

EFFECT OF METAL NANOPARTICLES USAGE ON OXIDATIVE STRESS INDICATORS AND ENDOTOXEMIA PARAMETERS UNDER DMH-INDUCED CARCINOGENESIS

S. B. KRAMAR¹✉, I. Ya. ANDRIICHUK², N. V. OHINSKA¹, Yu. V. SOROKA³,
Z. M. NEBESNA¹, S. M. DYBKOVA⁴, L. S. RIEZNICHENKO⁴, N. Ye. LISNYCHUK²

¹Department of Histology and Embryology,

I. Horbachevsky Ternopil National Medical University, Ukraine;

²Central Research Laboratory, I. Horbachevsky Ternopil National Medical University, Ukraine;

³Department of Anaesthesiology and Intensive Care,

I. Horbachevsky Ternopil National Medical University, Ukraine;

⁴F.D. Ovcharenko Institute of Biocolloidal Chemistry,

National Academy of Sciences of Ukraine, Kyiv;

✉e-mail: kramarsb@tdmu.edu.ua

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One of the properties of nanoparticles is their ability to correct manifestations of oxidative stress and endotoxemia, which are critical factors in cancer development. Therefore, the work aimed to investigate the effect of the usage of Au/Ag/Fe nanoparticles on oxidative stress indicators and endotoxemia parameters in experimental colon carcinogenesis. The study was performed on 90 white male rats kept in standard vivarium conditions. The division into groups: I – intact animals; II – intact animals with 21 days NPs administration; III – animals injected with *N,N*-dimethylhydrazine dihydrochloride for 30 weeks; IV – animals to which Au/Ag/Fe nanoparticles were intragastrically administered daily for 21 days after induced adenocarcinoma. According to our results, the concentration of oxidative stress indicators significantly increases under DMH-induced carcinogenesis conditions. It was established that the 21-day intragastric administration of NP Au/Ag/Fe composition caused a significant ($P < 0.001$) decrease in the concentration of TBARS in the blood serum by 1.33 times, in the content of diene and triene conjugates by 1.63 and 1.98 times, respectively compared to the third experimental group. The introduction of NPs in the fourth experimental group reduces the concentration of the Schiff bases by 1.34 times ($P < 0.001$), decreases the content of POMP₃₇₀ and POMP₄₃₀ by 1.25 ($P < 0.001$) and 1.37 times ($P < 0.001$), respectively, compared to the third experimental group. We also observed the reduction of endotoxemia levels in a fourth experimental animal group based on a significant decrease in MMM index and EII percentage.

Key words: Au/Ag/Fe nanoparticles; oxidative stress; endotoxemia; *N,N*-dimethylhydrazine dihydrochloride; induced colon carcinogenesis.

Cancer is a leading cause of death in global statistics. It is projected that in 2024, there will be more than 2 million new cancer cases and over 611000 cancer deaths in the United States only. The most common (in terms of new cases) are breast, lung, colon, rectum, prostate, skin, and stomach cancers [1-3]. The development of a tumor process in the body is accompanied by disorders in intracellular homeostasis, increased production of ROS, and changes in the intensity of lipid peroxidation, which causes oxidative damage to cellular mac-

romolecules and the development of oxidative stress [4-7]. In addition, endogenous intoxication syndrome (EIS) develops during carcinogenesis, as well as in other pathological conditions [8-10]. The most promising indicators of EIS are the determination of the erythrocyte intoxication index (EII) and the concentration of medium molecular mass molecules (MMM), i.e. oligopeptides with a molecular weight of 300-500 to 5000 Da, which are protein toxins with a high content of dicarboxylic and low content of aromatic acids. A significant increase in the con-

tent of MMM in the blood in various pathologies is a prognostically unfavourable indicator of the course of the disease [9-11].

It is always important to search for agents that help reduce oxidative stress levels and the degree of endogenous intoxication in oncological pathologies, as these factors only exacerbate carcinogenesis [5, 7]. The usage of nanotechnology in various areas of human life is widespread, and nanomedicine is developing rapidly in particular [12]. Nanoparticles (NPs) of chemical compounds or elements are widely used in diagnosing and treating diseases and delivering drugs to their destination in the body [13]. Metal NPs have unique properties, particularly gold, titanium, iron, silver and copper [12].

