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Effect of a glutathione-containing dinitrosyl iron complex on the oxidative metabolic state and crystallogenic properties of rat blood plasma: a preclinical experimental study

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ABSTRACT

Background: The multifaceted regulatory role of nitric oxide in biological systems predetermines the high value of studying the possibilities of the external control of the compound level in organs and tissues. There are several fundamentally different ways of exogenous modulation of nitric oxide metabolism. The most promising option is the use of pharmacological donors. Dinitrosyl iron complexes (DNIC) with various ligands hold a prominent place among such donors as they are considered as a natural deposited form of nitric oxide. **Objective.** To study the effect of a glutathione-containing dinitrosyl iron complex on the oxidative metabolism parameters and crystallogenic activity of rat blood. **Methods.** A preclinical experimental randomized study was conducted on 60 sexually mature male Wistar rats weighing about 250 g. The animals were divided into 6 groups, each consisting of 10 individuals. Group 1 included intact (without any manipulations) individuals. In group 2, the rats were administered daily intraperitoneal injections of 1 ml. of 0.9% sodium chloride solution for 10 days. The rats included in the other four groups received daily intraperitoneal injections of 1 ml of dinitrosyl iron complexes with glutathione ligands in an isotonic sodium chloride solution with different agent concentrations: 0.15 mM for group 3; 0.30 mM for group 4; 0.45 mM for group 5; 0.60 mM for group 6. The final indicator of the study was the assessment of the oxidative potential and crystallogenic properties of blood under the conditions of administering various doses of glutathione-containing dinitrosyl iron complexes. The following parameters were used to assess the activity of pro- and antioxidant systems: lipid peroxidation intensity; the total activity of antioxidant systems, and malondialdehyde concentration. The parameters for intrinsic crystallization assessment included serum facies structural index, crystallizability, assessment of the marginal facies zone, and the destruction degree of facies elements. The obtained data calculation was performed using the software packages MS Office 2013 (Microsoft Corporation, USA) and Statistica 10 (StatSoft, USA). **Results**. The research established that glutathione-containing dinitrosyl iron complexes have an antioxidant effect. Moreover, the manifestation of these properties demonstrates a nonlinear dependence on their dose, with a possible optimum lying in the range of 0.3–0.45 mM. The study also revealed a tendency towards crystallogenic properties activation induced by this agent, corresponding to concentrations of 0.3 and 0.45 mM. **Conclusion.** The undertaken studies indicate the presence of an antioxidant effect in glutathione-containing dinitrosyl iron complexes. The manifestation of these properties demonstrates a dependence on their dose with a possible optimum varying from 0.3 to 0.45 mM. The research has established the activating effect of glutathione-containing dinitrosyl iron complex injections on the crystallogenic potential of the blood serum of healthy rats. This effect consisted in an increase in the density and complexity of crystalline elements. What is more, the maximal manifestation of this tendency (for metabolic indicators as well) corresponded to concentrations of 0.3 and 0.45 mM.

KEYWORDS: nitric oxide, dinitrosyl iron complex, blood plasma, free radical oxidation, crystallization

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Влияние глутатион-содержащего динитрозильного комплекса железа на состояние окислительного метаболизма и кристаллогенные свойства плазмы крови крыс: доклиническое экспериментальное исследование

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АННОТАЦИЯ

Введение. Многогранная регуляторная роль оксида азота в биологических системах предопределяет высокую ценность изучения возможностей внешнего управления уровнем соединения в органах и тканях. Существует несколько принципиально различных путей экзогенной модуляции метаболизма оксида азота. В частности, наиболее перспективным является применение фармакологических доноров. Особое место среди последних принадлежит динитрозильным комплексам железа с различными лигандами, рассматриваемым как естественная депонированная форма оксида азота. **Цель исследования** — изучение действия глутатион-содержащего динитрозильного комплекса железа на параметры окислительного метаболизма и кристаллогенную активность крови крыс. **Методы.** Доклиническое экспериментальное рандомизированное исследование проведено на 60 половозрелых крысах-самцах линии Wistar массой около 250 г. Животные были разделены на 6 групп, состоящих из 10 особей: группа 1 — интактная (без каких-либо манипуляций), группа 2 — в течение 10 дней ежедневно крысам осуществляли внутрибрюшинное введение 1 мл 0,9% раствора хлорида натрия. Крысам, включенным в остальные группы, в течение 10 дней ежедневно осуществляли внутрибрюшинное введение 1 мл динитрозильных комплексов железа с глутатионовыми лигандами в изотоническом растворе хлорида натрия с различной концентрацией агента: 0,15 мМ — группа 3; 0,30 мМ — группа 4, 0,45 мМ — группа 5 и 0,60 мМ — группа 6. Итоговым показателем исследования явилась оценка окислительного потенциала и кристаллогенных свойств крови в условиях введения различных доз глутатион-содержащих динитрозильных комплексов железа. В качестве оценочных параметров активности про- и антиоксидантных систем использовали: интенсивность перекисного окисления липидов; общую активность антиоксидантных систем, концентрацию малонового диальдегида. Параметрами оценки собственной кристаллизации служили: индекс структурности фасции, кристаллизуемость, оценка краевой зоны фасции и степень деструкции элементов фасции. Расчет полученных данных проводили при использовании программных комплексов MS Office 2013 (Microsoft Corporation, США), Statistica, v. 10 (StatSoft, США). **Результаты.** В ходе проведенных исследований установлено наличие антиоксидантного эффекта у глутатион-содержащих динитрозильных комплексов железа, причем выраженность этих свойств демонстрирует нелинейную зависимость от их дозы с возможным оптимумом, лежащим в диапазоне 0,3–0,45 мМ. Также выявлена тенденция к активации кристаллогенных свойств данным агентом, соответствующая концентрациям 0,3 и 0,45 мМ. **Заключение.** Проведенные исследования свидетельствуют о наличии антиоксидантного эффекта у глутатион-содержащих динитрозильных комплексов железа, причем выраженность этих свойств демонстрирует зависимость от их дозы с возможным оптимумом, лежащим в диапазоне 0,3–0,45 мМ. Установлено активирующее действие инъекций глутатион-содержащих динитрозильных комплексов железа на кристаллогенный потенциал сыворотки крови здоровых крыс, проявляющееся в увеличении плотности кристаллических элементов и их усложнении, причем, как и для метаболических показателей, максимальная выраженность данной тенденции соответствовала концентрациям 0,3 и 0,45 мМ.

КЛЮЧЕВЫЕ СЛОВА: оксид азота, динитрозильный комплекс железа, плазма крови, свободнорадикальное окисление, кристаллизация

ДЛЯ ЦИТИРОВАНИЯ: Мартусевич А.К., Суровегина А.В., Кононец В.В., Давыдюк А.В., Перетягин С.П. Влияние глутатион-содержащего динитрозильного комплекса железа на состояние окислительного метаболизма и кристаллогенные свойства плазмы крови крыс: доклиническое экспериментальное исследование. *Кубанский научный медицинский вестник.* 2023;30(6):28–40. https://doi.org/10.25207/1608-6228-2023-30-6-28-40

ИСТОЧНИКИ ФИНАНСИРОВАНИЯ: авторы заявляют об отсутствии спонсорской поддержки при проведении исследования.
КОНФЛИКТ ИНТЕРЕСОВ: авторы заявили об отсутствии конфликта интересов.
ДЕКЛАРАЦИЯ О НАЛИЧИИ ДАННЫХ: данные, подтверждающие выводы этого исследования, можно получить по запросу у корреспондирующего автора.

