

METHOD OF BIOCHEMICAL CHANGE CORRECTIONS IN THE BOAR ORGANISMS WITH TOXICANT-INDUCED REPRODUCTIVE DYSFUNCTIONS

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Summary. The pathogenetic mechanism of reproductive diseases is oxidative stress, which is manifested by an increase in lipid peroxidation and a decrease in antioxidant potential. The aim of the study was to develop a method for the correction of biochemical changes in the body of boars with toxicant-induced reproductive dysfunctions using drugs based on nanobiomaterials, based on reducing lipoperoxidation, neutralization of toxic substances by antioxidant protection increasing of animals and endocrine activity stimulating of their gonads. The article presents the results of research on the effectiveness of the complex drug 'Karafand+OV,Zn', which contains carotenoids, phytoandrogens and nanomaterials — nanoparticles of gadolinium orthovanadate, activated by europium, and zinc carbonate. Experimental toxicant-induced reproductive dysfunctions were caused by feeding sodium nitrate at a dose of 0.3 g NO₃/kg body weight. The drug was administered in a dose of 15 ml per male, orally, once a day for 14 days. Blood samples for test were taken before and on the 20th day after drug administration. Conventional biochemical methods were used, as well as chemiluminometry and enzyme-linked immunosorbent assay. There was a positive effect of the developed drug on the hormonal state (testosterone concentration increased by 91.8% (20.6 ± 0.32 nmol/l, p < 0.001), the content of vitamin A increased 1.3 times (0.65 ± 0.02 μmol/l, p < 0.001) and zinc by 47.6% (24.8 ± 0.86 μmol/l, p < 0.001), the dynamics of lipoperoxidation processes (the concentration of malonic dialdehyde in the serum was reduced by 53.2% (0.394 ± 0.01 μmol/l, p < 0.001)) and the system of antioxidant protection of boars (increased activity of catalase and superoxide dismutase in serum by 71.5% (41.4 ± 1.03 μmol/H₂O₂/l-min, p < 0.001) and 54.8% (8.98 ± 0.09 st. un./mgHb, p < 0.001), respectively), increased the content of reduced glutathione by 23.2%, indicators of the oxygen metabolism system (concentration 2,3-diphosphoglycerate increased 1.3 times (1.4 ± 0.03 mmol/l, p < 0.001), the activation of which reduces the hypoxic state. The total antioxidant activity of boars increased, as evidenced by a decrease in the light sum of chemiluminescence of serum by 47.6% (4.4 ± 0.15 un., p < 0.001). The results of research convincingly testify to the high efficiency of the use of the complex drug 'Karafand+OV,Zn' as a means of correction of toxicant-induced reproductive dysfunctions in boars and proves the possibility of its use in practical veterinary andrology

Keywords: complex drug, Karafand+OV,Zn, nanobiomaterials, lipoperoxidation, antioxidant protection, oxygen metabolism

Introduction. The negative impact of environmental factors on the body of males causes the development of a complex of pathological processes — reproductive diseases, characterized by biochemical, hormonal and structural changes and lead to dysfunction of the testes, in particular hypofertility (Koshevoi et al., 2015).

The pathogenetic mechanism of reproductive disorders is oxidative stress (Agarwal et al., 2014). Oxidative stress is manifested by an increase in lipid peroxidation and a decrease in antioxidant potential (Danchuk, Karpovskiy and Danchuk, 2016; Khariv et al., 2016). It is known that the high content of thiobarbituric acid reactants leads to a deterioration in the quality of sperm and its fertilizing ability (Chornozub, 2013).

One of the leading causes of such conditions is the action of toxic factors on the body of animals through food, for example, the receipt of toxic doses of nitrates from water and feed (De Celis, Pedrón-Nuevo and Ferial-Velasco, 1996; Hunchak et al., 2010). In an experimental model of chronic nitrate-nitrite toxicosis, we showed its effect on the dynamics of the prooxidant-antioxidant system, oxygen metabolism and sperm quality (Koshevoi et al., 2016; Naumenko, 2020).

The development of modern means of reproductive disorder corrections is based on the interdependent action on the processes of lipoperoxidation, neutralization of toxic substances by increasing the antioxidant protection of animals and stimulating the endocrine activity of their gonads (Koshevoi et al., 2015, 2016).

The aim of the study was to develop a method for the correction of biochemical changes in the body of boars with toxicant-induced reproductive dysfunctions using drugs based on nanobiomaterials.

