

## INFLUENCE EXERTED BY REDOX-ACTIVE METALS ON OXIDATIVE STRESS EVIDENCE IN AN EXPERIMENT

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*Our research goal was to study influence exerted by  $Fe^{2+}$  and  $Cr^{6+}$  cations on oxidative stress signs during an experiment on Wistar rats. We detected that when these metals were introduced into animals it caused free radical oxidation activation which became apparent through changes in chemiluminescence intensity in blood serum, in increased malonic dialdehyde and diene conjugants concentrations in blood serum and tissues (liver and pancreas), and in depression of antioxidant enzymes of superoxide dismutase and catalase erythrocytes. We showed that  $Fe^{2+}$  introduction with drinking water in a dose equal to maximum permissible concentration (MPC) could cause moderate activation of free radical oxidation as iron was a key element in active particles generation in biological media, including superoxide-anion-radical and most reactive hydroxyl radical. As we studied possible influence exerted by another redox-active metal, namely  $Cr^{6+}$ , in concentration equal to 1 MPC we also detected enhanced free radical processes in blood serum which became more intense as exposure duration grew. Luminescence sum representing total antioxidant blood serum activity was almost 2.5 times higher as per two experimental periods when  $Cr^{6+}$  was introduced in comparison with intact animals. Processes activation under chromium cations effects is determined by its direct influence on free-radical mechanisms.  $Cr^{6+}$  ions recover to  $Cr^{3+}$  in biological media; one-electron recovery process with intermediates forming at intermediate oxidation levels involves occurrence of active oxygen forms; it results in free radical processes enhancement.*

**Key words:** rats, redox-active metals, free radical oxidation, malonic dialdehyde, maximum permissible concentration, biological medium, impact.

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Relevance in studying negative effects on human health as a result of environment pollution with heavy metals is determined by the prevalence of these chemicals in atmospheric air, natural and drinking water, soil, food, and by various mechanisms of their influence on human body. Understanding the mechanisms of impact allows us to further assessing risks to human health, and taking preventive measures in order to minimize them. Literature shows the direct impact of eco-toxicants, including heavy metals, which have a pronounced redox-activity on human health [1, 2,

11-13]. Metal-mediated generation of free radicals initiates various processes, including an increased lipid peroxidation (LPO). Lipid peroxides formed by the action of radicals, provided further exposure to such metals as chromium and iron, can form malonic dialdehyde (MDA), 4-hydroxynonenal and other toxic products [3, 4, 7, 20]. Proceeding from above, it seems relevant to study the effect of iron and chromium cations on oxidative stress signs during an experiment in animals that the **present research work was targeted on.**

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**Materials and methods.** Experiments were performed on 68 mature male Wistar rats weighing 250-300 g. Animals were divided into 3 groups and kept on a standard diet; The 1<sup>st</sup> group ( $n = 24$ ) served as reference, these animals unlimitedly consumed water from local artesian sources. Drinking water for experimental group rats ( $n = 26$ ) during 45 days was added with  $\text{Fe}^{2+}$  in amount of 0.5 MPC. Animals of the other group ( $n = 32$ ) received  $\text{Cr}^{6+}$  at 1 MPC with drinking water during 45 and 90 days (SanPiN 2.1.4.1074-01 "Drinking water").