СООТВЕТСТВИЕ ПРИНЦИПАМ ЭТИКИ: проведение экспериментального исследования одобрено на заседании локального этического комитета федерального государственного бюджетного образовательного учреждения высшего образования «Нижегородский государственный агротехнологический университет» (пр. Гагарина, 97, г. Нижний Новгород, 603117, Россия), протокол № 2 от 17.02.2017 г. Условия содержания животных и работы с ними соответствовали руководству ARRIVE (Animal Research: Reporting of In Vivo Experiments) и правилам работы с животными на основе положений Хельсинкской декларации и рекомендаций, содержащихся в Директиве ЕС 86/609/ЕСС и Конвенции Совета Европы по защите позвоночных животных, используемых для экспериментальных и других научных целей.

ВКЛАД АВТОРОВ: А. К. Мартусевич, А. В. Суровегина, В. В. Кононец, А. В. Давыдюк, С. П. Перетягин — разработка концепции и дизайна исследования; А. В. Суровегина, В. В. Кононец, А. В. Давыдюк — сбор данных; А. К. Мартусевич, А. В. Суровегина — анализ и интерпретация результатов; А. К. Мартусевич, С. П. Перетягин — обзор литературы, проведение статистического анализа; А. К. Мартусевич, А. В. Суровегина — составление черновика рукописи и формирование его окончательного варианта; В. В. Кононец, А. В. Давыдюк, С. П. Перетягин — критический пересмотр черновика рукописи. Все авторы одобрили финальную версию статьи перед публикацией, выразили согласие нести ответственность за все аспекты работы, подразумевающую надлежащее изучение и решение вопросов, связанных с точностью и добросовестностью любой части работы.

✉**КОРРЕСПОНДИРУЮЩИЙ АВТОР:** Мартусевич Андрей Кимович, доктор биологических наук, доцент, руководитель лаборатории медицинской биофизики Университетской клиники федерального государственного бюджетного образовательного учреждения высшего образования «Приволжский исследовательский медицинский университет» Министерства здравоохранения Российской Федерации; профессор кафедры физиологии и биохимии животных и акушерства федерального государственного бюджетного образовательного учреждения высшего образования «Нижегородский государственный агротехнологический университет». Адрес: пл. Минина и Пожарского, д. 10/1, г. Нижний Новгород, 603000, Россия. E-mail: cryst-mart@yandex.ru.

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INTRODUCTION

The multifaceted regulatory role of nitric oxide (NO) in biological systems predetermines the importance of examining the possibilities of controlling the level of this compound in organs and tissues externally. Besides its most well-known biological effect, namely vasodilatory activity, the study also shows the participation of this compound in neurotransmission, modification of blood coagulation processes, intracellular killing during phagocytic *respiratory burst*, membranotropic activity, and so on [1–4]. Moreover, it is important to note the extremely short lifetime of a nitrogen monoxide molecule, averaging six seconds in the free state [5]. This imposes strict requirements on the metabolism regulation of the compound and highlights the need for the presence of substances that temporarily deposit NO or create conditions for its synthesis (if necessary) in the organism [7–8].

Currently, there are several fundamentally different ways of exogenous modulation of NO metabolism. These include administering the L-arginine substrate of NO synthase, using selective inhibitors of this enzyme which influence the release of the compound, as well as employing a wide range of pharmacological donors [9–11]. Dinitrosyl iron complexes (DNICs) with various ligands hold a special place among such donors since they are considered to be a natural deposited form of nitric oxide [12–15]. At the same time, the biological effects of exogenous DNICs are not sufficiently elaborated yet [16]. Experimental data obtained by *in vivo* studies suggest that DNICs have marked antioxidant properties [17,18]. This hypothesis was confirmed by modeling oxidative stress *in vitro* (by introducing highly concentrated ozonized saline solution into bodily fluid samples) and *in vivo* (by modeling thermal injury in rats) [19]. On the other hand, the abovementioned data need to be confirmed *in vivo* and in healthy animals.

The research aims to study the effect of a glutathione containing dinitrosyl iron complex on the oxidative metabolism parameters and crystallogenic activity of rat blood.

METHODS

Experimental animals

The experiment was conducted on 60 sexually mature male Wistar rats weighing about 250 g. The animals were obtained from the Stolbovaya breeding nursery, a branch of the Scientific Center for Biomedical Technologies of the Federal Medical-Biological Agency, in the autumn-winter period.

Housing and welfare

The animals were kept in the vivarium of the University Experimental Biological Clinic at Privolzhsky Research Medical University of the Ministry of Health of the Russian Federation (hereinafter referred to as the vivarium) in accordance with the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines and the rules for working with animals based on the provisions of the Declaration of Helsinki, the recommendations contained in EC Directive 86/609/ECC, and the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes. The animals were fed the standard water and food diet with free access to food and water.

Study design

The study was randomized. Drug administration and biological material collection were conducted in the vivarium. The laboratory stage of the research was performed at the Medical Biophysics Laboratory of the University Clinic at Privolzhsky Research Medical University of the Ministry of Health of Russia. Fig. 1 shows the block diagram of the study design.

Sample size

The animals were divided into 6 groups with 10 individuals in each group using the envelope method. Group 1 in-

cluded intact (without any procedures) individuals. In group 2, the rats were administered daily intraperitoneal injections of 1 ml. of 0.9% sodium chloride solution for 10 days. The rats included in the other four groups received daily intraperitoneal injections of 1 ml of dinitrosyl iron complexes with glutathione ligands in saline with different agent concentrations: 0.15 mM for group 3; 0.30 mM for group 4; 0.45 mM for group 5; 0.60 mM for group 6. The prespecified analysis of the normal distributions for the age and weight variables of the rats in the groups using the Shapiro-Wilk test showed that there is no normal distribution (Gaussian) law in three age groups ($p < 0.05$) and in one weight group ($p < 0.05$). To prove that the age and weight of the rats were uniform, a nonparametric comparison method, namely the Kruskal-Wallis test, was employed. Table 1 presents the central tendency data in the form of the median and quartiles (Q1 — the first quartile or the 25th percentile and Q3 — the third quartile or the 75th percentile).

For age and weight, the differences in the median values for different groups of rats were not statistically significant with $p = 0.253$ and $p = 0.778$, respectively.

Eligibility Criteria

Inclusion criteria

Two-month-old male Wistar rats weighing about 250 g, without visible physical development abnormalities and injuries were included in the study.

Exclusion criteria

Animals weighing more than 250 ± 1 g, aged less than 56 and more than 64 days, female individuals, as well as animals with visualized developmental abnormalities and injuries were not included in the study.

Randomization

60 animals were selected according to the inclusion and exclusion criteria. The animals were allocated to groups randomly, namely by envelope method. Each animal was assigned one of the six group numbers extracted from an opaque envelope containing 60 pieces of paper with the group numbers. Depending on the group number indicated in the envelope, all animals were divided into six groups of 10 animals each.

