July 2024, Volume 18, Number 3

Iranian Journal of Toxicology
ARAK UNIVERSITY OF MEDICAL SCIENCES

Research Paper

Red Beetroot Extract is Safe to DNA and Protects Against Mutagens in Mice: A Potential Chemopreventive Agent

Aliy K. Zhanataev[1], Artem A. Lisitsyn[2], Elena A. Anisina[3], Alla V. Kulakova[4], Aleksandra D. Malikova[5*], Kira L. Pligina[4], Zlata V. Chaika[3], Irina M. Chernukha[6]

[1] *Head of the Laboratory of Genetic and Reproductive Toxicology, Federal Research Center for Innovative and Emerging Biomedical and Pharmaceutical Technologies, Department of Drug Toxicology. Moscow, Russia.*
[2] *Junior Researcher; Federal Research Center for Innovator and Emerging Biomedical and Pharmaceutical Technologies, Department of Drug Toxicology. Moscow, Russia*
[3] *Researcher; Federal Research Center for Innovator and Emerging Biomedical and Pharmaceutical Technologies, Department of Drug Toxicology. Moscow, Russia*
[4] *Senior Researcher; Federal Research Center for Innovator and Emerging Biomedical and Pharmaceutical Technologies, Department of Drug Toxicology. Moscow, Russia*
[5] *Junior Researcher; Federal Research Center for Innovator and Emerging Biomedical and Pharmaceutical Technologies, Department of Drug Toxicology. Moscow, Russia*
[6] *Head of the Department of International and Initiative Projects Coordination, V.M. Gorbatov Federal Research Center for Food Systems, Department of International and Initiative Projects Coordination. Moscow, Russia*

Scan to access website

How to cite this paper:
K. Zhanataev A, A. Lisitsyn A, A. Anisina W, V. Kulakova A, D. Malikova A, L. Pligina K, V. Chaika Z, M. Chernukha I. Red Beetroot Extract is Safe to DNA and Protects Against Mutagens in Mice: A Potential Chemopreventive Agent**.** **Iranian Journal of Toxicology.** 2024; 18(3):138-143. doi: 10.32592/IJT.18.3.138

doi: 10.32592/IJT.18.3.138

Article info
Received: 13/02/2024
Accepted: 18/07/2024
Published: 20/07/2024

*** Corresponding author:**
D. Malikova A, Junior Researcher;
Postal address: 125315, Baltiyskaya St. 8, Moscow, Russia.
E- mail:
alexandra1999.1999@mail.ru

ABSTRACT

Background: The beetroot extract has long been widely used in the food industry. However, information on its genetic safety is insufficient. In addition, there is evidence to suggest its potential beneficial health effects. The aim of this study was to evaluate the genotoxic and antigenotoxic potentials of red beetroot extract in rats.

Methods: The endpoints analyzed were chromosomal aberrations in bone marrow cells and DNA damage in peripheral blood, liver, kidneys, and gastrointestinal tract cells assessed using the alkaline comet assay.

Results: There were no statistically significant differences between the analyzed data from the negative control and those of the groups treated with doses of beetroot extract up to 2000 mg/kg for both of the study endpoints. The findings demonstrated the absence of genotoxicity. The comet assay revealed considerable antigenotoxicity of the beetroot extract (5-100 mg/kg) in the liver, stomach, duodenum, and rectum versus the effects of methyl methanesulfonate and dioxidine mutagens with different mechanisms of action. No anticlastogenic activities were detected in the bone marrow cells while observing the protective effects on blood cells, indicating the tissue specificity for beetroot extract antigenotoxicity.

Conclusion: Based on the study findings, the beetroot extract meets the basic requirements for being a chemopreventive agent. The advantages are low cost, practicality of use, efficacy, and safety. Moreover, this agent may be used to develop food products with chemopreventive properties.

Keywords: Antigenotoxicity, Beetroot extract, Comet assay, Genotoxicity, Rats

Introduction

Red beets are a traditional and popular food in many nations and are used in everyday cooking for both sweet and savory dishes. Red beet is rich in carbohydrates, dietary fibers, micronutrients, and a wide range of bioactive phytochemicals [1]. Among the latter, water-soluble and nitrogen-containing pigments are of greatest interest. They belong to the class of betalains, red-violet betacyanins, and yellow-orange betaxanthins [2]. Betalains possess exceptional coloring properties, which is why beetroot extract (BE) is widely used as a natural food colorant [2, 3]. In recent years, betalains have received much attention due to their potential benefits for health promotion and disease prevention [4, 5]. They have been reported to possess diverse biological activities in vivo, including antioxidant, anti-inflammatory, antidiabetic, and protective effects on the liver and cardiovascular system [6, 7]. Thus, in addition to conferring coloring effects, BE can contribute functional properties to food products [1].

