EFFECT OF GAS-DISCHARGE PLASMA RADIATION ON THE BIOCHEMICAL PARAMETERS OF BLOOD AND URINE OF INTACT ANIMALS AND THOSE WITH ACUTE ALCOHOL INTOXICATION

S.V. Trofimova[1*], *A.A. Kupriyanova*[1], *A.M. Shumilova*[1], *I.M. Piskarev*[2], *I.P. Ivanova*[1]

[1] Institute of Biology and Biomedicine, Lobachevsky State University of Nizhny Novgorod, 23 Prospekt Gagarina, Nizhny Novgorod, 603022, Russia;
[2] Skobeltsyn Institute of Nuclear Physics of Lomonosov Moscow State University, 1(2) Leninskie gory, GSP-1, Moscow, 119991, Russia.

* Corresponding author: trofimova_s_v@mail.ru

Abstract. The effect of gas-discharge plasma radiation on the biochemical parameters of blood and urine of intact animals and animals with acute alcohol intoxication was investigated. Measurements of the content of medium-mass molecules (MMM), creatinine, urea, glucose, lactate and hemoglobin in the blood and 14 physicochemical indicators of the urine test were carried out. Acute alcohol intoxication (AAI) was simulated by intraperitoneal injection of 33% ethanol at a dose of LD50. The treatment was carried out by an experimental pulsed (10 Hz) device generating radiation of spark discharge plasma. It was revealed that exposure to plasma radiation does not lead to changes in the biochemical parameters of blood and urine of intact animals. In the model of AAI, the development of protein-creatininia, urobilinogenuria, and an increase in the specific gravity of rat's urine was observed. The total level of MMM and hemoglobin in the blood increased, lactic acidosis developed. After exposure to plasma radiation in animals with AAI, blood and urine parameters normalized, which is probably associated with the activation of the organism's adaptive and antioxidant reserves. Thus, the positive effect of spark discharge plasma radiation on the organism during acute ethanol intoxication has been shown.

Keywords: acute alcohol intoxication, ethanol, radiation of gas-discharge plasma, detoxification.

List of Abbreviations

MMM – medium-mass molecules,
AAI – acute alcohol intoxication,
LD50 – 50% lethal dose,
PR – plasma radiation of a spark discharge
AU – arbitrary units of optical density
Hsp70 – heat shock protein

Introduction

A nonequilibrium plasma is a weakly ionized gas that contains charged particles (electrons, positively and negatively charged ions), neutral particles (atomic and/or molecular radicals and non-radicals), and electric fields. The plasma discharge also emits radiation with wavelengths in the infrared, visible, and ultraviolet wavelengths. The biological effects of gas discharge plasma are primarily due to the formation of primary chemically active species such as hydroxyl ($OH^{\bullet}$), atomic oxygen ($O^{\bullet}$), singlet delta oxygen ($O_2(^1\Delta)$), superoxide ($O_2^{\bullet-}$), ozone (O_3), hydrogen peroxide (H_2O_2), nitrogen oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO^{\bullet}_2$), and also secondary and tertiary compounds such as nitrite (NO_2^-), nitrate (NO_3^-), peroxynitrite ($ONOO^-$), peroxynitrous acid (ONOOH), organic radicals ($RO^{\bullet}$) formed in reactions with biological objects (Piskarev, 2012; Laroussi, 2021). Despite the fact that the mechanisms of action of plasma on various objects are still being studied, it has already found its wide application in dermatology, cosmetology, dentistry, surgery, regenerative medicine, experimental and clinical oncology (Sladek *et al*., 2004, Stoffels *et al*., 2006, Fridman *et al*., 2008, Jo *et al.*, 2022; Chen *et al*., 2017; Keidar, 2015; Haralambiev *et al*., 2020; Tabuchi *et al*., 2016).

The plasma discharge acts directly in the area of contact with a biological or other object. The radiation of gas-discharge plasma acts at a distance from the discharge area, and according to research, it is able to penetrate the skin (Piskarev, 2017). When working with plasma

radiation, the exposure time can vary widely, since there is no heating of the treated surface. Exposure is dose-dependent. High doses have a cytotoxic effect. At the same time, lower doses of exposure by gas-discharge plasma are able to activate fibroblast proliferation, improve vascular microcirculation and stimulate antioxidant blood systems (Martusevich *et al*., 2022); this indicates the possibility of using plasma as a factor that stimulates the reserve and adaptive capabilities of the organism against the background of various pathological conditions, including poisoning of various etiologies.

