Antigenotoxic and antimutagenic effects of lignin derivative BP-C2 against dioxidine and cyclophosphamide in vivo in murine cells

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1. Introduction

Polyphenols represent one of the largest and diverse categories of plant components with known complexity to a comprehensive characterization [45]. Typical polyphenols are phenol-containing compounds with carbon skeleton backbones ranging from C1-C6 to C3-C6 and C6-C3-C6, but while some contain the N-functional constituent. The chemical structures of other atypical polyphenols are too complex to group together or categorize. A major medicinal advantage of multiple different polyphenols is their demonstrated ability to act on multiple molecular and cellular targets [44]. Polyphenols (flavonoids, tannins, curcumin, resveratrol, galactatechins, quercitin, etc.) are considered for cancer chemoprevention, and for treatment and management of commonly known adverse drug reactions associated with current cancer chemotherapeutic drugs [5,18]. Anticancer and antioxidant activities of polyphenols are related to the ability of polyphenols to counteract genetic susceptibility to some diseases, effects from exposure to environmental carcinogens or effects of various exacerbation factors (i.e. oxidative stress, inflammation, cell proliferation or apoptosis, cell migration and angiogenesis, metastasis) [10,21,24,30]. As comprehensively reviewed elsewhere [34] polyphenols and specifically combinations containing multiple different polyphenols are viewed as promising anti-cancer drugs with a risk to benefit ratio better than of the conventional anti-cancer medicines.

Lignin is considered as a rich source of natural polyphenols [8]. Its derivatives have a broad range of biological activity and reckoned as...
good candidates for the development of medicinal products [15].

BP-Cx-1 is a water-soluble lignin derivative [41] containing benzene polycarboxylic acids (e. g., 2-hydroxy-4-(3,5,7-trihydroxy-4-oxo-4H-chromen-2-yl)benzoic acid, 3-(benzoyl)-4,5-dihydroxybenzoic acid, biphenyl-2,2′,3′-3′-tetracarboxylic acid, etc.). It resembles the structure of plant flavonoids, sapogenins, and phenanthrenes [17] and is used in the family of pharmaceutical products: anticancer drug BP-C1, radio-protector BP-C2 and geroprotector BP-C3 [1]. BP-C2 is a composition of BP-Cx-1 with ammonium molybdate; in aqueous solutions, ammonium molybdate is hydrolyzed to molybdenum oxides.

Despite many scientific papers discussing beneficial effects of lignin derivatives, there are few evaluating their antigenotoxic potential. A study with sulfur-free beech lignin polymer demonstrated the ability of reducing genotoxic activity of ofloxacin in Euglena cells [26]. Ability of study with sulfur-free beech lignin polymer demonstrated the ability of significantly reduced genotoxicity of 3-adsorptive and antioxidative activities [27]. A lignin biopolymer significantly reduced genotoxicity of 3′-azido-2′-deoxythymidine assessed as single strand breaks of DNA in human hepatoma HepG2 and colon Caco-2 cells in comet assay [43]. Lignin isolated from black liquor waste, a major by-product of palm oil extraction, and commercial lignin were evaluated for antioxidant and genoprotective properties in bone marrow micronucleus test against cyclophosphamide (CPA). Both lignins exhibited rich antioxidant activities and similar protective effects against CPA induced genotoxicity and cytotoxicity [31]. Considering unique and complex chemical composition of each lignin derivative it is relevant to study genotoxic/antigenotoxic activity of exact product. Previously, we studied the genotoxic and antigenotoxic effects of BP-C2 in rats challenged with methyl methanesulfonate (MMS) [36]. BP-C2 administered at doses of 20, 100 and 200 mg/kg 1 h before MMS significantly mitigated DNA damage, showing a strong genoprotective effect in the liver cells.

The aims of the present study were to further characterize the anti-genotoxic activity of BP-C2 in vivo against two different DNA-damaging agents and to assess antimutagenic activity of the composition. The comet assay was chosen as a test system. The method is based on measuring the changes in electrophoretic mobility of DNA in the agarose gel [7]. This method is applied to assess the pathogenetic role of primary DNA damage in the development of a number of pathologies [12–14, 28]. To assess the antimutagenic effect we studied chromosomal aberrations in bone marrow cells of mice. Laboratory mice are the most relevant mammalian species for in vivo studies of genotoxicity, mutagenicity, and antimutagenicity of natural and synthetic compounds [12, 48].