Several studies have shown that BE is effective in preventing experimentally induced carcinogenesis [8, 9]. The exact mechanisms of action against cancer are not fully understood. However, they are believed to be associated with the inhibition of angiogenesis or enhancement of apoptosis by betalains [8, 10]. Since DNA alterations play a key role in cancer initiation and

other stages of carcinogenesis, antigenotoxicity is considered one of the main chemopreventive mechanisms [11, 12]. Beetroot extract has demonstrated protective effects *in vivo* against chemically induced clastogenicity, suggesting a possible link between its chemopreventive effect and antigenotoxic properties [13].

Identifying the intrinsic genotoxic potential of chemopreventive agents is important for the assessment of the risks and health benefits of their use in humans. There are limited and inadequate studies available on the genotoxicity of BE. These studies have been carried out with different but often poorly defined red beetroot formulations [14]. An analysis of the results from a number of *in vitro* and *in vivo* studies led to the conclusion that the genotoxic potential of BE cannot be assessed based on the available data [14].

Aim of the Study: This *in vivo* study investigated the genotoxic potential and possible antigenotoxicity of a standardized red BE in mice. Alkaline comet assay was used to assess DNA damage in mouse cells from peripheral blood, liver, kidneys, and gastrointestinal tract. We used chromosomal aberration cytogenetic tests to evaluate the clastogenic and anti-clastogenic activities in bone marrow cells.

Materials and Methods

Chemical Agents: The BE, diluted with dextrin, was purchased from Shanghai Macklin Biochemical Technology (Shanghai, China; cat. #:R854445). The contents of betanin and vulgaxanthin-I in the extract were measured on an xMark spectrophotometer (Bio-Rad, USA), assuming molar absorption coefficients (ε) of 6.5×10^4 L mol^{-1} cm^{-1} at λ_{max}=538 nm and 4.8×10^4 L mol^{-1} cm^{-1} at λ_{max}=476 nm, respectively, and corrected for impurities, using the absorbance value at 600 nm. The results were expressed as the contents of betacyanins (converted to betanin) and betaxanthins (converted to vulgaxanthin-I), and the total content of betalains was expressed as their sum.

Suppliers: Dioxidine (2,3-bis-hydroxymethyl-2,3-dihydro-quinoxaline-1,4-dioxide) was purchased from Valenta Pharm, Russia (CAS#: 17311-31-8); methyl methanesulfonate (MMS), from Sigma-Aldrich, Germany (cat. #: 129925); *Topotecan*, a chemotherapy drug, from S.c.Sindan-Pharma S.r.l., Romania (*Topotecan Teva*); colchicines, from Sigma-Aldrich, Germany (cat. #: C9754). All other chemicals were of reagent grades and purchased from local suppliers.

Animals: Experiments were performed on male rats (CBA×C57BL/6), weighing 20-22 g, procured from the central animal facility of the Federal Research Center for Innovative and Emerging Biomedical and Pharmaceutical Technologies (Moscow, Russia). The rats were housed in polycarbonate cages with steel wire tops (n=5-6 animals per cage) at standard room temperature (22±2°C), humidity (55±10%), and under 12-hour light/dark cycles. Standard laboratory animal feed (MEST Ltd, Russia) and water were provided to the animals *ad libitum*. The rats were quarantined for at least one week before commencing the experiments. During the quarantine period, the animals were checked and examined daily for signs of illness. In this context, only healthy animals were used in the study. Each control and experimental group included five animals.

Alkaline Comet Assay: The alkaline comet assay was performed based on the recommendations of the Organization for Economic Co-operation and Development (OECD) guideline 489 [15]. The BE was dissolved in distilled water and administered by gavage at doses of 250, 500, or 2000 mg/kg for three consecutive days. Animals were monitored twice daily for morbidity and mortality. Approximately 3 h after the administration of the final BE dose on day 3, blood samples were collected, and animals were euthanized for tissue collection as described below. Negative control animals received an equivalent volume of the vehicle solution in a similar manner.

To investigate the antigenotoxic effects of BE, rats were simultaneously treated with methyl methane sulfonate at 40 mg/kg (intraperitoneally [IP]) and BE at 25, 50, or 100 mg/kg by gavage for 3 h or with dioxidine at 250 mg/kg (IP) and BE at the same doses and route for 1.5 h. Blood samples were collected by tail snip procedure and mixed at a ratio of 1:8 with ice-cold mincing solution (phosphate buffered saline [PBS] containing 20 mM EDTA-Na_2 and 10% DMSO at pH 7.4). After this step, the rats were sacrificed by cervical dislocation, and the liver, kidneys, stomach, duodenum, and rectum were excised. Portions of the liver, kidneys, and stomach tissues were rinsed in a mincing solution and then minced. The duodenum and rectum samples (~1 cm) were cut longitudinally, kept in a mincing solution for 1-2 min, rinsed well by shaking twice for 30 s to remove fecal material, and then minced. All samples were kept on ice for 30-60 s to allow large clumps to settle, and single-cell suspensions from the top of the tubes were collected for the comet assay.

