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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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NANOPARTICLES AND COLORECTAL CANCER: CAN THE USE OF METAL NANOPARTICLE COMPOSITIONS AFFECT OXIDATIVE STRESS MARKERS AND COLON HISTOLOGICAL CHANGES UNDER DMH-INDUCED CARCINOGENESIS

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Abstract.

Colorectal cancer (CRC) – a significant global health challenge. Exploring biological markers of oxidative stress is crucial, as they can play an essential role in initiating the transition from an organ's "healthy state" to a "malignant injury." There is substantial promise in investigating the level of 8-isoprostane (8-isoPGF2α) as a novel and dependable marker of oxidative stress. This paper presents that 8-isoprostane levels have been linked to the development of severe structural changes in the colon wall, accompanied by endogenic intoxication syndrome. The obtained results prove the strong connection between oxidative stress and carcinogenesis progression. Our research further illustrates the favorable and potentially beneficial impact of the Au/Ag/Fe NPs composition, which can find utility in a diverse range of contemporary applications.

Key words. Colon adenocarcinoma, colon morphology, 8-isoprostanes, metal nanoparticles.

Introduction.

Colorectal cancer (CRC) became a great challenge for millions of doctors and scientists and a significant problem of modernity. CRC has become increasingly widespread, although it is a well-studied malignancy with a slow progression, established risk factors, and detectable and treated pre-neoplastic lesions. In fact, colon cancer is the 4th most common cancer among men and the 3rd most common cancer in women. Despite the fact that scientists know more than ever about the genetic and cellular mechanisms that promote or inhibit cancer progression, it continues to be one of the world's leading health concerns [1-3].

Most colorectal cancer cases are nonhereditary and sporadic; early detection is critical. In the early stages of the disease, malignant neoplasms are usually asymptomatic. Many colon carcinogenesis markers are well-known nowadays [4,5]. However, there are two negative aspects of these diagnostic methods. Firstly, an increase in the concentration of almost all tumor markers, e.i. CA 242 – the most commonly used in Ukraine, is diagnostically significant in the later stages of the disease when the malignant oncological formation is sufficiently developed. Secondly, determining tumor markers is quite expensive and, in some countries, can be conducted only in certain laboratories. Even in developed countries, such testing might be difficult for a patient due to expensive tests are sometimes not covered by insurance. All of these restrict the patient's and doctor's ability to detect cancer early. When the pathological process has progressed to the point where first-line treatment is no longer viable, the patient visits a specialist for a palliative cure.

As a result, the search for more cancer markers continues. Scientists worldwide are still trying to figure out how to diagnose cancer in a way that will allow them to catch it early. Researchers have concentrated their efforts on developing new screening targets to detect neoplastic lesion growth at an early stage to improve patient survival rates and quality of life and minimize the number of colorectal cancer cases [6].

Numerous pathological processes consist of free radical-mediated oxidative stress, e.i. malignancy, cardiovascular, neurodegenerative, pulmonary illnesses etc. Lipid peroxidation is one of the most significant elements associated with oxidative stress. The measurement of lipid peroxidation products has been used to evaluate oxidative stress *in vivo*. Different oxidative stress markers *in vitro* are now available, but they are either insensitive or not specific, or they need invasive procedures. Free-radical oxidation processes that got out of antioxidant protection control may be the cause of carcinogenesis' fast progression. One of the most significant stages toward diagnosing the range of oxidative syndromes supposedly caused by reactive oxygen species (ROS) is the development of effective tools for assessing oxidative stress [7,8].

Several studies have demonstrated that quantitative evaluation of isoprostane levels, formed during oxidative stress, can be utilized as a useful marker of pro-oxidative status in various disease pathogenesis [9]. Isoprostanes have various advantages over other oxidative stress indicators, when used as oxidative stress markers [10,11]. First of all, researchers have drawn attention to this indicators, because they are chemically stable markers formed *in vivo*. Thus, we decided to observe changes in this indicator in the dynamics of the development of DMH-induced colon adenocarcinoma *in situ*.

Understanding the pathophysiology of CRC requires an understanding of changes in colon morphology. Deterioration of colon morphological structure in the dynamics of its neoplastic lesion, unfortunately, are insufficiently studied in the scientific investigations. That's why we also focused our attention on suctrural and substructural changes of colon under the condition of experimentally induced CRC. Despite considerable improvements in treatment, high morbidity remains a challenge. The effectiveness of widespread treatment methods is limited due to tumor cell resistance to chemotherapeutic bioactives, dose-related toxicities, and other major side effects. One of the new ways to circumvent such limitations seems to be the use of nanotechnology-based therapy [12,13].

Previous multiple explorations established positive effect of metal nanoparticles (NP) application in experimental oncology. The most impressive outcome was noticed when using each of the three metal NP: gold NP (Au NP), silver NP (Ag NP), and iron NP (Fe NP) [14-17].

Based on data obtained from the impressive results of different studies regarding the positive effect of the use of Au, Ag, and Fe NP, we were able to make an assumption, that the composition of these three nanometals will have no less and possibly more expressive and positive influence on various links of CRC pathogenesis, such as immunological disorders, anemia, dysbiosis, etc. [18-20].

So, we set ourselves the following research tasks: to investigate the degree of peroxidation processes development by the levels of its products (TBARS, 8-isoprostane) in the dynamics of the colonic neoplasia development; to analyze changes in the histoarchitectonics of the colon`s wall during the development of its adenocarcinoma in situ; evaluate the effect of the of metal nanoparticles composition (Au/Ag/Fe NPs) on changes of all the links of CRC pathogenesis which mentioned above.

