work.

Material and methods. The study on rats used methods for assessing the state of the central nervous system, including behavioral tests, electroencephalography, morphology of the cerebral cortex, cardiac tissue, testes and liver. Chemical research methods included the determination of the content of CO and $PM_{2.5}$ in the air of the exposure chamber. Genotoxicity on blood cells was determined by the DNA comet method.

Results. The experiment showed that exposure to peat smoke with a level of CO=99±2.5 mg/m^3 and $PM_{2.5}$=0.72±0.3 mg/m^3 for 30 minutes had no pronounced adverse effect on male rats. The results of studying the motor research behavior of animals, EEG parameters, the structure of cardiomyocytes, epicardium, myocardium and pericardium did not differ from those in control rats. In the tissue of the sensorimotor cortex of the rats of the experimental group, an increase in the number of acts of neuronophagy was revealed, and in the liver tissue – a decrease in the number of polynuclear hepatocytes. In animals exposed to peat smoke, a decrease in the spermatogenesis index was revealed, which demonstrates a violation of the formation of germ cells of the final stages of spermatogenesis. No genotoxic effect was detected. Collectively, the identified changes may indicate a compensatory reaction of the rat body when ingested with toxicants.

Conclusion. The toxicity of a multicomponent mixture of forest biomass combustion products with short-term exposure, containing a CO concentration of 100 mg/m^3, may be underestimated when predicting long-term effects on the reproductive potential of the adult population.

Limitation. It is caused by a number of uncertainties related to insufficient information about the short-term effects of high concentrations of CO and solid particles $PM_{2.5}$ in the smoke of landscape fires on the nervous, reproductive, genome/epigenome system of male rats.

Keywords: *landscape fires; white rats; toxicity; carbon monoxide; EEG; histology*

Compliance with ethical standards. The study was carried out in accordance with the rules of bioethics approved by the European Convention for the Protection of Vertebrates Used for Experimental and Other Purposes and approved by the Local Independent Ethical Committee of the Federal State Budgetary Scientific Institution "East Siberian Institute of Medical and Ecological Research" (Protocol No. 32 of 10.01.2023).

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Introduction

One of the main directions of preventive toxicology is the justification of safe levels of exposure to environmental and industrial factors. Unfortunately, there are none for the liquidators involved in extinguishing landscape fires. At the same time, this is a large group of employees of the Ministry of Emergency Situations and fire protection services, whose work is accompanied by prolonged exposure to chemical factors, inaccessibility of extinguishing sites, and irregular work schedules during the fire season. The chemical factor is represented by a mixture of gases formed during smoke, their concentrations are highest at a distance of up to 2 meters from the ground surface under the condition of a grass or peat fire. That is, in the breathing area, liquidators are most adversely affected by smoke containing such toxic components as carbon monoxide, sulfur and nitrogen dioxides, hydrogen sulfide, acetaldehyde, formaldehyde, benzene, toluene, chloromethane, benzpyrene, vanadium pentoxide, ultrafine particles and many others, including those that are potential repro- and genotoxicants [1–5].

Assessment of the level of exposure of the chemical factor to persons engaged in extinguishing landscape fires is a complex process due to the difficulty of sampling in the source of smoke, differentiation of the composition of a multicomponent mixture of gases. The recommended safe levels of carbon monoxide for short-term acute exposure for 15 minutes, according to WHO global recommendations on air quality, are 100 mg/m^3, and the maximum single concentration of $PM_{2.5}$ is 0.16 mg/m^3 [6]. It is known that liquidators and firefighters during extinguishing landscape fires may be exposed to high concentrations of carbon monoxide and particulate matter for a short time at levels close to or exceeding the recommended occupational exposure limits, especially in cases where respiratory protection equipment is not used due to the visual impression of low smoke intensity. It should not be forgotten that they do not act in isolation, but are part of a multicomponent smoke mixture.

When planning these studies, we hypothesized the adverse short-term effects of sublethal concentrations of peat smoke on the functional state of the body. The indicators were the content of carbon monoxide and ultrafine particles $PM_{2.5}$ in the air.

The aim of the research was to assess the functional state of the cardiovascular, nervous and reproductive systems, as well as the degree of DNA fragmentation in white rats when exposed to peat thermal degradation products in sublethal concentrations.