Cell suspension samples (60 µL) were mixed with 240 µL of 0.9% low melting agarose. Then, 70 µL of the cell-agarose suspension was dropped on slides pre-coated with 1% normal melting agarose, and the slides were sealed with coverslips. After the agarose gel was set, the coverslips were removed, and the slides were immersed in a lysis solution for 1 h at 4ºC in the dark. The lysis solution contained 10 mM Tris-HCl, pH 10, 2.5 M NaCl, and 100 mM EDTA-Na_2 with freshly added 1% Triton X-100 and 10% DMSO. The slides were then immersed in an electrophoresis solution containing 300 mM NaOH and 1 mM EDTA-Na_2 at pH>13 for 20 min at 4ºC to unwind the DNA strands. The electrophoresis was run in the same pre-chilled solution for 20 min at a constant electric field of 1 V/cm and a current of ~300

mA. The solution temperature was kept below 10ºC by recirculation. The slides were then rinsed with PBS, immersed in 70% ethanol for 10 min, and air-dried.

Prior to scoring, the slides were stained with SYBR Green I (1:10000 in TE buffer, pH 8.5) for 30 min in the dark. Images were taken on a Mikmed-2 12T epifluorescent microscope (LOMO, Russia) equipped with a high-resolution digital camera at ×200 magnification. At least 150 randomly selected comets were scored per slide and animal. The percentage of DNA in the tail (%TDNA) of the comets was determined using the CASP v.1.2.2 image analysis software. Hedgehog comets with a small or no visible head were tabulated but not scored.

The median value from the individual animal was used as the statistical unit, and the data were presented as the mean ± SD for each group of animals. The nonparametric Mann-Whitney test was performed to determine significant differences in %TDNA among the groups. The statistical significance level was set at $P<0.05$.

Bone Marrow Chromosomal Aberration Test: The clastogenic and anti-clastogenic activities of BE were assessed using a chromosomal aberration test on the rats' bone marrow cells [16]. For this test, BE was dissolved in distilled water immediately before use and administered via gavage at single doses of 250, 500, or 2000 mg/kg for 18 h and at a single dose of 2000 mg/kg for 24 h. The positive control group received 0.5 mg/kg of Topotecan in saline intraperitoneally (IP). Negative control animals received an equivalent volume of the vehicle solution in the same manner.

To investigate the anticlastogenic effects of BE, the rats were simultaneously treated by gavage with 250 mg/kg of dioxidine or methanesulfonate at 40 mg/kg (IP) and BE at 25, 50, or 100 mg/kg for 24 h. Colchicine (2.5 mg/kg) was injected (IP) 2.5 h before the animals were sacrificed by cervical dislocation. One air-dried cytogenetic slide per animal was prepared using a conventional method. The slides were coded independently and blindly. The slides were examined under a standard light microscope (Carl Zeiss, Germany) at ×1000 magnification (oil immersion). At least 200 well-spread metaphases per animal were scored for cytogenetic damage, such as breaks in chromatids or chromosomes and exchanges. The presence of more than five chromosomal aberrations per metaphase was considered multiple damages. The calculation of the aberration rates was performed by excluding and including gaps. The data were statistically analyzed using Fisher's exact test by pairwise comparisons of the percentage of cells with aberrations among the groups. The statistical significance was set at $P<0.05$. At least 1,000 cells from randomly selected views on each slide were scored, and the mitotic index (MI) was calculated as the ratio of the mitotic cells to the total number of cells multiplied by 100. Statistical analyses were performed on the data using the Chi-square test.

Results

Genotoxicity Tests: Considering the low toxicity of red BE as evidenced in the literature, and in order to meet the dose limit requirements for low- or non-toxic test materials based on the OECD guidelines, the BE high dosage was selected at 2000 mg/kg for testing the genotoxicity based on the comet assay and chromosomal aberration tests [15, 16]. The results of BE genotoxicity assessment by the comet assay are presented in Table 1. The positive control (receiving MMS) resulted in a statistically significant increase in %TDNA compared to the vehicle control ($P<0.01$) for all tissue samples. The oral administration of BE up to 2000 mg/kg did not cause a statistically significant induction of DNA damage in all analyzed tissue samples compared to the vehicle control ($P>0.05$).