The aim of the investigation was to study the effect of gas-discharge plasma radiation on the biochemical parameters of blood and urine of intact animals and those with acute alcohol intoxication.

Materials and Methods

24 adult male albino rats weighing 275 ± 30 g were examined for the experimental part of the research. The researches were made in accordance with rules of execution of works and use of experimental animals established by European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes of March 18, 1986; and by normative documents represented in "Guide for care and use of laboratory animals. ILAR publication, 1996, National Academy Press", and by the Local Ethics Committee of Institute of Biology and Biomedicine «Lobachevsky State University of Nizhny Novgorod».

Animals were divided into the following groups: 1 – intact animals «Control» (n = 6); 2 – animals treated with plasma radiation of a spark discharge «Control + PR» (n = 6); 3 – animals with acute alcohol intoxication «AAI» (n = 6); 4 – animals with acute alcohol intoxication treated with plasma radiation of a spark discharge «AAI + PR» (n = 6).

Acute alcohol intoxication in the «AAI» and «AAI + PR» groups was modeled by a single intraperitoneal injection of a 33% ethanol solution at a dose of LD50. Animals in the «Control» and «Control + PR» groups were given a single intraperitoneal injection of a physiological solution instead of ethanol in a similar volume.

During the early toxigenic phase, after the onset of the narcotic effect of ethanol, plasma radiation was applied to the area of the abdominal wall of rats in the «Control + PR» and «AAI + PR» groups for 600 seconds at a distance of 2 centimeters from the skin surface to the electrodes.

The generation of the discharge plasma radiation was carried out by the pulsed device «Pilimin», which has the following characteristics: 100 μs - the duration of one pulse, 11 kV – high voltage, 5.9 x 10^{-2} J – the energy in one pulse, 10 Hz – the pulse frequency. The electrodes of the plasma radiation generator with a diameter of 2 mm and a length of 15 mm (3 mm is the approximate distance between the electrodes) are made of stainless steel. The duration of the leading edge of the spark discharge is 50 ns. The plasma spark discharge cord emitted in a wide optical range of 200-800 nm with a maximum at 220 nm. The Pilimin device was developed by I.M. Piskarev, a leading researcher at the Skobeltsyn Institute of Nuclear Physics of Lomonosov Moscow State University in 2011 (Piskarev, 2012).

24 hours after treatment, a single portion of urine was taken from the animals, then the animals were injected with heparin intraperitoneally and blood was obtained for analysis.

To assess the level of endogenous intoxication molecules of medium mass (MMM) were determined spectrophotometrically at wavelengths (λ) of 254, 260 and 280 nm in the plasma blood and erythrocytes (Akimenko *et al*, 2019) The concentration was expressed in arbitrary units (AU) of optical density

The concentration of urea and creatinine was studied using test kits manufactured by «DIACON JSC» «UREA KT DDS» by the endpoint urease method and «CREATININE DDS» by the Jaffe kinetic method without deproteinization, respectively.

All spectrophotometric measurements were carried out on a spectrophotometer of the SF-2000 type (Russia, OKB Spektr).

Using of an automatic analyzer SUPER GL COMPACT (Dr. Müller, Germany), the molar concentration of glucose and lactate was measured by the electrochemical method and the

mass concentration of hemoglobin by the photometric method with sodium dodecyl sulfate.

Urinalysis was carried out by dry chemistry using «DIRUI H14-Ca» reagent strips and a Dirui H-100 urine analyzer (DIRUI, China). 14 parameters were tested: leukocytes, nitrites, urobilinogen, protein, pH, blood, specific gravity, ketones, glucose, bilirubin, microalbumin, ascorbic acid, creatinine, calcium.

The data obtained were processed statistically using the Statistica 10.0 for Windows software package. The calculation included determining the mean values and standard error of the mean. Differences were considered significant at a significance level of p<005 The results are presented as the mean and standard error.