Material and methods

The experiment was performed on 40 outbred white male rats weighing 200–220 g. Inhalation priming with peat smoke was carried out in a 200-liter exposure chamber in a dynamic mode using a device for simulating biomass combustion products*. Peat was used as a substrate, samples of which were taken at the site of drained peat bogs in the Angarsk region. The duration of exposure was 30 minutes. The required level of smoke was achieved in inhalation chambers with a volume of 200 liters by diluting the smoke with clean air. The carbon monoxide (CO) content was determined using a GANK-4 gas analyzer (NPO "Pribor", Russian Federation). The concentration of solid particles with a diameter of less than 2.5 microns ($PM_{2.5}$) was evaluated using a DustTrak 8533 analyzer (TSI Inc., USA).

The animals were examined 24 hours after the end of the exposure. The white rats of the experimental and control groups were tested in an "open field", electroencephalographic examination was performed, as well as analysis of the level of DNA fragmentation in blood cells and histological examination of the brain, liver, heart and testes.

The study was carried out in accordance with the rules of bioethics approved by the European Convention for the Protection of Vertebrates Used for Experimental and Other Purposes and approved by the Local Independent Ethical Committee of the Federal State Budgetary Scientific Institution "East Siberian Institute of Medical and Ecological Research" (Protocol No. 32 of 10.01.2023).

Laboratory animals were kept in standard vivarium conditions: temperature 22±3 °C, humidity 50–60%, 12-hour daylight/darkness cycle, free access to water and feed.

Methods of CNS research. The CNS study included testing animals in an "open field" with automatic recording of the distance traveled, time spent in the center of the arena, the studied area of the arena and vertical motor activity using the program "EthoStudio" (AISoftPro, Russian Federation) [7]. The recording and analysis of the electroencephalogram (EEG) were performed using the Neuron-Spektr-1/V electroencephalograph (Neurosoft, Russian Federation) using monopolar needle electrodes. The following neurodynamic parameters of the EEG were evaluated: the amplitude of each (δ, θ, α, $\beta1$ and $\beta2$), the index of each rhythm (the time of the presence of a certain rhythm (activity)

* Vokina V.A., Andreeva E.S., Novikov M.A., Sosedova L.M. Device for modeling intoxication in small laboratory animals by biomass combustion products. RF Patent: No. 2022107278; 2022.

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in relation to the entire time of EEG recording, expressed as a percentage).

Methods of histological research. Micro-preparations of liver, heart, brain, and testis biomaterials of experimental animals were prepared for histological examination using a standard histological technique. The total number of neurons, astroglia cells, degeneratively altered neurons, and cases of neuronophagy were evaluated on slices of the cerebral cortex stained using the Nissl method. The number of Kupffer's stellate macrophages and the number of polynuclear hepatocytes were recorded in liver sections stained with hematoxylin and eosin. The thickness of cardiomyocytes in the heart tissue was determined.

The morphological assessment of the seminal epithelium on stained gonadal sections was carried out according to the following indicators: the index of spermatogenesis, the relative number of Sertoli cells and Leydig cells, the number of seminal tubules in one field of view.

The DNA comet method. The level of DNA fragmentation in white rat leukocytes was assessed by the DNA comet method in the alkaline version [8]. DNA damage analysis was performed using the CASP 1.2.2. software (100 randomly selected cells per sample). The percentage of DNA in the tail of DNA comets was recorded.

Mathematical and statistical research methods. The results of the study were processed using the Statistica 6.1 Stat Soft Inc. (USA). The normality of the distribution was checked using the Shapiro–Wilk W-test. To assess the intergroup differences, the Student's t-test and the Mann–Whitney U-test were used, the differences were considered significant at $p \leqslant 0.05$.

Results

Characteristics of peat smoke exposure. To confirm/refute our hypothesis, an acute inhalation exposure to peat smoke for white rats was carried out experimentally using a previously developed device for simulating intoxication by biomass combustion products in small laboratory animals. During the period of smoke from wildfires, concentrations of CO and solid particles $PM_{2.5}$, which have the most pronounced toxic effects on the body, are most often used as criteria for air pollution. In this study, concentrations of CO and $PM_{2.5}$ were maintained in exposure chambers, commensurate with the actual conditions of short-term occupational exposure. The average concentration of CO was 99±2.5 mg/m^3, $PM_{2.5}$ – 0.72±0.3 mg/m^3. The dynamics of changes in the concentration of CO and $PM_{2.5}$ is shown in Fig. 1 (see on the insert).

Table 1

The results of testing in an "open field" of white rats, *Me* (Q_1; Q_3)

Indicators	Indicators	
	control (n = 20)	experimental (n = 20)
Time in the center, %	0,4 (0; 3,8)	0,4 (0; 2,6)
The studied area of the arena, %	37,4 (34,7; 42,3)	42,5 (35,4; 45,9)
The distance traveled, см	545,4 (464,4; 614,8)	530,1 (395,9; 607,5)
Vertical movement activity, мм	19,8 (18,4; 31,1)	27,1 (17; 33,2)

Assessment of the effect of peat smoke on CNS parameters. When exposed to peat smoke, experimental animals showed no significant changes in the indicators of research and locomotor activity compared with the comparison group (Table 1).