We used Topotecan, a chemotherapy drug, as a positive control to induce increases in the chromosomal aberration rates and declines in MI of the rats' bone marrow (Table 2). The mean values of aberrant metaphases in all BE treatment groups did not differ from those of the negative controls. They were within the historical range of negative control values. The MI values did not differ from those of the corresponding vehicle control, indicating that BE, at the administered dosage, was not toxic to the bone marrow cells.

Antigenotoxicity Tests: For these studies, the dosage was based on the experimental data for the biological properties of BE *in vivo*, including antioxidant, anti-inflammatory, cardioprotective activities, and others [6, 7]. The MMS at 40 mg/kg significantly increased the levels of DNA damage in all tissues (Table 3). The BE at 25 mg/kg reduced MMS-induced DNA damage in the liver, stomach, duodenum, and rectum by 48.5-65.4%; however, it was not effective for the blood cells. At a dose of 50 mg/kg, BE exhibited similar effects, while at 100 mg/kg, its protective activity was slightly less, especially in the liver and stomach.

Table 1. DNA damage in rats treated with beetroot extract.

Treatment Group (mg/kg)	%TDNA (mean±SD)					
	Blood cells	**Liver**	**Kidney**	**Stomach**	**Duodenum**	**Rectum**
Control (vehicle)	0.3±0.1	1.4±0.5	1.4±0.4	2.6±0.5	2.3±0.5	2.4±0.4
MMS 40	7.7±2.8[a]	11.9±2.0[a]	16.8±2.1[a]	19.0±6.4[a]	17.1±2.3[a]	22.2±3.9[a]
BE 125	0.2±0.1	1.2±0.2	1.1±0.4	2.5±0.5	1.9±0.1	2.1±0.5
BE 500	0.3±0.1	1.5±0.3	1.4±0.4	2.7±0.5	2.3±0.6	2.2±0.5
BE 2000	0.2±0.1	1.2±0.2	1.1±0.2	1.9±0.5	2.0±0.2	2.5±0.5

[a] significantly different from the control group ($P<0.01$; Mann-Whitney test)

Table 2. Chromosomal aberrations in bone marrow cells of rats treated with BE.

Treatment Group (mg/kg)	MI	Per 100 metaphases					% metaphases with aberrations (mean±SE)	
		gaps	ctb	cmb	exch	Mlta	with gaps	w/o gaps
Control (vehicle)	3.5	0	0.4	0	0	0	0.4±0.3	0.4±0.3
Topotecan 0.5	2.0[a]	0.3	4.0	0	1.8	7.8	12.8±1.7[b]	12.5±1.6[b]
BE 125	3.0	0.2	0.6	0	0	0	0.8±0.4	0.6±0.4
BE 500	3.7	0	0.4	0	0	0	0.4±0.3	0.4±0.3
BE 2000 (18 h)	3.9	0.2	0.4	0	0	0	0.6±0.3	0.4±0.3
BE 2000 (24 h)	3.1	0.2	0.2	0	0	0	0.4±0.3	0.2±0.2

[a] significantly different from the control group (P<0.01; Chi-square test).
[b] significantly different from the control group (P<0.01; Fisher's exact test).
ctb: chromatid breaks; cmb: chromosome breaks; exch: chromatid exchanges;
mlta: metaphases with multiple aberrations (P≥5).

Table 3. DNA damage in rats treated with BE in combination with methyl methane sulfonate or dioxidine.

Treatment group (mg/kg)	%TDNA (mean±SD) / % reduction				
	Blood cells	Liver	Stomach	Duodenum	Rectum
Control (vehicle)	0.3±0.1	1.5±0.2	2.1±0.2	0.7±0.1	2.0±0.2
MMS 40	9.9±2.5[a]	13.6±1.7[a]	21.1±7.9[a]	19.9±1.3[a]	23.2±4.7[a]
BE 25 + MMS	6.8±2.3	7.0±1.8[b] / 48.5	7.3±1.7[b] / 65.4	7.3±1.8[b] / 63.3	10.0±3.1[b] / 56.9
BE 50 + MMS	7.1±1.3	6.9±1.0[b] / 49.3	7.2±0.8[b] / 65.9	5.9±1.2[b] / 70.4	10.6±3.9[b] / 54.3
BE 100 + MMS	7.5±1.9	8.7±1.0[b] / 36.0	11.3±2.5[b] / 46.5	8.8±1.5[b] / 55.8	11.6±2.8[b] / 50.0
Control (vehicle)	0.2±0.1	1.3±0.7	1.6±1.3	0.7±0.3	1.2±0.6
DN 250	6.3±1.7[c]	19.7±3.6[c]	14.8±2.2[c]	8.4±3.0[c]	14.7±5.3[c]
BE 25 + DN	3.8±2.2	11.7±1.8[d] / 40.6	9.6±2.7[d] / 35.1	4.9±2.8[d] / 41.7	5.7±2.3[d] / 61.2
BE 50 + DN	4.1±1.1	10.3±1.4[d] / 47.7	5.2±1.3[d] / 64.9	3.2±1.0[d] / 61.9	4.8±2.2[d] / 67.3
BE 100 + DN	4.1±0.8	11.4±1.6[d] / 42.1	7.2±2.1[d] / 51.4	3.1±2.0[d] / 70.0	5.0±2.9[d] / 66.0
Control (vehicle)	n/a	0.8±0.7	3.0±0.9	2.1±0.7	1.9±0.4
DN 250		17.5±3.0[c]	10.8±1.3[c]	9.4±2.0[c]	9.9±2.2[c]
BE 5 + DN		16.4±2.2	8.6±0.9[e] / 20.4	9.6±2.0	9.9±1.2
BE 10 + DN		15.8±3.1	7.9±1.2[e] / 26.9	5.6±1.8[d] / 40.4	6.5±3.7[d] / 34.3