At the end of experiment, animals were decapitated under etheric Rausch anesthesia, in accordance with ethical norms and recommendations on humanizing treatment of laboratory animals described in "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1985). Blood for separation into plasma and red blood cells was centrifuged at 2600 rpm during 10 minutes. In erythrocyte lysates, we determined activity of superoxide dismutase (SOD) by adrenaline auto-oxidation rate into adrenochrome, and catalase activity using kinetic method by direct recording of hydrogen peroxide decomposition [8, 21, 22]. Studies were performed with Genesys 5 spectrophotometer (USA). Intensity of lipid peroxidation processes in blood serum and heart, liver and spleen tissues was determined by the level of diene conjugates (DC) and malonic dialdehyde (MDA) by its reaction with thiobarbituric acid using spectrophotometer [16, 17]. Heart and liver tissues were homogenized with a micro-grinder at a temperature of 4°C; homogenate was centrifuged at 500 G to settle out the intact cells and tissue fragments. In supernatant, DC and MDA were determined following the above-mentioned procedures; the MDA content was calculated per protein gram. Intensity of free radical processes in blood serum was analyzed by chemiluminescence (CL) method with HLM-003 apparatus, using the following parameters: spontaneous luminosity, characterizing the initial level of free radical oxidation (FRO), fast flare ( $h$ ) for the concentration of lipid hydroperoxides, and luminescence sum of slow flare ( $S$ ) to charac-

terize maximum possible intensity of LPO induced by  $\text{Fe}^{2+}$  ions [9, 10]. Results were statistically processed using Student t-test and Mann-Whitney  $U$ -criterion.

**Results and discussion.** As can be seen from the data (Table 1), that reflect lipoperoxidation processes intensity under  $\text{Fe}^{2+}$  cations, DC concentration in serum increased by 18% and MDA concentration – by 14% in comparison with the intact group.

The review of CL parameters in blood serum of rats receiving  $\text{Cr}^{6+}$  revealed general tendency to increasing intensity of FRO (Table 2) at all exposure stages. Thus, it is shown that, comparing to the reference, spontaneous luminosity is slightly reducing by the 45<sup>th</sup> day of experiment with the subsequent increase on the 90<sup>th</sup> day. Fast flare, which reflects hydroperoxides content in serum, also decreased by the 45<sup>th</sup>, and increased 6.5 times by the 90<sup>th</sup> day, compared to the reference. Luminescence sum level, reflecting total antioxidant activity of serum, when consuming  $\text{Cr}^{6+}$ , was almost 2.5 times higher during two periods of experiment, in relation to the intact animals.

Table 1  
 $\text{Fe}^{2+}$  cations' effect on intensity of lipid peroxidation processes in rats serum, liver, heart,  $M \pm m$

Parameter	Reference	Iron ( $2^+$ )	Statistical significance
MDA, serum, Mmo/l	181,54 ± 35,731	206,75 ± 50,512	$p > 0,05$
MDA, heart, Mmo/l	0,423 ± 0,029	0,471 ± 0,058	$p > 0,05$
MDA, liver, Mmo/l	0,355 ± 0,031	0,416 ± 0,048	$p > 0,05$
DC, serum, Mmo/l	456,11 ± 3,011	537,50 ± 57,590	$p > 0,05$
DC, heart, absorbency unit	0,455 ± 0,037	0,472 ± 0,045	$p > 0,05$
DC, liver, absorbency unit	0,475 ± 0,105	0,545 ± 0,090	$p > 0,05$
SOD, relative unit/gHb	257,0 ± 26,192	157,81 ± 9,031	$p > 0,01$
Catalase, relative unit/gHb	200,77 ± 28,489	131,11 ± 9,202	$0,01 < p < 0,05$

Table 2  
Effect of chromium on FRO processes intensity in serum of Wistar rats, per exposure dates

Group	Spontaneous luminosity, relative units	Rapid flare, relative units	Luminescence sum of slow flare, relative units
Reference	0,33 ± 0,05	0,75 ± 0,22	2,01 ± 0,32
45 days	0,25 ± 0,03	0,36 ± 0,02	<b>4,60 ± 1,27</b>
90 days	0,39 ± 0,10▲	<b>4,87 ± 2,59</b>	5,10 ± 2,08

Note: Indication for statistical significance ( $p < 0.05$ ): bold print – compared with the reference; ▲ – 45 and 90 days ( $p < 0.05$ ).

