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MEAT PRODUCTS WITH BEETROOT EXTRACT REDUCE DNA DAMAGE IN MOUSE INTESTINES

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Abstract
Red beetroot (Beta vulgaris L.) is known as the popular vegetable in Russian cuisine, as well as a plant food that protects human health. Beetroot is rich in chemical compounds with antioxidant, anticarcinogenic, anti-inflammatory and other health-beneficial properties. Using the DNA-comet method (alkaline version), the effect of dry red beetroot extract was assessed as part of a meat product on spontaneous and induced DNA damage and presence of "abnormal comet" indicators in cells of various parts of the gastrointestinal tract (GIT) and liver of male F_1 mice C.B.A. x C57 Bl /6. The obtained results showed that intraperitoneal administering of alkylating agent methyl methanesulfonate (MMS) to the mice at a dose of 40 mg/kg of the animal body weight statistically significantly increased the numerical value of DNA damage (% DNA in the tail) and the number of atypical DNA-comet in the liver, stomach, small and large intestines. Introduction of meat product with added red beetroot extract (20 g/kg of finished product) into the diet of the animals did not provide any significant effect on the scope of DNA damage caused by MMS in liver cells, but it led to a statistically significant decrease of this parameter by 58%, 59% and 48% in cells of the stomach, duodenum and rectum, respectively. The significant decrease of 29–54% in the release of atypical DNA-comet in the cells of all organs studied has been also confirmed, which proves decreasing in the cytotoxic effect of MMC in the gastrointestinal tract and liver. Thus, the antigenotoxic and cytoprotective effects of a meat product with addition of dry red beetroot extract have been recorded. This finding is able to have significant practical application, since the complications and issues in the gastrointestinal tract often occur and secondary tumors are induced in its parts during the chemotherapy of cancer located beyond the gastrointestinal tract. This result shows the potential ability of the developed meat product in protecting gastrointestinal tract cells from the genotoxic and cytotoxic effects of alkylating anticancer drugs.

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Introduction

According to WHO data presented by the UN, in 2022 about 20 million people in the world were diagnosed with cancer and about 10 million patients succumbed to cancer. Among the top five of most common cancers there are colorectal cancer (9.6%) and gastric cancer (4.9%), they are noted as the types of cancer most often leading to death in patients [1].

Based on various experimental approaches, it has been demonstratively proven that the trigger of carcinogenesis is genotoxic cell damage, i. e. DNA damage.

Stochastic depurination and deamination of bases, the hydrolysis of the n-glycosyl bond, disruptions in meiotic and mitotic recombination or topoisomerases functioning, exposure to random thermal fluctuations and many other spontaneous processes lead to the typical DNA damage; chemical modification or loss of purine and/or pyrimidine bases, their oxidative damage, various cross-links, single-strand and double-strand breaks of DNA chain. Single-strand DNA breaks are considered to be the predominant damage [2]. The overwhelming majority of spontaneous DNA damages are fixed by cellular repair systems [3].

The induction of DNA breaks and chemical modification of the macromolecule increases dramatically under the influence of chemical genotoxicants. They can have both exogenous and endogenous origin.

The reactive oxygen species (ROS), which cause oxidation of bases and DNA breakage, are generally acknowledged as endogenous genotoxicants. ROS are the products of normal cell metabolism that normally are not dangerous. However, under the conditions of oxidative stress, when the production of ROS increases extremely and it quantity exceeds the compensatory capabilities of the body's antioxidant protection, oxidative damage to DNA bases, single-strand and double-strand breaks of the macromolecule occur.

Oxidative stress goes along with many human diseases. These are viral and bacterial infections, bronchial asthma, diabetes, malignant neoplasms and many other diseases. Oxidative stress is also induced by smoking, physi-

cal stress, emotional stress, chronic alcoholism and other harmful addictions. In all diseases accompanied with the oxidative stress, increasing of the level of DNA damage has been recorded, which is proven by DNA-comet method, and these diseases are associated with carcinogenic risk increase [3,4,5,6].

Exogenous genotoxicants are usually divided into physical, biological and chemical agents or, depending on the source of origin, classified as household, industrial, medicinal and other genotoxicants.

Natural, industrial or diagnostic ionizing radiation, as well as natural ultraviolet radiation, is the reason of breaks of single-strand and double-strand DNA chains, DNA-DNA cross-links, DNA-protein cross-links and their other modifications [3,5].

Breaks of single-strand and double-strand DNA breaks, as well as various modifications of this molecule, are caused by numerous chemical factors. This are fumes and smokes of various origin, industrial emissions, exhaust gases from internal combustion engines, certain pesticides and medications. For example, the chemotherapeutic agents bleomycin and cisplatin cause DNA damage by their interfering with the activity of the enzyme DNA topoisomerase and alkylating DNA bases [3,5,6].