The results of an EEG examination of the brain of white rats exposed to peat smoke are presented in Table. 2 and 3. There were no statistically significant changes in the spectral and amplitude characteristics of the EEG in white rats after a single exposure to peat smoke compared with the control group. No interhemispheric asymmetry was observed.

In the animals of the experimental group, a tendency to decrease the maximum amplitude was observed, mainly due to the delta rhythm (Table 3).

A single exposure to peat smoke at high concentrations of CO had no pronounced effect on the indices of δ-, θ-, α-, β1- and β2-EEG rhythms, there were no statistically significant differences in these indicators between the experimental and control groups (Table 4).

Histological analysis did not show any structural changes in the epicardium, pericardium and endocardium. The color of cardiomyocytes is uniform. There were no violations of the normal rheology of the blood, the blood filling of the tissues was uniform. There were also no violations in the structure of the tissue as a whole. The thickness of cardiomyocytes did not differ from the values of the control group (Fig. 2 (see on the insert), Table 4).

In the liver tissue of the white rats of the experimental group, there was a tendency to decrease the number of polynuclear hepatocytes in the experimental group compared with the control group (p=0.096, see Table 4, Fig. 3 (see on the insert)). When comparing the number of Kupffer cells in the experimental and control groups, no statistically significant differences were found. An increase in the number of nuclei in hepatocytes is a compensatory

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Table 2

The maximum electroencephalogram amplitude (μV) of peat smoke exposed rats, *Me* (Q_1; Q_3)

Frequency range of the electroencephalogram	Study groups			
	experimental (*n* = 10)		control (*n* = 10)	
	hemisphere of the brain			
	left	right	left	right
δ	126,7 (103,9; 217,4)	179,3 (126,9; 340,3)	246 (195,7; 329,2)	278 (185,6; 331,7)
θ	138,9 (97; 212,4)	130,1 (108,5; 171,8)	149,6 (124,5; 232,8)	124,5 (117,1; 175,9)
α	122 (89,9; 156,5)	111 (80,6; 151,2)	121,1 (99,5; 137,8)	115,7 (90,7; 141,2)
β1	91,6 (56,8; 131,2)	73,2 (57,8; 118,8)	97,9 (63,5; 345,1)	122,3 (83; 298)
β2	56,3 (41,5; 64,8)	37,4 (32,1; 59,3)	47,7 (42,7; 111,5)	49 (40,9; 83,3)

Table 3

The values of the indices of the main EEG rhythms of rats (%), *Me* (Q_1; Q_3)

Frequency range of the electroencephalogram	Study groups			
	experimental (*n* = 10)		control (*n* = 10)	
	hemisphere of the brain			
	left	right	left	right
δ	1 (0,6; 1,5)	1 (0,6; 1,5)	2 (0,6; 2,4)	1,1 (0,9; 2)
θ	9,6 (8,5; 11,8)	10,3 (9,1; 13,3)	9 (8,2; 11,3)	9,6 (8,3; 12,1)
α	27,2 (24,1; 29,1)	26,9 (24,3; 29)	22,9 (18,9; 24,8)	21,4 (20; 27,6)
β1	17,6 (16,8; 19,2)	17,1 (15,2; 20)	16,9 (15,8; 18,4)	15,9 (15; 18,3)
β2	17,1 (13,9; 25)	19,3 (15,4; 22,9)	26,3 (18,6; 29,1)	26,3 (19,8; 27,6)

Table 4

Morphometric parameters of heart, liver and brain tissue of white rats, *m* ± *s*

Parameter	Study groups	
	experimental (*n* = 8)	control (*n* = 8)
Kupffer Cells	122,9 ± 18,5	128,3 ± 13,7
Polynuclear hepatocytes	8,9 ± 1,6	13,6 ± 2
Number of normal neurons	197,7 ± 21	214,3 ± 13,8
Number of gliocytes	120,2 ± 10,9	145,3 ± 10,9
Degeneratively altered neurons	1,8 ± 0,1	1,35 ± 0,71
Neuronophagy	1,3 ± 0,2*	0,31 ± 0,08
The number of swollen neurons	2,3 ± 1	0,43 ± 0,26
Thickness of cardiomyocytes, microns	12,2 ± 1	11,1 ± 0,5

Note. * – the differences are statistically significant when compared with the control group ($p < 0.05$).

process that makes it possible to increase the synthetic capabilities of the cell. Perhaps, a decrease in the number of polynuclear hepatocytes indicates a change in compensatory and adaptive capabilities in the organ.