Blinding

The head of the study, A. K. Martusevich, had information about the allocation of animals to groups. The author team

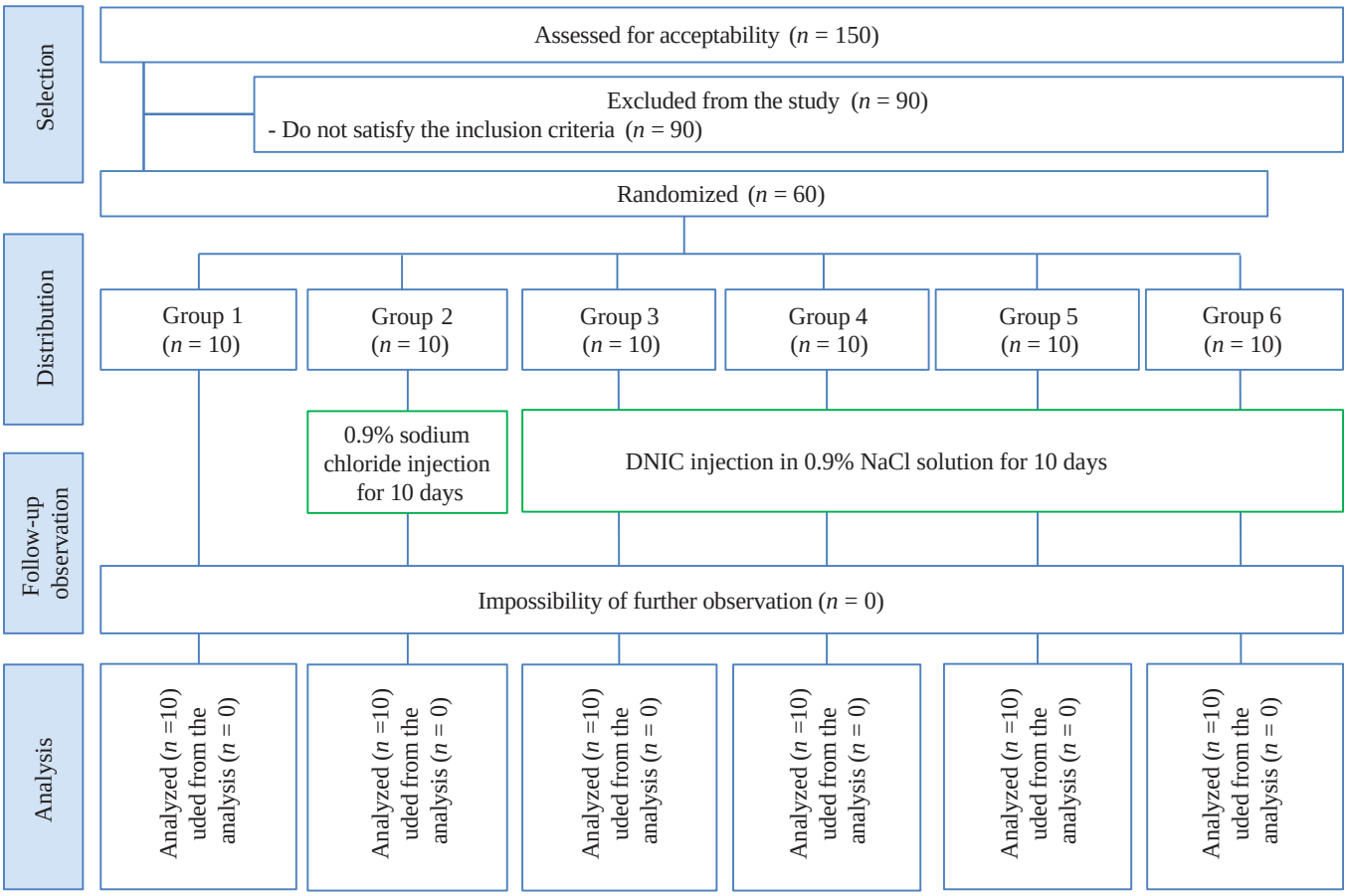

Fig. 1. Block diagram of the research design

Note: the block diagram was created by the authors (in compliance with the ARRIVE quidelines). Abbreviations: NaCl — saline solution; DNICs — dinitrosyl iron complexes with glutathione ligands.

Рис. 1. Блок-схема дизайна исследования

Примечание: блок-схема составлена авторами (согласно рекомендациям ARRIVE). Сокращения: NaCl — изотонический раствор хлорида натрия; ДНКЖ — динитрозильные комплексы железа с глутатионовыми лигандами.

Table 1. Medians, first and third quartiles (*Me* (*Q1-Q3*)) for the age and weight of rats in the study groups
Таблица 1. Медианы, первый и третий квартили (*Me* (*Q1-Q3*)) для возраста и веса крыс в исследуемых группах

Variables	Groups						Kruskal-Wallis test
	1	2	3	4	5	6	
Age (days)	**57.0** (57.0–60.3)	**59.0** (58.0–63.0)	**57.0** (56.3–57.0)	**57.5** (57.0–60.5)	**60.0** (57.0–62.0)	**58.5** (57.0–60.5)	0.253
Weight, g	**249.8** (249.6–250.2)	**250.2** (249.8–250.4**)**	**250.3** (250.0–250.4)	**250.2** (250.0–250.4)	**250.0** (249.7–250.3)	**250.2** (250.2–250.3)	0.778

Note: the table was compiled by the authors.
Примечание: таблица составлена авторами.

assessed the results and analyzed the obtained data without introducing additional persons.

Outcome measures

The study outcome was the assessment of the crystallogenic properties and oxidative potential of blood under conditions of injecting various DNIC doses.

Experimental procedures

The crystallogenic properties and oxidative potential of blood were assessed in bodily fluid (blood). In the animals of all groups, blood samples were obtained from the sublingual vein. In the rats of the first (intact) group, blood samples were collected once, whereas in the representatives of the other groups — twice (before and immediately after the dosing cycle). 1 ml of the studied solutions was administered daily for 10 days.

DNICs with glutathione ligands were synthesized according to the method of A. F. Vanin [20]. The compound concentration in the saline solution was determined spectrophotometrically at wavelengths of 310 and 360 nm by known extinction coefficient and amounted to 3.1 mmol/L.

The activity of pro- and antioxidant systems was studied in rat blood plasma using Fe^{2+}-induced biochemiluminescence (BChL-06, Russia). The following evaluation parameters were used: biochemiluminescence light sum for 30 s, which is usually considered as an indicator of lipid peroxidation (LPO) intensity (arbitrary units). The other parameters included the total antioxidant systems activity (AOA, arb. units) considered as the intensity criterion, as well as the slope of the chemiluminescence kinetic curve tg 2б, and malondialdehyde (MDA) concentration in blood plasma (mmol/L).

The crystallogenic properties of blood serum were studied by classical crystalloscopy. The results of the bodily fluid intrinsic structure were criterially evaluated. We used a specialized system of parameters [21] including crystallizability (CR, points), i. e. the density of the crystalline elements in the specimens mounted on microscope slides; structural index (SI, points), which characterizes the complexity of emerging structures (from amorphous solids to highly branched dendrites); the degree of facies destruction (DFD, points), which indicates the destruction level of the elements of the specimens on microscope slide; the marginal zone pronouncedness (M_z, points).

Animal care and monitoring

The animals were kept under standard vivarium conditions with free access to food and water. At the end of the research, the animals were removed from the experiment under general anesthesia with tiletamine hydrochloride (60 mg/kg) and xylazine hydrochloride (6 mg/kg) administered intramuscularly.

