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EFFECT OF CRYOPRESERVED PLACENTA EXTRACT ON SOME BIOCHEMICAL INDICES OF THERAPEUTIC EFFICIENCY AND TOXICITY OF DICLOFENAC SODIUM IN ADJUVANT-INDUCED EXPERIMENTAL ARTHRITIS

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Relevance. Non-steroidal anti-inflammatory drugs are among the top requested ones in the clinic of internal medicine. However, these drugs are associated with a wide range of adverse reactions involving a number of organs and systems, in particular the gastrointestinal tract, cardiovascular system and kidneys.
The aim of the study is to characterize the effect of the combined use of cryopreserved placenta extract and diclofenac sodium on the prooxidant-oxidative system, the activity of inflammatory, destructive and cytolytic processes, as well as protein and lipid metabolism in rats with experimental rheumatoid arthritis.
Results. The administration of diclofenac sodium and cryopreserved placenta extract to rats with adjuvant arthritis normalized the level of active products of thiobarbituric acid and hence was indicative of the neutralization of an arthritis-induced oxidative stress. A statistically significant (p=0.01) increase ~~of~~ in a superoxide dismutase activity (by 30.6% relative as compared with rats of the control group) has also been established. An increase in the anti-inflammatory properties of diclofenac sodium in the combined use of diclofenac sodium with a cryopreserved placenta extract has been found out. The level of C-reactive protein decreased (p<0.001) by 61.1% as compared with the untreated rats, and the level of seromucoid has been significantly (p<0.01) decreased by 17.1% as compared with the rats of the monotherapy group treated with the studied NSAIDs. It was shown that alanine aminotransferase and aspartate levels were significantly lower (by 38.9%, p<0.01 and by 37.9%, p<0.01, respectively) as compared with those of the animals that had been administrated with diclofenac sodium. Their indices were by 16.7% (p=0.02) and 17.2% (p<0.001) lower than the indices of the control group rats with untreated adjuvant arthritis. The established changes of aminotransferases levels indicate the ability of a cryopreserved placenta extract to level not only an arthritis-induced cytolytic syndrome, but also a diclofenac-induced one. The combined use of cryopreserved placenta extract and diclofenac sodium was accompanied by the normalization of the total lipids level and phospholipids in the blood serum of rats against the background of experimental rheumatoid arthritis. Thus, the content of phospholipids in the lipid pool statistically significantly (p=0.02) increased by 22.6% as compared with the indices of the animals with adjuvant arthritis without treatment.
Conclusion. The study showed that the combined use of diclofenac sodium and cryopreserved placenta extract leads to the restoration of the balance of the prooxidant-antioxidant system that is more pronounced than monotherapy with diclofenac sodium. A decrease in the activity of inflammatory, destructive and cytolytic processes, as well as the restoration of lipid metabolism in the rats with experimental rheumatoid arthritis, has also been observed.
Keywords: cryopreserved placenta extract; adjuvant arthritis; anti-inflammatory activity; nonsteroidal anti-inflammatory drugs; diclofenac sodium
Abbreviations: AlAT – alanine-aminotransferase; AsAT – aspartate aminotransferase; AA – adjuvant arthritis; AOS – antioxidant system; COX – cyclooxygenase; CPE – cryopreserved placenta extract; C-RP – C- reactive protein; DS – diclofenac sodium; GGTP – gamma glutamyl traspeptidase; GI tract – gastrointestinal tract; i/g – intragastrically; i/m – intramuscularly; IPC & C NAS of Ukraine – Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine; LP – lipid peroxidation; LP-AOS – lipid peroxidation-antioxidant system; NSAIDs – non-steroidal anti-inflammatory drugs; OS – oxidative stress; RA – rheumatoid arthritis; SOD – superoxide dismutase; TBA-AP – active products of thiobarbituric acid; 95% CI – 95% Confidence interval

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ВЛИЯНИЕ КРИОКОНСЕРВИРОВАННОГО ЭКСТРАКТА ПЛАЦЕНТЫ НА ОТДЕЛЬНЫЕ БИОХИМИЧЕСКИЕ ПОКАЗАТЕЛИ ЛЕЧЕБНОЙ ЭФФЕКТИВНОСТИ И ТОКСИЧНОСТИ ДИКЛОФЕНАКА НАТРИЯ ПРИ АДЪЮВАНТ-ИНДУЦИРОВАННОМ АРТРИТЕ В ЭКСПЕРИМЕНТЕ

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Актуальность. Нестероидные противовоспалительные препараты являются одними из наиболее востребованных классов лекарственных средств в клинике внутренней медицины. Однако применение указанных препаратов ассоциируется с широким спектром нежелательных реакций с вовлечением ряда органов и систем, в частности желудочно-кишечного тракта, сердечно-сосудистой системы и почек.

Цель. Охарактеризовать влияние криоконсервированного экстракта плаценты и диклофенака натрия при их комбинированном применении на состояние прооксидантно-оксидантной системы, активность воспалительных, деструктивных и цитолитических процессов, а также состояние белкового и липидного обмена по данным биохимических исследований у крыс с экспериментальным ревматоидным артритом.

Результаты. Введение диклофенака натрия и криоконсервированного экстракта плаценты крысам с адъювантным артритом привело к нормализации уровня активных продуктов тиобарбитуровой кислоты, что указывает на нивелирование признаков артрит-индуцированного оксидативного стресса. Также выявлено статистически достоверное ($p=0,01$) повышение активности супероксиддисмутазы на 30,6% относительно значений у крыс контрольной группы. Установлено усиление противовоспалительных свойств диклофенака натрия на фоне комбинированного применения диклофенака натрия с криоконсервированным экстрактом плаценты, так как уровень С-реактивного белка снизился ($p<0,001$) на 61,1% относительно нелеченых крыс, а уровень серомукоида статистически достоверно ($p<0,01$) снизился на 17,1% относительно показателей крыс группы монотерапии исследуемым нестероидным противовоспалительным препаратом. Показано, что уровень аланин-аминотрансферазы статистически достоверно ($p<0,01$) был ниже на 38,9%, а аспартат-аминотрансферазы – ниже на 37,9% ($p<0,01$) относительно показателей животных, которым вводили диклофенак натрия, что соответственно на 16,7% ($p=0,02$) и 17,2% ($p<0,001$) было ниже показателей крыс контрольной группы с нелеченым адъювантным артритом. Установленные изменения со стороны аминотрансфераз указывают на способность криоконсервированного экстракта плаценты нивелировать не только артрит-индуцированный цитолитический синдром, но и диклофенак-индуцированный. Комбинированное применение криконсервованого экстракта плаценты и диклофенака натрия сопровождалось нормализацией уровня общих липидов и фосфолипидов в сыворотке крови крыс на фоне экспериментального ревматоидного артрита. Так содержание фосфолипидов в пуле липидов статистически достоверно ($p=0,02$) выросло на 22,6% относительно показателей животных с адъювантным артритом без лечения.

Заключение. Исследование показало, что комбинированное применение диклофенака натрия и криконсервированного экстракта плаценты приводит к восстановлению равновесия прооксидантно-антиоксидантной системы, более выраженному, чем при монотерапии диклофенаком натрия, снижению активности воспалительных, деструктивных и цитолитических процессов, а также восстановлению липидного обмена у крыс с экспериментальным ревматоидным артритом.

Ключевые слова: криоконсервированый экстракт плаценты; адъювантный артрит; противовоспалительная активность; нестероидные противовоспалительные препараты; диклофенак натрия

Список сокращений: АА – адъювантный артрит; АлАТ – аланин-аминотрансфераза; АОС – антиоксидантная система; АсАТ – аспартат-аминотрансфераза, в/ж – внутрижелудочно, в/м – внутримышечно; ГГТП – гамма-глутамилтраспептидаза; ДН – диклофенак натрия; ЖКТ – желудочно-кишечный тракт; ИПКиК НАН Украины – Института проблем криобиологии и криомедицины Национальной академии наук Украины; КЭП – криоконсервированный экстракт плаценты; НПВП – нестероидные противовоспалительные препараты; ОС – оксидативный стресс; ПОЛ – перикисное окисление липидов; ПОЛ-АОС – перекисное окисление липидов-антиоксидантная система; СОД – супероксиддисмутаза; С-РБ – С-реактивный белок; ТБК-РП – активные продукты тиобарбитуровой кислоты; ЦОГ – циклооксигеназа; 95% ДИ – 95% доверительный интервал

INTRODUCTION

Among the great number of drugs that clinicians have in their arsenal, non-steroidal anti-inflammatory drugs (NSAIDs) occupy a special place. This is due to the presence of nonspecific anti-inflammatory and analgesic properties in them, that justifies the annual increase in their sales against the background of the population aging, since, the frequency of their use is known to increase with age. NSAIDs play a special role in symptomatic therapy in patients with rheumatic diseases – rheumatoid arthritis (RA), ankylosing spondylitis, gout, etc. [1–5].

However, despite the generally recognized effectiveness of NSAIDs, their use in clinical practice is significantly limited by the risk of developing unwanted side effects on various organs and systems, in particular – the gastrointestinal tract (GI tract), cardiovascular system, liver, kidneys [2–6]. In addition, in 25.0–35.0% of cases, patients use NSAIDs irrationally and do not take into account the presence of risk factors for the development of undesirable effects.