Materials and Methods.

Animals:

The research was carried out on 148 white mature outbred male rats with body weight 190 ± 5 g. The animals were kept in standard vivarium conditions. Bodyweights and survival were monitored throughout. Rats had free access to drinking water and basal diet *ad libitum*. Animal experiments conducted in this study conformed to internationally accepted standards and were approved by the Bioethical Committee of Ternopil National Medical University (Ternopil, Ukraine). All manipulation with animals was performed according to the requirements of the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" [21].

The rats were randomly allocated into 4 groups: 1st – 68 control animals,

2nd – 80 animals with modeled colorectal adenocarcinoma *in situ*. Afterwards 24 of affected animals received NP Au/Ag/Fe intragastrically for 21 day (3rd group). 4th group – 12 control animals received NP Au/Ag/Fe similarly.

Every 30 days, 24 hours after the each last scheduled DMH administration, equal numbers of rats from each experimental group were deeply anesthetized with Thiopental (50 mg/kg, intraperitoneally, Arterium, NUA/3916/01/02) and sacrificed by cervical displacement and exsanguination. At the end of the experimental period, colon adenocarcinoma *in situ* was histologically identified in all DMH-treated rats.

Experimental animals who received NP composition were deeply anesthetized with Thiopental (50 mg/kg, intraperitoneally, Arterium, NUA/3916/01/02) and sacrificed by cervical displacement and exsanguination after three days since the last NP administration.

Colorectal Cancer Model.

One of the most frequently applied chemicals is N,N-dimethylhydrazine (DMH) in animal models that has been used for colon neoplastic injury modelling. The selected model has similar morphological and molecular features with human sporadic CRC [22].

DMH (Sigma-Aldrich Sp. z o.o, Poland, series D161608) was dissolved in 0,9 % NaCl solution in 1:100 ratio and injected subcutaneously (the interscapular region) at a dose of 7.2 mg/kg of rats' body weight once a week for 30 weeks. Animals of control group got subcutaneous injections of physiological saline in the same manner to avoid the probable effects of stress.

Oxidative stress markers.

Using a commercial enzymatic immunoassay 8-Isoprostane ELISA Kit #516351 (Cayman Chemical, USA), the concentration of 8-isoprostane (8-iso-$PGF_{2\alpha}$) in the blood and colon tissue of experimental animals was calculated in pg/ml.

Histopathology.

Samples of colonic tissues were collected from the animals and fixed in 10 % neutral buffered formalin overnight. The tissue processing procedure was done with the use of histoprocessor LOGO Sone (Milestone). For histological analysis, colon paraffin slices (5 μm thickness) were stained with Hematoxylin and Eosin (Biognost) and examined under a light microscope (Nikon Eclipse Ci).

Nanoparticles dosage and administration.

Administration of NPs composition was started three days after the last injection of DMH. White mature outbred male rats had an intragastrically administrated composition of spherical silver, gold, and iron nanoparticles (Ag/Au/Fe NPs) once a day for 21 days at a dose of 0.842 mg Ag, 0.053 mg Fe, and 1.625 μg Au per 1 kg of body weight. The concentration of metals in 1 ml of initial water dispersion was 1.6 mg Ag, 0.1 mg Fe, 3.1 μg Au. Before the intragastric administration, the initial water mixture of NP Au/Ag/Fe was diluted by sterile distilled water at a ratio of 1:10.

Initial water dispersion of the used Ag NP was synthesized via reduction of silver nitrate ($AgNO_3$) by tannin (tannic acid) at the presence of potassium carbonate (K_2CO_3); Au NP were synthesized via reduction of the tetrachloroauric (III) acid ($HAuCl_4 \cdot 3H_2O$) (≥ 99.9 % trace metals basis, Sigma-Aldrich) by sodium citrate tribasic dehydrate at the presence of potassium carbonate; Fe NP were synthesized via reduction of iron (III) chloride by sodium borohydride. Composition of the

Au/Ag/Fe NP was received via the mechanical mixture of the water dispersions of silver, gold and iron nanoparticles.

Used NP were specified in size using laser-correlation spectroscopy (Zetasizer-3, Malvern Instruments Ltd, UK) and transmission electron microscopy (JEM-1230, JEOL, Japan). The diameter of silver and gold NP was 30 nm, iron NP – 40 nm. Single metal NP, as well as a made composition, were characterised as biosafe according to the criteria of genotoxicity (comet assay), cytotoxicity (MTT-test, crystal violet colour), mutagenicity (Allium-test) and immunotoxicity (using peritoneal macrophages of rats) under *in vitro* tests [23,24].

Statistical Analyses.

All data were presented as the mean±standard deviation (M±SD). The significant difference between the two experimental groups were measured using Student's t-test and evaluated with Prism 5.0 software (GraphPad software Inc., USA). Distinctions in average means were considered reliable when the probability of an alternative hypothesis was not less than 0.95.

Results.

As modeling of adenocarcinoma *in situ* of the colon lasts for 30 weeks (7.5 months) and a material collection was performed every 30 days, we divided the terms of the experiment and process of collection of material into 7 stages (each step refers to 30 days of the experiment) for the convenience of the presentation of the results.

Colon histopathology:

The histological structure of the intact animals` colon wall was typical and had no species features (Figure 1).