Histological analysis in the brain tissue of experimental animals revealed activation of the process of disposal of dead neurons, as evidenced by a significant increase in the number of acts of neuronophagy compared with the control group (p=0.002; see Table 4, Fig. 4 (see on the insert)). Quantitative analysis did not reveal statistically significant differences in the number of normal neurons and astroglial cells in animals experimental and control groups. The blood filling of the vessels of the brain substance and the condition of the vascular walls in animals of the experimental and control groups had no pathological changes.

In the testis samples of experimental animals, a statistically significant decrease in the spermatogenesis index was noted (p=0.037, Fig. 5, 6 (see on the insert)).

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There were no statistically significant changes in the relative number of Sertoli cells and Leydig cells.

The level of DNA damage in the blood cells of male rats in the control group averaged 5.38±0.21%, in experimental animals – 5.61±0.31%, which indicates the absence of a genotoxic effect of peat smoke with short–term exposure at the studied concentration (p=0.59). According to the structure of the distribution of cells with varying degrees of DNA fragmentation, there were no statistically significant differences between the experimental and control groups (Fig. 7, see on the insert).

Discussion

The research was due to a number of uncertainties related to insufficient information about the short-term effects of high concentrations of CO and solid particles of $PM_{2.5}$ in the smoke of landscape fires on the nervous, reproductive, and genome/epigenome systems of male rats. Thus, the results of the study showed that exposure to peat smoke for 30 minutes with a CO level of 99±2.5 mg/m^3 and a $PM_{2.5}$ level of 0.72±0.3 mg/m^3 exceeding the maximum allowable one-time 4.5 times does not have a pronounced adverse effect on male rats. The indicators of motor research behavior, EEG parameters, and the structure of cardiomyocytes, epicardium, myocardium, and pericardium of animals in the experimental group had no significant differences when compared with similar indicators of individuals in the control group. At the same time, a decrease in the number of polynuclear hepatocytes was detected in the liver tissue of animals exposed to smoke, and an increase in the number of acts of neuronophagy was observed in the cerebral cortex. Collectively, the revealed changes may indicate a compensatory reaction of the rat body upon ingestion of toxicants. At the same time, attention is drawn to the decrease in the index of spermatogenesis in animals of the experimental group, which indicates a high sensitivity of the spermatogenic testicular tissue to the effects of biomass combustion products and a high probability of disruption of the germ cell formation process at the final stages of spermatogenesis.

The level of smoke exposure used by us in the modeling process is often found in real conditions when smoke is caused by wildfires [9–13]. Researchers from the USA have shown that in about 50% of cases, smoke exposure from landscape fires exceeded the limits of short-term occupational exposure by up to three times (TWA=50ppm) [10]. The importance of assessing short-term impacts is also confirmed by the results of the McCammon and McKenzie studies, which present measurements of the average and peak levels of carbon monoxide exposure to personnel during firefighting. While the average CO concentration per shift was 3.3 ppm (0–22 ppm), the maximum exposure level reached 88 ppm (160.6 mg/m^3) [14]. The authors also found that 20% of the measured CO levels exceeded 200 ppm (365.1 mg/m^3), and 25% of the measured CO were above 125 ppm (228.2 mg/m^3). In a study by S.N. Orlovsky and Yu.T. Tsai at experimental sites, it was found that the concentration of CO during burning of the edge of a forest fire in dead-cover forest groups depends on the distance from the soil surface, while the maximum levels of carbon monoxide are observed at an altitude of 0.5 m and amount to 99–183 mg/m^3. The average concentrations in the work area of forest fire liquidators at a height of 2 m are 42–49 mg/m^3 [15]. Thus, even at low or moderate average daily levels of CO exposure during fire extinguishing operations, acute short-term exposure to combustion products may pose a potential hazard.

As a result of the study, our hypothesis was not fully confirmed, since no significant disorders were detected in the body of white rats with short-term exposure to peat smoke in sublethal concentrations. The observed changes indicated adaptive compensatory reactions of the body to the toxic effects of biomass combustion products. Exposure to smoke in male rats at the indicated concentrations for the main components of the mixture did not cause DNA damage in blood cells. However, the decrease in the spermatogenesis index in animals exposed to peat smoke should not be ignored. Thus, based on the results obtained, it was found that a multicomponent mixture of peat smoke gases, concentrations of CO and $PM_{2.5}$, in which 99±2.5 mg/m^3 and 0.72±0.3 mg/m^3 are 0.72±0.3 mg/m^3, has reproductive toxicity even with a single exposure for 30 minutes.