Chemical genotoxicants are able to interact directly with DNA — these are DNA-reactive genotoxicants; or they are able to provide damaging effect indirectly, without penetrating the cell nucleus and without direct interaction with DNA — these are DNA-non-reactive genotoxicants. The individual representatives of each of these groups require metabolic activation in order to obtain a genotoxic function, and are denoted as indirect genotoxicants.

Several mechanisms make up the action of majority of genotoxicants. Most often, this is the induction of oxidative stress and alkylating effect, which is typical for more than 90% of known chemical genotoxicants [3].

The assurance in the presence of connection between the genotoxic and carcinogenic activity of chemical compounds originates from two groups of facts. First, most known carcinogens have genotoxic properties. For example, the genotoxicant cyclophosphamide induces tumors of the bladder and hematopoietic system; and azothiopril induces tumors of the skin and lymphatic system. Secondly, patients suffering from hereditary DNA repair defect syndromes have a tenfold higher risk of developing tumors in comparison with healthy people [3].

Thus, the prevention of genotoxicity can serve as an efficient method to combat the socially significant disease as cancer. Attention is focused on the idea that up to one third of the cancer cases can be prevented by mere change of nutrition. The attempts to create food products for cancer prevention have been so far based on the idea of the unconditional benefits of food antioxidants for the human health [7].

Most antioxidants feature antigenotoxic properties, it means that they are capable to reduce the damaging effects of genotoxicants in experiments or in the clinic. This corresponds well with the concept of oxidative stress as the driving mechanism of genotoxic damage. Several dozens of natural compounds and plant products have been identified that possess antigenotoxic properties and are able to reduce the risk of malignant tumors. These are, in particular, vitamins of groups A, C, E, natural flavonoids, phenols, epigallates, catechins, etc. Based on natural antigenotoxic compounds or food raw materials that contain them, it is possible to create functional food products for the prevention of genotoxicity and associated cancer conditions [3,8,9].

In this regard, red beetroot (*Beta vulgaris L.*) deserve the most serious attention. This plant belongs to the botanical family *Amaranthaceae* and is commercially cultivated. It perfectly combines nutritional and medical value. Beetroot is rich in proteins (1.68 g), carbohydrates (9.96 g), fat (0.18 g), amino acids (1.22 g), fatty acids (0.12 g), phytosterols (0.03 g), minerals (0.48 g) and fiber (2.0 g) per 100 g fresh weight. It contains vitamins in amount of 4.8 mg per 100 g of fresh weight, and in dry beet extract there are such biologically active substances as betalains (3.9 g / 100 g), betacyanins (2.1 g / 100 g), betaxanthins (1.9 g/100 g) and phenols (0.19 g/100 g) [10].

The preventive and therapeutic effects of beetroot in many diseases are traditionally related with its profound antioxidant activity [10,11]. It is worth to note that antioxidant activity is inherent in beetroot not only in their fresh form, but also as part of processed food products. For example, Ali et al. [12] proved that fruit jelly sweets consisting of 75% strawberries and 25% beetroot have high antioxidant activity (more than 52%).

The possibility of using beetroots as part of functional food products with preventive properties, and the grounded feasibility of their production, were previously determined in the independent works [7,10,11]. In these works, as well as in many other researches, it was underlined that the mechanisms of the protective effects of beetroot and the technology for its use as the ingredient of the functional food are just at the very beginning of their development.

Earlier beetroot was already considered by us [13] as a possible source of antigenotoxicants. Methodological achievements that made it possible to use the method "DNA-comet" to assess DNA damage *in vivo,* in particular, to record DNA damage in cells of the various parts of the gastrointestinal tract (GIT) [3,4] opened up the future prospects for researching the use of beetroots for reducing the genotoxic effect in GIT cells, which function is exceptionally important in view of the above-cited information on genotoxicity as a trigger of carcinogenesis and the statistical prevalence of gastrointestinal types of cancers.

The purpose of this study is to assess the effect of red beetroot extract as part of a meat product on DNA damage in the cells of stomach, duodenum, rectum and liver of the experimental mice.

Objects and methods

Object of study and chemical reagents

The object of research was a ready-to-eat meat product — a boiled sausage. The product is based on the recipe of the sausages "Piknichok", developed in accordance with state standard GOST R 58110–2018[1]. The recipe for the experimental product contained poultry meat (chicken breast), pork back fat, water, salt, spices and dry beet extract (Specification No. KW/1001–11/2005, manufacturer: Parzew 14 63–220 Kotlin, Poland), in amount of 20 g per 1 kg of raw meat.

The recipe for the control food product was fully analogous to the recipe for the sausages "Piknichok".

The experimental sample and the control sample were produced on the same day on the same equipment from the same batch of the raw materials. The product was developed according to the modes described in GOST R58110–2018[1].