Histological analysis of the colon`s wall samples showed minor changes at the first three stages of the experiment (0-90 days DMH administration) compared with the colon tissue of control group animals. Histological changes of the colon`s wall in this period of observation were characterized by: the crypt lumen narrowed due to the accumulation of mucus in cylindrical epitheliocytes; lympho- and histiocytic infiltration of the crypt stroma led to an increase of crypts` depth; thickness of the mucosa slightly decreased because of the crypt's extension; mucosal folds were thickened due to the stagnation of blood in the vessels and perivascular edema of the submucosa; an increase in lymphocytic infiltration of the lamina propria was noticed.

The severity and progression of destructive changes in the colon wall increased until the end of the 3rd observation stage (Figure 2A-E).

The 4th stage of the experiment was a turning point in the severity of histological changes in affected animals' colon wall: lymphoid tissue of the solitary follicle began to spread out from the submucosa to lamina propria, which lead to the extension of their area; irregular blood filling of vessels with the dominance of perivascular edema was established in the submucosa layer; the rice of hyperplasia of solitary follicles lymphocytes was a specific feature of pathological process development in that period of the experiment (Figure 3).

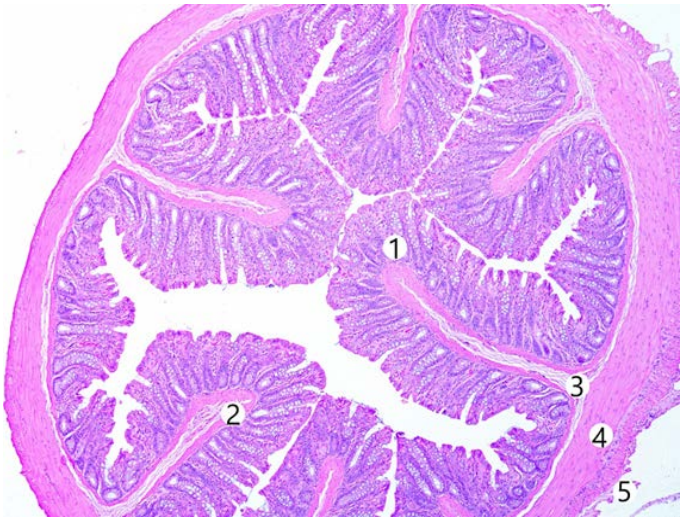


Figure 1. *Histological state of the colon of an intact animal. Crypts (1), muscularis mucosa (2), submucosa (3), muscular layer (4), serous tunic (5). Hematoxylin and eosin staining. x 40.*

Structural changes of the colon wall in the 5th – 6th stages of the experiment were characterized by the desquamation of surface epithelium with the formation of micro-erosions. For this period of time, it is also specific that lymphocytes were spreading by lymphatic vessels with the development of lymph stagnation. The most significant hyperplasia of solitary lymphatic follicles and lymph stagnation was observed in the 6th stage of the experiment. Furthermore, lymphocytic infiltration was spreading locally to the whole thickness of the mucosa (Figure 4A,B).

In the 7th stage of experimental cancerogenesis, severe epithelial dysplasia of the colon's mucosa layer was observed. The presence of hyperchromatic nuclei and the disorganisation of epithelium cell rows was a typical feature for violation of the ordinary microscopic organisation of the colon wall. One more feature of this term of neoplasia development was the presence of atypical cells with hyperchromic nuclei of different shapes and sizes, along with changes in the nuclear-cytoplasmic ratio towards the nuclei. The integrity deterioration of the basement membrane was also specific to that period of the experiment. All the above-described histopathologic features that developed at the 7th stage of colon adenocarcinoma *in situ* modeling corresponded to the classic histological changes for the development of colon neoplasia in humans: cytologic atypia, basal nuclei with chromatin condensation around the nuclear envelope, easily distinguishable nuclei hypercellularity, hyperchromic nuclei, heterogeneity of nuclear stratification and loss of polarity [25,26]. Histological analysis did not reveal such signs of invasive adenocarcinoma as invasion through the muscularis mucosae/submucosa, differing degrees of gland formation, desmoplasia.

Based on the facts mentioned above, we can state that *adenocarcinoma in situ* has developed in the colon. (Figure 5A,B).

8-iso-PGF2α concentration in investigated biological samples.

Serum:

It is important to highlight that level of 8-isoprostanes as markers of physiological oxidative processes slightly increased even in animals of the control group because of age progression of organism ($p \geq 0,05$).

In the 1st stage of neoplastic lesions modelling this indicator exceeded the indicator of the control group animals 1.4 times. On the 2nd stage of DMH administration, it exceeded the control indicator 1.5 times. Starting from the 3rd stage of the experiment the level of 8-isoprostanes increased significantly, comparing to the control group: on 3rd month – in 1.7 times, on 4th month – in 3.7 times, on 5th month – in 4.2 times, on 6th month – in 4.7 times, on 7th month – in 5.04 times (Figure 6).

Colon tissue

The level of 8-isoprostanes physiologically increased in homogenates of the colon of rats of the control group, representing ageing reorganization of the body ($p \geq 0,05$).