Dioxidine (DN) and CPA were used as genotoxicants for the studies of antigenotoxic/antimitogenic activity [13,14,48]. DN (1,4-di-N-oxide of 2,3-bis-(hydroxymethyl) quinoline), a wide-spectrum antibacterial drug [23,37], is also used as a prooxidant genotoxic agent [11,42]. DN is enzymatically reduced in vivo to yield a radical intermediate that causes DNA strand breaks. DNA cleavage also is mediated by hydroxyl radical derived from homolytic fragmentation of the drug radical [23].

CPA (N′-bis-(b-chloroethyl)-N′-O-trimethyl ester of phosphoric acid diamine) – is an alkylating anticancer drug and an immunosuppressive agent [39]. The main effect of CPA is due to its metabolite phosphoramide mustard. This metabolite is only formed in cells that have low levels of aldehyde dehydrogenase enzymes. Phosphoramide mustard forms DNA-crosslinks both between and within DNA strands at guanine N-7 positions (known as interstrand and intrastrand crosslinkages, respectively) [20].

2. Materials and methods

2.1. Animal husbandry

Experiments were performed on 184 male BALB/c mice (22–24 g, 6–8-week-old) originating from “Stolbovaya” animal breeding facility of the FSIS “Scientific Center of Biomedical Technology of the Federal Medical-Biological Agency of Russia”, Russia. The animals were kept under controlled room environment (20–24 °C, 45–65% relative humidity, 8–10 room volumes per hour). The 12 h light/12 h dark cycle was maintained in animal housing rooms using white luminous lamps. The mice were housed 10–12 per conventional polycarbonate cage 1290D (Tecniplast, Italy) with stainless steel mesh lid with built-in anti-feeding funnel. Wood chips of deciduous trees was used as bedding. Standard laboratory animal chow (CJSC “Tosnensky feed mill”, Leningrad region, Russia) and purified water were provided ad libitum.

Animals had one-week quarantine before the beginning of the experiment and were observed daily for the signs of morbidity. Only healthy animals were used in the study.

2.2. Ethics statement

The study was approved by the Local Bioethics Committee of FSBI “Zakusov Institute of Pharmacology” (Protocol #8 dated 17 June 2020).

2.3. Drugs and reagents

2.0% aqueous solution of BP-C2 composition (batch M3080420D3 valid till Apr 2021, Nobel Ltd., Saint-Petersburg, Russia) was used for the study. Dose selection was based on the previous study of radioprotective and radiomitigative effects of BP-C2 in total body irradiated mice [3]. In that study dose dependency has not been observed with regards to the BP-C2 doses of 1/650, 1/50 and 1/25 (limited by volume oral dose ~160 mg/kg) of LD10 established in male mice. Thus, for current study we selected two doses closest to the most effective one (80 mg/kg), a lower dose (60 mg/kg) and a higher dose (120 mg/kg) to check dose-dependent effects. Dilutions of BP-C2 were prepared ex tempore in all series of experiments. Deionized water was used as a vehicle for oral administration to animals. As the toxicity of BP-C2 is rather low (LD50 of oral BP-C2 in mice is ~8 g/kg, unpublished data) and BP-C2 did not exert genotoxicity in rats in the dose range of 20–2000 mg/kg [36] we avoided the use of groups treated with BP-C2 alone in order to reduce the total number of animals.

DN (OJSC Novosibkhimpharm, Novosibirsk, Russia), CPA (Sigma-Aldrich, Germany), SYBR Green I (Invitrogen, USA), EDTA-Na₂, Trizma base, Dimethyl sulfoxide anhydrous 99.9%, NaCl, Triton X100, normal and low melting agarose from Panreac (Spain), phosphate buffered saline (Gibco, Lot 1178515, United Kingdom) were used in the experiments.