Statistical procedures

Principles of sample size determination

No preliminary sample size calculation was made.

Statistical methods

The numerical samples were tested for compliance with the normal (Gaussian) distribution law using the Kolmogorov-Smirnov or Shapiro-Wilk tests. In case of departure from normality, descriptive statistics were presented as the median and the first and third quartiles of *Me* (*Q1–Q3*). In case of normality, descriptive statistics were presented as mean and standard deviation ($M \pm SD$). When analyzing the influence of a factor on all groups, one-way analysis of variance (ANOVA) was used according to the *F*-test for normally distributed samples. Pairwise comparisons were made using Student's *t*-test for independent samples. When analyzing the influence of a factor on all groups, one-way ANOVA was employed using the Kruskal-Wallis test for samples with a distribution departing from normality. Pairwise comparisons were made using the Mann-Whitney *U* test for independent samples. The statistical significance level was $p \leq 0.05$. Calculations were made using software packages MS Office 2013 (Microsoft Corporation, USA) and Statistica 10 (StatSoft, USA). The values were normalized. The average indicator values for the group of untreated animals are taken as 100%. The data are presented as histograms.

RESULTS

It was found that infusions of saline solution not containing the studied substance had no significant effect on both the intensity of lipid peroxidation in rat blood plasma and on its total antioxidant activity. Conversely, employing a physiological nitric oxide donor in all used dosages changed the values of the abovementioned parameters (Table 2). In particular, the intensity of lipid peroxidation demonstrated a marked statistically significant dependence on the administered DNIC concentration (according to the *F*-test for one-way ANOVA with $p = 0.049$, where the concentration of the DNIC solution acts as a factor).

Table 2. Mean values ($M \pm SD$) of oxidative metabolism parameters in the compared animal groups
Таблица 2. Средние значения ($M \pm SD$) показателей окислительного метаболизма в сравниваемых группах животных

Groups	Oxidative metabolism indicators		
	LPO (arb. units)	**AOA (arb. units)**	MDA (mmol/L)
Gr. 1 intact (n = 10)	10.57 ± 1.89	0.45 ± 0.06	0.96 ± 0.32
Gr. 2 control (n = 10)	10.88 ± 1.98	0.46 ± 0.07	1.02 ± 0.25
Gr. 3 (0.15 mM) (n = 10)	10.32 ± 2.61	0.48 ± 0.07	0.89 ± 0.17
Gr. 4 (0.30 mM) (n = 10)	8.76 ± 2.24	0.56 ± 0.12	0.73 ± 0.10
Gr. 5 (0.45 mM) (n = 10)	7.53 ± 2.45	0.59 ± 0.08	0.83 ± 0.13
Gr. 6 (0.60 mM) (n = 10)	9.61 ± 3.31	0.51 ± 0.05	0.91 ± 0.11
Significance level	**p = 0.049**	**p = 0.005**	**p = 0.050**

Note: the table was compiled by the authors. Abbreviations: Gr. — group; arb. units — arbitrary units of measurement; LPO — lipid peroxidation; AOA — total activity of antioxidant systems; MDA — malondialdehyde concentration.
Примечание: таблица составлена авторами. Сокращения: Гр. — группа; у.е. — условные единицы измерения; ПОЛ — перекисное окисление липидов; АОА — общая активность антиоксидантных систем; МДА — концентрацию малонового диальдегида.

Thus, when the animals were administered a minimal dose of the compound (1 ml of 0.15 mM solution), no significant indicator deviations from the indicator for the intact animal group were observed (p = 0.940). When the concentration of the compound in the solution (0.3 mM and above) was increased, a decrease in the intensity of lipid peroxidation processes was observed, reaching a minimum during a course of infusions of a 0.45 mM DNIC solution, i. e. in the animals of group 5 ($p < 0.05$ by Student's t-test for independent samples between the indicator pairs for group 5 and the indicators for the first, second, and third groups of animals, except for groups 4 and 6). A further increase in the dose of the administered NO donor had a less marked effect on the parameter level, which may be caused by the formation of an excess of the substance due to the partial destruction of complexes with nitric oxide release and the transformation of the latter into peroxynitrite, one of the most powerful oxidizing bioradicals [19, 21].

The mean values of the total blood plasma antioxidant activity were found to be dependent on the DNIC solution concentration. In particular, no significant differences in the indicator were observed in the rats receiving only saline infusions (according to Student's t-test, p = 0.915). When DNIC was added to it in any of the studied concentrations, an increase in this parameter value was observed (according to the F-test for one-way ANOVA, p = 0.005, where the DNIC solution concentration acts as a factor) (Table 2). This tendency was least pronounced for the minimum compound dose (0.15 mM). Thus, in the range of 0.15–0.45 mM DNIC, an increase in the total antioxidant activity of plasma was recorded: for the 0.15, 0.30, and 0.45 mM concentrations it was 1.07, 1.24, and 1.31 times, respectively, relative to the indicator values for the group of the untreated animals. For group 6 the increase amounted to 1.13 times. According to Student's t-test, the differences are statistically significant ($p < 0.05$) for groups 4, 5, 6. A further increase in the amount of the administered compound (up to 0.6 mM) produced the opposite effect. In this case, the total antioxidant activity only increased by 13% compared to the healthy animals ($p < 0.05$). We believe that the mechanism of these shifts is similar to the one presented above with regard to the dynamics of lipid peroxidation processes with the considered nitric oxide donor.

The biochemiluminescent analysis results characterizing the oxidative metabolism components were additionally verified by assessing the concentration of a stable lipid peroxidation product, namely malondialdehyde (MDA), in the blood plasma of the animals in the formed groups (Table 2). In particular, we detected no significant dynamics of this parameter in the rats that only received saline injections (p = 0.655). Moreover, minor changes in the indicator values were recorded in the group of the animals that were administered the minimal DNIC concentration (p = 0.533). At the same time, a twofold increase in the effective concentration of the compound (up to 0.3 mM) significantly enhanced the malondialdehyde level reduction in blood plasma (-24%; p = 0.049 compared with the healthy individuals). Similar behavior was observed when using a concentration of 0.45 mM (-14%; but p equaled 0.247 and was not statistically significant). What is more, a further increase in the DNIC dose (up to 4 times the minimal dose) contributed to a less marked decrease in the level of the studied metabolite of lipid peroxidation (-5%; p equaled 0.717 and was also statistically not significant). The change in the malondialdehyde value according to one-way ANOVA, where the factor is the DNIC solution concentration, is statistically significant (p = 0.050).

Since the values of oxidative metabolism indicators in absolute units differed from each other by several orders of magnitude, all values were normalized. The average values of the indicators for the group of untreated animals were taken as 100%. We obtained a histogram (Fig. 2–4) for all oxidative metabolism indicators. The most statistically significant changes (p = 0.005) were for the AOA indicator (Fig. 3).

It is found that administering saline solution containing no natural nitric oxide donor to animals had no significant effect on the intrinsic crystallization parameters of the bodily fluid. Thus, the differences in the median values for the indicators between groups one and two were not statistically significant.

According to the Mann-Whitney *U* test, *p* equaled 0.650 for the SI indicator, 0.705 for the CR indicator, 0.706 for the DFD indicator, and 0.571 for the M_z indicator (Table 3).

At the same time, the use of DNIC solutions changed the values of these indicators in comparison with the intact animals. However, the influence of different solution concentrations as a factor was not statistically significant for all indicators.