In the colonic homogenates, 8-isoprostanes increased proportionally throughout the simulation. As a result of the

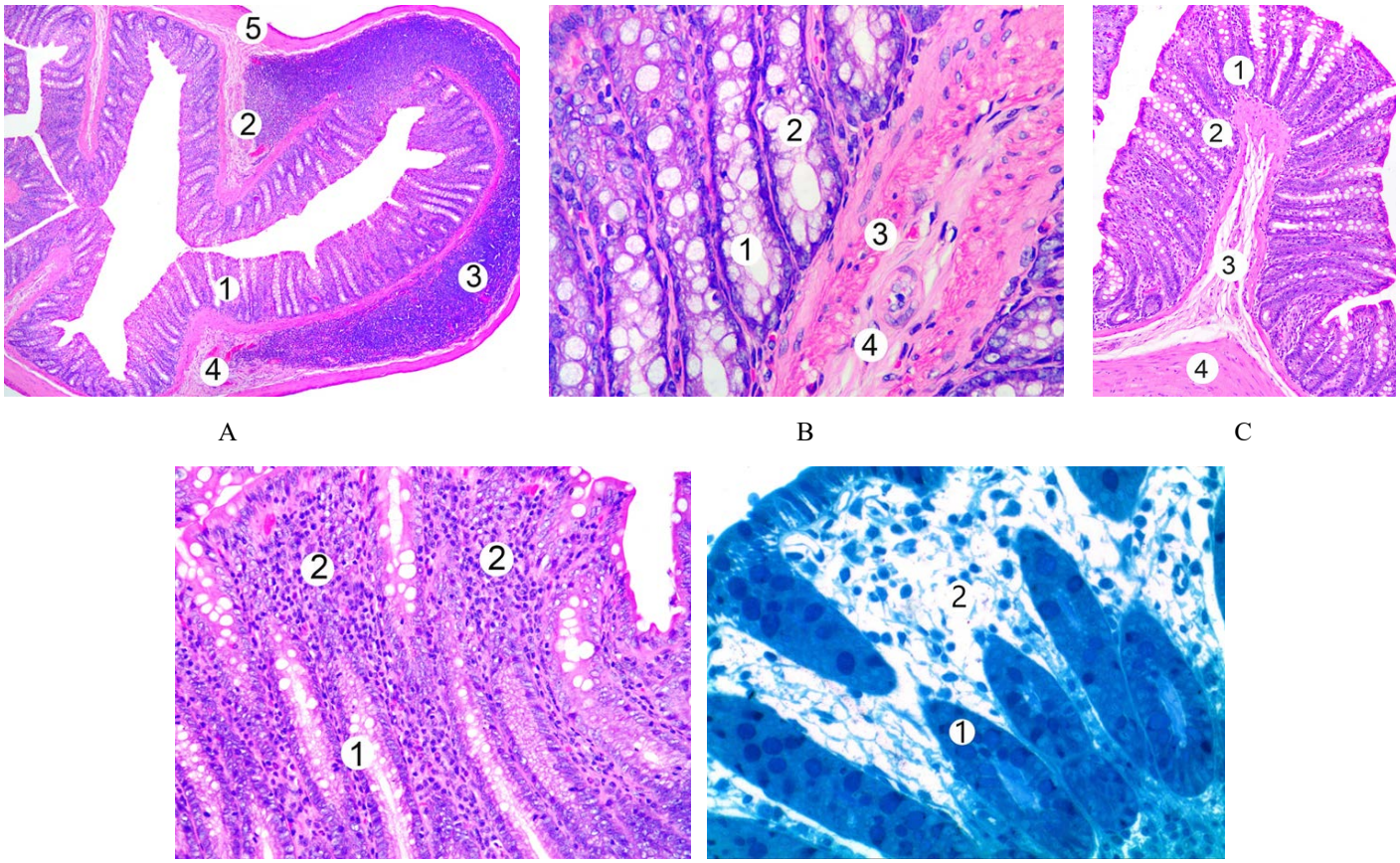


Figure 2. *Microscopic changes of the animals' colon on the 1st, 2nd, and 3rd stages of chronic DMH effect. Hematoxylin and eosin.*
A – mucosa (1), submucosa (2), solitary lymphatic follicle (3), full-blooded vessels (4), muscularis externa (5). x 40.
B – crypt (1), goblet cells (2), muscularis mucosa (3), submucosa (4). x 400.
C – mucosal fold (1), crypts (2), edema of the submucosa (3), muscularis externa (4). Hematoxylin and eosin. x 100.
D – crypts (1), lymphohistiocytic infiltration of the lamina propria (2). Hematoxylin and eosin. x 200.
E – crypts (1), lymphohistiocytic infiltration and edema of lamina propria (2). Semithin section. Methylene blue staining. x 200.

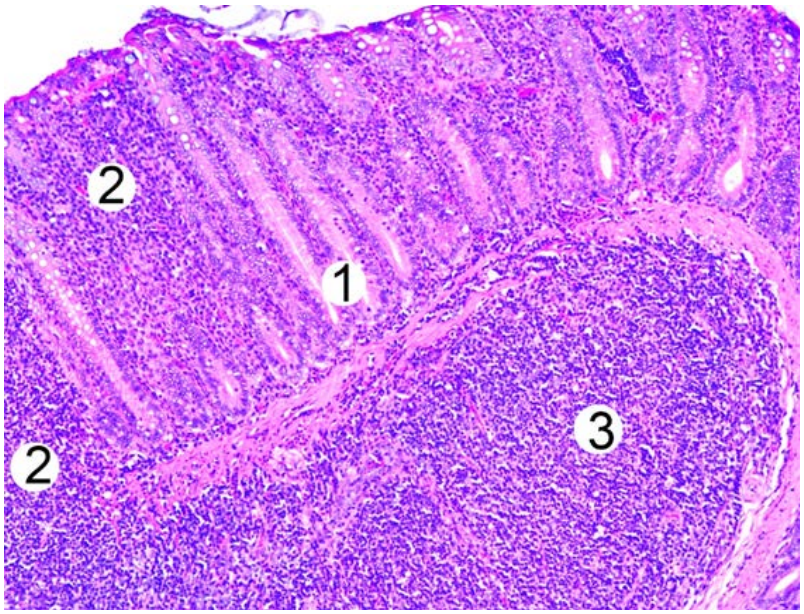


Figure 3. *Histological changes of the animals' colon on the 4th stage of chronic DMH effect. Crypts (1), lymphocytic infiltration (2), solitary follicle (3). Hematoxylin and eosin. x 100.*