It is known that the maximum risk of cardiovascular complications occurs when taking drugs with high selectivity to cyclooxygenase (COX) type 2 (coxibs – celecoxib, etoricoxib, etc.), while the risk of complications from GI tract occurs when taking non-selective NSAIDs (diclofenac, indomethacin, ibuprofen, etc.) [3, 4]. Thus, in the first 7 days of taking celecoxib, the increased risk of myocardial infarction is 92.0–99.0% [3, 7]. Adverse GI tract effects are the most common pathology caused by the inhibition of COX-1 and, as a consequence, a decrease in the protective potential of the mucous membrane of the digestive tract [8, 9]. Nonselective COX inhibitors increase the risk of GI bleeding by an average of 4 times, and selective inhibitors – by 3 times [4, 5].

The problem of GI-tolerance of widely used NSAIDs substantiates the relevance of the search for effective cytoprotective drugs that can increase the resistance of the gastrointestinal mucosa [10, 11]. The treatment of NSAID-induced esophagogastroenterocolonopathy is classically directed at acid suppressive therapy (proton pump inhibitors, H2-histamine blockers, etc.), the use of gastrocytoprotectors (de-nol, sucralfate, etc.) and the drugs that restore the content of prostaglandins in the mucous membrane, etc.) [5, 12–14]. However, none of these groups of drugs sufficiently satisfies clinicians, since they are mainly aimed at treating NSAID gastropathy, while NSAIDs are able of causing lesions, including the distal parts of the GI tract, and have their own side effects [13–19].

One of the promising areas of therapy for GI tract pathologies is the use of drugs of the biological origin, the advantages of which are their complex pharmacological action and high tolerability. [20–22].

As a means of correcting the ulcerogenic effect of NSAIDs, the authors' attention was drawn to a cryopreserved human placenta extract (CPE), which has a multivector spectrum of its biological activity [23–26].

For the first time, cryopreserved preparations of human placental tissue were obtained by scientists from the Institute for Problems of Cryobiology and Cryomedicine (IPC & C) of the National Academy of Sciences of Ukraine (IPC & C NAS of Ukraine), who developed and put into practice a unique technique for its long-term storage in a low-temperature environment. This ensured the primacy of Ukraine among European countries in the experience of cryopreservation of biological tissues [26–31].

In previous studies, it has been shown that CPE is able to neutralize the ulcerogenic effect of NSAIDs, in particular, of diclofenac sodium, meloxicam, ibuprofen, etc. [32–34]. At the same time, according to the literature data, CPE has its own anti-inflammatory properties [35], the mechanism of which is apparently associated with the action of hormones in it – progesterone, estradiol, prolactin, gonadotropin, etc.

In addition, CPE has pronounced antioxidant properties, and its anti-inflammatory effect is possibly associated with the elimination of the pro-inflammatory modulation of the blood system reactions by reactive oxygen species and products of lipid peroxidation (LP) as a result of stimulation of the physiological antioxidant system [35].

All of the forgoing facts justify the need for an in-depth study of the mechanisms of the therapeutic, in particular, the anti-inflammatory effect of CPE when used in combination with NSAIDs.

THE AIM of the study was to characterize the effect of cryopreserved placenta extract and diclofenac sodium in their combined use on the state of the prooxidant-antioxidant system, the activity of inflammatory, destructive and cytolytic processes, as well as the state of protein and lipid metabolism according to biochemical studies in rats with experimental rheumatoid arthritis.

MATERIALS AND METHODS

The study was conducted at the Department of Experimental Cryomedicine on the basis of IPC & C NAS of Ukraine. The work was performed in the frame of the planned research work "Destructive and regenerative processes in tissues in vivo after exposure to low temperatures and biologically active substances" (code 2.2.6.113, state registration number 0117U001049).

The study was carried out on 28 sexually mature nonlinear male rats obtained from the IPC & C NAS of Ukraine vivarium, weighing 200–220 g, divided into 4 groups of 7 animals each: I – intact rats (n=7); II (control) – rats (n = 7) with experimental RA without treatment; III – rats (n=7) with experimental RA administrated with DS (8 mg/kg [36], intragastrically (i/g)); IV (n=7) – rats with experimental RA administrated with DS (8 mg/kg, i/g) and CPE (0.16 mg/kg [37], intramuscularly (i/m)).

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Dose schedule of the investigational agents

AA treatment was carried out from 14 to 28 days. DS (Pr. JSC Chimfarmzavod Krasnaya Zvezda, ZAO "Zdorovye" Pharmaceutical Company, Ukraine) was administered i/g at the dose equal to ED_{50} for the anti-inflammatory activity in the carrageenin-induced edema model – 8 mg/kg in the form of an emulsion on polysorbate "Twin-80" [35, 37]. The indicated dose of DS corresponds to a single human dose of 88 mg (1.25 mg/kg), which is consistent with the clinical recommendations for the use of DS in patients at the dose of 75–100 mg/day in the long-term use and 1.7 times lower than its maximum daily dose of 150 mg [8].

According to the instructions, the CPE drug "Cryocell cryo-extract of the placenta" (State Enterprise "Interdepartmental Scientific Center of Cryobiology and Cryomedicine of the National Academy of Sciences, National Academy of Medical Sciences and the Ministry of Health of Ukraine", Ukraine), is used in patients parenterally in a single dose of 1.8 ml. Accordingly, a single dose for rats was (1.8 ml/70 kg) × 6.35 = 0.16 ml/kg of body weight [37]. Before using the "Cryocell cryo-extract of the placenta" preparation, a single dose (0.16 ml/kg) was extemporally diluted in a 0.9% NaCl solution at the rate of 0.1 ml of 0.9% NaCl solution/100 g of body weight. At the rate of at 0.16 ml / kg of body weight, CPE was injected i/m with an interval of 2 days (5 injections in total), on days 14, 17, 20, 23 and 26, respectively. That corresponded to the instructions for its clinical use – i/m by 1,8 ml with an interval of 2–3 days, a course of 1–5 injections.

Chronic immune inflammation model

To reproduce the conditions of NSAIDs administration to rats corresponding to their clinical application, a model of experimental rheumatoid arthritis (RA) – adjuvant arthritis (AA) in rats was chosen. It had all the morphofunctional signs of RA and was accompanied by a typical reaction, the main link of which is T-cell immunity [38–40]. AA was modeled by a subplantar injection of complete Freund's adjuvant (PAF, Thermo Fisher Scientific, USA) into the hind right limb at the rate of 0.1 ml per rat. The day of the administration of the adjuvant was taken as the "0" day of the experiment [40–42]. The maximum manifestation of the local inflammatory reaction, which was accompanied by a significant increase in the volume of the limb, was determined on the 12^{th}–14^{th} days, and then the activity of the inflammatory process gradually decreased.[1]

On the 28^{th} day of the experiment, the animals were withdrawn from the experiment, and after decapitation of the animals, blood samples were taken into tubes with a pre-introduced anticoagulant (2-substituted potassium salt of ethylenediaminetetraacetic acid). Plasma was separated by centrifugation for 15 min at 3000 revol/min. To obtain serum, blood was collected in glass tubes without anticoagulant and kept at room temperature (20–26°C) until complete separation of serum, which was taken into a test tube and centrifuged for 15–20 min at 3000 revol/min.

Biochemical methods of blood tests[2]

1. Content of active products of thiobarbituric acid (TBA-AP) in blood serum was determined spectrophotometrically by the method of Asakawa T. et al. [43] according to the reaction with TBA and was calculated from the optical density indices determined from the light absorption at the wavelength of λ=535 nm. The molar extinction coefficient of the red-colored complex, which is 1.56×105 mol-1/cm-1 and expressed in μmol/L, was taken into account.
2. The activity of superoxide dismutase (SOD) in blood serum was determined spectrophotometrically by the method of Kostyuk V.A. et al. [44], based on the suppression of the oxidation reaction of quercetin, according to the optical density, determined by the light absorption at the wavelength of λ=406 nm. The SOD activity was expressed as a percentage inhibition of quercetin oxidation.
3. The content of seromucoid in blood serum was determined spectrophotometrically by the method of Weimer H.E. and Moshin R.J., which consists in the precipitation of blood serum proteins with a 1.8 M solution of perchloric acid (HClO4), the isolation of seromucoid from the filtrate using phosphoric tungstic acid, and a further quantitative determination by the difference in light absorption at the wavelength of λ=260 nm and λ=280 nm. The content of seromucoid was determined in units of optical density (optical density units) and expressed in mmol/L (conversion factor = 1; 1 optical density unit = 1 mmol/L).
4. The content of C-reactive protein (C-RP) in blood serum was determined by the degree of agglutination and turbidity according to the instructions for using the latex diagnostic test for detecting C-reactive protein in blood serum "C-RP latex test" (NPL Granum LLC, Ukraine), and expressed in mg/L.
5. The activity of alanine aminotransferase (AlAT) in blood serum was determined spectrophotometrically by the method of Reitman S. and Frankel S. and expressed in μmol/(ml×h). The studies were carried out using kits for biochemical studies "ALT (Reitman-Frenkel with a calibrator)" (ZAO SPE "Filisit-Diagnostics", Ukraine).
6. The activity of aspartate aminotransferase (AsAT) in blood serum was determined spectrophotometrically by the method of Reitman S. and Frankel S. and expressed in μmol / (ml × h). The studies were carried out using kits for biochemical studies "AsAT (Reitman-Frenkel with a calibrator)" (ZAO SPE "Filisit-Diagnostika", Ukraine).

[1] Stefanov OV. Preclinical studies of drugs: Guidelines. Kiev.»Avitsena». 2001. 527 p.