Gold NPs have excellent optical and electronic properties, convert optical energy into heat, are biocompatible, colloidal stable, low toxicity, are easy to synthesize, and can be used as drug carriers [14-16]. Silver NPs have unique antimicrobial properties, which is why they are used in various industries (cosmetics, medicine, renewable energy, textiles) [17, 18]. Iron NPs ease of synthesis, have superparamagnetic properties, and a favorable surface-to-volume ratio [19], have an anti-anemic effect and contribute to the normalization of intestinal microflora in animals with modeled iron deficiency anemia [20, 21].

Despite the numerous positive properties of metal nanoparticles and their pharmacological activity, their cytotoxic effects on cells, particularly as activators of oxidative stress, have been observed [22]. However, the toxic properties of nanoparticles are related to their size and shape, preparation method, structure, and dose. The negative impact can be minimised by properly selecting these parameters [12, 13, 15, 23].

Many studies illustrate the effect of exposure to nanoparticles of a particular metal, but the results of their complex use as composites are insufficiently studied.

Therefore, our work aimed to investigate the effect of using the composition of Ag/Au/Fe nanometals on oxidative stress and endotoxemia parameters in experimental colon carcinogenesis.

Materials and Methods

Animals. The research was accomplished on 90 white outbred male rats with a body weight 190 ± 5 g. The animals had been retained in standard conditions of vivarium. Experimental animals had

unfastened access to drinking water and basal food regimen *ad libitum*. All manipulations with animals throughout this experiment have been conformed according to internationally accepted requirements and accredited by the Bioethical Committee of Ternopil National Medical University (protocol No 75, 01.11.2023). All experiments were carried out in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Different Medical Functions".

Animals were divided into groups: I – intact animals; II – intact animals with 21 days NPs administration; III – animals injected with N,N-dimethylhydrazine dihydrochloride for 30 weeks; IV – animals to which Au/Ag/Fe NP composition was intragastrically administered daily for 21 days after induced adenocarcinoma.

Colorectal cancer model. N,N-dimethylhydrazine-induced (DMH-induced) colon adenocarcinoma *in situ* was modelled by introducing N,N-dimethylhydrazine hydrochloride (Sigma-Aldrich Chemie, Japan, series D161802) dissolved in an isotonic sodium chloride solution. The chemical carcinogen was administered subcutaneously into the interscapular area once per week for a duration of 30 weeks, with a single dose of 7.2 mg/kg of body weight (primarily based on the active substance). Animals in the control group received 0.1 ml of physiological saline following the same frequency and procedure. At 30 weeks of DMH administration, colon adenocarcinoma *in situ* was histologically diagnosed in DMH-treated rats.

NP dosage and administration. In this study, the composition of spherical silver ($d = 30$ nm), gold ($d = 30$ nm) and iron ($d = 40$ nm) NPs was used. Individual sterile aqueous dispersions of spherical silver, gold and iron NPs were synthesized at the Ovcharenko Institute of Biocolloidal Chemistry of the National Academy of Sciences of Ukraine and characterized by size, shape and elemental composition [24-26]. Silver NP initial water dispersion was synthesised by the tannin (tannic acid) reduction of silver nitrate (AgNO_3) in the presence of potassium carbonate (K_2CO_3). The synthesis of gold NPs was performed via reduction of the tetra chloroauric (III) acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) ($\geq 99.9\%$ trace metals basis, Sigma-Aldrich) by sodium citrate tribasic dehydrate in the presence of potassium carbonate. Iron NPs have been synthesised via the reduction of iron (III) chloride by sodium borohydride (NaBH_4). The aqueous dispersions of metal nanoparticles were steri-

lised by autoclaving (121°C, 15 min). The composition of Au/Ag/Fe NPs was obtained by mechanical mixing of aqueous dispersions of monopreparations under aseptic conditions. The mixture of nanoparticles was aggregatively stable in time.

The final concentration of nanoparticles in the mixture was 1.6 mg Ag; 0.1 mg Fe; 3.088 µg Au per 1 ml by metal. The concentrations of metal nanoparticles in the mixture were selected based on the results of *in vivo* toxicity and biological effects studies performed earlier [25–28]. Metal NPs used to receive the experimental mixture, as well as the obtained composition, also was characterized as safe according to the criteria of cytotoxicity (MTT-test), genotoxicity (comet assay), mutagenicity (Allium-test) and immunotoxicity under *in vitro* tests [29].