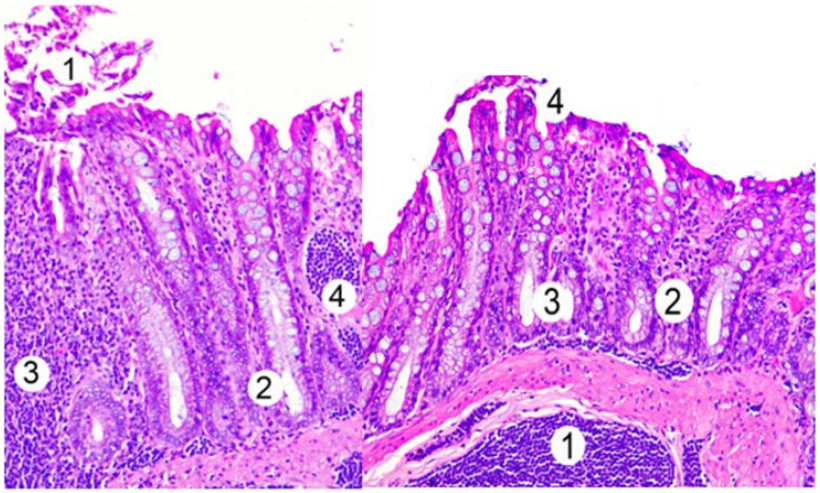


Figure 4. *Microscopic changes of the animals' colon on the 5th and 6th stage of chronic DMH effect. Hematoxylin and eosin. x 200.*
A – desquamated epithelial cells (1), crypts (2), lymphocytic infiltration of mucosa (3), lymph stagnation in lymphatic vessels (4).
B – lymph stagnation (1), lymphocytic infiltration of mucosa (2), crypts (3), desquamated epithelial cells (4).

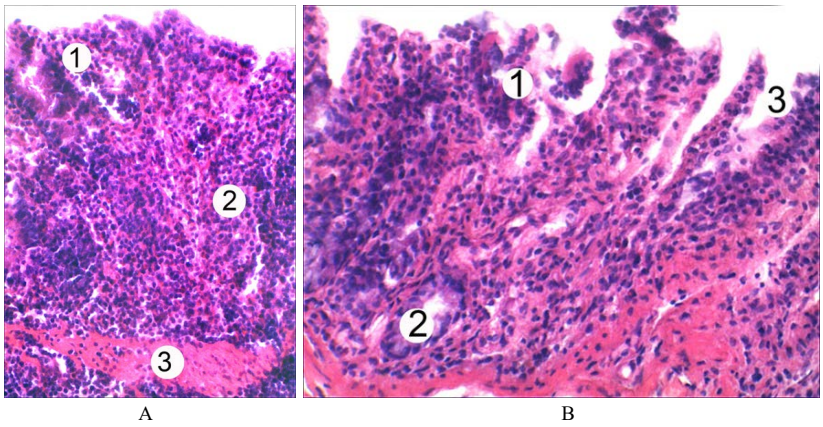


Figure 5. *Histological changes of the animals' colon on the 7th stage of chronic DMH effect. Hematoxylin and eosin. x 200*
A – atypical cells of mucosa layer (1), lymphocytic infiltration (2), muscularis mucosa (3).
B – erosion of the mucosal epithelium (1), crypt (2), epithelial dysplasia of the mucosa layer (3).

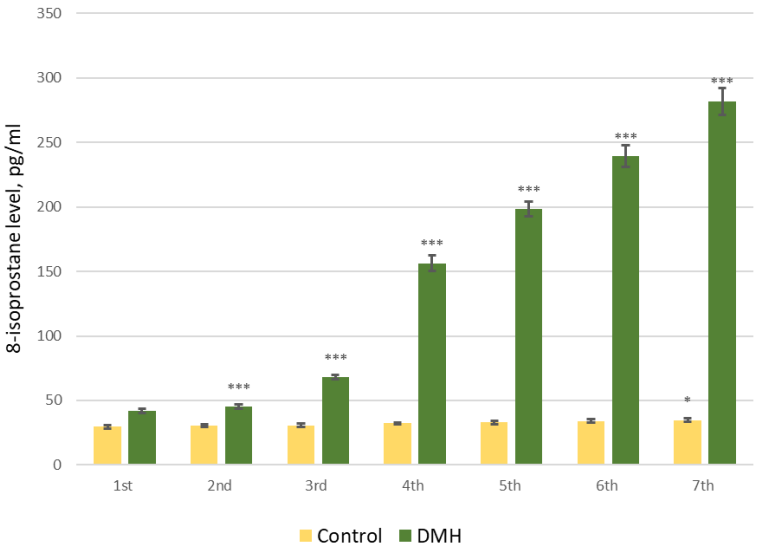


Figure 6. *Changes in 8-isoprostanes concentration in serum of rats in dynamics of DMH-induced colon adenocarcinoma in situ modelling.*
*Note. *** - significant changes between the indexes of control and DMH-affected animal group ($p<0,001$).*
** – moderate changes between the indexes of 1st and 7th experimental stages of the control animals group because of the age progression of the organism ($p\geq0,05$).*

analysis, we were able to track an evident increase in the levels of this indicator, which indicated the worsening of oxidative stress. Thus, in the 1st stage of DMH-induced neoplasia, this indicator exceeded the indicator of the control group animals 2,7 times while in the 2nd stage of DMH administration, it was 3 times higher than the one in the control group. The 3rd stage of the experiment became a turning point of 8-isoprostane in colon tissue homogenates level. The indicator began to increase, compared to the control group, as well as in the case of serum isoprostane levels. Raise of this oxidative stress marker was from 3.4 times on the 3rd month to 3.9 times on the 7th month (*** represents $p<0.001$) was indicated in the homogenate of the colon tissue compared to similar indicators of the control group of animals (Figure 7).