[2] Kamyshnikov VS. Handbook of clinical and biochemical research and laboratory diagnostics. Moscow. «MEDpress-inform». 2009. 896 p.

7. The activity of gamma-glutamyl traspeptidase (GGTP) in blood serum was determined spectrophotometrically and expressed in U/L. The studies were carried out using kits for biochemical studies "GTP" (ZAO SPE "Filisit-Diagnostics", Ukraine).
8. The activity of alkaline phosphatase in blood serum was determined spectrophotometrically and expressed in μmol/h×l. The studies were carried out using kits for biochemical studies "Alkaline phosphatase" (ZAO SPE "Filisit-Diagnostics", Ukraine).
9. The content of total protein in blood serum was determined spectrophotometrically by biuret reaction and was expressed in g/l. The studies were carried out using kits for biochemical studies "General protein" (ZAO SPE "Filisit-Diagnostics", Ukraine).
10. The content of total lipids in blood serum was determined spectrophotometrically by the color reaction with the sulfophosphovaniline reagent, which is based on the fact that the decomposition products of unsaturated fatty acids formed after acid hydrolysis of lipids, interact with the phosphoricaniline reagent to form colored complexes with a maximum absorption at the wavelength of λ=530 nm. Lipid extracts were obtained according to the method of Bligh E.G. and Dyer W.I. [45]. Phospholipids were fractionated by the method of Svetashev V.I. and Vaskovsky V.E., by two-dimensional micro-ball chromatography [46]. Phospholipids were identified by the method [47] and their content was expressed by the level of inorganic phosphorus in g/L.

Bioethical aspects of research

The animals were kept in the conditions of the IPC&C NAS of a Ukraine vivarium. For 14 days, the rats were in quarantine (in accordance with the sanitary standards of "The structure and content of experimental biological clinics" (Order No. 755 of 08/12/1997)) on a water-food ration with free access to food and water (according to the Appendix dated 12/04/1977 to Order No. 163 of 03/10/1996 "About daily feeding rates for laboratory animals and producers")). All experimental studies on laboratory animals were carried out in accordance with the requirements of good laboratory practice and in compliance with the main provisions of the Council of Europe Convention on the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes dated 18 March 1986; Directive of the European Parliament and of the Council of the European Union 2010 / 63 / EC dated 22 September 2010 on the protection of animals used for scientific purposes; Order of the Ministry of Health of Ukraine dated December 14, 2009 No. 944 "On approval of the procedure for conducting preclinical study of drugs and examination of materials for preclinical study of drugs" and the Law of Ukraine dated February 21, 2006 No. 3447-IV "On the protection of animals from cruelty".

The comprehensive research program was reviewed and approved by the Bioethics Committee at IPC&C NAS of Ukraine (extract from Protocol No. 2 dated March 11, 2020).

Statistical processing

Statistical processing of the obtained results was carried out using the application program for working with electronic tables "Microsoft Office Excel 2003; 2013" (Microsoft Corporation, USA) using the "Real Statistics" extension (http://www.real-statistics.com/). The character of the values distribution in each group of the sample was assessed using the Shapiro-Wilk test. The homogeneity of the dispersions was determined according to the Levene's test. To assess the significance of the revealed differences in the studied indicators under different experimental conditions, a statistical analysis was carried out using parametric and nonparametric criteria. With a normal distribution of independent values, the differences between the groups were determined in pairs by Student's t-test. In the case of an abnormal distribution of at least one of the groups of independent values, the differences between them were determined in pairs using the Mann-Whitney nonparametric U-rank test. The obtained values were compared with the critical ones at the confidence level above 95.0% ($p<0.05$), above 99.0% ($p<0.01$), above 99.5% ($p<0.005$) and above 99.9% ($p<0.001$); and a conclusion about the error probability was made. The numerical data in the case of a normal distribution of values are given as "M±m" (M±SE), where M is the arithmetic mean, m(SE) is the standard error of the arithmetic mean, or M (95%CI:5%–95%), and 95% CI is a 95% confidence interval. In case of an abnormal distribution of the obtained values, the data are presented in the form of Me [LQ; UQ], where Me is the median, [LQ; UQ] – the upper border of the lower quartile (LQ) and the lower border of the upper quartile (UQ).

RESULTS AND DISCUSSION

Biochemical studies of the peripheral blood of rats with AA (control group) showed a statistically significant ($p<0.001$) increase in the TBA-AP content by 53.7% relative to the indices of intact animals (Fig. 1). That indicates an LP activation, which is probably due to the development of a systemic inflammatory process [47, 48].

LP products are known to promote platelet aggregation, decrease the synthesis of prostaglandins with an anticoagulant effect, form cytolysis syndrome, release blood coagulation factors, and suppress cell division and regeneration. TBA-AP acts as an endogenous aldehyde, which is a clinical and laboratory marker of oxidative stress (OS) and is widely used to control the effectiveness of treatment of a number of diseases – RA, osteoarthritis, coronary heart disease, etc. [49–51].

Monotherapy of AA rats with DS, led to a statistically significant ($p<0.01$) decrease in the level of TBA-AP by

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19.0% relative to the indicators of animals in the control group in the same period of the study. The combined use of DS and CPE was accompanied by a more pronounced decrease in the intensity of LP processes, which was indicated by a decrease in the content of TBA-AP in rats with AA by 31.7% relative to the indices of rats with AA without treatment. That was 1.7 times higher than similarly directed changes against the background of using only DS.

It should be noted that in the animals with AA, which had been injected with DS and CPE, on the 28-th day of the experiment, the level of TBA-AP was practically compared with the indices of the intact rats and it was 4.3 [3.6; 4.5] μmol/L (Fig. 1). That indicates the signs leveling of AA-induced OS and can be regarded as one of the mechanisms of the therapeutic action of the studied drugs.

The established LP activation in the rats against the background of AA development was consistent with the parallel depletion of the antioxidant system (AOS), which indicated a statistically significant ($p<0.01$) decrease in the SOD level by 27.9% relative to the indices of intact animals (Fig. 2).

Together with other AOS enzymes (catalase, ceruloplasmin, glutathione peroxidase, glutathione reductase, etc.), SOD is known to be an integral component of LP-AOS. The assessment of the functional state of AOS, determines the level of the compensatory and adaptive response of the organism in conditions of the possible development of OS [51, 52].

Monotherapy of AA rats with DS, led to a statistically significant ($p=0.03$) increase in the SOD activity by 20.4% relative to the indices of untreated animals (group II), but this value (59.0% inhibition of quercetin oxidation) was still 13.2% lower than the indices of the animals without AA (68.0 [64.0; 76.5]%).

Against the background of the combined use of DS and CPE, a statistically significant ($p=0.01$) increase in the SOD activity by 30.6% relative to the values in rats of the control group was noted, which was only 5.9% lower than the indices of the intact rats, and indicated the most pronounced restoration of the AOS activity.

To assess the state of connective tissue structures in rats against the background of AA development, the level of seromucoids was studied, since it is this group of glycoproteins that enters the blood plasma upon destruction, degradation or damage of connective tissue. In addition, seromucoids are usually considered acute phase markers of inflammation, which can serve indicators of the severity of the inflammatory process, since their life span is about 5 days [53–56].

It was found out that on the 28th day of the experiment, the rats with AA showed a statistically significant ($p<0.001$) increase in the seromucoid level by 2.2 times relative to the indices of intact rats (Fig. 3).

Monotherapy of AA rats with DS, led to a statistically significant ($p<0.01$) decrease in the level of the studied parameter by 31.4% relative to the level of the untreated animals. The combined use of DS and CPE, led to a statistically significant ($p<0.01$) even greater decrease in the seromucoid content by 17.1% relative to the indicators of rats in the DS monotherapy group, which amounted to 0.18 [0.17; 0.18] mmol/L.

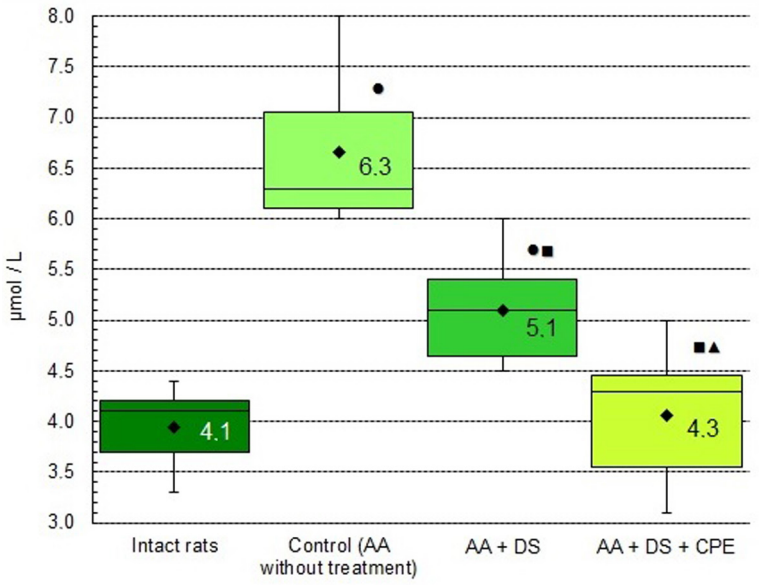

Figure 1 – Effect of DS and its combination with CPE on the level of TBA-AP in peripheral blood in rats with AA on the 28th day of the experiment