Results

In research practice, the study of endogenous intoxication indicators is used to identify and assess the degree of metabolic disorders in the organism. After exposure of gas-discharge plasma radiation in the organism of intact animals (the «Control + PR» group), there were no statistically significant differences in the content of MMM with the «Control» group, which was not exposed (Fig. 1A, B). In the simulation of acute alcohol intoxication in the blood plasma of animals of the «AAI» group, a general trend towards an increase in the level of MMM was found, statistically significant changes were revealed at wavelengths of 254 nm and 280 nm (Fig. 1A). In the erythrocyte samples of animals of the «AAI» group, the opposite trend towards a decrease in MMM was revealed, statistically significant changes were registered at a wavelength of 260 nm (Fig. 1B).

It is known that the pool of endogenous toxins (medium-mass molecules - MMM) is divided into three fractions: toxic, nuclear and aromatic. At a wavelength of 254 nm, a toxic fraction is determined, consisting of creatinine, urea, amino acids, nucleotides, and other non-protein substances of various nature, which are intermediate metabolism products, including products of free radical oxidation. At 260 nm, the nuclear fraction is determined, which is represented by nucleoprotein degradation products (L-guanosine, 6-thioguanine) (Akimenko *et al*., 2019). The increase in the nuclear fraction may be due to the accumulation of nucleic acid residues in the blood as a result of cell destruction (Bohan *et al*., 2012). The aromatic fraction is determined at a wavelength of 280 nm and is mainly represented by proteins containing aromatic amino acids such as tryptophan, tyrosine, and phenylalanine (Akimenko *et al*., 2019). Normally, aromatic amino acids are decarboxylated in the intestines under the action of bacterial decarboxylases with the formation of biologically active amines, which, entering through the portal vein to the liver, undergo oxidative deamination. It is likely that an increase in the level of endogenous plasma blood toxins at this wavelength may indicate a violation of the detoxification functions of the liver (Pavlova *et al*., 2011).

As endotoxins are normally removed from the organism by the kidneys through glomerular filtration, their accumulation in the blood plasma may indicate impaired renal excretory function on the one hand, and on the other hand, such changes may be a consequence of a decrease in the detoxification function of the liver against the background of acute alcohol intoxication. Also known is that under the influence of endogenous toxins, the ability of erythrocytes to sorb increases significantly, which is necessary for the redistribution of endotoxins between plasma and erythrocytes and constitutes one of the stages of endogenous intoxication formation. The general trend towards the accumulation of a catabolic pool in the blood plasma of animals and a decrease in MMM on the erythrocyte membrane may be associated with a decrease in the sorbtion capacity of erythrocytes due to the fluidizing and hemolytic effect of ethanol (Belov, 2013).

In the study of the effect of gas-discharge plasma radiation on rats with acute alcohol intoxication of the «AAI + PR» group has been found that treatment of animals in the early toxigenic phase of acute ethanol poisoning prevents the development of endotoxicosis: the content of MMM in the blood plasma and erythrocytes of the animals of the «AAI + PR» group did not differ statistically significantly from the values of the «Control» group (Fig. 1A, B).