The alkylating agent — methyl methanesulfonate (MMS), produced by the company Sigma (USA), was used as a genotoxicant.

Fluorescent dye — SYBR Green I (Thermofisher, USA).

While running the lysis and electrophoresis of isolated cells, the following reagents produced by the company AppliChem GmbH (Germany) were used: ris-HCl (pH 10), NaCl, EDTA-Na_2, Triton X-100, dimethyl sulfoxide, NaOH.

Animals

For the experiment the male mice of hybrids F1 CBAxC57Bl/6 raised in "Stolbovaya" nursery (the branch of the Scientific Center of Biomedical Technologies of Federal Medical and Biological agency of Russia) were used.

Two weeks before the beginning of the experiment, the animals were kept in the vivarium of the Federal State Budgetary Institution "Federal Research Center for Original and Prospective Biomedical and Pharmaceutical Technologies" on a twelve-hour light regime, 5 animals per each group in polycarbonate cages (235 × 155 × 140). They had free access to water and feed for the rodents "Profgryzun" (Russia). At the beginning of the experiment, the weight of the mice varied in the range of 20–22 g, and their age was 8–9 weeks.

The experimental researching, parameters of environment, housing and keeping the animals were approved by the Bioethical Commission of the Scientific and Research Institute of Pharmacology n. a. V. V. Zakusov, which is a structural subdivision of the Federal State Budgetary Institution "Federal Research Center for Original and Prospective Biomedical and Pharmaceutical Technologies" and conformed with GOST 33215–2014[2].

[1] GOST R58110–2018 "Sausage cooked goods from poultry meat (offal) for children nutrition. Specifications" Retrieved from https://docs.cntd.ru/document/1200159009 Accessed February 14, 2024

[2] GOST 33215–2014 "Guidelines for accommodation and care of animals. Environment, housing and management" Retrieved from https://docs.cntd.ru/document/1200127789 Accessed February 14, 2024

DNA-comet method

The method is used in its neutral and alkaline versions. In the first case, only double DNA breaks are recorded. In the alkaline version, the method allows to reliably detect single-strand and double-strand DNA damage, which are the predominant damage that happens under the influence of physical and chemical genotoxicants [3,4]. Moreover, during alkaline denaturation, the numerous chemical modifications of DNA, collectively known as alkaline-labile sites, are implemented as the single-strand DNA breaks, which allows integrative assessing of DNA macromolecule damage in the cell in comparison with the corresponding control sample [4,14,15].

The DNA damage was tested with the DNA-comet method in its alkaline version. The method is based on measuring the electrophoretic mobility of DNA of the individual cells in agarose gel. During the electrophoresis, the DNA of a cell has formed more or less pronounced figure, consisting of a nucleus and a tail, which in general looks like a comet (Figure 1). The generally accepted indicator of DNA damage is the DNA content in the comet tail, where DNA gets if it has been loosened and has acquired mobility as a result of single-strand and double-strand breaks [4].

The method is applicable to cells of any tissue. The main problem when studying the another tissue sample is to obtain high-quality microscopic preparations of isolated cells suitable for digital analysis. This problem has been successfully solved in this work in regards to gastrointestinal tract cells, as illustrated by the images shown below in the Figure 1. The procedure of isolating the gastrointestinal tract cells is described below.

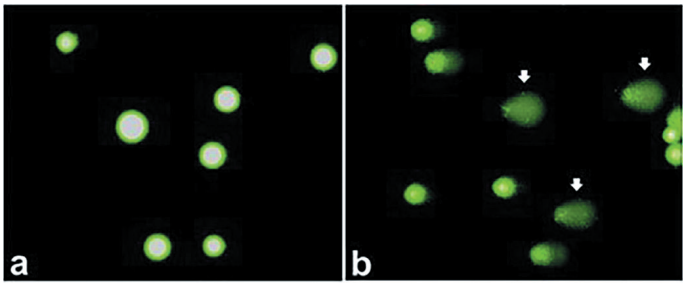

Figure 1. Digital images of the preparations of DNA-comets of rectal cells: a) undamaged cells b) typical DNA-comets (red arrows) and atypical comets (vaguely expressed nucleus and diffuse tail) are marked with white arrows. SYBR dye Green I, magnification × 200

Preparation of meat product and genotoxicant for their administering to the animals

Samples of the meat product weighing 5 g were finely cut with scissors and ground in a porcelain mortar with 10 ml of distilled water until a homogeneous mixture was obtained.

Methylmethane sulfonate (MMS) was administered as distilled water solution.

Experimental groups

Four experimental groups were formed, 5 animals per group. The animals in the control group got intragastric

administering of distilled water at a rate of 10 ml/kg, three times, with an interval of 24 hours.

The animals of MMS group got the intragastric administering of the distilled water in the same mode. The last administering was combined with an intraperitoneal injection of an alkylating genotoxicant — methylmethanesulfonate (MMS) at a dose of 40 mg/kg of animal body weight. The choice of genotoxicant dose was based on own experience and literature data [15,16].