Note: the distribution of values is abnormal. Boxes include results from the 25th to 75th percentile, vertical lines outside the boxes are the minimum and maximum values. The horizontal line inside the box is the median (► – the median is 25 (75) percentile), ♦ – the average value; ● – $p<0.05$ relative to the indices of intact rats, ■ – $p<0.05$ relative to the indices of rats with AA without treatment; ▲ – $p<0.05$ relative to the indices of AA rats treated with DS

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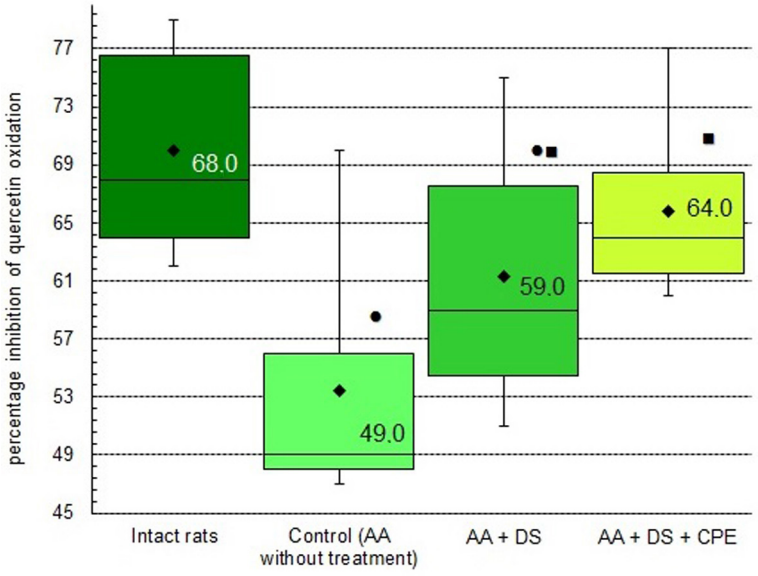

Figure 2 – Effect of DS and its combination with CPE on the level of SOD in peripheral blood in rats with AA on the 28th day of the experiment

Note: the distribution of values is abnormal. Boxes include results from the 25th to 75th percentile, vertical lines outside the boxes are the minimum and maximum values. The horizontal line inside the box is the median (► – the median is 25 (75) percentile), ♦ – the average value; ● – p<0.05 relative to the indices of intact rats, ■ – p<0.05 relative to the indices of rats with AA without treatment; ▲ – p<0.05 relative to the indices of AA rats treated with DS

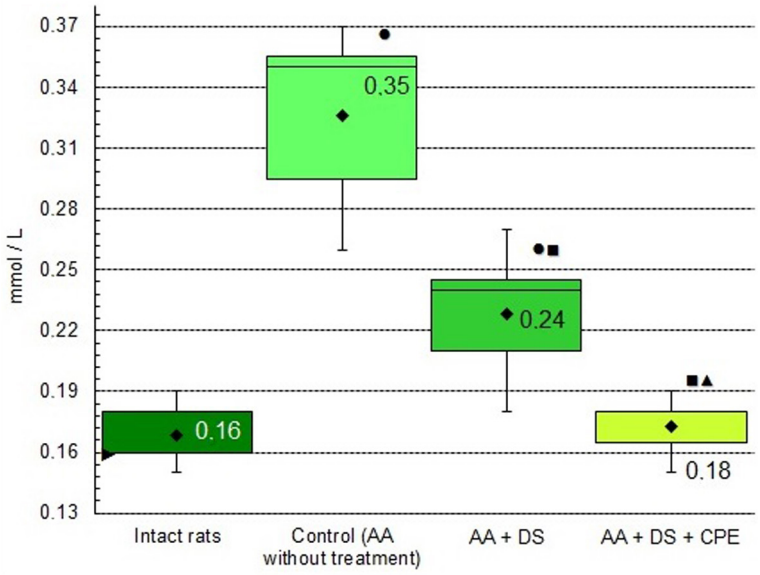

Figure 3 – Effect of DS and its combination with CPE on the level of seromucoid in peripheral blood in rats with AA on the 28th day of the experiment

Note: the distribution of values is abnormal. Boxes include results from the 25th to 75th percentile, vertical lines outside the boxes are the minimum and maximum values. The horizontal line inside the box is the median (► – the median is 25 (75) percentile), ♦ – the average value; ● – p<0.05 relative to the indices of intact rats, ■ – p<0.05 relative to the indices of rats with AA without treatment; ▲ – p<0.05 relative to the indices of AA rats treated with DS

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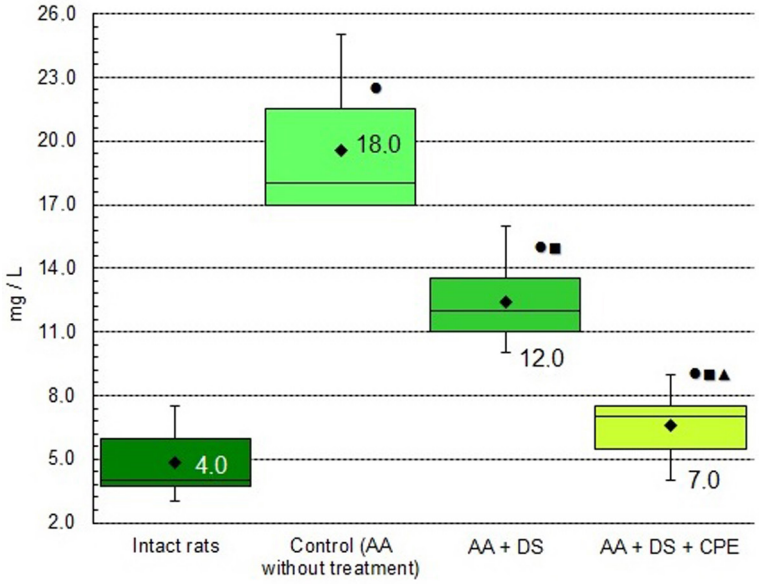

Figure 4 – Effect of DS and its combination with CPE on the level of C-RP in peripheral blood in rats with AA on the 28th day of the experiment

Note: the distribution of values is abnormal. Boxes include results from the 25th to 75th percentile, vertical lines outside the boxes are the minimum and maximum values. The horizontal line inside the box is the median (► – the median is 25 (75) percentile), ♦ – the average value; ● – p<0.05 relative to the indices of intact rats, ■ – p<0.05 relative to the indices of rats with AA without treatment; ▲ – p<0.05 relative to the indices of AA rats treated with DS

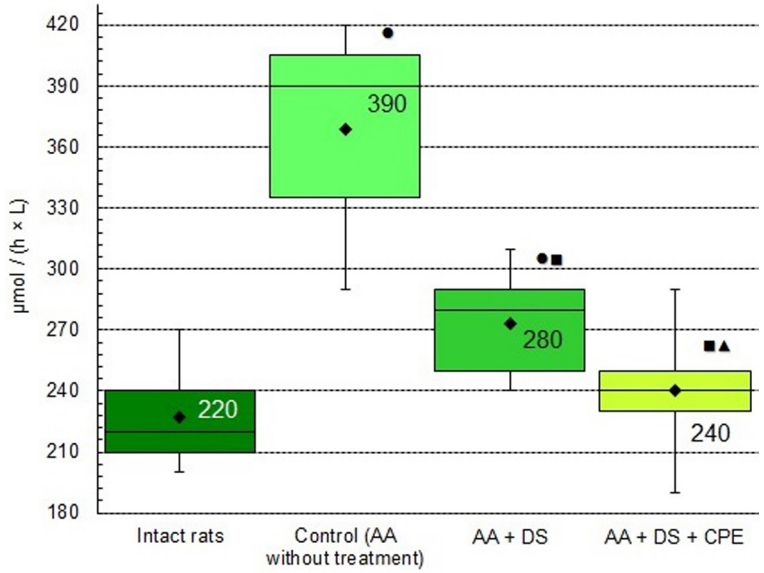

Figure 5 – Effect of DS and its combination with CPE on the level of alkaline phosphatase in peripheral blood in rats with AA on the 28th day of the experiment

Note: the distribution of values is abnormal. Boxes include results from the 25th to 75th percentile, vertical lines outside the boxes are the minimum and maximum values. The horizontal line inside the box is the median (► – the median is 25 (75) percentile), ♦ – the average value; ● – p<0.05 relative to the indices of intact rats, ■ – p<0.05 relative to the indices of rats with AA without treatment; ▲ – p<0.05 relative to the indices of AA rats treated with DS

Table 1 – Effect of DS and its combination with CPE on markers of cytolysis in the peripheral blood of rats with AA on the 28th day of the experiment (M ± m (95% CI) or Me [LQ; UQ]; n = 28)

Investigated indicator, units of measurement	Experimental conditions			
	Group I	Group II	Group III	Group IV
	Intact rats	Control (AA without treatment)	AA + DS	AA +DS + CPE
n	7	7	7	7
Alanine aminotransferase (AlAT), µmol/ml/h	1.4 [1.3; 1.6]	1.8 [1.6; 1.9] p_{1-2}=0.01	2.2 [2.1; 2.3] p_{1-3}<0.01 p_{2-3}=0.01	1,5 [1.5; 1.6] p_{1-4}=0.17 p_{2-4}= 0.02 p_{3-4}<0.01
Aspartate aminotrans-fe-times (AsAT), µmol/ml/h	2.3 [2.1; 2.4]	2.9 [2.9; 3.5] p_{1-2} < 0.001	3.5 [3.3; 2.4] p_{1-3} < 0.01 p_{2-3}= 0.2	2.4 [2.3; 2.5] p_{1-4}= 0.14 p_{2-4}<0.001 p_{3-4}< 0.01
Gammaglutamyl transpepti-dase (GGTP), U/L	22.3±1.81 (95% CI: 18.7–25.8)	36.1±0.80 (95% CI: 34.6–37.7) p_{1-2}<0,001	27.9±1.30 (95% CI: 25.3–30.4) p_{1-3}= 0.03 p_{2-3}<0.001	23.9±1.10 (95% CI: 21.7–26.0) p_{1-4}=0.5 p_{2-4}<0.001 p_{3-4}=0.04