For instance, the median value of the serum facies structural index, or the SI index, differed in the compared groups with respect to the Kruskal-Wallis test, but $p = 0.306$ did not show statistical significance. This parameter reflects the structure complexity of facies elements. The range from 1 to 2 arb. units is characterized by the presence of both single-crystalline and dendritic elements in the specimen on the microscopic slide. Moreover, the increase in the indicator value testifies to an increase in the proportion of the latter in the crystallogram. The maximum median value of the structural index was determined when rats were administered a saline solution containing 0.3 mM of DNIC (Table 3 and Fig. 5). In this case, the median value of the parameter exceeded the median for the group of animals with physiological values by 2.0 times ($p = 0.029$), which is statistically significant. Besides, the indicator value achieved at an agent concentration of 0.15 mM was also 2.0 times higher, but not statistically significant ($p = 0.098$). It should be noted that at a DNIC concentration of 0.6 mM, this indicator value, on the one hand, was higher than the median value characteristic of the intact rats. On the other hand, it was lower than the median value for the group of animals treated with a 0.3 mM DNIC solution.

We also recorded changes in relation to the crystallizability of blood serum facies, or the CR index, which is the main quantitative criterion for assessing the intrinsic crystallization of blood serum (Table 3 and Fig. 6). For this indicator, changes in accordance with one-way ANOVA using the Kruskal-Wallis test showed greater statistical significance of the influence of the DNIC solution concentration factor ($p < 0.001$).

In this regard, it is significant that the structural index and crystallization changes manifested in an increase in both parameters during intraperitoneal administration of DNIC to animals are unidirectional and indicate activation of the bodily fluid crystallogenic properties. At the same time, if the highest median value of the structural index was observed when using DNIC at a concentration of 0.3 mM, then the highest median value of crystallizability was registered with the introduction of 0.45 mM of DNIC. Thus, the difference in the crystallizability median between group 5 and the intact animals is statistically significant, $p = 0.002$. It should be noted that when using other agent concentrations, this indicator changes are significant.

The effect of the concentrations of the physiological nitric oxide donor on the destruction degree of crystalloscopic facies, or the DFD indicator in the form of medians, appears to be not statistically significant according to the Kruskal-Wallis test as $p = 0.102$ for all comparison groups (Table 3 and Fig. 7).

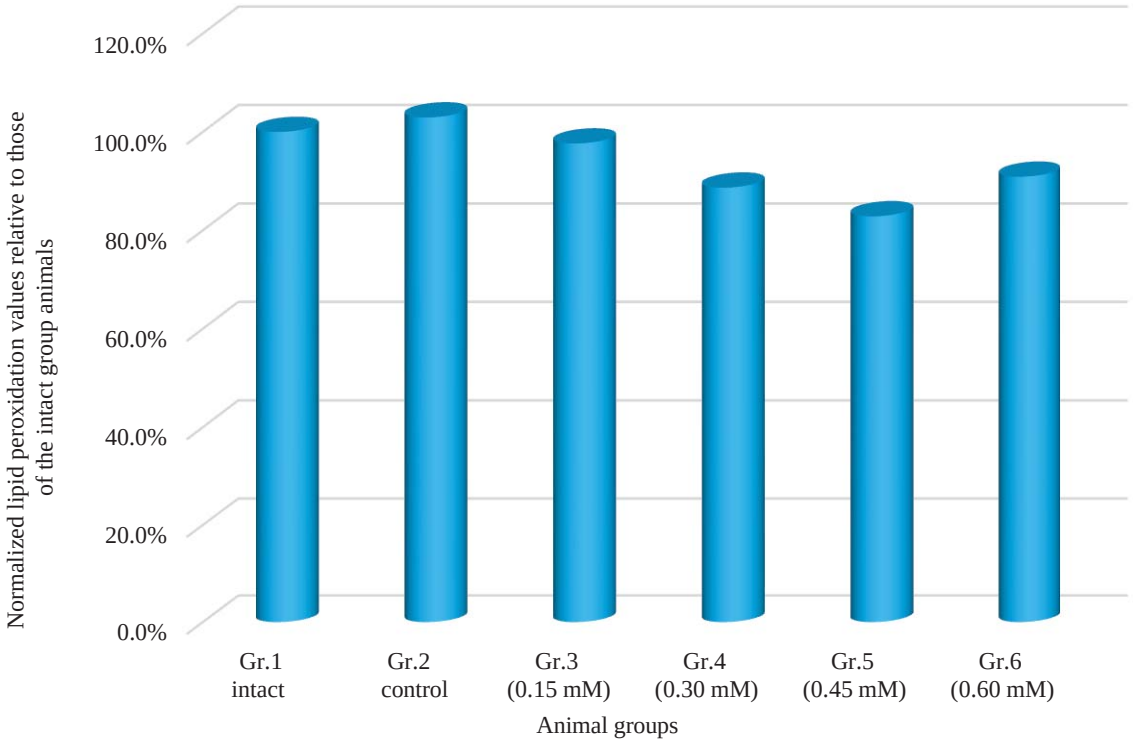

Fig. 2. Normalized lipid peroxidation values relative to those of the intact group animals
Note: the figure was created by the authors. Abbreviation: Gr. — group.
Рис. 2. Нормированные показатели перекисного окисления липидов относительно показателей животных интактной группы.
Примечание: рисунок выполнен авторами. Сокращение: Гр. — группа.

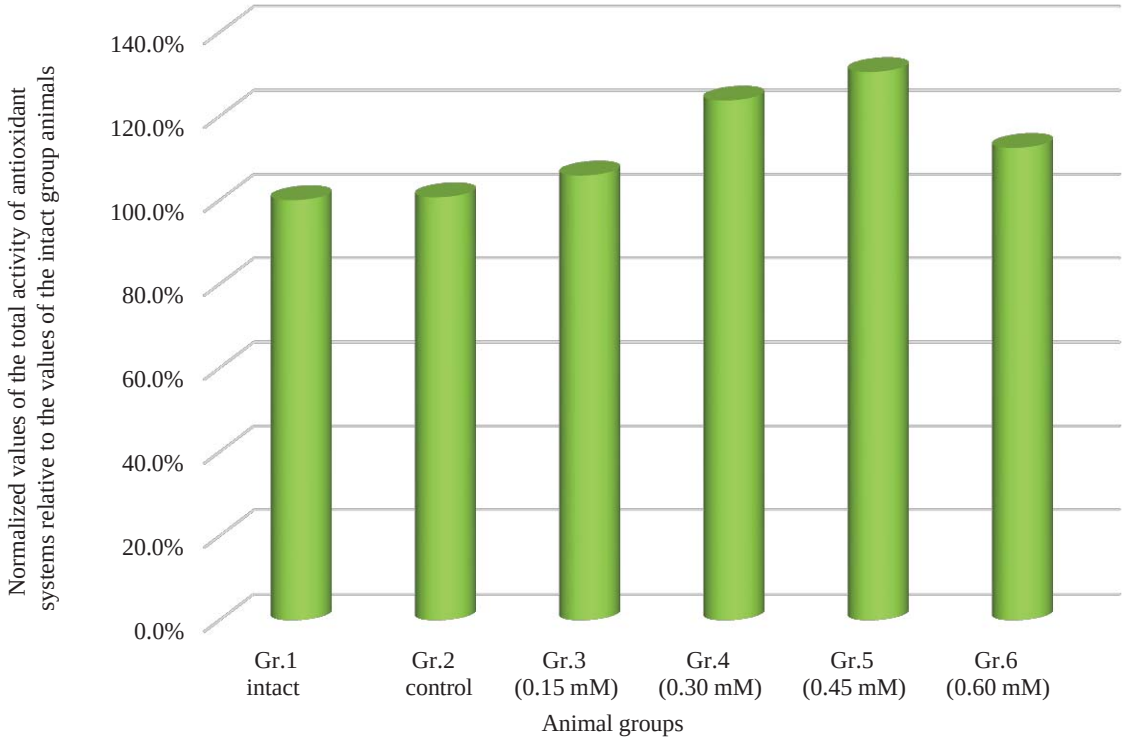

Fig. 3. Normalized values of the total activity of antioxidant systems relative to the values of the intact group animals
Note: the figure was created by the authors. Abbreviation: Gr. — group.