2.4. Selection of optimal dose of DN for antigenotoxic activity assessment

DN has low toxicity: in mice intravenous LD50 is 1031–1212 mg/kg depending of age and sex of animals. Our own experience of studies by the method of registration of chromosome aberrations shows that the range of DN doses of 100–300 mg/kg is optimal to achieve the level of damaged metaphases (10–30%) for assessing antimutagenicity [13,14,32,48]. Thus, the same dose range was used to select the optimal dose for assessing antigenotoxic activity. DN at 100, 200 and 300 mg/kg was injected intraperitoneally once. Mice of negative control group received intraperitoneal injection of an equivalent volume of physiological saline. After 1.5 h animals were euthanized, cell suspensions were obtained from blood, bone marrow and liver. Sampling time was chosen based on data from previous studies using the fluorometric analysis of DNA unwinding (FADU) technique (unpublished data), which showed that the maximum level of DNA damage induced by DN occurs after 1.5
h, followed by disappearance at the 6th hour.

2.5. Preparation of slides and conditions of electrophoresis (comet test)

The in vivo alkaline and neutral comet assay was used to detect DNA damage [9,33]. Whole blood was mixed in proportion 1:8 with 20 mM EDTA-Na2 solution. The bone marrow cells were isolated from the femur and perfused with 2 mL of ice-cold PBS containing 20 mM EDTA-Na2 and 10% DMSO (pH 7.4). A portion of the liver was removed, washed in the same buffer and minced to obtain a cell suspension. Cell suspensions were processed as described earlier [47]. Briefly, 60 μL of cell suspension was mixed with 240 μL of 1% low-melting agarose in phosphate buffer saline heated to 42 °C (microthermostat “Termir,” Russia). Then 35 μL of cell-agarose suspension was applied to the glass slide precoated with 1% normal-melting agarose, sealed with coverslip and placed on ice. All further steps were carried out in a dark room with yellow light.

After solidification of agarose (5–10 min) coverslip was removed and the slide was placed into a glass cuvette (Schifferdecker type) filled with ice. All further steps were carried out in a dark room with yellow light. With prechilled to 4 °C 1% normal-melting agarose, sealed with coverslip and placed on ice. All further steps were carried out in a dark room with yellow light.

When performing the alkaline comet test, after the lysing step slides were transferred into 4 °C alkaline electrophoretic buffer (300 mM NaOH, 1 mM EDTA-Na2, [pH>13]) and incubated for 20 min to unwind the DNA. Then the slides were placed into electrophoresis chamber (SubCell GT, Bio-Rad) filled with fresh 4 °C electrophoretic buffer and electrophoresis was performed during 20 min (1 V/cm, ~300 mA, buffer recirculation rate 75 mL/min). After electrophoresis, the slides were washed with phosphate buffer saline for 10 min, fixed in 70% ethanol for 15 min, air-dried, coded and stored at room temperature.

When performing the neutral comet test, after the lysing step slides were transferred into electrophoresis chamber filled with Tris-borate buffer (445 mM Tris-HCl [pH=8.5], 445 mM boric acid, 10 mM EDTA-Na2) and electrophoresis was performed during 10 min (1 V/cm, ~12 mA). Then, slides were washed out, fixed and dried as described above.

Immediately before microscopy, the slides were stained with SYBR Green I (1:10000 in TE buffer; TE: 10 mM Tris-Cl [pH=10], 100 mM EDTA-Na2, 1% Triton X-100% and 10% dimethyl sulfoxide) and incubated for at least 1 h in the dark at 4 °C.

When performing the alkaline comet test, after the lysing step slides were transferred into electrophoresis chamber filled with Tris-borate buffer (445 mM Tris-HCl [pH=8.5], 445 mM boric acid, 10 mM EDTA-Na2) and electrophoresis was performed during 20 min (1 V/cm, ~300 mA, buffer recirculation rate 75 mL/min). After electrophoresis, the slides were washed with phosphate buffer saline for 10 min, fixed in 70% ethanol for 15 min, air-dried, coded and stored at room temperature.