The resulting suspension of the meat product was administered to the animals of the experimental groups "MMS + Mstd" (sample of the meat product without beetroot extract (*std*) and "MMS + M*be*" (sample of a meat product with beetroot extract (*be*) intragastrically at rate of 10 ml/kg, which corresponded to 100 mg of red beetroot extract per kilogram of animal body weight. The suspensions were administered three times, with an interval of 24 hours. The last administering was combined with an intraperitoneal injection of MMS at a dose of 40 mg/kg.

Preparation of cell suspensions, cells lysis and electrophoresis

Three hours after the last administering of the solutions, the animals were euthanized by decapitation. The liver, stomach, duodenum and rectum were taken out as quickly as possible and crushed in 3 ml of phosphate-buffered saline pre-cooled down to a temperature of 4 °C containing 20 mM EDTA-Na_2, and of 10% dimethyl sulfoxide (pH 7.5). The tissue samples were pestled in the glass vials with a glass pestle to obtain cell suspensions and kept for 1–2 minutes for sedimentation of large fragments of tissue.

30 µl of a suspension of cells from one or another part of the gastrointestinal tract was added into the prepared glass tubes containing 120 µl of 1% solution of low-melting agarose in phosphate-buffered saline heated up to temperature 42 °C (in microthermostat "Termit", "DNA-technology", Russia). After resuspension, 30 µl of cell suspension in low-melting agarose was applied onto the slides coated with 1% universal agarose, placed under coverslips, and put onto ice. The next actions were carried out under yellow light, the solutions used were cooled down to the temperature of 4 °C.

After 5–10 minutes of exposure to low temperature, after the agarose got solidified, the coverslips were taken off and the microslides were placed into a glass cuvette (Schiffendecker type) for lysis, which lasted for 1 hour in a buffer containing 10 mM Tris-HCl (pH 10), 2.5M NaCl, 100 mM EDTA-Na2 1% Triton X-100 and 10% dimethyl sulfoxide of 4 °C.

After completion of lysis the microslides were kept for 20 min in electrophoresis buffer containing 300 mM NaOH, 1 mM EDTA-Na2 (pH>13) and were transferred to a horizontal electrophoresis chamber (Bio — Rad (USA), Sub-Cell type, model 192, 25 x 10 cm), filled with fresh buffer of the same composition. Electrophoresis was run for 20 minutes (field strength was 1 V/cm, current ~300 mA). Then the preparations were washed with phosphate-buffered saline and fixed with 70% ethyl alcohol for 15 minutes.

Staining and analysis of the microslides

The obtained preparations were stained with fluorescent dye SYBR Green I (dilution 1:10000 in TE buffer (pH 8.5) in 50% glycerol) for 30 minutes long in the dark room right before microscopy. For microscopy (×200) an epifluorescence microscope Mikmed-2 12T (Lomo, Russia) with a digital camera of high-resolution (VEC-335, EVS, Russia) was used.

The digital images of DNA-comets were computed via CASP 1.2.2 software [17]. In accordance with the existing recommendations [4], DNA damage was assessed by its percentage in the "tail" of DNA-comets (% of DNA in the tail). At least 200 DNA comets were analyzed per each experimental point. As an additional indicator the atypical DNA-comets were taken into consideration also. They featured vague, undefined nucleus and a peculiar diffuse tail. They are regarded as an indirect indicator of cytotoxicity [18].

Assessment of antigenotoxic effect

The antigenotoxic effect (AE) was expressed as a percentage and was calculated using the following formula:

$$AE = 100 - \frac{\%\ \text{of DNA in the tail}_{s+g}}{\%\ \text{of DNA in the tail}_{g}} \times 100, \qquad (1)$$

where: % of DNA in the $tail_{s+g}$ — parameter % of DNA in the tail, recorded during the assessment of the mice that received sausage and genotoxicant; $\%DNA_g$ — parameter % of DNA in the tail, registered after the action of the genotoxicant.

Statistical processing

While confirming the distribution normality and dispersion homogeneity with the help of the Shapiro-Wilk test and Bartlett's test, respectively, the data obtained from the experimental groups were compared pairwise out in accordance with the Mann-Whitney test.

Otherwise, in accordance with existing recommendations [19], the original values were logarithmically converted, followed by the Mann-Whitney test.

When comparing the proportions of atypical DNA-comets, Fisher's exact test was used.

Results and discussion

In a control series of experiments the spontaneous levels of DNA damage in cells of certain parts of the gastrointestinal tract and liver were determined, as well as the number of atypical DNA comets (ADC) was found, which phenomena are the evidence of cytotoxicity according to the overwhelming majority of opinions [4,18]. The relatively larger amount of ADC observed in the cells of the analyzed parts of the gastrointestinal tract compared to liver tissue is explained by the physiologically determined intensive renewal of cells of the gastrointestinal mucosa, which undergo intensive exfoliation into the lumen of intestines (Table 1).