Рис. 3. Нормированные показатели общей активности антиоксидантных систем относительно показателей животных интактной группы.
Примечание: рисунок выполнен авторами. Сокращение: Гр. — группа.

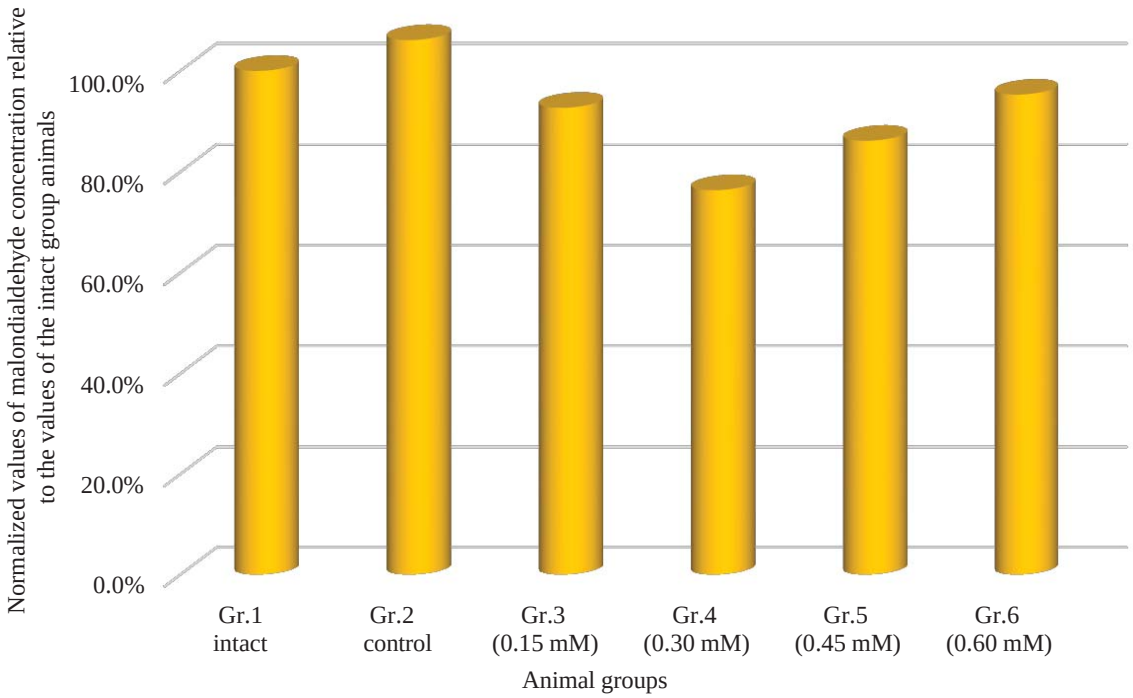

Fig 4. Normalized values of malondialdehyde concentration relative to the values of the intact group animals
Note: the figure was created by the authors. Abbreviation: Gr. — group.

Рис. 4. Нормированные показатели концентрации малонового деальдегида относительно показателей животных интактной группы.
Примечание: рисунок выполнен авторами. Сокращение: Гр. — группа.

Table 3. Medians, first and third quartiles (*Me* (*Q1-Q3*)) of intrinsic crystallization values in the compared animal groups
Таблица 3. Медианы, первый и третий квартили (*Me* (*Q1-Q3*)) показателей собственной кристаллизации в сравниваемых группах животных

Groups	Intrinsic crystallization indicators			
	SI (points)	CR (points)	DFD (points)	M_z (points)
Gr. 1 intact (*n* = 10)	1.0 (0.0–2.0)	0.0 (0.0–1.0)	0.0 (0.0–0.8)	3.0 (2.3–3.0)
Gr. 2 control (*n* = 10)	1.0 (1.0–2.0)	0.5 (0.0–1.0)	0.0 (0.0–1.0)	2.5 (2.0–3.0)
Gr. 3 (0.15 mM) (*n* = 10)	1.0 (1.0–2.0)	1.0 (1.0–1.8)	1.0 (0.0–1.0)	2.0 (2.0–3.0)
Gr. 4 (0.30 mM) (*n* = 10)	2.0 (2.0–2.0)	1.0 (1.0–2.0)	1.0 (0.3–1.0)	3.0 (2.3–3.0)
Gr.5 (0.45 mM) (*n* = 10)	1.5 (1.0–2.0)	1.5 (1.0–2.0)	1.0 (0.0–1.0)	2.5 (2.0–3.0)
Gr. 6 (0.60 mM) (*n* = 10)	1.5 (1.0–2.0)	1.0 (1.0–2.0)	1.0 (1.0–1.0)	3.0 (1.5–3.0)
Significance level	*p* = 0.306	***p* < 0.001**	*p* = 0.102	*p* = 0.258

Note: the table was compiled by the authors. Abbreviations: Gr. — group; arb. units — arbitrary units of measurement; SI — structural index; CR — crystallizability; DFD — degree of serum facies destruction; M_z — pronouncedness of the marginal facies zone on the microscope slide.
Примечание: таблица составлена авторами. Сокращения: Гр. — группа; у.е. — условные единицы измерения; ИС — индекс структурности; КР — кристаллизуемость; СДФ — степень деструкции фации; Кз — выраженность краевой зоны микропрепарата.