Conclusion

The results of the study show that the toxicity of multi-component mixtures of the products of combustion of forest biomass at short-term influence, with the concentration of CO=99±2.5 mg/m^3 and $PM_{2.5}$=0,72±0.3 mg/m^3, can be overlooked while forecasting the long-term effects on the reproductive potential of the adult population. In general, research needs to be continued to assess the safe level of exposure of forest biomass smoke on the reproductive potential and health of future generations.

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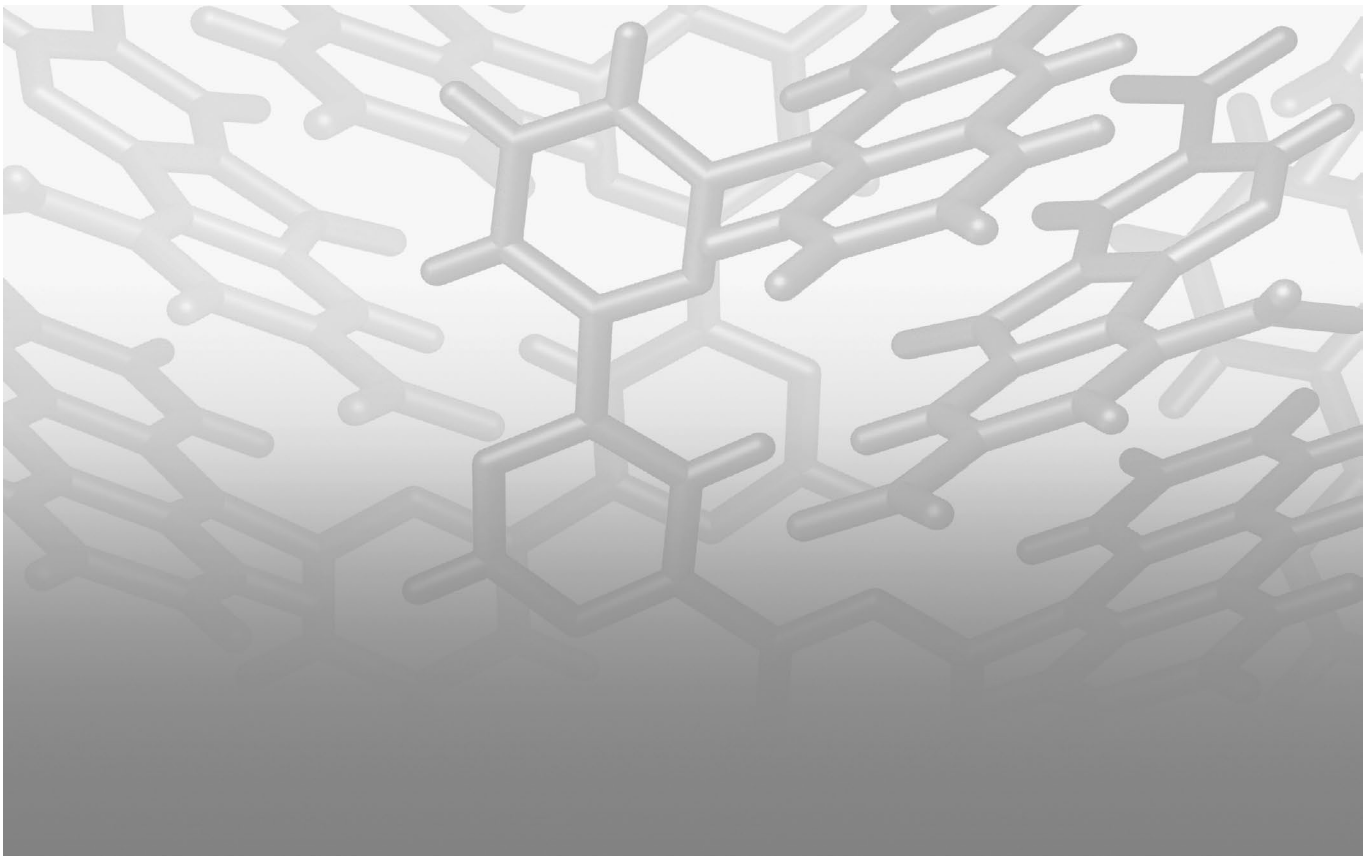