Animals received NP Au/Ag/Fe water dispersion intragastrically once a day for 21 days at a dose of 0.842 mg Ag/0.0526 mg Fe/1.625 µg Au per 1 kg of rat body weight. The initial water mixture of NP Au/Ag/Fe was diluted with sterile distilled water at a ratio of 1:10 before the intragastric administration.

Endogenous intoxication syndrome markers and oxidative stress indicators. The severity of the endogenous intoxication syndrome (EIS) was assessed by changes in erythrocyte intoxication index (EII, %), the content of middle mass molecules (MMM₂₃₈, MMM₂₅₄, MMM₂₆₀, MMM₂₈₀) and their indices (distribution index $\text{MMM}_{280}/\text{MMM}_{254}$); the peptide-nucleotide index ($\text{MMM}_{238}/\text{MMM}_{260}$); aromaticity index ($\text{MMM}_{238}/\text{MMM}_{280}$). EII was determined by the amount of colouring agent (methylene blue) absorbed by erythrocyte membranes. The method is based on the idea of the erythrocyte as an adsorbent, i.e. the ability of the erythrocyte membrane to absorb and pass coloured substances [30, 31]. The content of the MMM was determined by isolating the acid-soluble MMM fraction and then detecting the tenfold diluted supernatant at 238 nm (low molecular mass peptides with a molecular mass of up to 2000 Da), 254 nm (low- and medium-molecular mass peptides with a molecular weight of 500 Da to 5000 Da), 260 nm (nucleotide fraction) and 280 nm (high-molecular-mass fraction, aromatic amino acids) [30].

As markers of oxidative stress, changes in the concentrations of thiobarbituric acid reactive substances (TBARS), diene and triene conjugates (DC, TC), Schiff bases, and aldehyde and ketone derivatives products of neutral (POMP₃₇₀) and basic (POM₄₃₀) origin were studied in the blood serum.

Their concentration was detected with the generally accepted methods [32].

Statistical analysis. Statistical processing of the obtained quantitative data was performed using the method of variational statistics, one-factor analysis of variance ANOVA in the “Excel” software (Microsoft, USA). Data are presented as the mean and standard error of the mean ($M \pm \text{SEM}$). If $P < 0.05$, differences were considered significant.

Results and Discussion

The induction of carcinogenesis contributes to the development of oxidative stress in the body, with excessive ROS formation. The latter are involved in further developing the neoplastic process through the activation or inhibition of transcription factors, are the molecular basis for the survival of cancer cells, and are the initiators of the destruction of lipid molecules [4, 5].

To verify the biosafety of the Au/Ag/Fe NP composition and its effect on the state of oxidative processes in the body of unaffected animals, the level of oxidative stress markers and EIS indicators in the second experimental group was studied. According to the results, there were no statistically significant differences between the studied parameters. Due to this, we focused on comparing the levels of

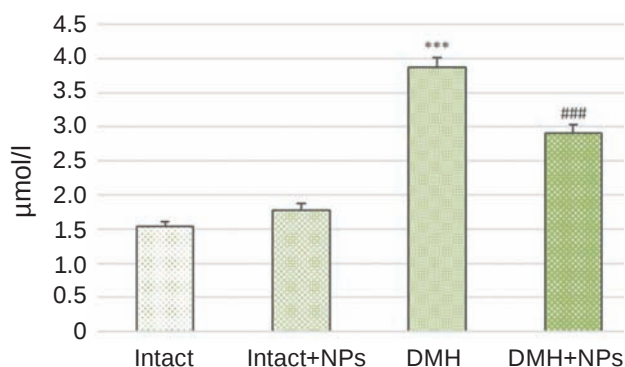


Fig. 1. Changes in the concentration of TBARS in the blood serum of: intact – unaffected animals; intact+NPs – unaffected animals with 21 days NP administration; DMH – animals with DMH-induced colon adenocarcinoma; DMH+NPs – affected animals treated with NPs during 21 days. ***Value that differs from similar indicators of the intact group of animals with a significance of $P < 0.001$; ###value that differs from similar indicators of the DMH-affected animals with a significance of $P < 0.001$