Estimation of the 8-isoprostanes levels in the blood and colon tissue demonstrated its statistically significant increase in animals with induced CRC, compared to the control group, during all terms of the experiment. Due to the above-described changes, we can state that the development of non-invasive adenocarcinoma, even in the early stages, is accompanied by the progression of oxidative stress, and 8-isoprostane is a prominent marker of redox balance disorders.

Evaluation of the effectiveness of Au/Ag/Fe NPs composition use in conditions of simulated colon adenocarcinoma *in situ*

Colon histopathology

Microscopic studies demonstrated a positive effect of the applied corrective method on the condition of the structural components of the colon wall under DMH-induced lesion.

The continuous epithelial lining of the crypt's apical portion with columnar cells without signs of atypia prevails in the mucous membrane of the examined organ. Crypts are deep with narrow lumens. The epithelial plate of the crypts was rich with goblet cells. A slight thickening of the muscular plate of the intestinal mucosa was also indicated (Figure 8A). Moderate diffuse lymphocytic infiltration and edema of the mucous membrane connective tissue and submucosal base were observed. The submucous base was unevenly expanded with the loosening of collagen fibers due to edema (Figure 8B).

The state of the hemocapillary bed also improved. The blood vessels lumens of the colon`s wall submucosal base were moderately full-blooded. Sludge effects were indicated in a small part of the vessels (Figure 9).

The conducted morphological essay determined that the state of the colon wall in animals with DMH-induced carcinogenesis has improved significantly. Its histological composition corresponded to state inflammatory lesion – chronic colitis without signs of dysplasia of the covering epithelium and crypt epithelium after using Au/Ag/Fe NPs composition.

Au/Ag/Fe NPs and oxidative stress

The Au/Ag/Fe NPs composition usage demonstrated effective antioxidant properties that can help to prevent oxidative damage progression. This corrective method led to a significant decrease in the levels of 8-isoprostanes (both in blood serum and colon tissue) compared with the group of animals with DMH-induced colon adenocarcinoma in situ. Our results showed no significant difference in the 8-isoprostane concentrations of the control group animals with NPs application. (Figure 10).

It was found that the level of 8-isoprostane after correction of DMH lesions with Au/Ag/Fe nanoparticles significantly decreased by 1.5 times both in blood serum and in the colon,

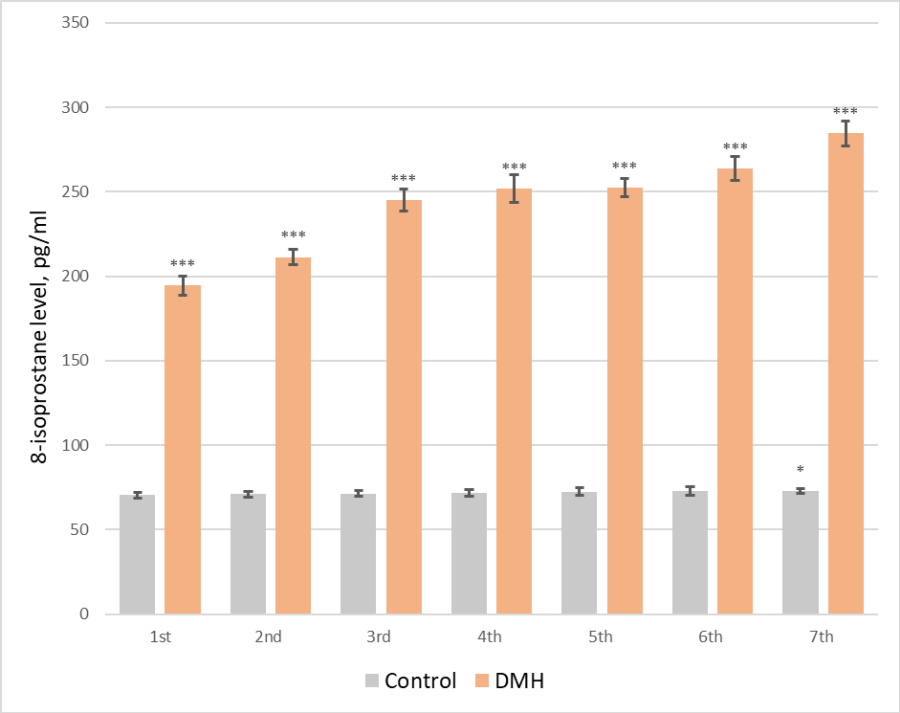
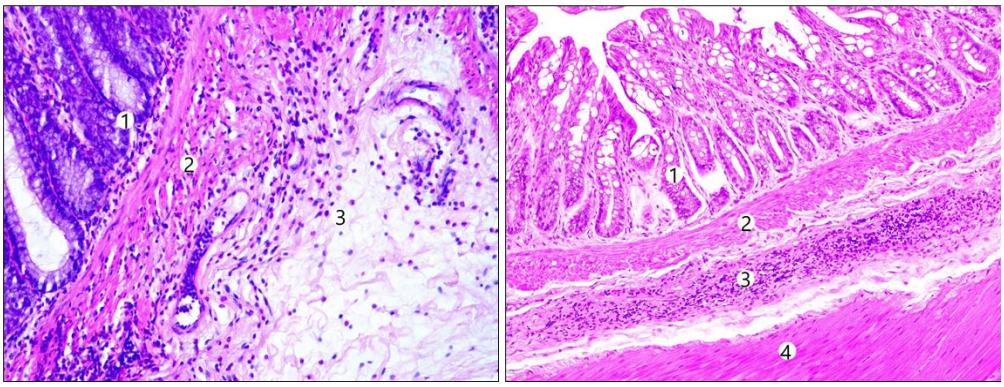