Table 2 – Effect of DS and its combination with CPE on the indices of protein homeostasis in the peripheral blood of rats with AA on the 28th day of the experiment (M ± m (95% CI) or Me [LQ; UQ]; n = 28)

Investigated indicator, units of measurement	Experimental conditions			
	Group I	Group II	Group III	Group IV
	Intact rats	Control (AA without treatment)	AA + DS	AA +DS + CPE
n	7	7	7	7
Total protein, g/l	78.9±1.45 (95% CI: 76.0–81.7)	74.6±1.36 (95% CI: 71.9–77.2) p_{1-2}=0.05	74.7±0.92 (95% CI: 72.9–76.5) p_{1-3}=0.03 p_{2-3}=0.9	81.0±1.50 (95% CI: 78.1–83.9) p_{1-4}=0.3 p_{2-4}=0.01 p_{3-4}<0.01
Albumin, g/l	35.3±1.69 (95% CI: 32.0–38.6)	25.1±1.03 (95% CI: 23.1–27.2) p_{1-2}<0.001	27.9±1.30 (95% CI: 25.3–30.4) p_{1-3}<0.01 p_{2-3}= 0.1	37.4±1.90 (95% CI: 33.7–41.2) p_{1-4}=0.01 p_{2-4}<0.001 p_{3-4}<0.01
Globulins, g/l	45.7±1.54 (95% CI: 42.7–48.7)	49.6±1.29 (95% CI: 47.0–52.1) p_{1-2}=0.08	46.7±1.19 (95% CI: 44.4–49.0) p_{1-3}=0.6 p_{2-3}=0.1	40.4±0.65 (95% CI: 39.2–41.7) p_{1-4}<0.01 p_{2-4}<0.001 p_{3-4}<0.001
Albumin / globulin ratio	0.78±0.039 (95% CI: 0.70–0.85)	0.51±0.028 (95% CI: 0.46–0.57) p_{1-2}<0.001	0.60±0.035 (95% CI: 0.53–0.67) p_{1-3}<0.01 p_{2-3}=0.07	0.93±0.045 (95% CI: 0.84–1.02) p_{1-4}= 0.03 p_{2-4}<0.001 p_{3-4}<0.001

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Table 3 – Effect of DS and its combination with CPE on lipid homeostasis indices in the peripheral blood of rats with AA on the 28th day of the experiment (M ± m (95% CI) or Me [LQ; UQ]; n = 28)

Investigated indicator, units of measurement	Experimental conditions			
	Group I	Group II	Group III	Group IV
	Intact rats	Control (AA without treatment)	AA + DS	AA +DS + CPE
n	7	7	7	7
Total lipids, g/l	3.0 [2.7–3.3]	5.1 [4.5–5.2] p_{1-2}<0.001	4.4 [4.2–4.5] p_{1-3}<0.001 p_{2-3}=0.07	3,3 [3.2–3.4] p_{1-4}= 0.1 p_{2-4}<0.001 p_{3-4}<0.001
Phospholipids, g/l	2.1±0.05 (95% CI: 2.0–2.2)	2.5±0.09 (95% CI: 2.3–2.7) p_{1-2}<0.001	2.0±0.05 (95% CI: 1.9–2.1) p_{1-3}= 0.6 p_{2-3}<0.001	2.1±0.05 (95% CI: 2.0–2.2) p_{1-4}= 0.5 p_{2-4}<0.01 p_{3-4}=0.2
Phospholipids relative to total lipids,%	70.9±5.35 (95% CI: 60.4–81.4)	53.2±3.60 (95% CI: 46.2–60.3) p_{1-2}=0.02	46.9±1.80 (95% CI: 43.3–50.4) p_{1-3}<0.01 p_{2-3}=0.1	65.2±2.47 (95% CI: 60.3–70.0) p_{1-4}= 0.4 p_{2-4}=0.02 p_{3-4}<0.001

For a good reason, C-RP is considered the "golden" marker of inflammation in almost all pathological processes, since it tends to increase significantly in tissue damage, infections of a bacterial or viral nature, inflammation and malignant neoplasms; in tissue necrosis, it can even increase up to 300 mg/l during the day [47, 57–61].

It has been found out that the development of the experimental RA in rats led to a statistically significant (p<0.001) increase in the C-RP level by 4.5 times relative to the indices of the intact animals (Fig. 4).

On the photo of monotherapy with DS, this indicator decreased (p<0.001) by 33.3%, and against the background of the combined administration of DS and CPE, it decreased (p<0.001) by 61.1% relative to the values in the rats with AA without treatment. The data obtained indicate pronounced anti-inflammatory properties of DS, which are potentiated when combined with CPE, but may also be associated with the intrinsic anti-inflammatory activity of CPE.

The established biochemical signs of an active inflammatory process and the resulting imbalance in the LP-AOS system substantiate the advisability of a detailed analysis of the activity of cytolysis markers against the background of treatment of rats with AA – AlAT, AsAT and GGTP [62, 63].

The study showed that the development of AA in rats was accompanied by an increase in the content of AlAT by 28.6% (p=0.01), AsAT – by 26.1% (p<0.001) and GGTP – by 62.2% (p<0.001) relative to the indicators of the intact rats (Table 1).

Aminotransferases are complex enzyme proteins that catalyze transamination processes and ensure the synthesis of individual amino acids in the body. It has been proven that under the influence of damaging factors, the energy metabolism of hepatocytes is disrupted, which leads to an increase in the permeability of cell membranes and the appearance of cytoplasmic components in the blood serum.

Since the marker of the cellular damage, GGTP, is found to the greatest extent in the kidneys, liver and small intestine, it can be assumed that there is an inflammatory process in these organs. That is compared with the literature data on the systemic character of the inflammatory process in RA, which is analogous to AA in animals [50].

The use of DS led to an increase in cytolytic processes, which was indicated by an even greater increase in the studied markers in the peripheral blood – so, AlAT was higher by 22.2% (p<0.01), and AsAT – by 20.7% (p=0, 2) relative to the indices of rats with AA without treatment. That was 57.1% (p<0.01) and 52.2% (p<0.01), respectively, higher than the indices of the intact animals (Table 1). No mortality in the group treated with a combined use of DS and CPE, had been noted. These changes in the activity of aminotransferases are consistent with the data on the hepatotoxic effect of DS [3, 8, 64–66].

It was also found out that against the background of the use of DS, GGTP statistically significantly (p<0.001) decreased by 22.9% relative to the indices of the animals in the control group (Table 1).

Against the background of the combined use of DS and CPE, the indices of cytolytic processes in the rats with AA are of particular interest (Table 1). Therefore,

it was found out that AlAT was statistically significantly ($p<0.01$) lower by 38.9%, and AsAT was lower ($p<0.01$) by 37.9% relative to the indices of the animals injected with DS, which, respectively, were by 16.7% ($p=0.02$) and 17.2% ($p<0.001$) lower than the indices of the rats in the control group with untreated AA. The changes established on the part of aminotransferases, indicate the ability of CPE to level not only AA-induced cytolytic cider, but also concomitant cytolysis, enhanced by DS. That indicates the universality of its cytoprotective activity, probably due to its ability to stabilize cell membranes, as well as its antioxidant properties.

In addition, it was found out that the combined use of DS and CPE, was also accompanied by a statistically significant ($p<0.001$) decrease in the GGTP level by 28.6% relative to the untreated rats with AA, which was 11.4% lower ($p=0.01$) than in the rats treated with DS alone (Table 1).

The assessment of the level of alkaline phosphatase in the peripheral blood in the rats with AA showed that the development of a systemic autoimmune inflammation was accompanied by a statistically significant ($p<0.001$) increase in the indicated metalloprotein by 77.3% relative to the indices of the intact rats, and amounted to 390 [335; 405] µmol/h×L (Fig. 5).

Alkaline phosphatase is a heterogeneous enzyme consisting of separate isoenzymes, each of which is concentrated in a specific organ: liver, bone tissue, intestines, placenta. Localized on the cell membrane, the enzyme is connected to the transport of biologically important compounds, primarily phosphorus. Alkaline phosphatase is actively involved in the processes of phosphorus-calcium metabolism in the body. An increase in the activity of alkaline phosphatase indicates the activation of bone remodeling processes, since its bone fraction is synthesized by osteoblasts and their precursors, and is involved in the mineralization of the bone matrix [50, 67–69]. An increase in the activity of this enzyme may indicate an increase in an osteoblastic activity and an increase in destructive changes in the liver or the occurrence of cholestasis in the rats with AA [3].

DS monotherapy led to a statistically significant ($p<0.01$) decrease in the activity of alkaline phosphatase by 28.2%, and the combined use of DS and CPE was accompanied by a more pronounced decrease in the activity of this enzyme by 38.5% ($p<0.01$) relative to the indicators of the untreated rats with AA (Fig. 5).

The study of the protein spectrum of the blood of rats with AA showed that the level of total protein practically did not differ in the intact rats and the rats with AA (Table 2).