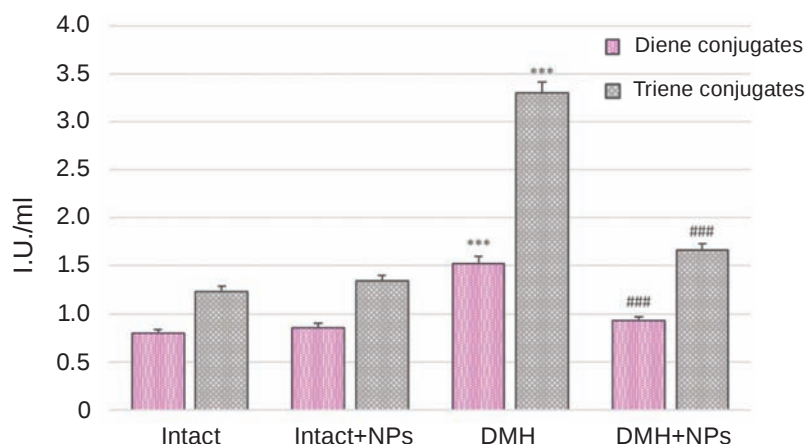


Fig. 2. Changes in the concentration of diene and triene conjugates in the blood serum of: intact – unaffected animals; intact+NPs – unaffected animals with 21 days NP administration; DMH – animals with DMH-induced colon adenocarcinoma; DMH+NPs – affected animals treated with NPs during 21 days. ***Values that differ from similar indicators of the intact group of animals with a significance of $P < 0.001$; ###values that differ from similar indicators of the DMH-affected animals with a significance of $P < 0.001$

indicators between the first, third and fourth experimental groups.

One of the secondary products of lipid peroxidation in the reaction with 2-thiobarbituric acid is TBARS. According to our results, under DMH-induced carcinogenesis conditions, the TBARS concentration in the animal's blood serum is 2.51 times higher ($P < 0.001$) than in intact animals. On the contrary, 21-day intragastric administration of NP Au/Ag/Fe composition caused a significant ($P < 0.001$) decrease in the concentration of TBARS in the blood serum by 1.33 times compared to the third experimental group (Fig. 1).

The concentration of diene and triene conjugates under carcinogenesis conditions increases significantly ($P < 0.001$) and exceeds the intact value by 1.91 and 2.69 times, respectively. After 21 days of NP Au/Ag/Fe composition administration to animals of the fourth experimental group, a significant decrease in the content of these compounds was observed compared with the third experimental group by 1.63 and 1.98 times, respectively (Fig. 2).

DC and TC decompose very rapidly, leading to the appearance of toxic products, one of which is Schiff bases. Currently, a lot of research are focused on the antimicrobial, antifungal and antitumour properties of Schiff bases [33].

Biochemical analysis of the Schiff bases content in the blood serum of DMH-affected animals shows a significant ($P < 0.001$) increase in its concentration by 3.83 times compared with intact values. However,

the introduction of NPs reduces their concentration by 1.34 times ($P < 0.001$) (Fig. 3).

Protein oxidative modification products (POMP) are more stable and specific than lipid peroxidation products. Determination of changes in their concentration is a convenient diagnostic and prognostic marker for the development of pa-

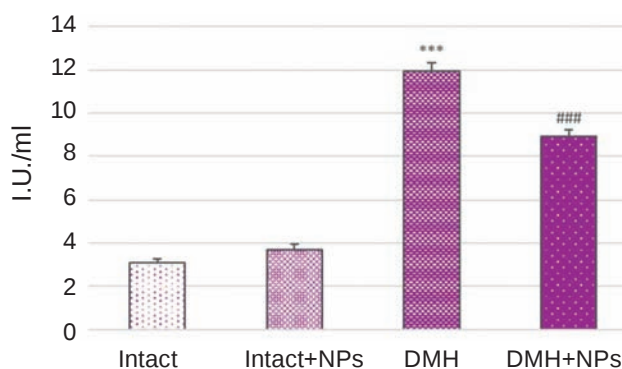


Fig. 3. Changes in the concentration of the Schiff bases in the blood serum of: intact – unaffected animals; intact+NPs – unaffected animals with 21 days NP administration; DMH – animals with DMH-induced colon adenocarcinoma; DMH+NPs – affected animals treated with NPs during 21 days. ***Value that differs from similar indicators of the intact group of animals with a significance of $P < 0.001$; ###value that differs from similar indicators of the DMH-affected animals with a significance of $P < 0.001$

