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GENERAL GENETICS

Antigenotoxic Activity of Biologically Active Substances from *Inula britannica* and *Limonium gmelini*

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Abstract—The antigenotoxic and antioxidant activities of biologically active substances of extracts from *Inula britannica* L. and *Limonium gmelinii* (Willd.) Kuntze in *E. coli* strains MG1655 (pColD-*lux*), MG1655 (pSoxS-*lux*), and MG1655 (pKatG-*lux*) were studied by the bioluminescent test. Plant extracts from *I. britannica* and *L. gmelinii* in all used concentrations (0.5, 5.0, 50.0, and 500.0 μg/mL) had no genotoxic or oxidant activity. The extracts statistically significantly reduced the bioluminescence intensity of the pColD-*lux*, pKatG-*lux*, and pSoxS-*lux* sensors ($p < 0.05$) induced by 4-NQO and dioxidine, hydrogen peroxide, and paraquat, respectively. The activity of the extracts depended on their concentration; the greatest antigenotoxic and antioxidant effects were detected at a concentration of 500.0 μg/mL.

Keywords: lux biosensors, biologically active substances, antigenoxicant, oxidative stress, antioxidant
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INTRODUCTION

Chemical environmental pollutants are known to be able to exert genotoxic action on living organisms resulting in mutagenesis, carcinogenesis, and other specific toxic effects. According to the International Agency for Research on Cancer (IARC), genotoxicity involves direct and indirect effects on DNA [1]. This means that genotoxicity not only results from the direct interaction of a chemical substance with DNA molecule but also occurs owing to some indirect exposures on DNA molecule. They include activation of intracellular free radical generation and inhibition of activity of reparation enzymatic systems [2, 3]. Therefore, it is accepted in genetic toxicology to detect both mutagenic effects and a wider range of genetic effects by assessing induction of the SOS response in bacterial cells, repair synthesis, and DNA breaks in mammal cells [1].

Avoidance of human contacts with genotoxic environmental factors is almost impossible. Therefore, a high priority is the search for protectors, i.e., antigenotoxicants against the action of these factors. These protective substances also include antimutagens when a chemical is tested for its ability to induce mutations in test systems that detect inherited genetic variations. Thus, antigenotoxicity and antimutagenicity are test-specific terms similar to genotoxicity and mutagenicity. Antigenotoxic properties of many natural biologically active substances (BASs), which include vitamins, plant flavonoids, phytohormones, polypeptides, amino acids, and others, have been revealed. Most of them are antioxidants and can enhance the resistance of an organism to the genotoxic action of a wide range of pollutants. The study of medicinal plants as prospect sources of BASs possessing antigenotoxicity has intensified considerably in recent years owing to the low toxicity and low allergenicity of natural BASs, their complex influence on the organism, and the possibility of long-term use without adverse effects [4–6]. Antigenotoxicity of natural substances is closely associated with their antioxidant activity. Moreover, they can inhibit certain biochemical processes and exert antimetabolic properties. Many BASs exert an antigenotoxic action through several mechanisms to provide protection of genetic structures against diverse mutagens. Most of the protective agents either react with a mutagen or the generated free radicals and reactive oxygen species or are involved in inactivation of cytochrome P450-mediated metabolism or inactivation of reactive metabolites. It is noted that the ability of biologically active substances to influence a genotoxic factor through several different mechanisms at once raises substantially the effectiveness of an antigenotoxicant

[7]. Hence, it is important to search for natural BASs for management of genotoxic effects caused by the widespread use of xenobiotics in production activity and use of these BASs as prophylactic means to provide protection of genetic structures against mutagen exposure. The most notable of these substances are vitamins, amino acids, phenols, and polyphenols present in vegetables, fruits, and herbs at various concentrations. In this regard, the British yellowhead (*Inula britannica*) and Siberian statice (*Limonium gmelinii*) are candidate plants for production of high-value phytopreparations. Many plants of the family Compositae possess a range of medicinal properties. Phytopreparations from plants of the genus *Inula* have anti-inflammatory, antibacterial, broncholytic, antiallergic, secretolytic, cholagogic, expectorant, wound healing, and diuretic properties [8]. The chemical composition of plants of the genus *Limonium* of the family Plumbaginaceae includes phenolic and polyphenolic compounds, amino acids, and vitamins known to suppress free radical processes.