When performing the neutral comet test, after the lysing step slides were transferred into electrophoresis chamber filled with Tris-borate buffer (445 mM Tris-HCl [pH=8.5], 445 mM boric acid, 10 mM EDTA-Na2) and electrophoresis was performed during 10 min (1 V/cm, ~12 mA). Then, slides were washed out, fixed and dried as described above.

Immediately before microscopy, the slides were stained with SYBR Green I (1:10000 in TE buffer; TE: 10 mM Tris-Cl [pH=8.5], 1 mM EDTA-Na2, 50% glycerol) for 30 min in the dark. Epifluorescent microscope Mikmed-2 12 T (LOMO, Russia) equipped with a high-resolution digital camera (VEC-335, Russia) was used to capture images at ×200 magnification. Images were assayed with CASP 1.2.2 software [25]. At least 100 DNA comets were analyzed per slide. The DNA fragment intensity in the tail (TDNA, %) was used as a measure of DNA damage and calculated as described [36].

2.6. Investigation of the antigenotoxic activity of BP-C2 composition in vivo in murine cells

BP-C2 was administered to mice via gavage at 60, 80 and 120 mg/kg once 1 h before single intraperitoneal injection of a genotoxic agent. Mice of the negative control group received intraperitoneal injection of an equivalent volume of physiological saline. Each experimental group, as well as the negative control group, were composed of 5 animals. Mice were euthanized 24 h after administration of a single dose of DN or CPA. 2.5 h prior to the euthanasia the mice were administered colchicine 4 mg/kg intraperitoneally. Cytogenetic preparations of the femoral bone marrow (one slide per animal) were prepared with the standard air-drying method [38]. All slides were coded (blinded) and stained with freshly prepared 2% Giemsa stain and microscopically examined (×1000 optical magnification). During the cytogenetic study 100 metaphase spreads from each animal/slide (500 per study group) were analyzed and cells with achromatic gaps, chromatid and chromosome breaks and various exchanges were counted. Cells with multiple abnormalities (metaphases with more than 5 chromosomal aberrations) were assigned to a separate category.

2.7. Chromosome aberrations in murine bone marrow cells

BP-C2 was administered to mice via gavage at 60, 80 and 120 mg/kg once 1 h before single intraperitoneal injection of DN (200 mg/kg) or CPA (20 mg/kg). Mice of the negative control group received intraperitoneal injection of an equivalent volume of physiological saline. Each experimental group, as well as the negative control group, were composed of 5 animals. Mice were euthanized 24 h after administration of a single dose of DN or CPA. 2.5 h prior to the euthanasia the mice were administered colchicine 4 mg/kg intraperitoneally. Cytogenetic preparations of the femoral bone marrow (one slide per animal) were prepared with the standard air-drying method [38]. All slides were coded (blinded) and stained with freshly prepared 2% Giemsa stain and microscopically examined (×1000 optical magnification). During the cytogenetic study 100 metaphase spreads from each animal/slide (500 per study group) were analyzed and cells with achromatic gaps, chromatid and chromosome breaks and various exchanges were counted. Cells with multiple abnormalities (metaphases with more than 5 chromosomal aberrations) were assigned to a separate category.

2.8. Statistical analysis

Data were analyzed with the Two-way ANOVA with Tukey’s multiple comparison post-test and Fisher exact test (GraphPad Prism 6.0).

3. Results

Results of selection of optimal dose of DN for the comet assay are presented in Table 1. In the alkaline comet test, DN at 100 mg/kg increased DNA damage only in the bone marrow cells. At 200 and 300 mg/kg DN increased level of DNA damage in all studied tissues. When DN was used at 300 mg/kg, more pronounced genotoxic effect was observed in liver cells. Thus 300 mg/kg was selected as an optimal dose for the assay.

3.1. Investigation of the antigenotoxic activity of BP-C2 in vivo in murine cells

The DNA-damaging effects of DN and antigenotoxic activity of BP-C2 are presented on Fig. 1 and in Table 2. When evaluated in the alkaline comet test DN at 300 mg/kg significantly increased the level of DNA damage in all studied tissues. When DN was used at 300 mg/kg, more pronounced genotoxic effect was observed in liver cells. Thus 300 mg/kg was selected as an optimal dose for the assay.