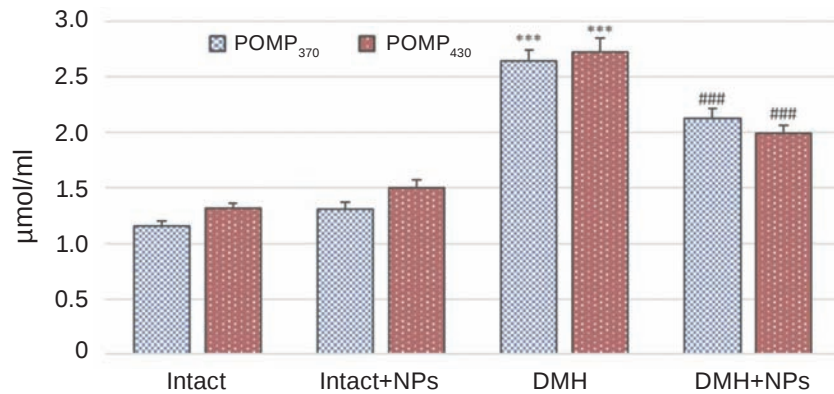


Fig. 4. Changes in the concentration of the POMP in the blood serum of: intact – unaffected animals; intact+NPs – unaffected animals with 21 days NP administration; DMH – animals with DMH-induced colon adenocarcinoma; DMH+NPs – affected animals treated with NPs during 21 days. ***Values that differ from similar indicators of the intact group of animals with a significance of $P < 0.001$; ###values that differ from similar indicators of the DMH-affected animals with a significance of $P < 0.001$

thologies as an indicator of the intensity of oxidative stress [34].

According to the results of our studies, the concentration of POMP in the blood plasma of white rats injected with DMH during seven months significantly increased. The concentration of neutral aldehyde and ketone derivatives (POMP₃₇₀) was statistically significantly ($P < 0.001$) higher than that of intact animals by 2.28 times. The content of basic derivatives (POMP₄₃₀) was also significantly ($P < 0.001$) higher than that of the first group by 2.10 times. After 21 days of NP administration to animals of the fourth group, a decrease in the content of POMP₃₇₀ and POMP₄₃₀ was observed by 1.25 ($P < 0.001$) and 1.37 times ($P < 0.001$), respectively, compared to the third experimental group (Fig. 4).

Based on the concept of “metabolic intoxication” syndrome, in the long course of various pathologies, including carcinogenesis, toxic metabolic products accumulate in the body’s biological fluids, most of which are medium molecular weight substances [10]. Under the conditions of DMH-modeled colorectal cancer, the content of various fractions of MMM in the blood plasma increased significantly. Thus, the concentration of MMM₂₃₈ (low molecular weight peptides) in animals of the third group increased by 3.92 times ($P < 0.001$) compared to the normal value, MMM₂₅₄ (total integral index of low and medium molecular weight substances) increased by 2.59 times ($P < 0.001$), MMM₂₆₀ (nucleotide fraction) – by 1.31 times ($P < 0.001$), MMM₂₈₀ (aromatic amino acids) – by 6.79 times ($P < 0.001$). These re-

sults are consistent with the findings of other scientists who have studied endotoxemia in pathologies of various genesis [9-11, 35]. The heterogeneity in the growth of the MMM fractions can be explained by the fact that each of them is eliminated from the body by different organs. For example, the most significant increase in our studies was observed in the fraction of aromatic amino acids, which are eliminated from the body mainly by the liver [11]. It is known that under conditions of DMH-induced carcinogenesis, this organ undergoes significant morphological and functional changes [36].

The NP administration significantly reduced the level of MMM fractions in animal blood of the fourth group compared to the third group: MCM₂₃₈ – by 1.88 times ($P < 0.001$), MMM₂₅₄ – by 2.06 times

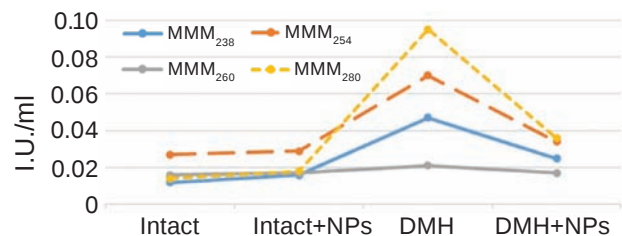


Fig. 5. The concentration of the middle mass molecules in the blood serum of: intact – unaffected animals; intact+NPs – unaffected animals with 21 days NP administration; DMH – animals with DMH-induced colon adenocarcinoma; DMH+NPs – affected animals treated with NPs during 21 days

($P < 0.001$), MMM_{260} – by 1.24 times ($P < 0.01$), MMM_{280} – by 2.64 times ($P < 0.001$) (Fig. 5).