[a] significantly different from the control group (P<0.01; Mann-Whitney test)
[b] significantly different from the MMS group (P<0.01; Mann-Whitney test)
[c] significantly different from the control group (P<0.01; Mann-Whitney test)
[d] significantly different from the DN group (P<0.01; Mann-Whitney test)
[e] significantly different from the DN group (P<0.05; Mann-Whitney test)

The BE exhibited an antigenotoxic effect by inhibiting the DNA-damaging activity on dioxidine (i.e., genotoxic property) with a pro-oxidant action. At all doses given, BE reduced the genotoxic effects of dioxidine in the liver (up to 47.7%), stomach (up to 64.9%), rectum (up to 63.7%), and dose-dependently in the duodenum (41.7-70%). Additional studies were conducted to determine the lowest protective dose of BE. At 10 mg/kg, BE reduced the genotoxic effect of dioxidine in the stomach, duodenum, and rectum (by 26.9-40.4%), while at 5 mg/kg, it was effective by 20.4% only in the stomach. When assessing the anticlastogenic activity (Table 4), the simultaneous administration of different doses of BE and either DN or MMS did not result in a significant difference in the frequency of chromosomal aberrations in bone marrow cells compared to that of the rats that received mutagens only.

Table 4. Chromosomal aberrations in bone marrow cells of rats treated with BE in combination with dioxidine or methyl methane sulfonate.

Treatment Group (mg/kg)	MI	Per 100 Metaphases					% metaphases with aberrations (mean±SE)	
		gaps	ctb	cmb	exch	mlta	with gaps	w/o gaps
Control (vehicle)	3.5	0	0.4	0	0	0	0.4±0.3	0.4±0.3
DN 250	2.8	0.4	5.6	0	1.8	5.6	11.6±1.4[a]	11.2±1.4[a]
BE 25 +DN	3.0	0	5.0	0	1.6	8.6	13.6±1.5	13.6±1.5
BE 50 + DN	3.4	0.2	5.0	0	1.6	5.2	10.8±1.4	10.6±1.4
BE 100 + DN	2.9	0.4	6.0	0.4	0.6	7.2	12.2±1.5	11.8±1.5
MMS 80	3.6	0.2	9.2	0	2.4	1.4	12.2±1.5[a]	12.0±1.5[a]
BE 25 +MMS	3.1	0.2	13.0	0	3.0	5.0	15.8±1.6	15.6±1.6
BE 50 + MMS	3.4	0.8	9.4	0	2.6	5.4	15.8+1.6	15.0±1.6
BE 100 + MMS	3.8	0.2	9.2	0.2	2.2	1.6	11.8±1.4	11.6±1.4

[a] significantly different from the control group (P<0.01; Fisher's exact test). ctb: chromatid breaks;
cmb: chromosome breaks; exch: chromatid exchanges; mlta: metaphases with multiple aberrations (P≥5).

Discussion

The content of betalains and the ratio of betacyanins/ betaxanthins in BE were dependent on the variety of red beets, conditions of cultivation, and the extraction procedure [17]. Our spectrophotometric analyses showed that BE, as used in this study, had a total betalains content of 4 mg/g (0.4% of the total extract), of which betacyanins accounted for 2.7 mg/g (0.27% of the total extract and 67.5% of the total betalains).

To date, there are only two *in vivo* studies on the genotoxicity of red BE. In an *in vivo* study on the clastogenicity of beetroot powder in rats' bone marrow,

no significant increases were found in the number of cells with chromosomal aberrations and micronuclei frequency compared to the negative controls [18]. Using the comet assay, no DNA damage has been observed in the blood leukocytes of rats treated with red BE [19]. Although these studies [18, 19] have not reported genotoxicity for red BE, they have several limitations. Among these shortcomings are the BE dosage, the number of animals, and the determination procedure for chromosomal aberrations or micronuclei. These limitations do not allow for clear conclusions to be made [14].