The aim of this paper is to study the antigenotoxic activity of BASs extracted from plants of wild flora of Kazakhstan of the genus *Inula* of the family Compositae and the genus *Limonium* of the family Plumbaginaceae using *lux* biosensors.

MATERIALS AND METHODS

The antigenotoxic activity was tested using aqueous solutions of extracts from the underground and aboveground parts of the British yellowhead (*Inula britannica* L., family Compositae) and Siberian statice plants (*Limonium gmelinii* (Willd.) Kuntze, family Plumbaginaceae) at concentrations of 0.5, 5.0, 50.0, and 500.0 μg/mL.

BASs were extracted from *I. britannica* and *L. gmelinii* plants using 70% aqueous ethanol. The qualitative and quantitative composition of BASs of the plants under study was determined previously. Saponin, tannins, and polysaccharides dominated in the extracts of *I. britannica*, while tannins, flavonoids, polysaccharides, and carotenoids dominated in *L. gmelinii* extracts [9, 10].

Distilled water was used as a negative control. The genotoxicants (positive control) were as follows: 1,4-dioxido-2,3-quinoxaline dimethanol (dioxidine, $C_{10}H_{10}N_2O_4$) and 4-nitroquinoline-1-oxide (4-NQO, $C_9H_6N_2O_3$); the oxidant substances (positive control) were paraquat ($C_{12}H_{14}Cl_2N_2$) and hydrogen peroxide (H_2O_2).

The study was performed using genetically modified strains of *E. coli* which contain plasmids bearing the *lux*CDABE operon of a marine phytobacteria *Photorhabdus luminescens* under the control of corresponding promotors—katG, soxS, colD: *E. coli* MG1655 (pSoxS-*lux*), *E. coli* MG1655 (pKatG-*lux*), *E. coli* MG1655 (pColD-*lux*). This operon is responsible for luciferase function and provides bioluminescence, which was used as the reporter function in this test. The strains were kindly provided by G.B. Zavilgelskii and A.V. Manukhov (State Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow). Genotypes of the strains, constructs of recombinant plasmids, and the threshold biosensor sensitivity of *E. coli* MG1655 (pSoxS-*lux*) and *E. coli* MG1655 (pKatG-*lux*) to the action of paraquat and hydrogen peroxide are presented in studies in [11–13].

The PcolD promotor was activated using 4-NQO at a concentration of 75.0 μg/mL and dioxidine at a concentration of 0.01 μg/mL since these concentrations were established to be optimal for induction of ColD operons in biosensor strains. The PkatG promotor was activated using hydrogen peroxide (Ferrain) at a concentration of 0.005 μg/mL. The PsoxS promotor was activated using paraquat (1,1'-dimethyl-4,4'-bipyridilium dichloride, Sigma) at a concentration of 5.0 μg/mL.

Experiments with a bioluminescent test to reveal the antigenotoxic potential of plant extracts under study included the following variants: control group (distilled water); positive control (dioxidine, 0.01 μg/mL; 4-NQO, 75.0 μg/mL); group with an addition of the plant extracts; group with a combined addition of extracts and genotoxicants.

Experiments with a bioluminescent test to study the oxidant and antioxidant potential of BASs included the following variants: control group (distilled water); positive control group (paraquat, 5.0 μg/mL; hydrogen peroxide, 0.005 μg/mL); group with an addition of the plant extracts; group with a combined addition of the plant extracts and oxidants.