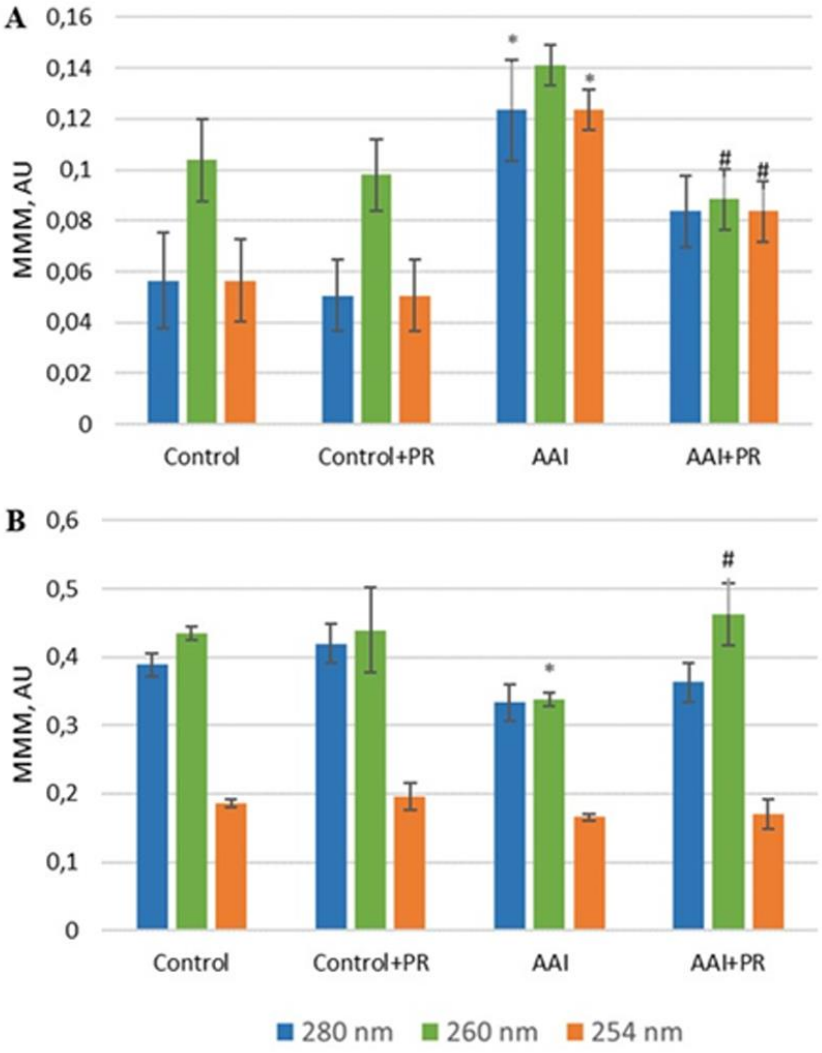

Fig. 1. MMM content in blood plasma (A) and erythrocytes (B) of rats
* – the differences are statistically significant relative to the «Control» group ($p < 0.05$)
# – the differences were statistically significant relative to the «AAI» group ($p < 0.05$)

Urea and creatinine are the end products of protein catabolism and are excreted from the organism by the kidneys. Thus, the level of these biomarkers directly reflects how productively the kidneys cope with their excretory function, and their increase in blood indicates a decrease in glomerular filtration. When assessing the concentration of creatinine by the Jaffe method and urea by the urease method in the blood plasma of animals, statistically significant changes were not revealed in any of the experimental groups (Fig. 2). However, there was a tendency to increase the concentration of creatinine in the «AAI» group relative to the «Control» group by 23%. Thus, it can be concluded that, firstly, the effect of gas discharge plasma radiation does not have a nephrotoxic effect, and secondly, modeling acute alcohol intoxication does not lead to suppression of excretory function, despite the fact that some pathological changes in the kidneys are indicated in the literature with acute alcohol intoxication (Kovalev *et al*., 2022). Also, based on the obtained results, it can be concluded that the increase in the content of MMM in the blood plasma on the background of acute alcohol intoxication, registered spectrophotometrically at 254 nm (Fig. 1A), occurred not due to urea and creatinine, but mainly due to amino acids, nucleotides and products of free radical oxidation.