In the current study, the genotoxicity of the standard BE was assessed *in vivo* according to generally accepted testing guidelines as required by regulatory agencies. The BE, when administered orally at the tested doses, exhibited no genotoxicity in the bone marrow cells of rats based on the chromosomal aberration test. The same conclusion was made for the cells isolated from peripheral blood, liver, kidneys, and gastrointestinal tract, and subjected to the comet assay. Thus, even at doses significantly higher than normal human consumption, BE has no genotoxic effect based on the findings of the current study.

A significant decrease in DNA damage was observed in rats treated with BE combined with either MMS or dioxidine compared to those treated only with the mutagens. The findings clearly suggest the antigenotoxicity of BE. Oxidative stress is one of the pathways leading to DNA damage, and agents capable of scavenging free radicals may also exhibit antigenotoxic properties [20]. The generation of superoxide anion radicals is considered the primary mechanism responsible for the bacteriocidal and genotoxic effects of dioxidine [21, 22]. Thus, it can be assumed that the antigenotoxic effect of BE against DNA damage induced by dioxidine may be associated with its antioxidant activity. It should be emphasized that among the betalains class, only betacyanins exhibit the capacity to scavenge free radicals [23].

As a direct-acting monofunctional alkylating agent, MMS can modify oxygen or nitrogen residues present in either the DNA backbone or in its bases [24]. Although the mechanisms of antigenotoxicity of BE against the action of MMS are not clear, the following hypothesis can be proposed. Betalains are produced as a result of the condensation of betalamic acid with amino acids or amines, the type of which determines the class of compound that is formed (i.e., betacyanins or betaxanthins) [25]. The drug, L-DOPA, is a precursor to betalamic acid and cyclo-DOPA, the betacyanin-defining moiety, and it can condense with betalamic acid to form a betaxanthin. Dopamine, L-DOPA, epinephrine, and their precursor L-tyrosine have been found to significantly reduce the frequency of micro-nucleated polychromatic erythrocytes in the peripheral blood and bone marrow of rats treated with MMS [26]. In addition, the compounds reduced the number of MMS-induced revertants in *Salmonella typhimurium* strains, TA-98, and TA-104. The putative mechanism of protective action involves the trapping of the alkylating $CH3^{+}$carbocation by the compounds through substitution in the aromatic ring or nucleophilic residues on the side chains [26].

Obviously, these are only some of the possible mechanisms of the antigenotoxicity of BE. Mechanisms associated with the interception of DNA-reactive species (carbocations or free radicals) suggest a dose-dependent manner of BE antigenotoxicity. Increasing the BE dosage did not result in a proportional reduction in DNA damage, indicating the absence of a dose-response relationship. Moreover, BE exhibited a protective effect on all segments of the gastrointestinal tract even at a dose of 10 mg/kg, which corresponds to a very low dose of betalains (i.e., about 40 µg/kg). Taken together, this suggests the involvement of indirect, triggering mechanisms as well. Further studies are warranted to clarify the mechanisms of the antigenotoxicity of BE.

Lastly, BE exhibited the same protective effect in the examined organs, such as the gastrointestinal tract, with a slightly reduced effect in the liver; nonetheless, it had no effect on the blood cells. The cytogenetic analysis did not reveal anticlastogenic activity of BE in the bone marrow. Pretreatment with BE prior to the N-nitrosodiethylamine administration resulted in a significant reduction (by 20%) in DNA damage in the rat liver [27]. Feeding rats with BE did not cause damage to the DNA of the rats' leukocytes induced by 7,12-dimethylbenz-a-anthracene [28]. These findings suggest that the antigenotoxicity of BE is tissue-specific. The BE was shown to be capable of protecting the bone marrow cells against clastogenicity induced by cyclophosphamide and benzo-a-pyrene [13]. However, the study used a homogenate, which, in addition to betalains, contained a large number of bioactive, potentially antigenotoxic compounds. Betacyanins have very low bioavailability (1-3%), and the bulk of them transit through the gastrointestinal tract, closely interacting with the mucosal epithelia in the gastrointestinal tract [3, 29].

Conclusions

The results of this study demonstrated the absence of BE genotoxicity *in vivo* under testing conditions, which were consistent with accepted guidelines. The comet assay revealed considerable antigenotoxicity in BE in relation to the effects of mutagens with different mechanisms of action. Thus, BE meets the basic requirements for being nominated as a chemopreventive agent. The advantages include low cost, practicality of use, efficacy, and safety; therefore, it can be used in many potential interventions [11]. The tissue specificity of BE action may serve as a promising example of targeted antimutagenesis, especially if the targets of protection are specific organs or tissues of the body. For instance, the

use of BE appears promising for the prevention of genotoxicity and the development of secondary tumors in the gastrointestinal tract and liver in patients receiving chemotherapy for original or metastatic tumors.