Figure 7. *Changes in the level of 8-isoprostanes in colon tissue caused by oxidative stress due to the development of organ neoplasia.*
*Note. *** - significant changes between the indexes of control and DMH-affected animal group ($p<0,001$).*
** – moderate changes between the indexes of 1st and 7th experimental stages of control animals group because of age progression of organism ($p\geq0,05$).*



A B

Figure 8. *Histological changes in the colon of chronic DMH effect after the application of the Au/Ag/Fe nanoparticles composition.*
A – crypts (1), diffuse lymphocytic infiltration, and edema of the muscularis mucosa (2) and the submucosa (3). Hematoxylin and eosin. x 200
B – deformation of the crypts (1), muscularis mucosa (2), lymphocytic infiltration of the submucosa (3), muscularis externa (4). Hematoxylin and eosin. x 100.

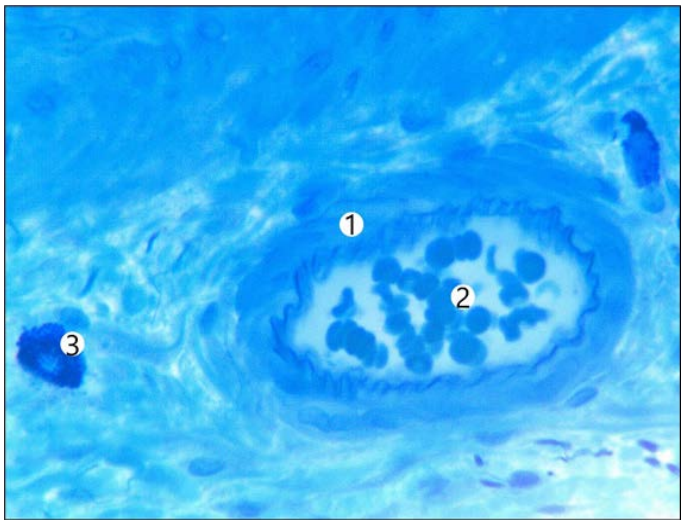


Figure 9. *Histological features of the submucosal base of the colon wall of injured animals after the administration of Au/Ag/Fe nanoparticles composition. Arteriole (1) with erythrocytes (2), basophil (3). Semithin section. Methylene blue. x 400.*

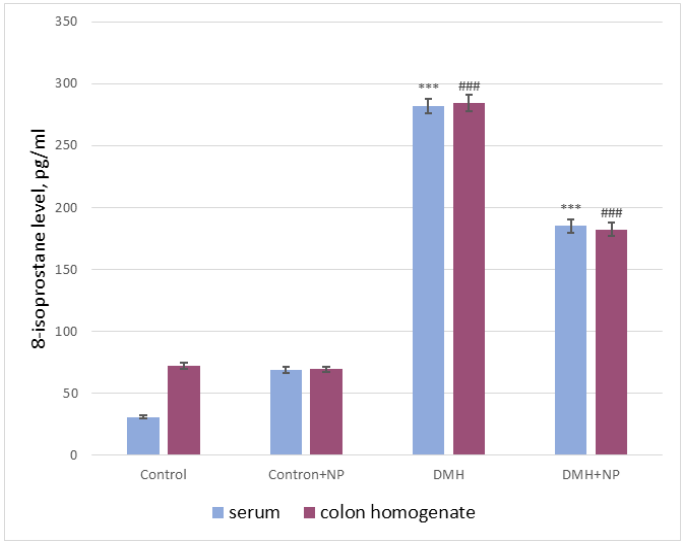


Figure 10. *Effect of Au/Ag/Fe NPs composition on 8-isoprostanes levels in blood serum and colon homogenates of animals in terms of experimental carcinogenesis.*
*Note. *** - significant changes between the indexes of control and DMH-affected animal group (p<0,001).*
- significant changes between the indexes of the DMH-affected animal group and animal group with correction Au/Ag/Fe NPs composition (p<0,001).

homogenate compared to a similar indicator in the group of animals without correction.

All described-above changes indicate that the Au/Ag/Fe NPs usage composition causes the reduction of oxidative stress manifestations in experimental animals with induced carcinogenesis.

Discussion.

DMH belongs to the hydrazine group and acts as a potent DNA-altering agent. DMH is vastly used as a cancer-causing agent to induce colon cancer in animal models. The subcutaneous injection of DMH is highly efficient in induced colorectal cancer in small rodents and has a variety of similar features to human CRC. Investigation into the progression of colon carcinogenesis induced by DMH in rodent models provides valuable insights into the biochemical and histological mechanisms involved in various stages of colon cancer development. Following a series of metabolic reactions, DMH eventually reaches the colon and generates the ultimate carcinogen and reactive oxygen species (ROS). These substances further initiate the progression of colon carcinogenesis. The study of preneoplastic lesions and the histopathological examination of colon tumors induced by DMH contribute to a comprehensive understanding of this disease in rodents and humans. DMH-induced CRC has been studied in large-scale experiments [27], but the step-by-step development of carcinogenesis from the earliest stages was not widely and clearly defined.