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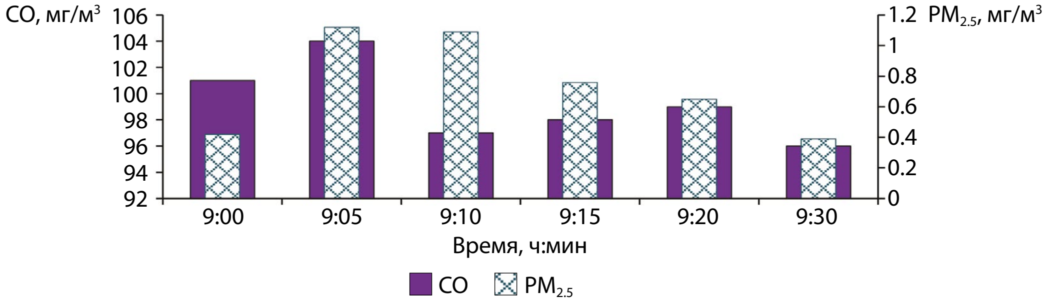
К статье Л.М. Соседовой и соавт.

To the article by Larisa M. Sosedova et al.

Рис. 1. Содержание CO и $PM_{2,5}$ в экспозиционных камерах при моделировании торфяного пожара.

Fig. 1. CO and $PM_{2,5}$ content in exposure chambers during peat fire simulation.



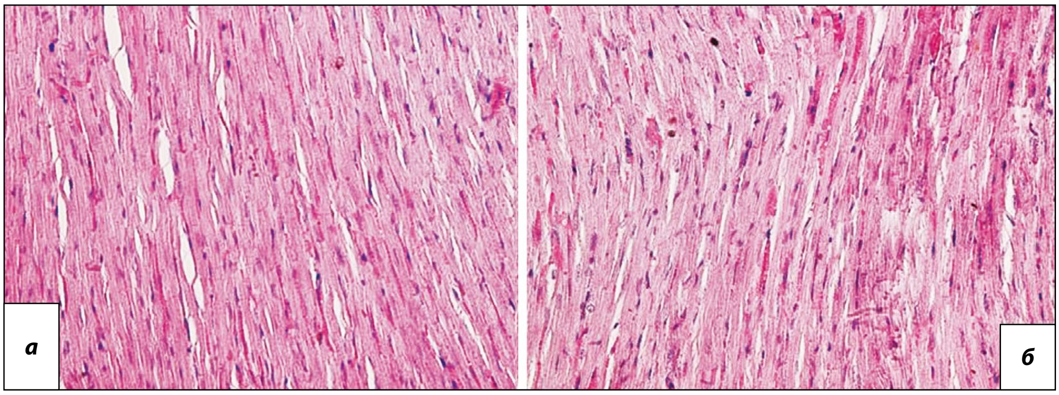
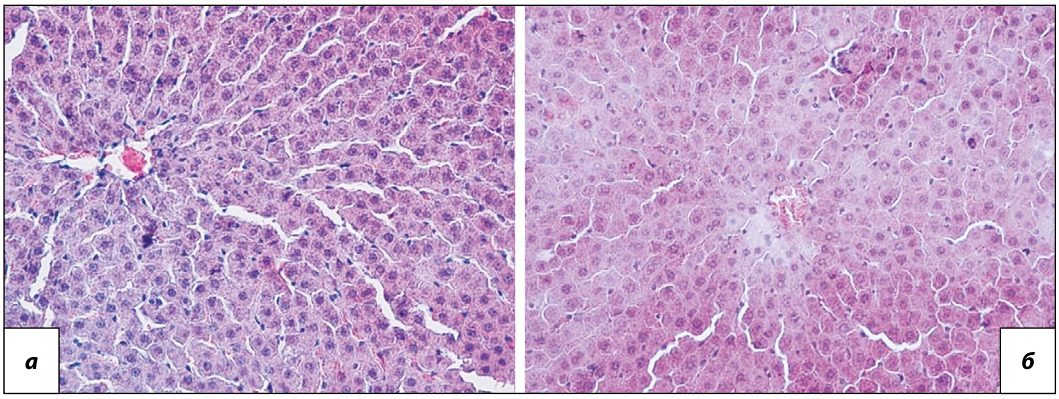


Рис. 2. Кардиомиоциты миокарда белых крыс, после затравки торфяным дымом: *а* – опыт; *б* – контроль. Увеличение ×400. Окраска гематоксилин-эозин.

Fig. 2. Cardiomyocytes of the myocardium of peat smoke exposed white rats: *а* – experimental group; *б* – control group. Hematoxylin-eosin staining. ×400.