Cell cultures of *E. coli* were grown in Luria-Bertani (LB) complete medium [14]. An antibiotic ampicillin was added to both liquid and solid media (100.0 μg/mL). Bacteria were cultured in liquid medium at 37°C to an early or middle log phase. The night culture was diluted with fresh medium to a density of 0.01–0.1 McFarland units (a concentration of 3×10^7–3×10^6 cells/mL). Measurements were performed using a DEN-1B densitometer (Biosan). The suspension was grown for 2 h at 37°C at 200 rpm to an early log phase. Aliquots of this culture (180–190 μL each) were transferred to sterile wells (in well plate strips) and 10 μL of tested BASs and/or 10 μL of an oxidative stress inductor (except for control wells) was added according to the experimental variant; 10.0 μL of distilled water was added to control wells.

For genotoxic and oxidant activity evaluation, 10.0 μL of BASs or 10.0 μL oxidative stress inductors was added separately in each culture aliquot. For evaluating antigenotoxic and antioxidant potential, 10.0 μL of BASs and 10.0 μL oxidative stress inductors were added together in each culture aliquot.

After treatment, a well plate with samples was placed into an Infinite M1000 microplate reader

Table 1. Influence of extracts (μg/mL) from the aboveground and underground parts of the British yellowhead (*Inula britannica*) on luminescence* of bacteria of the *E. coli* MG1655 (pColD-*lux*) strain induced by 4-NQO and dioxidine

Variant of the experiment	Aboveground part					Underground part				
	0	0.5	5.0	50.0	500.0	0	0.5	5.0	50.0	500.0
Extract (control)	1414.63 ± 14.37	978.56 ± 72.54	897.47 ± 67.65	797.92 ± 42.18	544.64 ± 27.26	1990.34 ± 65.03	1931.11 ± 85.08	1324.17 ± 40.82	1207.33 ± 27.42	790.92 ± 37.17
4-NQO, 75.0 μg/mL + extract	3845.89 ± 114.09	4029.86 ± 220.28	3347.94 ± 47.63	3043.14 ± 73.27	891.08 ± 14.13	3063.17 ± 89.40	3158.83 ± 112.04	2658.32 ± 137.21	2383.85 ± 262.92	1456.54 ± 385.06
Dioxidine, 0.01 μg/mL + extract	4795.14 ± 258.62	3809.17 ± 296.62	3664.19 ± 202.03	3999.08 ± 232.70	1292.89 ± 70.39	4814.1 ± 117.18	4387.42 ± 151.77	3771.83 ± 172.72	4593.19 ± 177.93	2015.83 ± 174.08

* In relative light units—RLU.

(Tecan, Austria) and incubated at 30°C. The bioluminescence intensity was measured every 10 min and expressed in relative light units (RLU).

The significance of luminescence induction was judged from statistically significant elevation of L_e over L_c estimated using a *t*-test, where L_e is the intensity of luminescence in the experimental sample (relative units) and L_c is the intensity of luminescence in the control sample (relative units). The index of the antigenotoxic potential, or the protector activity (A, %), was estimated using the formula $A = \left(1 - \frac{L_a}{L_p}\right) \times 100$, where L_a is the luminescence intensity with a toxicant and extract, L_p is the luminescence intensity with a toxicant without extract, and 100 is the coefficient of conversion to percent. The antigenotoxic effect was considered moderate at a 25–40% inhibition of the induced luminescence and strong at more than 40%; the effect was considered weak at an inhibition of the induced luminescence of less than 25% and the result was not considered positive.

All the experiments were performed in four independent replicates. The mean value of A during the whole period of measurements was used as a characteristic of protector activity of the extract concentration.