Table 1

| Table 1 | DNA-damaging effect of DN in cells of BALB/c mice in vivo in the alkaline comet test (TDNA%) | | | |
|----------|---------------------------------|---|---|---|
| Tissue   | Control | DN, 100 mg/kg | DN, 200 mg/kg | DN, 300 mg/kg |
| Bone marrow | 1.6     | 3.1 ± 0.6*    | 5.7 ± 1.3*    | 6.8 ± 0.9** ,# |
| Liver    | 3.0     | 3.9 ± 0.7     | 6.2 ± 1.8     | 12.3 ± 6.2*** ,** |
| Blood cells | 3.0     | 3.9 ± 1.0     | 8.3 ± 0.9**   | 5.4 ± 1.1     |

DN – dioxidazine. * , **, *** - p < 0.1, p < 0.01, p < 0.001 vs Control group, #, ## - p < 0.1, p < 0.01 vs DN 100 mg/kg group. Data presented as mean with standard deviation.

The DNA-damaging effects of DN and antigenotoxic activity of BP-C2 are presented on Fig. 1 and in Table 2. When evaluated in the alkaline comet test DN at 300 mg/kg significantly increased the level of DNA damage in tissues (bone marrow cells TDNA% 7.7 ± 1.3 vs 2.3 ± 0.4 in

RTI = 100 – TDNA%exp /TDNA%con × 100

where: TDNA%exp – DNA damage index in the experimental group; TDNA%con – DNA damage index in the control group.
Tukey’s multiple comparison test. *, **, *** – p < 0.05, p < 0.01, p < 0.001 vs control, #, ##, ### – p < 0.05, p < 0.01, p < 0.001 vs DN, 300 mg/kg.

Table 2
Relative antigenotoxic effect of BP-C2 against DNA damage induced by DN in tissues of BALB/c mice in vivo (%).

| Tissue                  | DN, 300 mg/kg | DN, 300 mg/kg + BP-C2, 60 mg/kg | DN, 300 mg/kg + BP-C2, 80 mg/kg | DN, 300 mg/kg + BP-C2, 120 mg/kg |
|-------------------------|---------------|---------------------------------|---------------------------------|----------------------------------|
| A. Alkaline comet Assay |               |                                 |                                 |                                  |
| Bone marrow             | 100           | 57 (–43)                        | 80 (–20)                        | 65 (–35)                         |
| Liver                   | 100           | 64 (–36)                        | 59 (–41)                        | 86 (–14)                         |
| Blood cells             | 100           | 71 (–29)                        | 55 (–45)                        | 47 (–53)                         |
| B. Neutral comet Assay  |               |                                 |                                 |                                  |
| Bone marrow             | 100           | 59 (–41)                        | 51 (–49)                        | 68 (–32)                         |
| Liver                   | 100           | 66 (–34)                        | 39 (–61)                        | 73 (–27)                         |
| Blood cells             | 100           | 42 (–58)                        | 50 (–50)                        | 74 (–26)                         |

DN – dioxidine. Numbers in parentheses indicate relative difference from the positive control taken as 100%.

Control, p < 0.0001; liver TDNA% 14.1 ± 1.5 vs 1.6 ± 0.6 in Control, p < 0.0001; blood cells TDNA% 6.3 ± 0.6 vs 0.9 ± 0.5 in Control, p < 0.0001). BP-C2 at 60 mg/kg decreased DNA damage induced by DN in the bone marrow cells, liver cells and blood by 43 (TDNA% 4.3 ± 0.9, p < 0.01 vs DN, 300 mg/kg), 36 (TDNA% 9.0 ± 3.5, p < 0.0001 vs DN, 300 mg/kg) and 29% (TDNA% 4.5 ± 0.7, p = 0.339 vs DN, 300 mg/kg), respectively. At 80 mg/kg BP-C2 reduced genotoxic effect of DN in the liver and blood cells by 41 (TDNA% 8.3 ± 2.8, p < 0.0001 vs DN, 300 mg/kg) and 45% (TDNA% 3.5 ± 0.5, p < 0.05 vs DN, 300 mg/kg), respectively, while for bone marrow cell the effect was also decreased by 20% but it was statistically insignificant (TDNA% 6.2 ± 2.1, p = 0.522 vs DN, 300 mg/kg). At 120 mg/kg BP-C2 decreased genotoxic effect of DN by 35 (TDNA% 5.0 ± 1.4, p = 0.053 vs DN, 300 mg/kg) and 53% (TDNA% 3.0 ± 0.5, p < 0.01 vs DN, 300 mg/kg) in the bone marrow cells and in the blood, respectively, but not in the liver (TDNA% 12.1 ± 1.6, p = 0.239 vs DN, 300 mg/kg).