Materials and methods. The research was conducted on boars belonging to the FP 'Vlada' (Yurivka, Pavlohrad District, Dnipropetrovsk Region). The method of correction of toxicant-induced reproductive dysfunctions includes the use of a complex drug 'Karafand+OV,Zn', synthesized in the laboratories of the Department of Veterinary Reproductology of the Kharkiv State Zooveterinary Academy and the Nanostructured Materials Department of Institute for Scintillation Materials of the National Academy of Sciences of Ukraine under the agreement on scientific and practical cooperation. The developed preparation in 1.0 cm³ contains carotenoids (10.0 ± 0.75 mg), biologically active

substances from the rhizome of marsh calamus (1.0 ± 0.05 mg) and nanomaterials — nanoparticles of gadolinium orthovanadate activated by europium (0.00015 ± 0.00001 mg) and zinc carbonate (2.0 ± 0.1 mg), the basis of the pharmaceutical composition was refined oil.

The group of animals for production testing consisted of clinically healthy males ($n = 5$), live weight 291.0 ± 5.3 kg aged 4–6 years, kept on a standard diet and had free access to water. Experimental toxicant-induced reproductive dysfunctions were induced by feeding sodium nitrate at a dose of 0.3 g NO_3^-/kg body weight of the male. The drug was administered in a dose of 15 ml per male, orally, once a day for 14 days. Blood samples for test were taken before and on the 20th day after drug administration.

The effectiveness of the developed drug was determined by changes in the content of vitamin A, zinc, the dynamics of the prooxidant-antioxidant system, oxygen metabolism and hormonal state. In the laboratory of the Department of Veterinary Reproductology, the content of vitamin A was determined by the Bessey method in the modification by Levchenko et al. (Vlizlo, 2012). Indexes of oxygen metabolism were determined: the number of erythrocytes by photocolometric registration of the studied samples optical density on KFK-3 at a wavelength of 670 nm, the hemoglobin concentration was investigated by hemoglobin cyanide method followed by photocolometry at an optical path of 540 nm, the content of 2,3-diphosphoglycerate in the erythrocyte suspension was determined spectrophotometrically (Dyce method modified by Apukhovska). The content of zinc in the serum was determined by atomic adsorption spectrophotometry at the Department of Animal Internal Medicine of the Kharkiv State Zooveterinary Academy.

In boars the following parameters were spectrophotometrically determined: the dynamics of the prooxidant-antioxidant system was determined by the content of the final product of lipoperoxidation — malonic dialdehyde by reaction with thiobarbituric acid (Fedorova, Korshunova and Larsky, 1983), and the activity of superoxide dismutase by the degree of inhibition of the reaction by the enzyme to reduce nitroblue tetrazolium in the presence of NADH and phenazine methosulfate (Dubinina et al., 1990), catalase activity on the ability of hydrogen peroxide to form a stable complex with ammonium molybdate, the color intensity of which was measured at $\lambda = 410$ nm (Korolyuk et al., 1988), the amount of reduced glutathione was determined by the Butler method using Ellman's reagent (Vlizlo, 2012) at the Central Research Laboratory of the National University of Pharmacy.

The general antioxidant activity of boars was studied by chemiluminescent analysis in the laboratory of Institute for Scintillation Materials of the National Academy of Sciences of Ukraine.

The concentration of testosterone in the serum was determined in the State Institution 'V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine' using the method of enzyme-linked immunosorbent assay. Statistical processing of the results was conducted by Student's *t*-test (Rebrova, 2002).

Results. In the correction of toxicant-induced reproductive dysfunctions in boars, we found high efficiency of the developed drug. In particular, there was a positive effect on the hormonal state, the content of vitamin A and zinc (Table 1).

Table 1 — The effect of the complex drug 'Karafand+OV,Zn' on the content of vitamin A, zinc and testosterone concentration in boars ($M \pm m$)

Indexes	Before administration	After administration
Vitamin A, $\mu\text{mol/l}$	0.28 ± 0.012	$0.65 \pm 0.02^*$
Zinc, $\mu\text{mol/l}$	16.8 ± 0.374	$24.8 \pm 0.86^*$
Testosterone concentration, nmol/l	10.74 ± 0.214	$20.6 \pm 0.32^*$

Note. * — $p < 0.001$ compared to pre-introduction.

The content of vitamin A in boars increased almost 1.3 times (0.65 ± 0.02 $\mu\text{mol/l}$, $p < 0.001$), while the amount of zinc in the serum increased by 47.6% (24.8 ± 0.86 $\mu\text{mol/l}$, $p < 0.001$). Normalization of the hormonal state was noted — the concentration of testosterone was higher by 91.8% (20.6 ± 0.32 nmol/l , $p < 0.001$) compared to the indicators before administration.

The effect of the drug on the dynamics of lipoperoxidation processes and the system of antioxidant protection of boars was effective (Table 2).