Table 1. The influence of control and experimental samples of meat product on the genotoxic and cytotoxic effects of methyl methanesulfonate in the mice organs *in vivo*

Group/ organ	Control		MMS40 mg/kg		MMS + M_{std}		MMS + M_{be}	
	% of DNA in the tail	ADC (%)	% of DNA in the tail	ADC (%)	% of DNA in the tail	ADC (%)/ RE	% of DNA in the tail/AE	ADC (%)/ RE
Liver	0.6 ± 0.2	0.4 ± 0.6	7.4 ± 2.2[a]	6.5 ± 1.9[b]	7.3 ± 1.9 —	3.6 ± 1.0[c] ↓44.6%	6.2 ± 0.5 —	4.0 ± 1.7 ↓38.5%
Stomach	1.7 ± 0.4	13.2 ± 4.5	12.8 ± 2.9[a]	32.7 ± 1.0[b]	11.5 ± 1.9 —	20.0 ± 6.7[c] ↓38.8%	5.4 ± 0.8[d] ↓57.8%	19.3 ± 5.2[c] ↓41.0%
Duodenum	0.7 ± 0.2	19.6 ± 1.5	14.0 ± 2.5[a]	28.0 ± 3.8[b]	13.4 ± 0.7 —	25.7 ± 8.0 —	5.7 ± 1.2[d] ↓59.3%	20.3 ± 3.1[c] ↓28.5%
Rectum	0.7 ± 0.3	7.9 ± 4.9	14.6 ± 4.3[a]	29.7 ± 4.6[b]	13.9 ± 5.0 —	18.5 ± 7.5[c] ↓37.7%	7.6 ± 2.6[d] ↓48.0%	13.6 ± 4.2[c, e] ↓54.2%

Notes:
M_{std} — the sample of a standard meat product; M_{be} — sample of a meat product with beetroot extract; AE — a antigenotoxic effect; RE — reduction in the number of atypical comets, ADC — atypical DNA-comets; ↓ — decrease of ADC level in comparison with the effect of MMS;
[a] — $p < 0.01$ compared with the "Control" group (as per Mann-Whitney test); [b] — $p < 0.01$ compared with the "Control" group (Fisher's test); [c] — $p < 0.05$ compared with the "MMS40 mg/kg" group (Fisher test); [d] — $p < 0.01$ compared with the "MMS40 mg/kg" group (Mann-Whitney test); [e] — $p < 0.05$ compared with the "MMS+ Mst " group (Fisher test).

Alkylating genotoxicant MMS, recommended by the OECD as the positive control sample when using the DNA-comet method [16], demonstrated the profound DNA damaging and cytotoxic activity in all parts of intestine and liver researched. The results characterizing DNA damage during the use of the genotoxicant significantly exceeded the values recorded in the control sample by at least 11 times. In its turn, the number of ADC also significantly exceeded the control values in all versions of the experiment; the maximum increase in the yield of ADC under the influence of MMS was found in the liver, where 6.5 ± 1.9% of abnormal comets were recorded in comparison with 0.6 ± 0.2% in the control sample.

The results that mark the effects of MMS in the liver coincide with the data of historical control run by the Research Laboratory of Genetic and Reproductive Toxicology of Federal State Budgetary Institution Research Institute of Pharmacology n.a V. V. Zakusov "Federal Research Center for Original and Prospective Biomedical and Pharmaceutical Technologies" [3], data on the effects of genotoxicant action in the cells of the gastrointestinal tract were obtained for the first time.

Supplementing the diet of mice with the control meat product without beetroot extract provided no effect on the levels of MMS-induced DNA damage in the stomach, small intestine, rectum, and liver. Among the same animals of the same group, the significant decrease of MMS cytotoxicity was observed in the cells of liver, gastric and rectal mucosa, by 44.6%, 38.8% and 37.7% respectively. This effect can be hypothetically explained by enriching the animals' diet with substances in the base meat of the cooked sausage, which substances improve the constructive and energy metabolism in animals. Meanwhile, it is important to underline that the addition of the meat component into the diet provided no effect on DNA damage, which indicates the absence of antigenotoxic activity in the studied control sample.

A different situation was observed after feeding the animals with the sausage with beetroot extract added. In the mucosal cells of all examined parts of their intestine a significant decrease in DNA damage was recorded. In stomach cells the genotoxic effect of MMS was significantly diminished by 57.8%, in duodenal cells by 59.3%, and in rectum by 48.0% respectively. Eating of a meat product with added beetroot extract did not anyway affect DNA damage of liver cells.