RESULTS

Study of Antigenotoxic and Antioxidant Activities of BASs from Inula britannica

A bioluminescent test (*lux* biosensors) was used to study the abilities of BAS extracts of the British yellowhead to protect the *E. coli* MG1655 (pColD-*lux*) strain against DNA damage under the action of dioxidine and 4-NQO, inactivate superoxide anion under the action of paraquat in the *E. coli* MG1655 (pSoxS-*lux*) biosensor strain, and inactivate hydroperoxides and organic peroxides under action of hydrogen peroxide in the *E. coli* MG1655 (pKatG-*lux*) biosensor strain.

The results showed that BAS extracts from the underground and aboveground parts of the British yellowhead exert antigenotoxic and antioxidant activities; however, the degree of inhibition of the negative effect depended on the BAS concentration (Tables 1, 2; Fig. 1).

Extracts of BASs from the underground part of the British yellowhead exerted a weak antigenotoxic effect at concentrations of 0.5, 5.0, 50.0 μg/mL and a strong antigenotoxic effect at 500.0 μg/mL against the action of dioxidine and 4-NQO. The antigenotoxic activity of BASs from the underground part of the British yellowhead at a concentration of 500.0 μg/mL was 51.53 ± 14.13% against the action of 4-NQO and it was 57.81 ± 4.60% against the action of dioxidine (Fig. 1). BAS extracts from the underground part of the British yellowhead exerted a weak antioxidant effect at concentrations of 0.5 and 5.0 μg/mL and a moderate effect at a concentration of 50.0 μg/mL against the action of paraquat. Extracts at concentrations of 5.0 and 50.0 μg/mL exerted a weak antioxidant activity against the action of hydrogen peroxide. BASs at the highest concentration, 500.0 μg/mL, exerted a strong antioxidant activity against the action of both oxidants—hydrogen peroxide and paraquat. The antioxidant activity of BASs from the underground part of the British yellowhead at a concentration of 500.0 μg/mL was 44.87 ± 0.87% against the action of hydrogen peroxide and 55.15 ± 0.99% against the action of paraquat (Fig. 1).

BAS extracts from the aboveground part at concentrations of 0.5 and 50.0 μg/mL exerted a weak antigenotoxic effect and at a concentration of 5.0 μg/mL a moderate antigenotoxic effect against the action of dioxidine.

Table 2. Influence of extracts (μg/mL) from the aboveground and underground parts of the British yellowhead (*Inula britannica*) on luminescence* of bacteria of the *E. coli* MG1655 (pKatG-*lux*) strain induced by hydrogen peroxide and bacteria of the *E. coli* MG1655 (pSoxS-*lux*) strain induced by paraquat

Variant of the experiment		Aboveground part					Underground part				
		0	0.5	5.0	50.0	500.0	0	0.5	5.0	50.0	500.0
Extract (control)	*E. coli* MG1655 (pKatG-*lux)* strain	3775.29 ± 48.54	3386.88 ± 627.42	4296.81 ± 605.72	22127.94 ± 361.82	11744.38 ± 275.31	3632.75 ± 73.56	3379.33 ± 552.19	3385.50 ± 694.29	3970.58 ± 300.71	12328.67 ± 310.76
Hydrogen peroxide, 0.005 μg/mL + extract		34223.34 ± 591.04	35736.19 ± 1003.21	34094.31 ± 772.18	31697.38 ± 440.94	18212.56 ± 187.71	35386.04 ± 185.63	41674.33 ± 4144.05	32996.75 ± 852.21	31114.33 ± 1852.41	19504.58 ± 215.60
Extract (control)	*E. coli* MG1655 (pSoxS-*lux)* strain	7113.68 ± 121.63	5645.17 ± 122.83	5424.04 ± 87.43	4488.25 ± 60.64	3353.96 ± 85.53	7773.99 ± 91.24	5924.42 ± 51.75	6023.38 ± 136.42	5217.71 ± 91.91	3734.46 ± 109.94
Paraquat, 5.0 μg/mL + extract		8843.50 ± 143.94	8352.96 ± 169.50	8051.08± 158.77	5751.96 ± 61.22	4060.54 ± 77.56	10010.73 ± 61.08	9992.58 ± 209.51	9659.63 ± 105.89	7465.13 ± 118.00	4490.17 ± 103.77

* In relative light units—RLU.