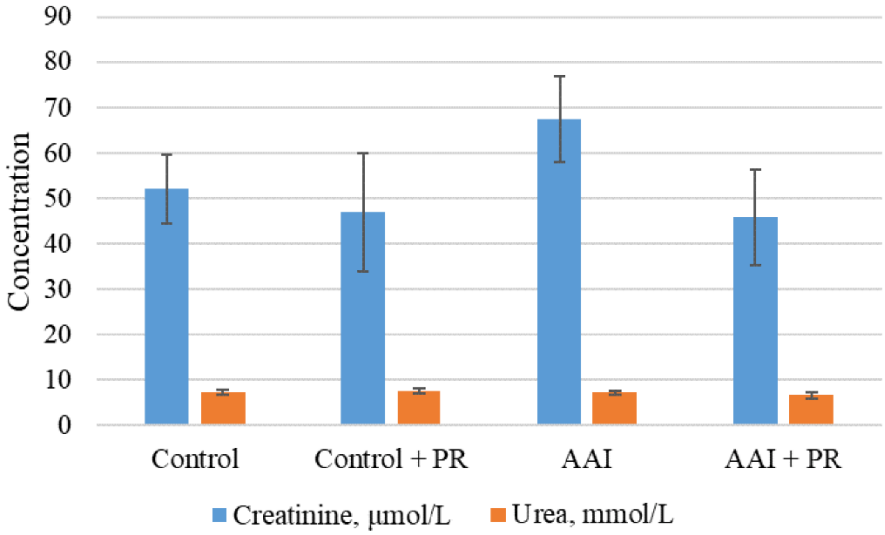

Fig. 2. Concentration of urea and creatine in the blood plasma of experimental animals

Using the SUPER GL COMPACT analyzer (Germany, Dr. Müller), the content of glucose, lactic acid and hemoglobin in the blood of experimental animals was determined. Glucose concentrations did not change in the experimental groups (Fig. 3). It was also found that the concentration of hemoglobin and lactic acid in the blood of intact animals of the «Control + PR» group did not change after treatment relative to the «Control» group (Fig. 3). In the group of animals «AAI» with acute alcohol intoxication, there was a 2-fold increase in the concentration of lactic acid and hemoglobin by 1.3 times compared to the «Control» group. The data obtained indicate the development of lactic acidosis against the background of acute alcohol intoxication, which is associated with an increase in the NADH/NAD^+ ratio and the conversion of pyruvic acid into lactic acid during ethanol metabolism (Kursov *et al*., 2012). The decrease in hemoglobin concentration during acute alcohol intoxication is likely due to the fact that ethyl alcohol alters the composition of the lipid membrane of erythrocytes - the ratio of cholesterol/phospholipids increases, which causes hemolysis of erythrocytes (Belov, 2013). Hemoglobin released from hemolyzed erythrocytes is subsequently metabolized to ferrous iron ions Fe^{2+}, which explains its low content in animals of the «AAI» group (Soboleva & Orlov, 2011).

After exposure by plasma radiation on animals in the early toxigenic phase of alcohol intoxication in the «AAI + PR» group, lactate values were 2 times lower, and hemoglobin values were 1.5 times higher compared to animals with acute alcohol intoxication in the «AAI» group that were not exposed, the values of these parameters corresponded to the values of the «Control» group (Fig. 3). Thus, exposure of gas-discharge plasma radiation in the early toxigenic phase prevents the development of toxic hemolytic anemia and lactic acidosis in the organism of animals against the background of acute alcohol intoxication. It is likely that normalization of biochemical parameters in the blood plasma of animals with acute alcohol intoxication after exposure of gas discharge plasma radiation occurs due to the activation of blood antioxidant systems (Martusevich *et al*., 2017) and indicates the stimulation of the body's detoxification systems. A decrease in the concentration of lactic acid in the blood may also occur due to an increase in the level of the oxidized form of NAD^+ after treatment of animals with plasma radiation (Dong *et al*., 2017).

Thus, exposure of gas-discharge plasma radiation on the abdominal wall of experimental animals does not cause changes in the studied biochemical parameters of blood, but at the same time normalizes the intensity of oxidative processes in the blood of animals against the background of acute alcohol intoxication.