When evaluated in the neutral comet test, DN significantly increased the level of DNA damage in all studied cell types (bone marrow cells TDNA% 18.2 ± 5.7 vs 4.6 ± 1.6 in Control, p < 0.0001; liver TDNA% 13.1 ± 2.4 vs 4.5 ± 1.6 in Control, p < 0.001; blood cells TDNA% 14.1 ± 4.4 vs 2.8 ± 0.6 in Control, p < 0.0001). BP-C2 at 60 mg/kg decreased DN-induced DNA damage in bone marrow, liver and blood cells by 41 (TDNA% 10.8 ± 2.4, p < 0.01 vs DN, 300 mg/kg), 34 (TDNA% 8.7 ± 1.2, p = 0.122 vs DN, 300 mg/kg) and 58% (TDNA% 5.9 ± 1.4, p < 0.001 vs DN, 300 mg/kg), respectively. At 80 mg/kg the DNA damage decreased by 49 (TDNA% 9.3 ± 3.7, p < 0.0001 vs DN, 300 mg/kg), 61 (TDNA% 5.1 ± 1.8, p < 0.001 vs DN, 300 mg/kg) and 50% (TDNA% 7.1 ± 3.8, p < 0.01 vs DN, 300 mg/kg) for bone marrow, liver and blood cells, respectively. While the higher dose of BP-C2 (120 mg/kg) was associated with lower antigenotoxic activity, the DNA damage decreased in this group by 32 (TDNA% 12.4 ± 3.9, p < 0.05 vs DN, 300 mg/kg), 27 (TDNA% 9.6 ± 1.3, p = 0.316 vs DN, 300 mg/kg) and 26% (TDNA% 10.4 ± 1.7, p = 0.278 vs DN, 300 mg/kg) in bone marrow, liver and blood cells, respectively.

We additionally studied antigenotoxic activity of BP-C2 using CPA as a DNA-damaging agent (Fig. 2, Table 3). CPA induces damage to the DNA molecule by alkylating guanine N-7 positions and forming cross-links between and within DNA strands. DNA crosslinks decrease its migratory capacity in the gel during electrophoresis. Therefore, in the present study, we used a modified method, in which the cells of control animals, animals treated with CPA and CPA with the BP-C2 were treated with methyl methanesulfonate after lysis in the gel to induce multiple
Fig. 2. The effect of BP-C2 on genotoxic activity of CPA in murine cells in vivo. All cells in the slides were processed with methyl methanesulfonate. CPA – cyclophosphamide. Two-way ANOVA with Tukey’s multiple comparison test. *, **** - p < 0.05, p < 0.0001 vs control, **, ###, #### - p < 0.01, p < 0.001, p < 0.0001 vs CPA, 40 mg/kg.

Table 3
The effect of BP-C2 on genotoxic activity of CPA in murine tissues in vivo (RTI, %).

| Tissue          | Control | CPA, 40 mg/kg | CPA, 40 mg/kg + BP-C2, 60 mg/kg | CPA, 40 mg/kg + BP-C2, 80 mg/kg | CPA, 40 mg/kg + BP-C2, 120 mg/kg |
|-----------------|---------|---------------|---------------------------------|---------------------------------|----------------------------------|
| Bone marrow     | 0 ± 0   | 46.1 ± 13.2***| 16.9 ± 11.6**                   | 46.1 ± 14.1****                 | 60.4 ± 12.1****                  |
| Liver           | 0 ± 0   | 33.2 ± 17.9***| 12.6 ± 13.9                     | 42.0 ± 13.9***                  | 55.9 ± 10.1****                  |
| Blood cells     | 0 ± 0   | 48.1 ± 17.4***| 15.3 ± 15.7***                  | 42.9 ± 14.5****                 | 57.1 ± 10.3****                  |

Note that all cells in the slides were processed with methyl methanesulfonate. CPA – cyclophosphamide. ***, **** - p < 0.001, p < 0.0001 vs control, #, ##, ### - p < 0.05, p < 0.01, p < 0.001 vs CPA, 40 mg/kg. Data presented as mean with standard deviation.