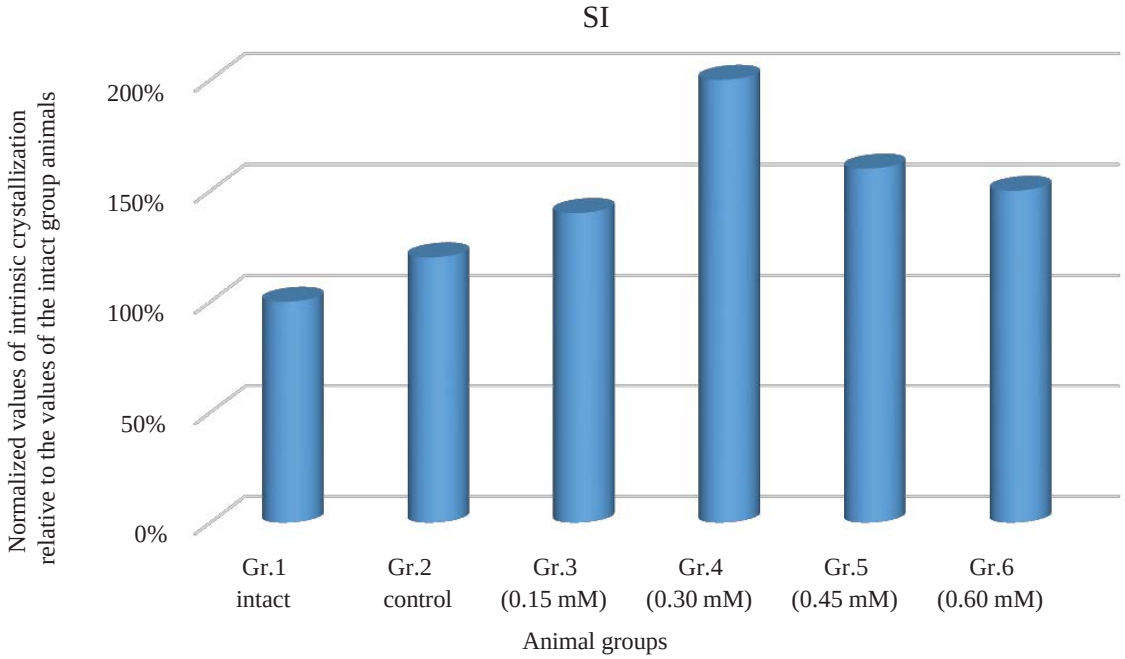

Fig. 5. Normalized values of intrinsic crystallization relative to the values of the intact group animals. Structural index indicator
Note: the figure was created by the authors. Abbreviations: Gr. — group; SI — structural index.
Рис. 5. Нормированные показатели собственной кристаллизации относительно показателей животных интактной группы. Показатель индекса структурности
Примечание: рисунок выполнен авторами. Сокращения: Гр. — группа; ИС — индекс структурности.

The values of this indicator became higher with increasing the DNIC dose, but they did not exceed the mean value of the indicator (0.7 arb. units) at all concentrations except 0.6 mM. Such a parameter level indicates a weak pronouncedness of destructive processes during the formation of crystalline facies elements, indirectly showing the absence of a significant toxic effect of the compound. Moderate destruction of the sample structures was observed only when the highest concentration of the used compound (0.6 mM) was administered to the rats.

We also revealed uniform pronouncedness of the marginal protein zone of the specimen on the microscope slide under the action of different DNIC concentrations (Table 3 and Fig. 8). Thus, at all doses of the compound used, a decrease in the median values of this indicator was recorded. However, the statistical significance of the influence of the factor in the form of DNIC concentrations on the marginal protein zone of the specimen, or the M_z indicator, was not revealed, because *p* equaled 0.258.

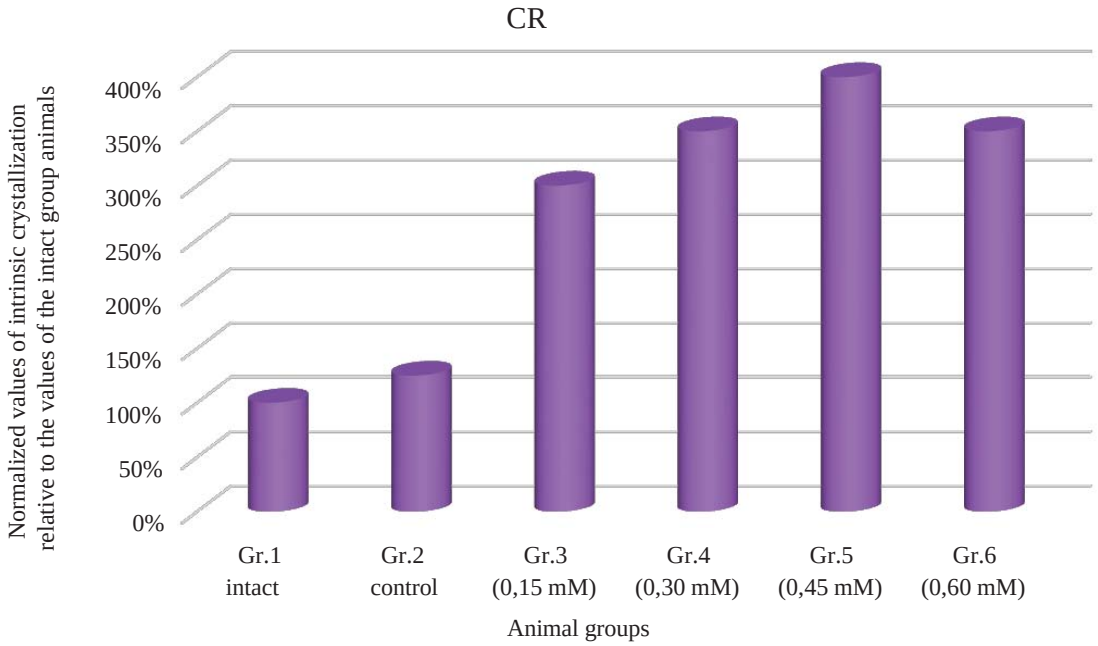

Fig. 6. Normalized values of intrinsic crystallization relative to the values of the intact group animals. Crystallizability index
Note: the figure was created by the authors. Abbreviations: Gr. — group; CR — crystallizability.
Рис. 6. Нормированные показатели собственной кристаллизации относительно показателей животных интактной группы. Показатель кристаллизуемости
Примечание: рисунок выполнен авторами. Сокращения: Гр. — группа; Кр — кристаллизуемость.