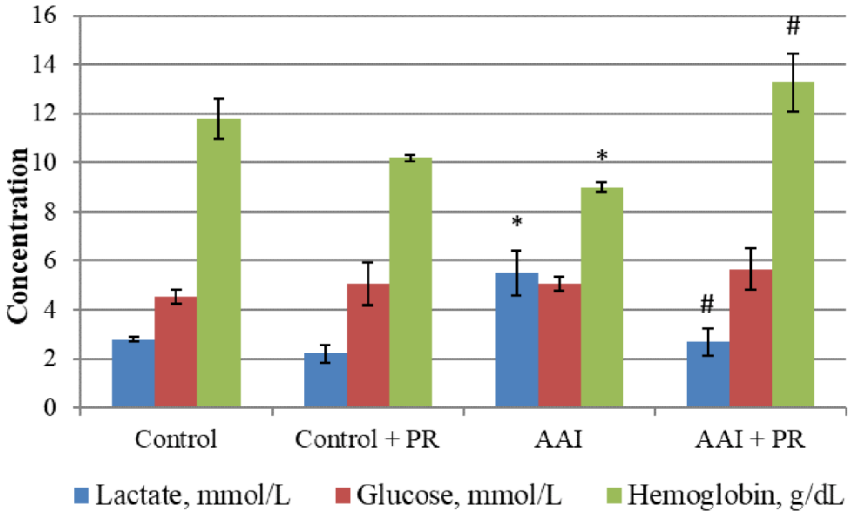

Fig. 3. Concentration of glucose, lactate and hemoglobin in whole blood of experimental animals
* – the differences are statistically significant relative to the "Control" group ($p < 0.05$)
# – the differences are statistically significant relative to the "AAI" group ($p < 0.05$)

With the help of urine analyzer H-100 (China, DIRUI), a clinical analysis of physicochemical and biochemical parameters of animal urine was performed. The analysis revealed no intergroup differences and deviations from the normal values of the following parameters: leukocytes, nitrites, pH, blood, ketones, glucose, bilirubin, microalbumin, ascorbic acid, calcium. It was found that exposure of gas-discharge plasma radiation did not lead to statistically significant changes in general clinical parameters in a single urine sample of animals of the «Control + PR» group compared to the «Control» group (Table 1). In the urine of animals with alcohol intoxication of the group «AAI», an increase in the concentration of creatinine by 2.2 times, urobilinogen by 2.4 times, and urine specific gravity from 1021 ± 292 to 1029 ± 1 compared to the control group of animals without exposure (Table 1). The concentration of protein in the urine of all animals of the «AAI» group exceeded 0.3 g/L, which indicates the development of moderate and pronounced proteinuria in 100% of animals in the group (Table 2). Exposure to radiation of gas-discharge plasma in the early toxigenic phase prevented the development of protein-, creatinine- and urobilinogenuria in animals under the influence of ethanol and its metabolites against the background of acute intoxication. In animal urine samples of the «AAI + PR» group, creatinine concentration values were registered 2.9 times lower, urobilinogen concentration values were 6.3 times lower than those of the «AAI» group, urine specific gravity decreased to the level of 1015 ± 0.43, protein concentration in a single portion of urine exceeded normal values only in 33% of animals and had a moderate character (Tables 1, 2).

Table 1

Indicators of general clinical analysis of urine for experimental animals

Parameters	Control	Control + PR	AAI	AAI + PR
Urobilinogen, μmol/L	8.84 ± 3.33	5.67 ± 2.27	21.25 ± 4.25*	3.4 ± 0#
Creatinine, mmol/L	8.8 ± 0	6.77 ± 2.4	19.73 ± 2.3*	6.75 ± 1.4#
Specific gravity	1021 ± 2.92	1020 ± 2.74	1029 ± 1*	1015 ± 0.43#

Note: * – the differences are statistically significant relative to the «Control» group ($p < 0.05$)
– the differences are statistically significant relative to the «AAI» group ($p < 0.05$)

Table 2

Percentage distribution of animals in groups (100%) by heaviness of proteinuria

Protein concentration in urine sample, g/l	Control	Control + PR	AAI	AAI + PR
0	16.7%	33.3%	–	16.7%
0–0.3	33.3%	66.7%	–	50%
0.3–1.0	16.7%	–	16.6%	16.7%
1–3.0	33.3%	–	50%	16.7%
≥ 3.0 g/l	–	–	33.3%	–

According to the literature, it is known that acute alcohol intoxication caused by a single injection of ethanol leads to the development of morphological changes in the liver and kidney tissue, indicating the toxic and destructive effects of ethanol. Pathological changes at the cellular, tissue, and organ levels are characterized by varying degrees of severity, directly depending on the dose of ethanol injected (Zoroastrov, 2004; Alyabyev *et al*., 2012). Its main target in the case of acute alcohol poisoning is the proximal tubules. Their damage leads to impaired protein reabsorption, and, consequently, the development of proteinuria, and an increase in the specific gravity of urine (Reine & Langston, 2005; McGlynn *et al.*, 2023).