Рис. 3. Ткань печени белых крыс после затравки торфяным дымом: *а* – опыт; *б* – контроль. Увеличение ×400. Окраска гематоксилин-эозин.

Fig. 3. Liver tissue of peat smoke exposed white rats: *а* – experimental group; *б* – control group. Hematoxylin-eosin staining. ×400.



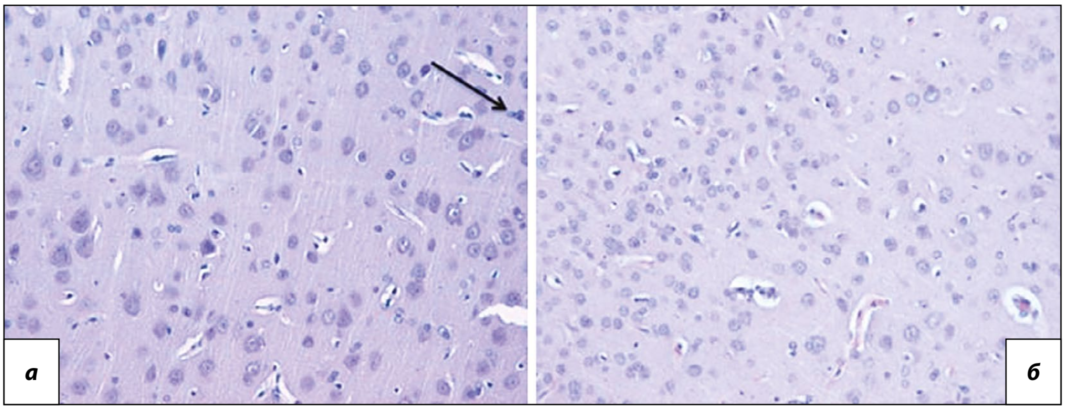


Рис. 4. Ткань головного мозга белых крыс после затравки торфяным дымом: *а* – опыт; *б* – контроль. ↑ – нейронофагия. Увеличение ×400. Окраска по Нисслю.

Fig. 4. Brain tissue of peat smoke exposed rats: *а* – experimental group; *б* – control group. ↑ – neuronophagy. Nissl staining. ×400.

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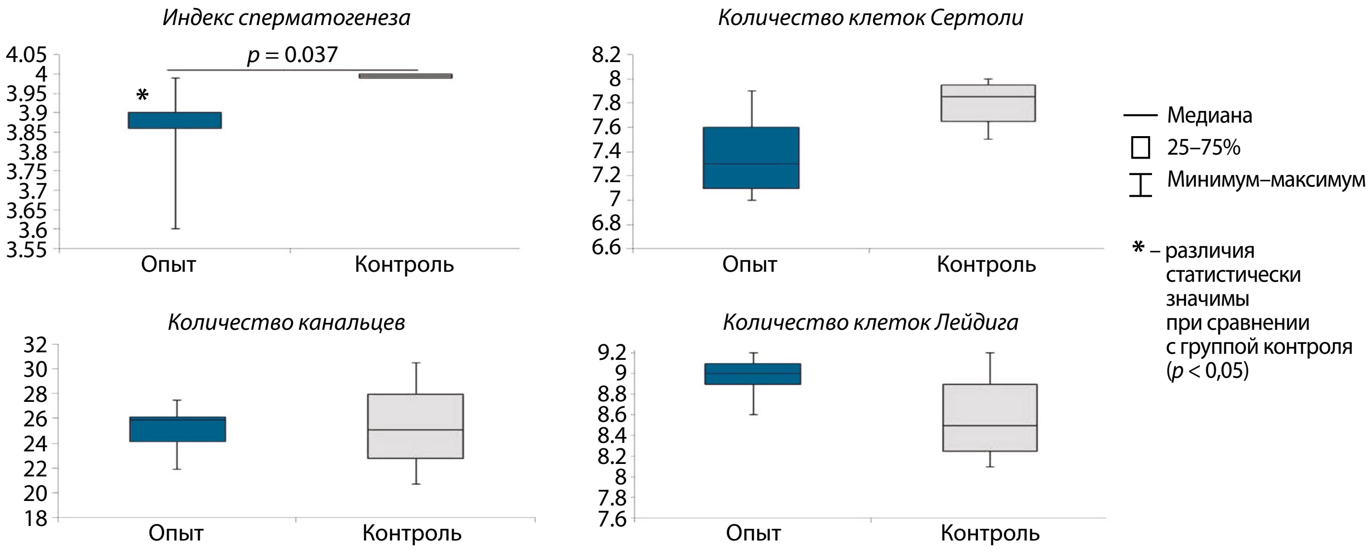

Рис. 5. Морфометрические показатели ткани семенников белых крыс.
Fig. 5. Morphometric parameters of testes tissue of white rats.