The content study of the individual protein fractions showed that the development of AA in the rats was accompanied by a statistically significant ($p<0.001$) decrease in the albumin content by 28.7% relative to the indices of the intact rats. Albumin is the most homogeneous fraction of proteins, the main function of which is to maintain colloidal osmotic pressure, as well as to transport carbohydrates, lipids, hormones and pigments. A patient's body with active RA, consumes albumin at the site of inflammation, resulting in hypoalbuminemia more actively.

In addition, it was noted that in the rats with AA, the content of globulins slightly increased by 8.4% ($p=0.04$) relative to the indices of the intact animals. As it is known, in a long subacute progressive process, hypergamaglobulinemia is primarily observed, which is associated with an increase in the level of autoantibodies [50, 51, 70, 71].

These changes led to a statistically significant ($p<0.001$) decrease in the albumin / globulin ratio of peripheral blood by 34.2% relative to the indices of intact animals (Table 3). The change in the ratio of protein fractions, as a rule, corresponds to the severity of RA and reflects the dynamics of the inflammatory process [50, 51, 70, 72].

DS monotherapy led to a statistically significant ($p=0.04$) decrease in the albumin / globulin ratio by 17.5% relative to the AA rats without treatment. The combined use of DS and CPE was accompanied by a complete increase ($p=0.01$) of the studied ratio by 19.5% higher than in the intact animals. That is due to an increase in the albumin level almost up to the level of the intact animals, and at the same time it is by 11.6% lower ($p<0.01$) than the values in the intact rats, the content of globulins (Table 3).

Lipids, which act as structural components of biomembranes, the energy substrate of the cell, participating in the reactions of signal transduction, exo- and endocytosis and the like, play an important role in the life of the cell. In addition, they participate in the fixation of proteins of the phospholipid bilayer and ensure their appropriate orientation in the cell membrane, are a non-polar medium for fat-soluble substrates and enzyme cofactors, cause their folding, and also act as regulators and modulators of an enzymatic activity [69, 73].

The study showed that against the background of the AA development, in the rats, there was a statistically significant ($p=0.02$) decrease in the proportion of phospholipids in the total lipid pool by 25.0%. At the same time, there was an increase in the total lipid level ($p<0.001$) by 1.7 times and a disproportionate increase in the content of phospholipids ($p<0.001$) by 1.2 times, which is due to the activation of the cyclooxygenase pathway of membrane phospholipid metabolism (Table 3).

Against the background of DS monotherapy, a decrease in the level of total lipids ($p=0.07$) was noted by 1.3 times and a decrease in the level of phospholipids ($p <0.001$) was also noted by 1.3 times relative to the indicators of the animals with AA without treatment. That corresponded to the development of even greater imbalance in the content of phospholipids in the pool of total lipids - their content decreased by another 6.3%,

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however, these changes did not reach the level of statistical significance (p=0.1).

Against the background of the combined use of DS and CPE in the rats with AA, a statistically significant (p=0.02) harmonization of the lipid balance was noted – the content of phospholipids increased by 22.6% relative to the indicators of the animals with AA without treatment and amounted to 2.1±0.05, respectively (95% CI: 2.0–2.2) g/l. These changes were observed against the background of a decrease in the level of total lipids in the blood serum (p<0.001) by 35.3% relative to the indices of the untreated rats, and amounted to 3.3 [3.2–3.4] g/l, respectively, which was compared with the indices of the intact animals 3.0 [2.7–3.3] g/l.

CONCLUSION

The administration of diclofenac sodium and cryopreserved placenta extract to rats with adjuvant arthritis normalized the level of active products of thiobarbituric acid, which indicates leveling of signs of the arthritis-induced oxidative stress. A statistically significant (p=0.01) increase in the level of superoxide dismutase (by 30.6%) as compared with the values in the rats of the control group, was also revealed.

An increase in the anti-inflammatory properties of diclofenac sodium was found out in the combined use of diclofenac sodium with cryopreserved placenta extract. The level of C-reactive protein decreased (p<0.001) by 61.1% as compared with the untreated rats, and the level of seromucoid statistically significantly (p<0.01) decreased by 17.1% relative to the indices of the rats in the monotherapy group with the studied NSAIDs.

The level of alanine aminotransferases was significantly (p<0.01) lower by 38.9%, and aspartate aminotransferase was lower by 37.9% (p<0.01) relative to the parameters of the animals that had been injected with diclofenac sodium., It was, respectively, 16.7% (p=0.02) and 17.2% (p<0.001) lower than the indices of the rats in the control group with untreated adjuvant arthritis. The established changes on the part of aminotransferases indicate the ability of a cryopreserved placenta extract to level not only an arthritis-induced cytolytic cider, but also a diclofenac-induced one.

A combined use of cryopreserved placenta extract and diclofenac sodium was accompanied by the normalization of the level of total lipids and phospholipids in the blood serum of rats against the background of experimental rheumatoid arthritis. Thus, the content of phospholipids in the phospholipid pool statistically significantly (p=0.02) increased by 22.6% relative to the indicators of the animals with adjuvant arthritis without treatment.

Prospects for further research. For a correct assessment of the activity of the inflammatory process, it is advisable to determine the level of matrix metalloproteinases and homocysteine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Fedor V. Hladkykh – idea, concept and design of the study, coordination and participation in experimental work, collection of materials, statistical processing and analysis of the data obtained, writing the main text of the article and formulating conclusions; Mykola O. Chyzh – research concept, participation in experimental work, collection of materials, editing the text of the article; Anna O. Manchenko – participation in experimental work, collection of materials; Iryna V. Belochkina – participation in experimental work, collection of materials, search and analytical work; Iryna P. Mikhailova – participation in experimental work, collection of materials. All authors have read and approved the final version prior to publication.

REFERENCES

1. Zeng C, Wei J, Persson MSM, Sarmanova A, Doherty M, Xie D, Wang Y, Li X, Li J, Long H, Lei G, Zhang W. Relative efficacy and safety of topical non-steroidal anti-inflammatory drugs for osteoarthritis: a systematic review and network meta-analysis of randomised controlled trials and observational studies. British Journal of Sports Medicine. 2018;52(10):642–50. DOI: https://doi.org/10.1136/bjsports-2017-098043.
2. Balabantseva AP, Karateev AE. Frequency and clinical and endoscopic features of combined NSAID-induced gastrointestinal injuries. Modern rheumatology. 2018;12(4):95–100. DOI: https://doi.org/10.14412/1996-7012-2018-4-95-100. Russian
3. Ushkalova EA, Zyryanov SK, Zatolochina KE. Safety and tolerance of NSAIDs: focus on aceclofenac. Medical Council. 2019;9:110–20. DOI: https://doi.org/10.21518/2079-701X-2019-9-110-120. Russian