A weak antigenotoxic activity was also revealed against the action of 4-NQO, but at concentrations of 5.0 and 50.0 μg/mL. BASs at a concentration of 500.0 μg/mL exerted a strong antigenotoxic activity against the action of both dioxidine and 4-NQO. Antigenotoxic activity of BASs from the aboveground part of the British yellowhead at a concentration of 500.0 μg/mL was 76.75 ± 0.93% against the action of 4-NQO and 74.14 ± 1.28% against the action of dioxidine (Fig. 1). BAS extracts from the aboveground part exerted a weak antioxidant activity at concentrations of 5.0 and 50.0 μg/mL and a strong activity at 500.0 μg/mL against the action of hydrogen peroxide. Antioxidant activity of extracts from the aboveground part also depended on the concentration used. Thus, BAS exerted a weak activity at concentrations of 0.5 and 5.0 μg/mL, a moderate activity at a concentration of 50.0 μg/mL, and a strong activity at a concentration of 500.0 μg/mL against the action of paraquat. Antioxidant activity of BASs from the aboveground part of the British yellowhead at a concentration of 500.0 μg/mL was 46.76 ± 0.56% against the action of hydrogen peroxide and 60.49 ± 6.02% against the action of paraquat (Fig. 1). A comparative analysis of the protective activity in BAS extracts from the underground and aboveground parts of the British yellowhead showed

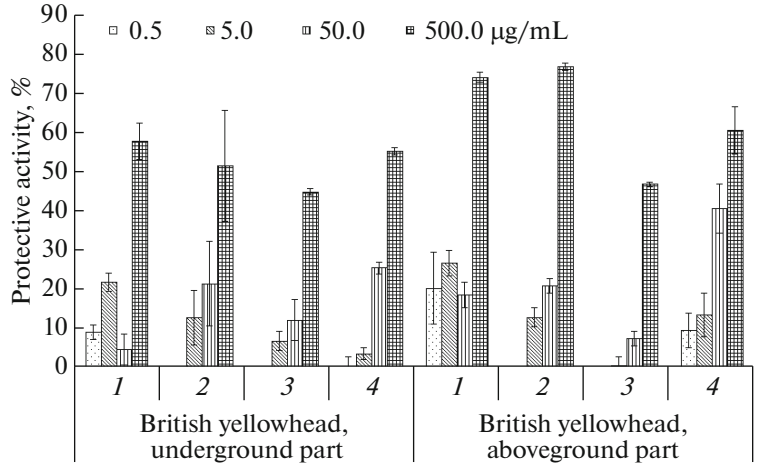

Fig. 1. Protective activity of BASs from the underground and aboveground parts of the British yellowhead against the action of dioxidine (*1*), 4-NQO (*2*), hydrogen peroxide (*3*), and paraquat (*4*).

Table 3. Influence of extracts (μg/mL) from the aboveground and underground parts of Siberian statice (*Limonium gmelinii*) on luminescence* of bacteria of the *E. coli* MG1655 (pColD-*lux*) strain induced by 4-NQO and dioxidine

Variant of the experiment	Aboveground part					Underground part				
	0	0.5	5.0	50.0	500.0	0	0.5	5.0	50.0	500.0
Extract (control)	1062.84 ± 19.12	1228.28 ± 82.36	1250.86 ± 99.93	1142.22 ± 95.13	678.47 ± 62.96	1379.99 ± 46.03	967.83 ± 19.67	915.67 ± 25.33	817.58 ± 16.57	424.42 ± 13.67
4-NQO, 75.0 μg/mL + extract	10933.83 ± 337.23	7903.92 ± 550.084	8223.75 ± 432.794	7785.72 ± 198.087	1253.22 ± 18.18	2702.89 ± 46.20	2378.11 ± 35.91	2237.17 ± 32.48	2104.86 ± 81.27	531.14 ± 13.66
Dioxidine, 0.01 μg/mL + extract	7691.86 ± 221.22	4216.61 ± 159.37	5265.97 ± 237.40	9954.39 ± 300.68	1990.69 ± 107.39	3579.667 ± 122.44	2711.53 ± 65.89	2683.39 ± 42.83	3015.17 ± 304.77	838.78 ± 14.81