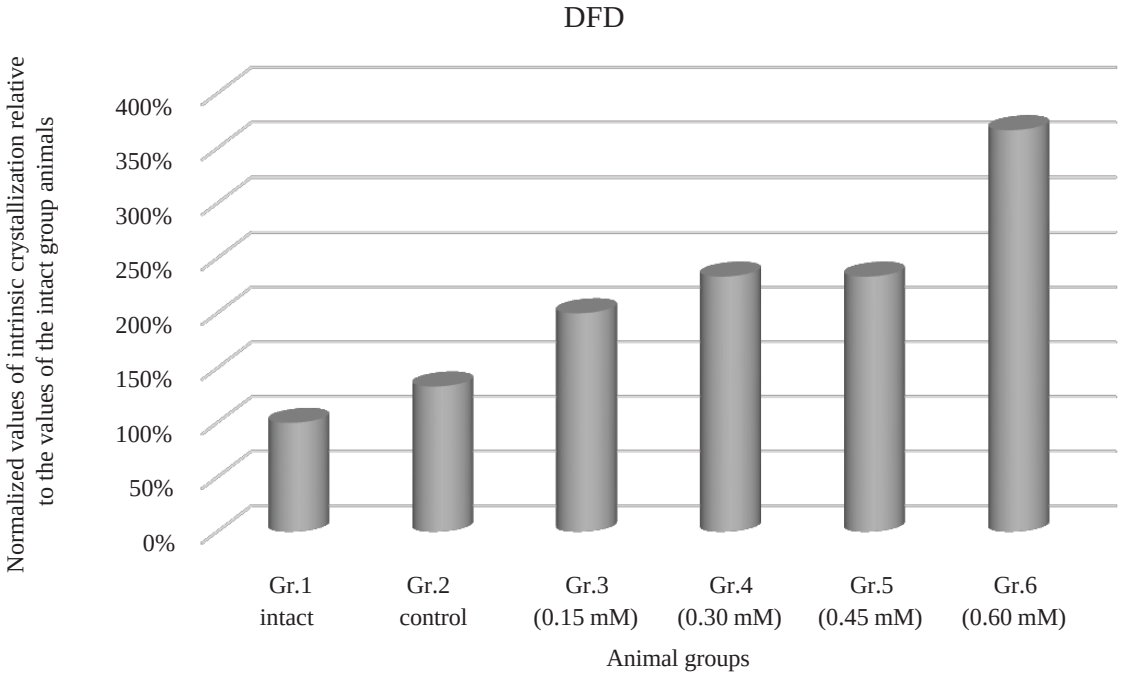

Fig. 7. Normalized values of intrinsic crystallization relative to the values of the intact group animals. Degree of facies destruction
Note: the figure was created by the authors. Abbreviations: Gr. — group; DFD — degree of facies destruction.
Рис. 7. Нормированные показатели собственной кристаллизации относительно показателей животных интактной группы. Степень деструкции фаций
Примечание: рисунок выполнен авторами. Сокращения: Гр. — группа; СДФ — степень деструкции фаций.

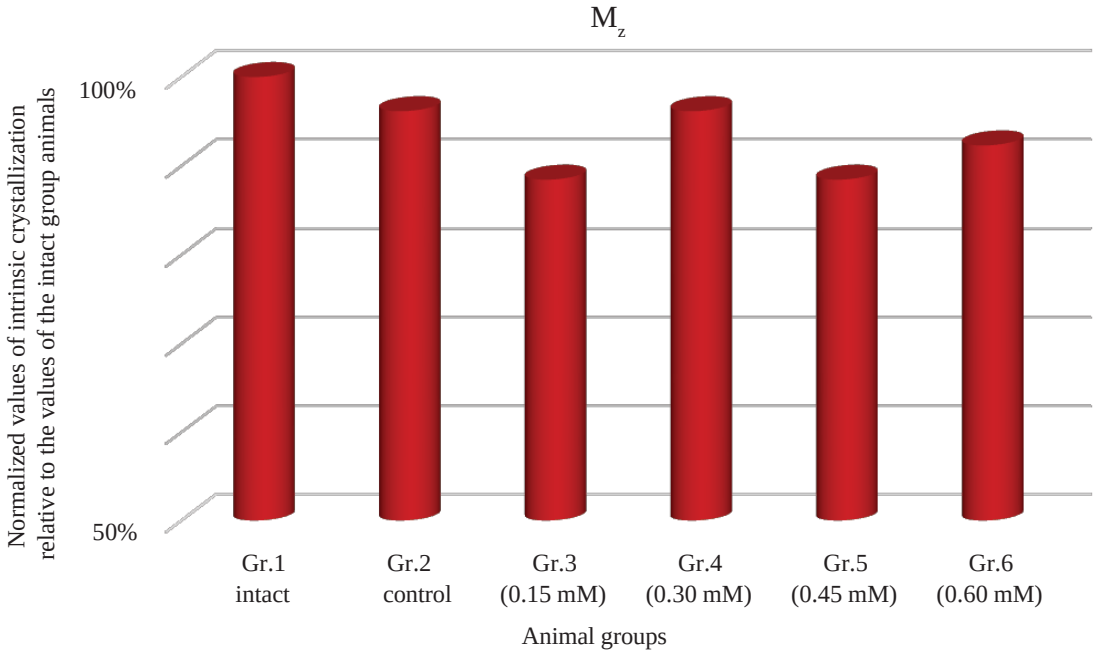

Fig. 8. Normalized values of intrinsic crystallization relative to the values of the intact group animals. Pronouncedness of the marginal serum facies zone

Note: the figure was created by the authors. Abbreviations: Gr. — group; M_z — pronouncedness of the marginal facies zone.

Рис. 8. Нормированные показатели собственной кристаллизации относительно показателей животных интактной группы. Выраженность краевой зоны

Примечание: рисунок выполнен авторами. Сокращения: Гр. — группа; Кз — выраженность краевой зоны.

DISCUSSION

Interpretation / scientific significance

Despite the already established numerous biological effects of DNICs associated with their ability to release nitrogen monoxide [1, 22–28], relatively little attention has been paid to the antioxidant effects of the compound. They were first discovered by us earlier in experiments performed on a thermal injury model [19, 21]. The present study conducted on healthy laboratory animals as a test bio-object, enabled us to visualize this effect. We believe that it includes two components, namely the direct antioxidant properties of dinitrosyl iron complexes themselves and the corresponding activity of glutathione ligands. Together, they can ensure the functioning of DNIC as a pharmacological agent with marked antioxidant potential.

Research limitations

Within the framework of the study, we considered the factor of injecting animals with saline solution without the studied agent (dinitrosyl iron complexes). Nevertheless, we chose a limited range of doses of the compound for testing (from 0.15 to 0.60 mM). Based on previous studies, it was assumed that this range corresponds to the most intense bioregulatory activity of the compound. Nevertheless, going beyond this range may provide additional information about the biological effects of DNICs over a wider dose range. Additionally, due to the need to minimize the number of animal groups for bioethical reasons, we only used 4 concentrations of the compound. By increasing the number of points within the considered range, additional data can be obtained to clarify the identified *dose-effect* relationships.

Extrapolation

It is known that DNICs, which are used as a natural nitric oxide donor, can stimulate antioxidant status correction through qualitative inhibition of free radical oxidation of lipids [18, 19, 21]. Our experiment has revealed that lipid peroxidation intensity values decreased dose-dependently in comparison with those in the intact animals as the DNIC agent concentration increased. However, the exception was the highest concentration used (0.6 mM), which affected the lipid peroxidation indicator in the blood plasma to a lesser extent than the previous DNIC dose (0.45 mM). Nevertheless, in this case there was also a decrease in the indicator values in comparison with those in the intact rats.

Blood plasma malondialdehyde tests also confirmed a decrease and weakening of LPO intensity with an increase in the administered DNIC concentration. A similar tendency was recorded in relation to the total antioxidant activity of blood plasma. The ability of DNIC to produce antioxidant effects is due to the ability of complexes to intercept free radicals in conjunction with the subsequent recovery of the oxoferryl form of myoglobin. Interception of O_2- formed during superoxide decomposition is characteristic of DNIC with thiol-containing ligands [29–30].

In regard to the crystallogenic properties of rat blood serum, we registered changes indicating a positive effect of adminis-

tering DNIC at concentrations of 0.3 and 0.45 mM. This was manifested in an increase in the key parameters, namely the structural index and facies crystallizability. At the same time, the indicators of the possible toxic effects of the agent used (the degree of facies destruction and the pronouncedness of the marginal protein zone) showed moderate deviations in comparison with those in the intact animals.

CONCLUSION

In general, the conducted studies indicate the presence of an antioxidant effect in glutathione containing DNICs. The pronouncedness of these properties demonstrates a dependence on their dose with a possible optimum lying in the range of 0.3–0.45 mM (agent dose — 2.86–4.29 μg/g of animal weight). The research has established the activating effect of glutathione containing DNIC injections on the crystallogenic potential of blood serum in healthy rats. This effect manifested itself in an increase in the density of crystalline elements and their complexity, and, as for the metabolic indicators, the maximum pronouncedness of this tendency corresponded to concentrations of 0.3 and 0.45 mM.

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