The EIS can be caused by increased toxin concentration in the blood and changes in the ratio between individual MMM fractions. Therefore, indices reflecting the ratio of extinctions at certain wavelengths were calculated. The distribution index (DI), which reflects the accumulation of peptides containing aromatic chromatophores in the blood, in animals of the third group significantly ($P < 0.001$) exceeded the intact index by 2.61 times. The administration of the NP Au/Ag/Fe composition led to a 1.28-fold ($P < 0.001$) decrease in this index compared to DMH-affected animals (Table).

The aromaticity index (AI) under the conditions of modelled carcinogenesis significantly ($P < 0.001$) decreased by 1.73 times compared to the intact value. Under the conditions of correction with NPs in the fourth experimental group, AI increased, exceeding the value of the third group by 1.4 times ($P < 0.001$). This indicates a significant shift in the MMM equilibrium toward an increase in the concentration of aromatic chromatophores. The most significant increase was observed in the peptide-nucleotide index (PNI). Its average value in the blood of animals with DMH-induced carcinogenesis exceeded the normal value by 3.17 times ($P < 0.001$), and under conditions of correction by the composition of Au/Ag/Fe NPs, this indicator decreased by 1.52 times ($P < 0.001$) compared to the data of the third group. The dominance of the nucleotide fraction and the increased aromaticity of the peptides that make up the MMM are unfavorable factors in the course of oncogenesis and contribute to EIS development. It should be noted that calculating the indices in the second experimental group, the values of DI and PNI

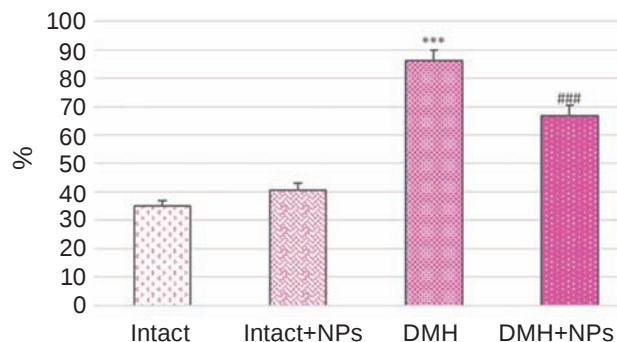


Fig. 6. Changes in erythrocyte membrane permeability in experimental groups of animals: intact – unaffected animals; intact+NPs – unaffected animals with 21 days NP administration; DMH – animals with DMH-induced colon adenocarcinoma; DMH+NPs – affected animals treated with NPs during 21 days. ***Value that differs from a similar indicator of the intact group of animals with a significance of $P < 0.001$; ###value that differs from a similar indicator of the DMH-affected animals with a significance of $P < 0.001$

were significantly ($P < 0.05$ and $P < 0.001$, respectively) higher than the norm, although changes in the concentration of individual MMM fractions did not differ significantly from the intact values. This may be due to the reaction of the animal body to the 21-day administration of the NP Au/Ag/Fe composition (Table).

Determination of the degree of erythrocyte membrane permeability is an important prognostic criterion for assessing the severity of endotoxemia [31]. Our study revealed its increase throughout the entire period of modelling neoplastic lesions of the colon. Thus, at the 30th week of observation, this in-

Table. Level of MMM indexes

Index	Experimental groups of animals			
	Intact	Intact+NPs	DMH	DMH+NPs
Distribution index 280/254	0.519 ± 0.023	0.621 ± 0.038*	1.357 ± 0.068***	1.059 ± 0.049###
Aromaticity index 238/280	0.857 ± 0.039	0.889 ± 0.031	0.495 ± 0.022***	0.694 ± 0.032##
Peptide-nucleotide index 238/260	0.706 ± 0.029	0.941 ± 0.045***	2.238 ± 0.115***	1.471 ± 0.068##

Notes: intact – unaffected animals; intact+NPs – unaffected animals with 21 days NP administration; DMH – animals with DMH-induced colon adenocarcinoma; DMH+NPs – affected animals treated with NPs during 21 days. *Values that differ from similar indicators of the intact group of animals with a significance of $P < 0.05$ (*) or $P < 0.001$ (***); ###values that differ from similar indicator of the DMH-affected animals with a significance of $P < 0.001$

indicator was 2.45 ($P < 0.001$) higher than the same in the group of unaffected animals (Fig. 6).