Table 3  
Cr<sup>6+</sup> effect on DC formation intensity (relative unit/ protein mg) and MDA (nmol/ protein mg) in spleen and liver of Wistar rats

Group	Day	Spleen		Liver	
		DC	MDA	DC	MDA
Reference		0,39 ± ± 0,01 (n = 28)	1,33 ± ± 0,09 (n = 28)	0,40 ± ± 0,02 (n = 6)	3,73 ± ± 0,53 (n = 32)
Chromе (VI)	45	<b>0,34 ± ± 0,01</b> (n = 10)	2,26 ± ± 0,40 (n = 8)	<b>0,36 ± ± 0,01</b> (n = 10)	<b>8,28 ± ± 1,71</b> (n = 8)
	90	<b>0,47 ± ± 0,01▲</b> (n = 8)	2,03 ± ± 0,32 (n = 12)	<b>0,57 ± ± 0,01▲</b> (n = 8)	3,86 ± ± 0,60▲ (n = 23)

Note: Indication for statistical significance ( $p < 0.05$ ): bold print – compared with the reference; ▲ – 45 and 90 days ( $p < 0.05$ ).

Studying the dynamics of DC and MDA formation in rats' spleen and liver (Table 3) revealed general direction to increase in their concentration, as in the case with Fe<sup>2+</sup> intake.

Thus, it was found that, comparing to the reference group, the chromium-consuming rats showed an increase in DC concentration by 1.2 times on the 90<sup>th</sup> day of the experiment, while MDA level did not change authentically.

In comparison with the reference group, in liver of rats receiving Cr<sup>6+</sup>, there was a 1.1 time decrease in DC concentration on the 45<sup>th</sup> day and, on the contrary, 1.4 times increase on

the 90<sup>th</sup> day of the experiment. MDA content in liver increased with the maximum of 45 days: 2.2 times.

Studying the antioxidant enzymes in rats treated with Cr<sup>6+</sup>, compared with the reference group (257.40 ± 8.49 rel.unit/gHb), revealed a decrease in catalase activity on the 45<sup>th</sup> day (218.68 ± 3.75 rel. units/gHb), while SOD activity decreased on the 90<sup>th</sup> day of exposure (123.39 ± 14.24 rel.units/gHb) comparing to the reference group (226.68 ± 25.58 rel.units/gHb).

Thus, experimental results showed that Fe<sup>2+</sup> intake with drinking water in a concentration corresponding to 0.5 MPC can cause moderate activation of free-radical oxidation. This metal in biological medium is a key element in generation of active particles, including superoxide-anion-radical and the most reactive hydroxyl radical formed mainly at hydrogen peroxide decomposition [13-15, 18]. Implementation of the given mechanism is accompanied with a decreasing activity of SOD antioxidant enzymes and catalase, which was proved by the performed research.

Study of the possible effect from another redox-active metal Cr<sup>6+</sup> at a concentration equal to 1 MPC on the intensity of free-radical processes in blood serum also showed their enhancement, progressing with the increasing duration of exposure. The activation of processes by chromium cations action is due to its direct effect on free-radical mechanisms. In biological medium, Cr<sup>6+</sup> ions are reduced to Cr<sup>3+</sup> mainly by the action of glutathione and vitamin C [5, 6, 19]. The one-electron reduction process with intermediates generation in medium oxidation states is associated with the formation of reactive oxygen intermediate, which results in enhancement of free-radical processes, probably due to interaction of Cr<sup>n+</sup> ( $6 \leq n \leq 3$ ) with hydrogen peroxide, according to Haber-Weiss and Fenton reactions. Inactivation effect of SOD enzymes and catalase shown by the study results also causes a pronounced activation of free-radical oxidation processes and oxidative stress.

Generally, effects of isolated exposure to iron and chromium ions shown in this study testified that, under multi-component envi-

ronmental factors, it is necessary to take into account not so much their concentrations with reference to the maximum permissible ones, as primarily the possibility to realize their presence in body through various mechanisms, giving also attention to an expectable potentiating action in cases of simultaneous intake.

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