4. Fischbach W. Drug-induced gastrointestinal bleeding. Internist. 2019;60(6):597-607. DOI: https://doi.org/10.1007/s00108-019-0610-y.
5. Trinus FP. Evolutionary evidence about the mechanism of non-steroidal anti-pyretic drugs and analgesics-antipyretics. Pharmacology and toxicology. 2012;2(27):68–72. Ukrainian
6. Hladkykh F. Multimodal analgesia: polypharmacy in anesthesia or rational use of non-steroidal anti-inflammatory drugs to prevent chronic pain. Traktoriya nauki: international electronic journal of science = Traektoriâ Nauki = Path of Science. 2020;6(7):4009–18. DOI: http://dx.doi.org/10.22178/pos.60-5. Russian
7. Bally M, Dendukuri N, Rich B, Nadeau L, Helin-Salmivaara A, Garbe E, Brophy JM. Risk of acute myocardial infarction with NSAIDs in real world use: bayesian meta-analysis of individual patient data. BMJ. 2017 May 9;357:j1909. DOI: 10.1136/bmj.j1909.
8. Karateev AE, Nasonov EL, Ivashkin VT, Martynov AI, Yakhno NN, Arutyunov GP, Alekseeva LI, Abuzarova GR, Evseev MA, Kukushkin ML, Kopenkin SS, Lila AM, Lapina TL, Novikova DS, Popkova TV, Rebrov AP, Skorobogatykh KV, Chichasova NV. Rational use of non-steroidal anti-inflammatory drugs. Clinical guidelines. Scientific and practical rheumatology. 2018; 56:1–29. DOI: https://doi.org/10.14412/1995-4484-2018-1-29. Russian
9. Cooper C, Chapurlat R, Al-Daghri N, Herrero-Beaumont G, Bruyere O, Rannou F, Roth R, Uebelhart D, Reginster JY. Safety of oral non-selective non-steroidal anti-Inflammatory drugs in osteoarthritis: what does the literature say? Drugs & Aging. 2019;36(1):15–24. DOI: https://doi.org/10.1007/s40266-019-00660-1.
10. Lazebnik LB, Golovanova EV, Alekseenko SA, Bakulina NV, Baranovskiy AYu, Belova GV, Bordin DS, Vyuchnova ES, Gaidukova IZ, Gimaeva ZF, Kashkina EI, Kozlova IV, Kokorin VA, Lapina ED, Li ED, Miguskina EI, Mironchev OV, Onuchina EV, Okhlobystin AV, Pasechnikov VD, Pakhomova IG, Pozdnyakova OYu, Putintseva IV, Sarsenbaeva AS, Sviridova TN, Simanenkov VI, Simonova ZhG, Sitkin SI, Tkachenko EI, Turkina SV, Khabarova YuA, Shevyakov MA, Yakovenko EP, Yakovlev AA, Yankovaya TN. Recommendations for the prevention and treatment of esophago-gastro-enterocolopathies induced by nonsteroidal anti-inflammatory drugs. Experimental and Clinical Gastroenterology. 2018;3(151):4–18. Access: https://www.nogr.org/jour/article/view/567/562. Russian
11. Yang JH, Lee BH, Eum KS, Suk KS, Park JO, Kim HS, Lee HM, Moon SH. Prevalence of gastrointestinal and cardiovascular risk in patients with degenerative lumbar spinal disease. Clinics in Orthopedic Surgery. 2020;12(3):343–52. DOI: https://doi.org/10.4055/cios20021.
12. Karateev AE. Do non-steroidal anti-inflammatory drugs have a pathogenetic effect? Modern rheumatology. 2012;4:13–22. Russian
13. Karateev AE. Selective inhibitors of cyclooxygenase-2 and "protected" non-steroidal anti-inflammatory drugs: two methods of preventing drug complications. Clinical Medicine. 2014;8:54–61. Russian
14. Davis A, Robson J. The dangers of NSAIDs: look both ways. British Journal of General Practice. 2016;66(645):172–173. DOI: https://doi.org/10.3399/bjgp16X684433.
15. Dovgan EV. Clinical pharmacology of non-steroidal anti-inflammatory drugs: a course towards safety. Russian medical journal. 2017;13: 979–85. Russian
16. Stepanyuk NH, Hladkykh FV, Basarab OV. Analysis of Adverse Reaction of Analgesics, Antipyretics and Non-Steroidal Anti-Inflammatory Drugs Prescribed by Physicians of Health Care Facilities in Podilskyi Region during 2015. Galician Medical Journal. 2016;2(23):60–3. Available from: http://ojs.ifnmu.edu.ua/index.php/gmj/article/view/545.
17. Bjarnason I, Takeuchi K. Intestinal permeability in the pathogenesis of NSAID-induced enteropathy. J Gastroenterol. 2009;44 Suppl 19:23-9. DOI: 10.1007/s00535-008-2266-6.
18. Golovanova EV. Protection of the mucous membrane of the gastrointestinal tract from the damaging effect of non-steroidal anti-inflammatory drugs. Clinical gerontology. 2017;1–2:47–51. Russian
19. Hladkykh FV. Preventive and therapeutic strategies for the pharmaco-correction of gastropathy induced by nonsteroidal anti-inflammatory drugs. Reviews on clinical pharmacology and drug therapy. 2017;4:14–23. DOI: http://dx.doi.org/10.17816/RCF15414-23. Russian
20. Kuksgauz IA, Shekunova EV, Kashkin VA, Faustova NM, Gushchin YaA, Makarova MN, Makarov VG. Study of the gastroprotective effect of the drug Alflutop on the model diclofenac-induced gastropathy in rats. Experimental and Clinical Gastroenterology. 2019;5:15–21. DOI: https://doi.org/10.31146/1682-8658-ecg-165-5-15-21. Russian
21. Gulevsky AK, Abakumova EU, Moiseeva NN, Dolgikh OL. Influence of umbilical cord blood fraction (up to 5 kDa) of cattle on biochemical parameters of blood in experimental subchronic gastric ulcer in rats. Ukrainian Biochemical Journal. 2008;80(2):120–7. Russian
22. Zharikov AYu, Lorenz SE, Bobrov IP, Mazko ON, Makarova OG Search for new oligopeptide molecules for pharmacological correction of NSAID-induced gastric ulcer. Biomedicine. 2019;15(3):90–7. DOI: https://doi.org/10.33647/2074-5982-15-3-90-97. Russian
23. Gulida MO, Miroshnichenko EB, Birch HI, Garyachiy EB. Application of placenta extract in the complex treatment of patients with rheumatoid arthritis. Experimental and clinical medicine. 2014;1(62):168–71. Russian
24. Kapustianska AA. Stagnation of the drug "Cryocell-cryoextract of the placenta" in the complex treatment of gouty arthritis with metabolic arthritis. Actual problems of contemporary medicine: Bulletin of the UMSA. 2010;10(30):54–8. Ukrainian
25. Pogozhykh O, Prokopyuk V, Figueiredo C, Pogozhykh D. Placenta and placental derivatives in regenerative therapies: experimental studies, history, and prospects. Stem Cells International. 2018;2018:1–14. DOI: https://doi.org/10.1155/2018/4837930.
26. Shepitko KV. Application of cryopreserved placenta preparations in the small intestine pathologies in rats for their further use in exigent conditions. Bulletin of problems biology and medicine. 2019;4(154):56–61. DOI: http://doi.org/10.29254/2077-4214-2019-4-2-154-56-61.
27. Petrenko YA, Petrenko AY, Martin I, Wendt D. Perfusion bioreactor-based cryopreservation of 3D human mesenchymal stromal cell tissue grafts. Cryobiology. 2017;76:150–3. DOI: http://doi.org/10.1016/j.cryobiol.2017.04.001.

DOI: 10.19163/2307-9266-2021-9-4-278-293

28. Rogulska O, Tykhvynska O, Revenko O, Grischuk V, Mazur S, Volkova N, Vasyliev R, Petrenko A, Petrenko Y. Novel Cryopreservation Approach Providing Off-the-Shelf Availability of Human Multipotent Mesenchymal Stromal Cells for Clinical Applications. Stem Cells International. 2019;2019:4150690. DOI: https://doi.org/10.1155/2019/4150690.
29. Pogozhykh D, Pogozhykh O, Prokopyuk V, Kuleshova L, Goltsev A, Blasczyk R, Mueller T. Influence of temperature fluctuations during cryopreservation on vital parameters, differentiation potential, and transgene expression of placental multipotent stromal cells. Stem Cell Research & Therapy. 2017;8(1):66. DOI: https://doi.org/10.1186/s13287-017-0512-7.
30. Kapustyanska AA The use of the drug "Cryocell-cryoextract of the placenta" in the complex treatment of exacerbation of gouty arthritis with metabolic arthritis. Actual problems of modern medicine: Bulletin of the Ukrainian Medical and Dental Academy. 2010.10.(30);54–8. Russian
31. Prokopyuk V. Yu., Falko OV, Musatova IB, Prokopyuk OS, Roenko OO, Terekhova OO, Chub OV. Critical preservation and low-temperature cure of placental biosynthesis. Problems of Criobiology and Criomedicine. 2015;25(4):291–310. Ukrainian
32. Hladkykh FV, Chyzh NA. Protection mechanisms of the gastric mucosa under conditions of placental cryoextract use in diclofenac-induced gastropathy. Collection of materials of the satellite remote scientific-practical conference of students and young scientists "Fundamental Science in Modern Medicine – 2021". Minsk: Foundation of education "Bilorussian State Medical University". 2021:436–40. Russian
33. Hladkykh FV, Chyzh NA, Sleta IV. Study of gastroprotective activity of cryopreserved placenta extract in ibuprofen-induced gastric lesion in experiment. Materials of the VII Republican Scientific-Pak conference of young scientists "Modern achievements of young scientists in medicine - 2020". Grodno. Grodno: Foundation of education "Grodno State Medical University". 2020:57–60. Russian
34. Hladkykh FV, Chyzh MO. Modulation of meloxicam-induced changes in gastrointestinal and motor activity of the stomach by applying placenta cryoextract. Proceedings of the Shevchenko Scientific Society. Medical Sciences. 2021;64(1):84–94. DOI: https://doi.org/10.25040/ntsh2021.01.08 Access: https://mspsss.org.ua/index.php/journal/article/view/400. Ukrainian
35. Gryshchenko NG, Klimenko NA, Gorgol NI, Tatarko SV. Effect of placental cryoextract on chronic inflammation of the ovaries in mice. Medicine today and tomorrow. 2010;2–3(47–8):7–17. Available from: http://dspace.nbuv.gov.ua/handle/123456789/44519
36. Brun LV, Makolinets VI. Investigation of the effect of low-intensity infrared laser radiation on the concentration of diclofenac sodium in the blood plasma of rats by high-performance liquid chromatography. Medical and clinical chemistry. 2016;18(2):54–9. Ukrainian. Available from: http://nbuv.gov.ua/UJRN/Medkh_2016_18_2_12
37. Rybolovlev YuR, Rybolovlev RS Dosing of substances for mammals according to the constants of biological activity. Reports of the USSR Academy of Sciences. 1979;247(6):1513–6. Russian
38. Orlovskaya IA, Tsyrendorzhiev DD, Shchelkunov SN Rheumatoid arthritis: laboratory models of the disease. Medical immunology. 2015;17(3):203–10. Russian
39. Gromyko MV, Gritsuk AI. Experimental models of rheumatoid arthritis. Problems of health and ecology. 2012;2(32):115–8. Russian
40. Freund J. Some aspects of active immunization. Annual Review of Microbiology. 1947;1:291–308.
41. Choudhary N, Bhatt LK, Prabhavalkar KS. Experimental animal models for rheumatoid arthritis. Immunopharmacology and Immunotoxicology. 2018;40(3):193–200. DOI: https://doi.org/10.1080/08923973.2018.1434793.
42. Alavala S, Nalban N, Sangaraju R, Kuncha M, Jerald MK, Kilari EK, Sistla R. Anti-inflammatory effect of stevioside abates Freund's complete adjuvant (FCA)-induced adjuvant arthritis in rats. Inflammopharmacology. 2020;28(6):1579–97. DOI: https://doi.org/10.1007/s10787-020-00736-0.
43. Asakawa T, Matsushita S. Coloring condition of thiobarbituric acid test for detecting lipid hydroperoxides. Lipids. 1980;15(3):137–40.
44. Kostyuk VA, Potapovich AI, Kovaleva ZhV. A simple and sensitive method for determining the activity of suprokid dismutase, based on the oxidation reaction of quercetin. Questions of medicinal chemistry. 1990;2:88–91. Russian
45. Bligh EG, Dyer WI. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology. 1959;37(8):911–7.
46. Svetashev VI, Vaskovsky VE. A simplified technique for thin layer microchromatography of lipids. Journal of Chromatography. 1972;67:376–8.
47. Vaskovsky VE, Kostetsky EY, Vasendin IM. A universal reagent for phospholipid analysis. Journal of Chromatography. 1975;114:129–41.
48. Mititelu RR, Padureanu R, Bacanoiu M, Padureanu V, Docea AO, Calina D, Barbulescu AL, Buga AM. Inflammatory and oxidative stress markers-mirror tools in rheumatoid arthritis. Biomedicines. 2020;8(5):125. DOI: https://doi.org/10.3390/biomedicines8050125.
49. Voitenko NG, Makarova MN, Zueva AA Variability of blood biochemical parameters and the establishment of reference intervals in preclinical studies. Communication 1: rats. Laboratory animals for scientific research. 2020;1:47–53. DOI: https://doi.org/10.29296/26187223X-2020-01-06. Russian
50. Hladkykh FV, Stepanyuk NH. Characteristics of the therapeutic effect of ibuprofen and the combination of hematological indicators on the model of adjuvant arthritis in rats. Lviv Medical Journal. 2015;4:64–70. Ukrainian. Available from: http://nbuv.gov.ua/UJRN/Lmch_2015_21_4_14
51. Podolskiy VV, Podolskiy VV. Peroxidation of lipids as a manifestation of oxidative stress in the organisms of women because of reproductive health symptoms on aphids of somatoform diseases due to disorders of vegetative homeostasis. Obstetrics and obstetrics. 2017;2:98–104. DOI: https://doi.org/10.11603/24116-4944.2017.2.8095. Ukrainian
52. Agrahari G, Sah SK, Nguyen CT, Choi SS, Kim HY, Kim TY. Superoxide dismutase 3 inhibits LL-37/KLK-5-mediated skin inflammation through modulation of EGFR and associated