Because the erythrocyte membrane is considered a prototype of other cell membranes, we can assume that under these pathological conditions, they are also damaged, and intracellular homeostasis is disturbed [31, 35].

Considering the antimicrobial and anti-inflammatory properties of the nanometal composition [15, 17, 19, 25, 27], it was assumed that they might indirectly affect endotoxemia markers. The use of NPs contributed to a decrease in EII: this indicator was 1.29 times ($P < 0.001$) lower compared to the same in the group of animals with induced colon adenocarcinoma.

Conclusions. The experimental study indicates a positive effect of the NP Au/Ag/Fe composition usage on the activity of oxidative processes and the severity of the endogenous intoxication syndrome caused by the development of DMH-induced colon carcinogenesis. A statistically significant decrease in the concentrations of lipid peroxidation products, protein oxidative modification products and plasma toxicity parameters was found, which contributes to the improvement of cell metabolism and the general condition of the affected organism.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВПЛИВ НАНОЧАСТИНОК МЕТАЛІВ НА ПОКАЗНИКИ ОКСИДАТИВНОГО СТРЕСУ ТА ЕНДОТОКСЕМІЇ ЗА УМОВ ДМГ-ІНДУКОВАНОГО КАНЦЕРОГЕНЕЗУ

С. Б. Крамар^{1✉}, І. Я. Андрійчук²,
Н. В. Огінська¹, Ю. В. Сорока³,
З. М. Небесна¹, С. М. Дибкова⁴,
Л. С. Резніченко⁴, Н. Є. Лісничук²

¹Кафедра гістології та ембріології, Тернопільський національний медичний університет імені І. Я. Горбачевського, Тернопіль, Україна;

²Центральна науково-дослідна лабораторія, Тернопільський національний медичний університет імені І. Я. Горбачевського, Тернопіль, Україна;

³Кафедра анестезіології та інтенсивної терапії, Тернопільський національний медичний університет імені

І. Я. Горбачевського, Тернопіль, Україна;

⁴Інститут біологічної хімії ім. Ф. Д. Овчаренка НАН України, Київ, Україна;

✉ e-mail: kramarsb@tdmu.edu.ua

Однією з властивостей наночастинок є їх здатність коригувати прояви критичних факторів розвитку раку – оксидативного стресу та ендотоксемії. Тому, метою роботи було дослідити вплив використання наночастинок Au/Ag/Fe на показники оксидативного стресу та ендогенної інтоксикації за експериментального канцерогенезу товстої кишки. Дослідження виконано на 90 білих щурах-самцях, яких утримували в стандартних умовах віварію. Розподіл на групи: I – інтактні тварини; II – інтактні тварини, яким протягом 21 дня вводили Au/Ag/Fe наночастинок; III – тварини, яким протягом 30 тижнів вводили N,N-диметилгідразин дигідрохлорид; IV – тварини, яким після закінчення індукування аденокарциноми щоденно внутрішньошлунково вводили наночастинок Au/Ag/Fe протягом 21 дня. Показано, що за умов ДМГ-індукованого канцерогенезу концентрація показників оксидативного стресу суттєво зростала. Встановлено, що введення композиції наночастинок Au/Ag/Fe протягом 21 дня зумовлювало достовірне

($P < 0,001$) зниження концентрації ТБК-активних продуктів у сироватці крові в 1,33 раза, вмісту дієнових і трієнових кон'югатів у 1,63 і 1,98 раза порівняно з дослідною групою III. Застосування наночастинок у дослідній групі IV також сприяло зниженню концентрації основ Шиффа в 1,34 раза ($P < 0,001$), зменшенню вмісту ОМБ₃₇₀ і ОМБ₄₃₀ в 1,25 ($P < 0,001$) і 1,37 раза ($P < 0,001$) порівняно з групою III. Ми також спостерігали зниження рівня ендотоксемії в групі тварин IV на основі достовірного зменшення індексу МСМ та відсотка ЕП.

Ключові слова: Au/Ag/Fe наночастинок; оксидативний стрес; ендотоксемія; N,N-диметилгідразин дигідрохлорид; індукована аденокарцинома товстої кишки.

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