Table 2 — Dynamics of the prooxidant-antioxidant system of boars under the action of a complex drug 'Karafand+OV,Zn' ($M \pm m$)

Indexes	Before administration	After administration
Erythrocyte content:		
Malon dialdehyde, $\mu\text{mol/l}$	46.4 ± 1.21	$37.6 \pm 0.81^{**}$
Catalase, $\mu\text{mol}/\text{H}_2\text{O}_2/\text{l-min}$	14.6 ± 0.68	$26.5 \pm 0.52^{**}$
Reduced glutathione, $\mu\text{mol/l}$	3.02 ± 0.16	$3.72 \pm 0.15^*$
Serum content:		
Malon dialdehyde, $\mu\text{mol/l}$	0.842 ± 0.02	$0.394 \pm 0.01^{**}$
Catalase, $\mu\text{mol}/\text{H}_2\text{O}_2/\text{l-min}$	24.14 ± 1.02	$41.4 \pm 1.03^{**}$
Superoxide dismutase, st. un./mgHb	5.8 ± 0.14	$8.98 \pm 0.09^{**}$
Light-sum of chemiluminescence, un.	8.4 ± 0.14	$4.4 \pm 0.15^{**}$

Notes: * — $p < 0.05$, ** — $p < 0.001$ compared to pre-introduction.

It was noted that the significant effect of the drug 'Karafand+OV,Zn' — was effectively reduced the amount of malonic dialdehyde in serum by 53.2% ($0.394 \pm 0.01 \mu\text{mol/l}$, $p < 0.001$) and erythrocytes by 19% ($37.6 \pm 0.81 \mu\text{mol/l}$, $p < 0.001$). Under the action of the drug revealed a significant increase in catalase activity in serum by 71.5% ($41.4 \pm 1.03 \mu\text{mol}/\text{H}_2\text{O}_2/\text{l-min}$, $p < 0.001$) and in erythrocytes by 81.9% ($26.5 \pm 0.52 \mu\text{mol}/\text{H}_2\text{O}_2/\text{l-min}$, $p < 0.001$). There was an increase in superoxide dismutase activity by 54.8% ($8.98 \pm 0.09 \text{ st. un.}/\text{mgHb}$, $p < 0.001$) compared with pre-administration. This is marked in the body of boars by a decrease in the activity of catalase and superoxide dismutase activity (Shostya et al., 2020). The content of reduced glutathione in erythrocytes was probably higher by 23.2% ($3.72 \pm 0.15 \mu\text{mol/l}$, $p < 0.05$) of the group of animals before drug administration. Similar results of the research of a decrease in the activity of antioxidant enzymes in boars in the testes and epididymis and increasing their values after correction (Tang et al., 2019). The total antioxidant activity of boars increased, as evidenced by a decrease in the light sum of chemiluminescence of serum by 47.6% ($4.4 \pm 0.15 \text{ un.}$, $p < 0.001$).

There was also a positive effect of this drug on the indicators of the oxygen metabolism system, the activation of which reduces the hypoxic state observed in the development of toxicosis (Table 3).

An increase in the number of erythrocytes by 44.9% ($7.42 \pm 0.14 \times 10^{12}/\text{l}$, $p < 0.001$), hemoglobin content by 25.65% ($106.8 \pm 1.93 \text{ g/l}$, $p < 0.001$) and probable increase in the concentration of 2,3-diphosphoglycerate in erythrocytes by 1.3 times ($1.4 \pm 0.03 \text{ mmol/l}$, $p < 0.001$).

Table 3 — The state of the system of oxygen metabolism of boars under the action of the complex drug 'Karafand+OV,Zn' ($M \pm m$)

Indexes	Before administration	After administration
Erythrocytes, $\times 10^{12}/\text{l}$	$5.12 \pm 0.09^*$	$7.42 \pm 0.14^*$
Hemoglobin concentration, g/l	85 ± 1.84	$106.8 \pm 1.93^*$
2,3-diphosphoglycerate concentration, mmol/l	0.6 ± 0.04	$1.4 \pm 0.03^*$

Note. * — $p < 0.001$ compared to pre-introduction.

Our results coincide with the use of antioxidant enzymes in the liver of wild boars against the background of selenium deficiency occurred during the winter (Jankowiak et al., 2015).

Conclusions. The results of research convincingly show the high efficiency of the complex drug 'Karafand+OV,Zn' as a means of correction of toxicant-induced reproductive dysfunctions in boars, in particular, its positive effect on the prooxidant-antioxidant system (reduction of malonic dialdehyde by 53.2% ($p < 0.001$) and increase in the activity of catalase by 81.9% ($p < 0.001$) and superoxide dismutase by 54.8% ($p < 0.001$), increase in the content of reduced glutathione by 23.2% ($p < 0.05$), oxygen metabolism (increase in concentration of 2,3-diphosphoglycerate by 1.3 times ($p < 0.001$)), homeostasis (increase in vitamin A and zinc by 1.3 times and 47.6%, respectively) and hormonal state (increase in testosterone concentration by 91.8% ($p < 0.001$)).

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