However, in our experiment, there was no increase in the concentration of urea and creatinine in the blood plasma, which indicates that there is no decrease in renal function. But, on the other hand, the concentration of urea and creatinine in the blood serum, although it reflects the glomerular filtration rate, is not an indicator of minor disorders of kidney function in the early stages of the pathological process. This is because the levels of these metabolites in the blood do not increase significantly until the kidneys have lost their function (to produce primary urine) by 50%. Creatinine is a «threshold-free» substance, so it is normally freely filtered by the glomeruli and excreted in the urine. There are no statistically significant changes in this indicator in the blood plasma (Fig. 2) when its concentration increases 2.2 times in the urine of animals in the «AAI» group, indicating a normal glomerular filtration rate, and probably related to alcohol-induced muscle damage (Mora *et al*, 2008). It can be assumed that toxic myolysis of skeletal muscles against the background of acute alcohol intoxication is accompanied by the release of creatinine and myoglobin from muscle fibers, which leads to the development of prerenal proteinuria (Kazantseva *et. al.*, 2012).

On the one hand, an increased level of urobilinogen in the urine of rats of the «AAI» group may indicate a violation of the functional state of the liver. It is formed from bilirubin, which in turn is formed in the body by the catabolism of red blood cells and the subsequent degradation of heme. Albumin transports bilirubin to hepatocytes, where it is converted to the water-soluble bilirubin diglucuronide and excreted in the bile. Gut bacteria break down bilirubin so that it can be reabsorbed or further metabolized into urobilinogen. Some of the urobilinogen is also reabsorbed from the intestine and enters the portal blood. Normally, most of the reabsorbed urobilinogen is excreted by the liver (Song *et al.*, 2021; Wang *et al.*, 2006). The first stage of liver parenchyma damage is characterized by the loss of the ability of liver cells to enzymatically break down urobilinogen, which enters through the portal vein, and the development of urobilinogenemia and urobilinogenuria (Chindakar *et al.*, 2014; Stisova & Jirsa, 2013). On the other hand, an increase in urobilinogen in the urine may be a consequence of increased hemolysis of red blood cells under the influence of ethanol (Belov, 2013).

Discussion

According to the results of the study, it was found that the radiation of gas-discharge plasma with the studied characteristics does not have a damaging effect on the organism of intact animals, as evidenced by the absence of changes in the blood and urine parameters of animals after treatment.

It was also shown that after the simulation of acute alcohol intoxication in rats, the level of MMM in blood plasma increased, lactic acidosis, hemolytic anemia, creatine-, protein- and urobilinogenuria developed.

Exposure of gas-discharge plasma radiation leads to normalization of the urinalysis, the level of lactate, hemoglobin and MMM in the blood of animals with of acute alcohol intoxication.

It is probable that a 10-minute single treatment with gas-discharge plasma radiation prevents the damaging effects of ethyl alcohol and its metabolites due to two mechanisms: stimulation of the organism's antioxidant systems and generation of non-toxic concentrations of NO•, which have a bioregulatory effect on most types of cells.

It is known that NO• provides a regulatory effect on the functional activity of the cardiovascular, immune, digestive, genitourinary systems, and plays a mediator function in various brain structures. NO• can act as a stress-limiting factor by limiting the release of pituitary stress hormones, the release of catecholamines in synaptic structures and from the adrenal glands. Also, nitric oxide can have a protective effect, exhibiting independent anti-oxidant properties, effectively intercepting radicals such as O2•–, OH•, ROO•, thiyl (GS•) and inhibiting Fe3+-mediated oxidative reactions. NO• increases the activity of antioxidant enzymes and the expression of genes, encoding them, and also activates the synthesis of protective proteins Hsp70 (Chesnokova *et al.*, 2006).

In addition, the oxide can improve renal hemodynamics, glomerular filtration, supports vasodilation, which has a positive effect on kidney function and can stimulate the excretion of ethanol and its toxic metabolites (Kuznetsova & Solovyova, 2015).

Thus, it can be concluded that the antioxidant and detoxification effects of gas-discharge plasma are most likely mediated through nitric oxide, however, this assumption requires additional research to better understand the mechanisms of biological action of spark discharge plasma radiation by in vivo experiments.

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