ADC accounting showed that consumption of a meat product with added beetroot extract significantly diminished the cytotoxicity of MMS in all organs under research. In the liver, a decrease in ADC was noted by 38.5%, in the stomach by 41.0%, in the duodenum by 28.5%, and in the rectum by 54.2%. It is worth to point out that the last result in the list above, recorded in the rectum, was significantly higher than the result recorded when consuming a meat product without beetroot extract, which directly proves the role of beetroot extract in the cytoprotective protection of liver and gastrointestinal cells.

So, in result of the experiments conducted it was found that poultry meat products enriched with beetroot extract possess profound antigenotoxic activity in the cells of various parts of the gastrointestinal tract, except for the liver cells. Significantly more expressed cytoprotective activity of a meat product enriched with beetroot extract was also revealed in comparison with the food product, not enriched with beetroot extract, in terms of ADC count in the liver and rectum cells.

It is logically reasonable to consider the data obtained in the experimental part of this study in the context of known information about the consumer and biological properties of red beetroot.

Historical review shows that beetroots have been long used not only as a food product and a valuable source of dye pigments of plant origin, but also as a folk curative remedy in Persian and Arabic medicine, in Serbia and Germany to prevent metastases and/or treat patients who suffer from gastrointestinal tumors. Evidence-based medicine data on the beneficial effects of red beetroot for the prevention and treatment of tumors is insufficient, but this

plant features antioxidant, anti-inflammatory and other functions potentially beneficial for the patients with cancer of certain stages, including using it for prevention of the chemotherapy side effects. [20]. Moreover, individual clinical studies have confirmed the benefits of beetroot for managing blood pressure and endothelial dysfunction, improving cognitive function and aiding for recovery after physical activity among the athletes [20,21,22].

In the predominant majority of cases whole variety of beetroot's positive health effects of are attributed to their antioxidant properties. The antioxidant activity of beetroot juice is higher than that of many other juices (tomato, carrot, orange, pineapple) and it only slightly ranks below to pomegranate juice [7].

However, two facts are left beyond the focus of due attention. Firstly, antioxidants, especially phenolic ones, tend, depending on their concentration, do typically change their antioxidant effect to the opposite pro-oxidant effect, i. e. they demonstrate the inversion of their effect. Perhaps this is the feature that explains the heightened mortality among the cancer patients treated with antioxidants, as well as the ability of these antioxidants to boost metastasis [3,7,23,24]. Secondly, antioxidant activity as a chemical property and the antioxidant status of an organism, organ or tissue are fundamentally different concepts, one is purely chemical concept inherent to a substance, the other concept is biological one; it is the antioxidant status of a body that determines the efficiency of its protection from the harmful effects of oxidative stress, which today is considered as one of the most widespread pathogenic mechanisms [3,25,26]. If to add to this effect the obvious genetic heterogeneity of the mankind and the diversity of dietary preferences among the people, it is easy to imagine that additional consumption of exogenous antioxidants can provide positive effect for some individuals and be negative for the others, or within one organism it can provide positive effect on functioning of one organ and be negative for another one. Many authors paid attention to the probable negative effect of antioxidants and the inadmissibility of their consumption [25,26]. It is therefore obvious that enriching food with antioxidants not in all cases would lead to the desired beneficial health-improving effect, and the declared health-improving and cancer-preventive properties of the developed functional products, as noted by the academicians Lisitsyn and Oganesyants of the Russian Academy of Sciences [27], must be proven in special researches and studies, similar in their program to preclinical and clinical studies of the medical drugs. This condition is especially relevant for the compounds which antioxidant functions were demonstrated *in vitro*, in model chemical systems.

The significant benefit of red beetroot is that its positive effect on the efficiency of functioning of mammals antioxidant system has been demonstrated *in vivo*. In particular, there is data [28] that in the liver of rats that were consuming beetroot extract for 7 days in a row and while this diet got injected with carcinogenic carbohydrate tetrachloride (CCl_4), a significant decrease in peroxidation products was observed in comparison with the corresponding positive control. The same authors point out the protective antioxidant properties of beetroot juice (8 ml/kg of rat's body weight for 28 days) in rats against the oxidizing effects of scopolamine and the famous carcinogen 7.12-dimethylbenzaantracene.

As possible mechanisms of the antioxidant effect of beetroot, the capability to maintain the antioxidant function of tissues is considered which function is due to restoration of glutathione, the important endogenous antioxidant, and affecting the antioxidant defense enzymes glutathione peroxidase and catalase, which decompose peroxides down to water and thereby prevent the formation of highly reactive forms of oxygen [29].

Based on the concept of genotoxicity of the products formed by oxidative stress, antioxidant and antigenotoxic properties are considered as mutually conjugated. Despite this, the research of the antigenotoxicity of beets *in vivo* is represented by only few studies with controversial results

In some studies beetroots did not affect the induction of DNA damage [28], in some other studies beetroot juice, administered orally to rats at rate of 8 ml/kg per day for 28 days, reduced by 20% max. the damage of DNA caused by a single injection of the carcinogen N-nitrosodiethylamine%, but increased the damaging effect of carbon tetrachloride in rat blood leukocytes by almost 40% [30,31].