inflammatory cascades. Journal of Investigative Dermatology. 2020;140(3):656–65. DOI: https://doi.org/10.1016/j.jid.2019.08.434.

53. Nguyen NH, Tran GB, Nguyen CT. Anti-oxidative effects of superoxide dismutase 3 on inflammatory diseases. Journal of Molecular Medicine. 2020;98(1):59–69. DOI: https://doi.org/10.1007/s00109-019-01845-2.

54. Suchdev PS, Williams AM, Mei Z, Flores-Ayala R, Pasricha SR, Rogers LM, Namaste SM. Assessment of iron status in settings of inflammation: challenges and potential approaches. The American Journal of Clinical Nutrition. 2017;106(6):1626–33. DOI: https://doi.org/10.3945/ajcn.117.155937.

55. Iwanczak B, Ruczka M, Matusiewicz M, Pytrus T, Matusiewicz K, Krzesiek E. Correlation between biomarkers (calprotectin, seromucoid, metalloproteinase-3 and CRP) and clinical and endoscopic activity of ulcerative colitis in children. Advances in Medical Sciences. 2020;65(2):259–64. DOI: https://doi.org/10.1016/j.advms.2020.03.004.

56. Namaste SM, Aaron GJ, Varadhan R, Peerson JM, Suchdev PS; BRINDA Working Group. Methodologic approach for the biomarkers reflecting inflammation and nutritional determinants of anemia (BRINDA) project. The American Journal of Clinical Nutrition. 2017;106(1):333–47. DOI: https://doi.org/10.3945/ajcn.116.142273.

57. Smith SA, Waters NJ. Pharmacokinetic and pharmacodynamic considerations for drugs binding to alpha-1-acid glycoprotein. Pharmaceutical Research. 2018;36(2):30. DOI: https://doi.org/10.1007/s11095-018-2551-x.

58. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. Frontiers in Immunology. 2018;9:754. DOI: https://doi.org/10.3389/fimmu.2018.00754.

59. Lapic I, Padoan A, Bozzato D, Plebani M. Erythrocyte sedimentation rate and C-reactive protein in acute inflammation. American Journal of Clinical Pathology. 2020;153(1):14–29. DOI: https://doi.org/10.1093/ajcp/aqz142.

60. Yao Z, Zhang Y, Wu H. Regulation of C-reactive protein conformation in inflammation. Inflammation Research. 2019;68(10):815–23. DOI: https://doi.org/10.1007/s00011-019-01269-1.

61. Avan A, Tavakoly Sany SB, Ghayour-Mobarhan M, Rahimi HR, Tajfard M, Ferns G. Serum C-reactive protein in the prediction of cardiovascular diseases: Overview of the latest clinical studies and public health practice. Journal of Cellular Physiology. 2018;233(11):8508–25. DOI: https://doi.org/10.1002/jcp.26791.

62. Li W, Cao T, Luo C, Cai J, Zhou X, Xiao X, Liu S. Crosstalk between ER stress, NLRP3 inflammasome, and inflammation. Applied Microbiology and Biotechnology. 2020;104(14):6129–40. DOI: https://doi.org/10.1007/s00253-020-10614-y.

63. Wang J, Xia J, Yan X, Yang Y, Wei J, Xiong Y, Wu W, Liu Y, Chen Y, Jia B, Chen Z, Zhang Z, Ding W, Huang R, Wu C. The gamma-glutamyl transpeptidase to platelet ratio predicts liver inflammation in chronic hepatitis B with normal or mildly elevated alanine transaminase. Clinics and Research in Hepatology and Gastroenterology. 2020;44(6):913–22. DOI: https://doi.org/10.1016/j.clinre.2020.01.011.

64. Ndrepepa G, Colleran R, Kastrati A. Gamma-glutamyl transferase and the risk of atherosclerosis and coronary heart disease. Clinica Chimica Acta. 2018;476:130–8. DOI: https://doi.org/10.1016/j.cca.2017.11.026.

65. Mohi-Ud-Din R, Mir RH, Sawhney G, Dar MA, Bhat ZA. Possible pathways of hepatotoxicity caused by chemical agents. Current drug metabolism. 2019;20(11):867–79. DOI: https://doi.org/10.2174/1389200220666191105121653.

66. Jung SH, Lee W, Park SH, Lee KY, Choi YJ, Choi S, Kang D, Kim S, Chang TS, Hong SS, Lee BH. Diclofenac impairs autophagic flux via oxidative stress and lysosomal dysfunction: Implications for hepatotoxicity. Redox Biology. 2020;37:101751. DOI: https://doi.org/10.1016/j.redox.2020.101751.

67. Vyas A, Purohit A, Ram H. Assessment of dose-dependent reproductive toxicity of diclofenac sodium in male rats. Drug and Chemical Toxicology. 2019;42(5):478–86. DOI: https://doi.org/10.1080/01480545.2017.1421659.

68. Yeh JC, Wu CC, Choy CS, Chang SW, Liou JC, Chen KS, Tung TH, Lin WN, Hsieh CY, Ho CT, Wang TM, Chang JF. Non-Hepatic Alkaline Phosphatase, hs-CRP and Progression of Vertebral Fracture in Patients with Rheumatoid Arthritis: A Population-Based Longitudinal Study. Journal of Clinical Medicine. 2018;7(11):439. DOI: https://doi.org/10.3390/jcm7110439.

69. Jo S, Han J, Lee YL, Yoon S, Lee J, Wang SE, Kim TH. Regulation of osteoblasts by alkaline phosphatase in ankylosing spondylitis. International Journal of Rheumatic Diseases. 2019;22(2):252–61. DOI: https://doi.org/10.1111/1756-185X.13419.

70. Hirschmugl B, Crozier S, Matthews N, Kitzinger E, Klymiuk I, Inskip HM, Harvey NC, Cooper C, Sibley CP, Glazier J, Wadsack C, Godfrey KM, Desoye G, Lewis RM. Relation of placental alkaline phosphatase expression in human term placenta with maternal and offspring fat mass. International Journal of Obesity. 2018;42(6):1202–10. DOI: https://doi.org/10.1038/s41366-018-0136-8.

71. Liao KP, Solomon DH. Lipids and cardiovascular risk through the lens of rheumatoid arthritis. Arthritis & Rheumatology. 2019;71(9):1393–5. DOI: https://doi.org/10.1002/art.40891.

72. Kril IY, Gavrilyuk AM, Stoyka RS, Chop'yak VV, Kit YYa. Characteristics of enzymatic activity and protein composition of serum of rats under the conditions of immunization-induced inflammation of the joints. Experimental and clinical physiology and biochemistry. 2014;2:15–23. Ukrainian

73. Hladkykh FV, Mikhailova IP, Manchenko AO. Infusion of sodium and diclofenac with this combination with a cryopreserved placental extract on the lynchial balance in the syrovatous blood in experimental rheumatoid arthritis. Materials of the XXVII International Scientific and Practical Conference of Young Graduates and Students "Topical issues of new medicines development" (March 18–19, 2021); Kharkiv: National Pharmaceutical University of the Ministry of Health of Ukraine. 2021:272–3. Ukrainian

ОРИГИНАЛЬНАЯ СТАТЬЯ

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