DNA single-strand breaks [40]. DNA crosslinks induced with CPA decrease migratory capacity of the DNA as compared to the DNA from cells of the negative control animals (Fig. 2).

The data on relative reduction of DNA migration in the gel are presented in Table 3. CPA at 40 mg/kg induced DNA damage in bone marrow, liver and blood cells with RTI of 46%, 33% and 48%, respectively. BP-C2 at 60 mg/kg co-administered with CPA reduced RTI to 17%, 13% and 15% for bone marrow, liver and blood cells, respectively. When BP-C2 was administered at 80 mg/kg, significant effect on the damaging activity of CPA was not observed. A significant increase of the genotoxic effect of CPA co-administered with BP-C2 at 120 mg/kg was observed in liver cells but not in bone marrow and blood cells.

3.2. Assessment of antimutagenic activity of BP-C2 in murine bone marrow cells in vivo

Single injection of CPA at 20 mg/kg to mice induced significant chromosomal aberrations in bone marrow cells (Table 4). BP-C2, co-administered with CPA at 60, 80 and 120 mg/kg significantly decreased mutagenic activity of the latter by 33%, 25% and 24%, respectively. This was marked by a decreased proportion of cells with multiple chromosome abnormalities, although the proportion of cells with single fragments was slightly higher in all three BP-C2 groups (17.2, 16.2, 15.8 for 60, 80, and 120 mg/kg, respectively), compared to the CPA group (13.8).

Single DN injection induced a significant mutagenic effect. BP-C2 co-administered with DN at 60, 80 and 120 mg/kg reduced mutagenic effect of DN by 25%, 49% and 49% (Table 4).

Thus, BP-C2, administered as a single dose, decreased the clastogenic effects of DN and CPA.

4. Discussion

We have studied the effect of 60–120 mg/kg BP-C2 on the DNA-
damaging and clastogenic effects of DN and CPA. 60 mg/kg BP-C2 demonstrated the most pronounced genoprotective effect. A decreased DN- and CPA-induced DNA damage was observed in bone marrow, liver and blood cells. At 80 mg/kg and 120 mg/kg BP-C2 produced less significant reduction of the DN-induced DNA damage and no modification of the CPA-induced DNA damage was observed when BP-C2 was co-administered with these agents at 120 mg/kg. When co-administered with 120 mg/kg BP-C2 CPA induced a higher genotoxic effect in liver cells.

80 mg/kg was established as the optimal radio-protective dose of BP-C2 in a total body irradiation model in mice [3]. Higher doses of BP-C2 were not associated with a higher radioprotective effect, which is consistent with the results of the present study where better genoprotective activity was observed with the lower doses of BP-C2. The assessment of the genotoxicity of BP-C2 is important as this agent is developed as a medical countermeasure for treatment of damage resulting from accidental or deliberate exposure to radiation. Evaluation of genotoxicity is also important in the context of assessment of risks of mutagenesis and carcinogenesis for subjects with prolonged exposure to genotoxic agents.