So, only the data obtained in this study convincingly demonstrate the ability of dry beetroot extract added as an ingredient of a meat product to diminish induced genotoxicity and for the first time demonstrate the functional manifestation of this ability in the cells of gastrointestinal tract.

The Table 2 presents the information we described in detail earlier [13], characterizing the content of natural compounds in beetroot which showed antigenotoxic activity in experiments or in direct observations in humans.

Most of the potential antigenotoxicants are found in beetroot in the quantities insufficient to form doses which have an antigenotoxic effect. Ascorbic acid the most well-known and widespread antigenotoxicant in plant materials is present in beetroot in amount of 13 mg/100 g, which is definitely not sufficient to demonstrate the antigenotoxic effect which is observed in doses of tens of milligrams per kg of animal weight [13]. Though there are two exceptions — betaine and betanin. However, betaine is synthesized in the body from choline, which suggests a dynamic balance between its intake and removal from the body for maintaining a physiological optimum. There are more grounds and reasons to consider betanin as the compound responsible for the antigenotoxicity.

Betanin (betanidin-5-O-β- glucoside) — is the representative of N- heterocyclic betalain pigments. This is a red dye known as E162; it is used in majority of countries and regarded as harmless. Earlier we described betanin in detail [13].

Table 2. Potential antigenotoxicants and their content in red beetroot.

ANTIMUTAGENS IN BEETROOT					
PHENOLS AND POLYPHENOLS		VITAMINS VITAMIN-LIKE SUBSTANCES		BETALAINS* (betacyanins and betaxanthins)	
Compound	Contents per 100 g of beets	Compound	Contents per 100 g of beets	Compound	Contents per 100 g of beets
4-hydroxybenzoic acid	12 mg	Ascorbic acid (vitamin C)	3.6÷13 mg	Betanin (betanidin 5-O-β-glucoside)	50–60 mg
Vanillic acid	5.1 mg	Betaine	Up to 500 mg		
Katekhin	38 mg	Carotene	0.01÷20 μg		
Quercetin	no data	Pantothenic acid (vitamin B_5)	130÷155 μg		
Kaemferol	no data	Pyridoxine (vitamin B_6)	67 μg		
Coffee shop acid	7.1 mg	Retinol (vitamin A)	2 μg		
Rutin	0.25 mg	Tocopherol (vitamin E)	40 μg		
Saponins	no data	Thiamine (vitamin B_1)	0.31÷31 μg		
Chlorogenic acid	1.8 mg	Folic acid	73÷109 μg		
Epicatechin	0.39 mg				

Note:
*** — some betalains are phenolic compounds, in particular — isobetanin, prebetanin and neobetanin, vulgaxanthin I, vulgaxanthin II and indicaxanthin.**
**** — Dry beet extract contains 3.976 g of betalains / 100 g of extract.**

It is noteworthy that the vast majority of studies engaged in betanin actually used beetroot extract. For example, in the study [29], betanin produced by ABCR GmbH is specified as the compound under study and even its structural formula is given, whereas according to the manufacturer's catalogue it is beetroot extract with the same name. Commercially available products marketed as "betanin" " (E162) are red beetroot extracts with dextrin, and consist of betalains and some non-coloring substances like minerals, organic acids, vitamins, etc. [32]. Being isolated in the form of a separate compound betanin is unstable and gets easily destroyed under the influence of heat, light and oxygen. The instability creates certain challenges for its storage and experimental works [33]. In this regard, we emphasize that in commercial products and in experimental studies, the name "betanin" is used to denote red beetroot extracts.

For the first time this work demonstrates the possibility of targeted protection of gastrointestinal tract cells from the damaging effects of alkylating agent *in vivo* as per the framework of the accepted methodology of searching and studying antigenotoxicants. The aggregate of the achieved results is shown in the Figure 2.

The action of the predominant majority of anticancer medications is based on their ability to damage DNA of tumor cells, but they affect healthy cells too. Cancer chemotherapy entails large number of side effects, including the development of new tumors, which formation is provoked by genotoxic effects of the applied chemotherapy [34,35]. Actively proliferating tissues, including gastrointestinal cells, are the most sensitive to the iatrogenic effects of chemotherapy; DNA damage plays significant role in these tissues damage [36,37,38,39].

The application of antigenotoxicants or cytoprotectors, usually antioxidants, for prevention of the side effects of chemotherapy has long been discussed [40]. However, while protecting healthy cells, they inevitably compromise the efficiency of chemotherapy [41], moreover, antioxidants taken *per se* out of the natural complexes are characterized by inversion of their effects [3,23,24,42].