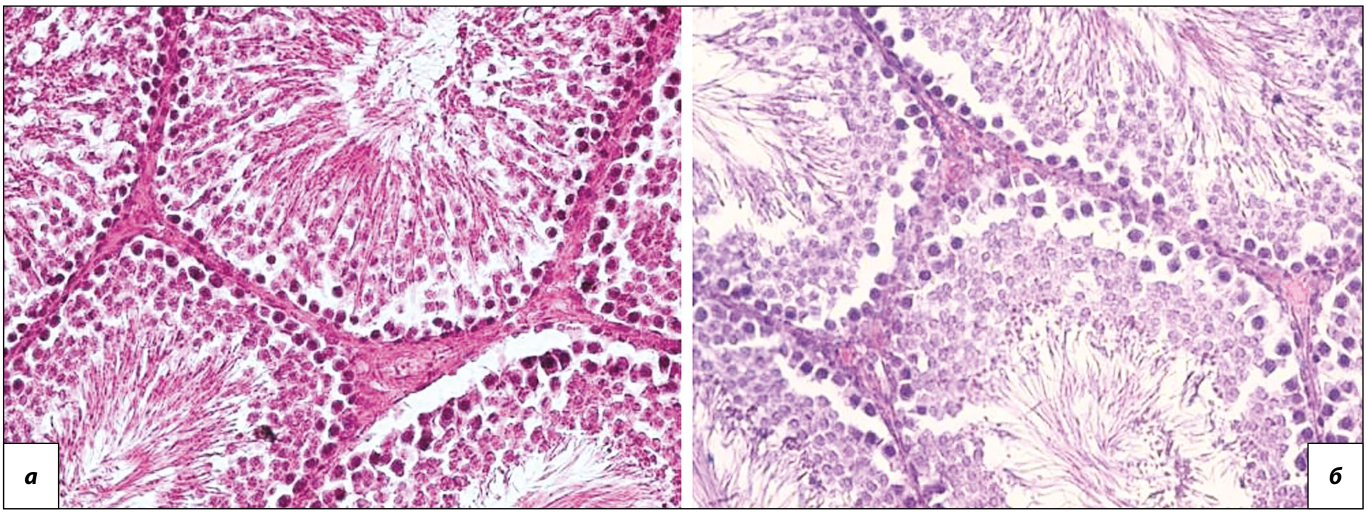

Рис. 6. Микрофотография участка семенника крысы. *а* – контроль; *б* – опыт. Окраска гематоксилин-эозин. Увеличение ×400.
Fig. 6. Microphotograph of a section of a rat testis. *а* – control group; *б* – experimental group. Hematoxylin-eosin staining. ×400.

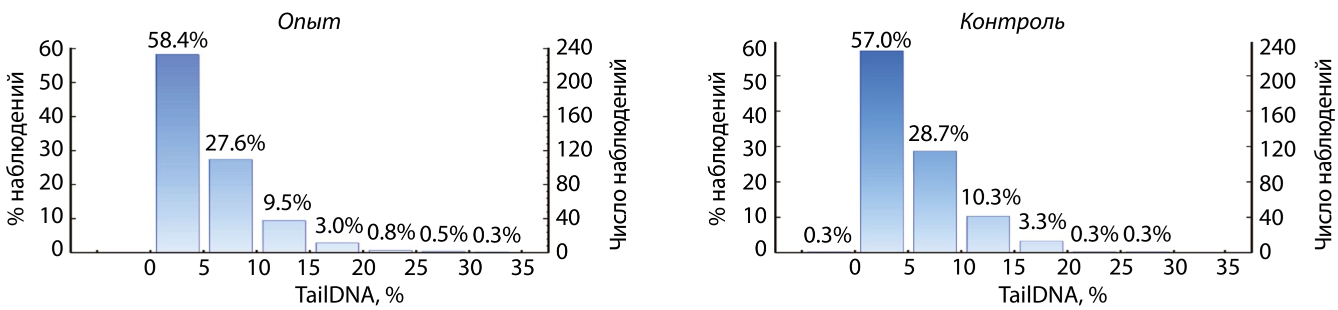

Рис. 7. Распределение клеток по степени фрагментации ДНК в клетках крови крыс.
Fig. 7. Distribution of cells according to DNA damage degree in the blood cells of rats.