Present study demonstrated significant antigenotoxic activity of BP-C2 against prooxidant and alkylating and DNA-crosslinking genotoxincants. The genoprotective activity of BP-C2 established in our experiments can apparently be attributed to its polyphenolic backbone (BP-Cx-1), which contains many biologically active compounds (i.e., flavonoids, sapogenins, phenanthrenes) [17]. The biological activity of the BP-C2 observed in vivo is considered to be associated with the ability of its organic core to bind to glucocorticoid receptor and serotonin receptor 5-HT1 [16]. As the exposure of test subjects to genotoxicants was relatively short in our experiments (several hours), anti-oxidant and not the anti-inflammatory activity was apparently the leading mechanism of genotoxicogenicity of BP-C2. This is further supported by the fact that the observed antigenotoxic effect of BP-C2 was higher in DN (prooxidant) than in CPA (alkylating and crosslinking agent) treated animals. Anti-inflammatory mechanism of action may be more relevant in cases of prolonged exposure to BP-C2 as a radioprotector/radiomitigator [3]. Previously, another composition, BP-C3, containing the same polyphenolic ligand BP-Cx-1 mitigated CPA-induced hematological toxicity in mice with benzo[a]pyrene-induced sarcomas [35]. Other polyphenols have been shown to reduce the toxicity of CPA. Thus, combination of curcumin and piperine exhibited profound cardioprotection against CPA-induced cardiotoxicity in rats [6]. For natural flavanone glycoside naringin inhibitory effect on CPA-induced toxicity was demonstrated and it was related to modulation of oxidative stress, inflammation, apoptosis, autophagy, and DNA damage in male rats [4].

The antimutagenic effect of BP-C2 can be mediated by various mechanisms. The adsorption of DNA-damaging agents by lignin derivatives has been reported [27,29] but direct interaction of BP-C2 with DNA or CPA appears very unlikely because BP-C2 and these agents were administered to mice via different routes (oral vs. intraperitoneal). Prevention of mutagen-produced changes can be also related to antioxidant properties of BP-C2 as the cytogenetic effect of CPA is also mediated by lipid peroxidation products [22] or could be realized via inhibition of P450 cytochromes playing a role in the metabolism of CPA.

Genoprotective effect of BP-C2 against CPA genotoxicity was observed at 60 mg/kg dose in all tissues tested but the protective activity was not significant at 80 mg/kg. Moreover, at dose 120 mg/kg BP-C2 potentiated CPA-induced DNA damage in liver cells. The reasons for this multidirectional effect are not clear. CPA has complex mechanism of DNA damage: strand breaks formation due to alkylation of bases, interstrand and intrastrand DNA-crosslinking, as well as DNA-protein cross-linking. Used modification of comet assay detects genotoxicity of CPA as relative reduction in DNA migration (RTI), which is the resultant of crosslinks and strand breaks. Theoretically, the decrease mainly in the level of DNA alkylation under the influence of BP-C2 at higher doses will lead to a decrease in the level of DNA breaks and respectively an increase of RTI.

Increased level of chromosomal aberrations significantly raises the risk of developing cancer [19]. Antimutagenic activity of the polyphenolic core of BP-C2 may explain the anticarcinogenic properties earlier reported for the related BP-C3 composition [2]. Antimutagenic effect demonstrated for BP-Cx-1 may be common to natural polyphenolic substances. The chemically related compound sodium humate in a concentration range of 50–1000 mg/l reduced clastogenic effects induced with DN (20 mg/l) [42]. Bark dry extract co-administered (single combined administration, 5-day pretreatment, and 5-day combined administration) with mutagens at 50, 150, and 450 mg/kg significantly decreased their cytogenetic effects [48].

Thus, in this study we established that BP-C2 exhibits antigenotoxic activity in the murine bone marrow, liver and blood cells in vivo, reducing the DNA-damaging effects of the prooxidant and alkylating genotoxic agents, DN and CPA. BP-C2 exerts the highest protective activity in relation to the genotoxic effects of DN and CPA when administered at 60 mg/kg. BP-C2 has a pronounced antimutagenic effect against DN and CPA in mouse bone marrow cells in the dose range of 60–120 mg/kg.

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CRediT authorship contribution statement

Aly K. Zhantaev: Methodology, Validation, Writing – review & editing. Sergey E. Pigarev: Writing – original draft, Conceptualization. Elena I. Fedoros: Project administration, Conceptualization. Andrey V. Panchenko: Data curation, Writing – original draft, Writing – review & editing. Elena A. Anisina: Investigation, Data curation. Zlata V. Chayka: Investigation, Data curation. Andrey D. Durnev: Resources, Software. Vladimir N. Anisimov: Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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