The problem of selective protection of the healthy cells from the genotoxic effects of chemotherapy while keeping up the antitumor effect within the tumor growth area still has no practical solution [37], although theoretically the solution lies in the path of creation of selective antigenotoxicants. They should protect healthy tissues that are most vulnerable to the genotoxic effects of chemotherapy, and should not provide protection for tumor cells. The obtained data indicate that the developed meat product with dry beetroot extract protects the cells of gastrointestinal tract from genotoxic effects. The similar effect has not been recorded for liver, so the consuming the meat product with a dry beetroot extract solves the task of antigenotoxic protection of gastrointestinal tract cells.

Moreover, it is known that DNA damage plays a leading role in the occurrence of colorectal cancer, which affects younger and younger people on incrementally larger scale

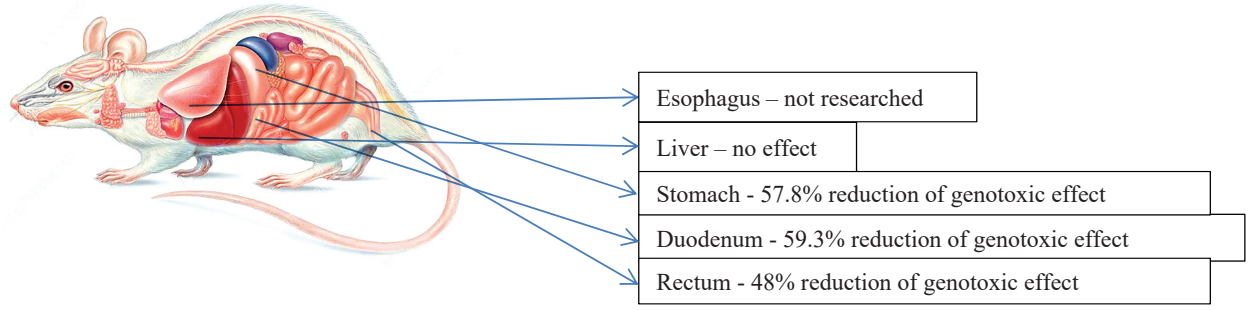

Figure 2. Antigenotoxic effect of the experimental meat product with addition of red beetroot extract.

[35]. Prevention of this disease with the help of antigenotoxic functional products with addition of beetroot extract can be considered as significant scientific direction at the intersection of food technologies and technologies of preventive medicine.

It is crucially important that beetroot extract, one of the few natural red dyes, has no dosage limitations; there is no upper limit for its consumption. Its application is limited only to current tasks and requirements to the organoleptic properties of the product, where it is used as a dye. This feature attaches particular value to the beetroot in terms of its use as part of functional antigenotoxic food products.

It is important to point out a large number of studies that demonstrate antioxidant, anti-inflammatory, antitumor, hypotensive properties of red beetroot, as well as its positive effect on cognitive functions [10], and now antigenotoxicity in the gastrointestinal tract as well. However, beetroot have not still taken their respected place among the raw materials for functional products, losing out to more exotic and expensive vegetables and fruit. This situation is caused by insufficient attention of the researchers to this valuable vegetable and the lack of evidence-based medicine data in the clinical setting demonstrating the beneficial effects of beetroot on human health. Those researches are impossible without the development of functional food products with addition of beetroot and beetroot products; this study is a fragment of such research.

Conclusion

The results of the conducted studies demonstrated the manifestation of antigenotoxic and cytoprotective properties of red beetroot extract (*Beta vulgaris*) used as part of a meat product. Taking into consideration the fact that dry extract of red beetroot is a widely used food production additive E162 ("Beetroot Red"), the revealing of this peculiar property of this food additive ensures it using in the composition of the functional and specialized food products. The above-specified food additive is allowed for use without restrictions, so the restrictions can only be determined by organoleptic aspects and consumer's perception. It is necessary to note that up to this date the antigenotoxic and cytoprotective properties of red beetroot in gastrointestinal tract cells *in vivo* have not been proven.

So, the above-presented results demonstrate the protective effect of a meat product — a boiled sausage — enriched with beetroot extract added in amount of 20 g per 1 kg of raw meat, on DNA damage induced by alkylating agent in stomach, duodenum and rectum cells. The obtained data confirm the anticarcinogenic properties of beetroot and reveal the mechanism of their actuation through the manifestation of antigenotoxic activity, provide the grounds for further research of the functional antigenotoxic food products based on beetroot extract and, in the future, their clinical tests. It was established that standard technological methods and modes of the boiled sausages production do not provide any negative impact on the studied properties of the red beetroot betanin.

The discovered capability of targeting the antigenotoxic effect of dry beetroot extract as part of a meat product opens up the opportunity of it using in economically efficient and natural diets aimed to prevention of primary and secondary gastrointestinal cancer.

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