Understanding the sequential progression of CRC and examining its morphological development is crucial for gaining valuable insights into early detection, biochemical mechanisms, and effective prevention strategies. This comprehensive understanding serves as a fundamental basis for enhancing patient outcomes, advancing the field of personalized medicine, and ultimately reducing the global impact of CRC.

The phased study of CRC morphological development is crucial for several reasons. First of all, it helps with early detection. Precancerous lesions and early-stage tumors can be identified by understanding the sequential evolution of CRC. This aids in creating efficient screening methods and diagnostic tools to find CRC at its most curable phases. Patient outcomes and survival rates are dramatically enhanced by early identification.

The sequential morphological development of CRC may also be correlated with specific biochemical changes. These morphological characteristics and the corresponding biomarkers can be used to create diagnostic or prognostic indicators for CRC. These biomarkers can help with disease progression tracking, risk assessment, early identification, and therapy selection.

Oxidative stress is crucial in various aspects of cancer, including tumor formation, treatment, and prevention [28]. When the oxidative processes caused by free radicals surpass the body's antioxidant defense, it can potentially accelerate the progression of carcinogenesis. The analysis of the data obtained from the study revealed a correlation between the development and progression of carcinogenesis in white rats and the presence of oxidative stress. Several significant findings emerged from this research. First of all, we observed not only the occurrence of oxidative stress but also found that the level of 8-isoprostane increased in line with the histological changes in the colon's wall during the development of colon adenocarcinoma *in situ*.

The use of Au/Ag/Fe nanoparticle compositions has been a research subject in various fields, including cancer therapeutics. The administration of silver (AgNPs) and gold nanoparticles for CRC treatment causes their accumulation in colon tumors, indicating their bioavailability. The AgNPs and AuNPs significantly enhanced cellular antioxidant enzyme levels, including catalase, superoxide dismutase, glutathione, glutathione peroxidase and reduced lipid peroxidation [15,17-19]. Gold nanoparticles (AuNPs) have been extensively studied in the context of different cancer treatments and are being explored as a potential alternative or complement to numerous non-specific chemotherapy drugs to enhance treatment effectiveness while minimizing adverse side effects [13,14]. Multi-functional AuNPs are highly stable and versatile scaffolds for drug delivery due to their unique size, chemical and physical properties. Surface bio-functionalization of AuNPs offers the potential to target specific cells, thereby facilitating the destruction of cancer cells [14,19,23]. Iron has many properties that make it particularly useful in nanoparticle form. Iron nanoparticles have been extensively researched due to their low toxicity and a distinctive magnetic characteristic known as superparamagnetism. Magnetic nanoparticles enclosed within a phospholipid bilayer, forming liposomes, have shown significant structural and pharmacokinetic benefits for drug delivery [16,20].

Many NPs are being intensively researched separately, but little is known about how they interact together as a composite. The impact of NPs composition usage depends on a wide range of variables that must be considered, including the size, shape, and particular production techniques of the NPs. Our investigation was also conducted to understand better Au/Ag/Fe NPs composition on different aspects of colorectal cancer progression. Although the positive impacts of metal nanoparticles (NPs) have been noticed, numerous inquiries have arisen. The first question pertains to the significance of NP properties in determining their beneficial effects, such as size, shape, concentration, and so on. The second query concerns the mechanism through which NPs exert these favorable effects. To address these inquiries, we conducted an experiment utilising a carcinogenesis model, which revealed increased levels of oxidative stress, mirroring observations in humans.

Special attention is paid to the relationship between particle size and its influence on the organism. It has already been studied that NPs have size-dependent properties. For example, small size and big surface areas with different physicochemical characteristics of NPs may reveal unpredictable genotoxic properties. Small-sized NPs can penetrate the nucleus and damage chromatin [29]. Ag NPs with lower than 10 nm diameter have the potential to bind with the cell walls in Escherichia coli bacteria and finally lead to cell death [30].

On the other hand, Butler, and Peeler state that the results of their research with AgNO3 suggest that silver ions are non-mutagenic [31].

The way of NPs administration also plays an important role. The oral and intraperitoneal modes of delivery of AuNPs were

shown to have the highest toxicity compared to the systemic route of administration [32].

Some studies have suggested that certain metal nanoparticles, including Au and Ag, possess antioxidant properties and can scavenge reactive oxygen species (ROS) in cells. By reducing oxidative stress, these nanoparticles may potentially mitigate the damage caused by ROS and modulate oxidative stress markers. Additionally, Fe NPs have pro-oxidant properties due to their ability to generate ROS through Fenton or Fenton-like reactions [33]. These reactions involve the interaction of iron with hydrogen peroxide, leading to the formation of hydroxyl radicals.

The administration of Au/Ag/Fe nanoparticle compositions can potentially modulate oxidative stress markers and histological changes in the colon.

Some studies have reported the potential protective effects of certain metal nanoparticles against oxidative stress and histological changes in various carcinogenesis models. However, more research is needed to determine the specific effects of Au/Ag/Fe nanoparticle compositions in the context of DMH-induced colon carcinogenesis. It's essential to consider the nanoparticle dosage, duration of treatment, route of administration, and potential toxicity concerns when evaluating their effects on oxidative stress markers and colon histological changes.

Conclusion.

All mentioned results provide an attractive explanation for the development of oxidative stress along with carcinogenesis progression and prove that the Au/Ag/Fe NPs composition has the potential to alleviate oxidative stress and restore deficiencies.

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