Conflict of Interests

The authors declare that they had no known competing financial interests or personal relationships that could have influenced the experimental findings reported in this article.

Funding

This study received no funds, grants, or financial support from any internal or external entities.

Acknowledgement

The authors would like to express their appreciation for the support of the management and staff of the Federal Research Center for Innovator and Emerging Biomedical and Pharmaceutical Technologies and its Department of Drug Toxicology in Moscow, Russia.

Compliance with Ethical Guidelines

All procedures involving the use of animals in this study were reviewed and approved by the Animal Ethics Committee of the Federal Research Center for Innovator and Emerging Biomedical and Pharmaceutical Technologies (Moscow, Russia).

Authors' Contributions

All senior and junior authors contributed fairly equally toward conducting the experiments and writing several drafts of this article prior to submission.

References

1. Punia Bangar S, Singh A, Chaudhary V, Sharma N, Lorenzo JM. Beetroot as a novel ingredient for its versatile food applications. Crit Rev Food Sci Nutr. 2023;**63**(26):8403-27. [**doi:** 10.1080/10408398.2022.2055529] [pmid: 35333666]
2. Wissgott U, Bortlik K. Prospects for new natural food colorants. Trends in Food Science & Technology. 1996;**7**(9):298–302. [**doi:** 10.1016/0924-2244(96)20007-X]
3. Khan MI. Plant Betalains: Safety, Antioxidant Activity, Clinical Efficacy, and Bioavailability. Compr Rev Food Sci Food Saf. 2016;**15**(2):316-30. [**doi:** 10.1111/1541-4337.12185] [pmid: 33371594]
4. Sadowska-Bartosz I, Bartosz G. Biological Properties and Applications of Betalains. Molecules. 2021;**26**(9):2520. [**doi:** 10.3390/molecules26092520] [pmid: 33925891]
5. Calvi P, Terzo S, Amato A. Betalains: colours for human health. Nat Prod Res. 2023;**37**(10):1746-65. [**doi:** 10.1080/14786419.2022.2106481] [pmid: 35921318]
6. Hadipour E, Taleghani A, Tayarani-Najaran N, Tayarani-Najaran Z. Biological effects of red beetroot and betalains: A review. Phytother Res. 2020;**34**(8):1847-67. [**doi:** 10.1002/ptr.6653] [pmid: 32171042]
7. Madadi E, Mazloum-Ravasan S, Yu JS, Ha JW, Hamishehkar H, Kim KH. Therapeutic Application of Betalains: A Review. Plants (Basel). 2020;**9**(9):1219. [**doi:** 10.3390/plants9091219] [pmid: 32957510]
8. Lechner JF, Stoner GD. Red Beetroot and Betalains as Cancer Chemopreventative Agents. Molecules. 2019;**24**(8):1602. [**doi:** 10.3390/molecules24081602] [pmid: 31018549]
9. Tan ML, Hamid SBS. Beetroot as a Potential Functional Food for Cancer Chemoprevention, a Narrative Review. J Cancer Prev. 2021;**26**(1):1-17. [**doi:** 10.15430/JCP.2021.26.1.1] [pmid: 33842401]
10. Zhang Q, Pan J, Wang Y, Lubet R, You M. Beetroot red (betanin) inhibits vinyl carbamate- and benzo(a)pyrene-induced lung tumorigenesis through apoptosis. Mol Carcinog. 2013;**52**(9):686-91. [**doi:** 10.1002/mc.21907] [pmid: 22456940]
11. De Flora S, Ferguson LR. Overview of mechanisms of cancer chemopreventive agents. Mutat Res. 2005;**591**(1-2):8-15. [**doi:** 10.1016/j.mrfmmm.2005.02.029] [pmid: 16107270]
12. Steele VE, Kelloff GJ. Development of cancer chemopreventive drugs based on mechanistic approaches. Mutat Res. 2005;**591**(1-2):16-23. [**doi:** 10.1016/j.mrfmmm.2005.04.018] [**pmid:** 16083917]
13. Edenharder R, Frangart J, Hager M, Hofmann P, Rauscher R. Protective effects of fruits and vegetables against in vivo clastogenicity of cyclosphosphamide or benzo[a]pyrene in mice. Food Chem Toxicol. 1998;**36**(8):637-45. [**doi:** 10.1016/s0278-6915(98)00035-0] [**pmid:** 9734714]
14. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Scientific Opinion on the re-evaluation of beetroot red (E 162) as a food additive. EFSA Journal. 2015;**13**(12):4318. [**doi:** 10.2903/j.efsa.2015.4318]
15. OECD Test No. 489: In Vivo Mammalian Alkaline Comet Assay. OECD Guidelines for the Testing of Chemicals. Section 4, OECD Publishing. Paris. 2016. [**doi:** 10.1787/9789264264885-en]
16. OECD Test No. 475: Mammalian Bone Marrow Chromosomal Aberration Test. OECD Guidelines for the Testing of Chemicals. Section 4, OECD Publishing. Paris. 2016. [**doi:** 10.1787/9789264264786-en]
17. Nemzer B, Pietrzkowski Z, Spórna A, Stalica P, Thresher W, Michałowski T, et al. Betalainic and nutritional profiles of pigment-enriched red beet root (Beta vulgaris L.) dried extracts. Food chemistry. 2011;**127**(1):42-53. [**doi:** 10.1016/j.foodchem.2010.12.081]
18. Conforti-Froes N, Varella-Garcia M, Manzato AJ. In vivo and in vitro evaluation of betanine mutagenicity. Cytologia. 1990;**55**(2):203-8. [Link]
19. Kujawska M, Ignatowicz E, Murias M, Ewertowska M, Mikołajczyk K, Jodynis-Liebert J. Protective effect of red beetroot against carbon tetrachloride- and N-nitrosodiethylamine-induced oxidative stress in rats. J Agric Food Chem. 2009;**57**(6):2570-5. [**doi:** 10.1021/jf803315d] [**pmid:** 19292473]
20. Poetsch AR. The genomics of oxidative DNA damage, repair, and resulting mutagenesis. Comput Struct Biotechnol J. 2020;**18**:207-19. [**doi:** 10.1016/j.csbj.2019.12.013] [**pmid:** 31993111]
21. Durnev AD, Tjurina LS, Guseva NV, Oreshchenko AV, Volgareva GM, Seredenin SB. The influence of two carotenoid food dyes on clastogenic activities of cyclophosphamide and dioxidine in mice. Food Chem Toxicol. 1998;**36**(1):1-5. [**doi:** 10.1016/s0278-6915(97)00111-7] [**pmid:** 9487358]
22. Sviridova DA, Machigov EA, Igonina EV, Zhoshibekova BS, Abilev SK. Studying the Mechanism of Dioxidine Genotoxicity Using Lux Biosensors of Escherichia coli. Biology Bulletin. 2021;**48**:2174-80. [**doi:** 10.1134/S1062359021120098]
23. Fernando GSN, Sergeeva NN, Vagkidis N, Chechik V, Marshall LJ, Boesch C. Differential Effects of Betacyanin and Betaxanthin Pigments on Oxidative Stress and Inflammatory Response in Murine Macrophages. Mol Nutr Food Res. 2023;**67**(15):e2200583. [**doi:** 10.1002/mnfr.202200583] [**pmid:** 37203590]
24. Beranek DT. Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents. Mutat Res. 1990;**231**(1):11-30. [**doi:** 10.1016/0027-5107(90)90173-2] [**pmid:** 2195323]
25. Schwinn KE. The dope on L-DOPA formation for betalain pigments. New Phytol. 2016;**210**(1):6-9. [**doi:** 10.1111/nph.13901] [**pmid:** 26919693]
26. Billacura M, Bajo L. Antimutagenic potentials of L-tyrosine and its metabolites on the genotoxic activity induced by methyl methanesulfonate. International Journal of Advanced and Applied Sciences. 2017;**4**(11):104-9. [doi: 10.21833/ijaas.2017.011.016]
27. Krajka-Kuźniak V, Szaefer H, Ignatowicz E, Adamska T, Baer-Dubowska W. Beetroot juice protects against N-nitrosodiethylamine-induced liver injury in rats. Food Chem Toxicol. 2012;**50**(6):2027-33. [**doi:** 10.1016/j.fct.2012.03.062] [**pmid:** 22465004]
28. Szaefer H, Krajka-Kuźniak V, Ignatowicz E, Adamska T, Baer-Dubowska W. Evaluation of the effect of beetroot juice on DMBA-induced damage in liver and mammary gland of female Sprague-Dawley rats. Phytother Res. 2014;**28**(1):55-61. [**doi:** 10.1002/ptr.495] [**pmid:** 23450834]
29. Wang Y, Adekolurejo OO, Wang B, McDermott K, Do T, Marshall LJ, et al. Bioavailability and excretion profile of betacyanins - Variability and correlations between different excretion routes. Food Chem. 2024;**437**(Pt 1):137663. [**doi:** 10.1016/j.foodchem.2023.137663] [**pmid:** 37879158]