* In relative light units—RLU.

that the antigenotoxic activity of BAS from the aboveground part against the action of 4-NQO and dioxidine was higher than that from the underground part. Antioxidant activity of BASs from the aboveground part against the action of hydrogen peroxide and paraquat was also higher than that from the underground part. However, a statistically significant difference was not revealed except for the protector potential of BASs from the aboveground part at concentrations of 50.0 and 500.0 μg/mL compared to the underground part against the action of dioxidine ($p < 0.05$).

Study of Antigenotoxic and Antioxidant Activities of BAS from Limonium gmelinii

A similar study of the antigenotoxic and antioxidant potential of BASs in plant extracts of Siberian statice was performed. It was established that extracts from the underground and aboveground parts of Siberian statice exert an antigenotoxic and antioxidant activity, though the degree of inhibition of the damaging effect of the used genotoxic and oxidant substances depended on the BAS concentration (Tables 3, 4). However, the extract from the aboveground part at a concentration of 50.0 μg/mL led to a 10-fold increase in the response of the pKatG-*lux* sensor to oxidative stress, indicating the presence of prooxidant properties in BASs from Siberian statice. BASs at concentrations of 0.5, 5.0, and 50.0 μg/mL from the underground part of these plants exerted a weak (against the action of 4-NQO) and moderate (against the action of dioxidine) antigenotoxic effect and a weak (against the action of paraquat) and moderate (against the action of hydrogen peroxide) antioxidant effect (Fig. 2). The greatest antigenotoxic and antioxidant effects were observed with BAS solutions at a concentration of 500.00 μg/mL. Thus, antigenotoxic activity of BASs from the underground part of the plant at a concentration of 500.00 μg/mL was 76.52 ± 0.52% against the action of dioxidine and 80.32 ± 0.76% against the action of 4-NQO. The antioxidant activity of BASs from the underground part of Siberian statice at a concentration of 500.0 μg/mL was 61.15 ± 1.97% against the action of hydrogen peroxide and 67.91 ± 0.33% against the action of paraquat (Fig. 2).

BAS extracts at concentrations of 0.5, 5.0, and 50.0 μg/mL from the aboveground part of Siberian statice exerted a moderate antigenotoxic effect against the action of 4-NQO. The results that reflect the ability of BAS extracts from the aboveground part of Siberian statice to protect DNA of the *E. coli* MG1655 (pColD-*lux*) strain against the genotoxic action of dioxidine were controversial. At a concentration of 50.0 μg/mL, the plant extracts increased the level of DNA damage caused by dioxidine; they exerted a moderate antigenotoxic activity at a concentration of 5.0 μg/mL and a strong activity at concentrations of 0.5 and 500.0 μg/mL. BAS extracts from the aboveground part of Siberian statice at concentrations of 0.5, 5.0, and 50.0 μg/mL exerted a weak antioxidant activity against the action of hydrogen peroxide; they exerted a weak antioxidant activity at concentrations of 0.5 and 5.0 μg/mL and moderate activity at a concentration of 50.0 μg/mL against the action of paraquat. Plant extracts at a concentration of 500.00 μg/mL exerted a strong antigenotoxic and antioxidant activity against the action of all the used genotoxicants and oxidants. Thus, the protector activity of BASs from the aboveground part of Siberian statice against the action of 4-NQO was 88.51 ± 0.36% and it was 74.06 ± 1.58% against the action of dioxidine. The antioxidant activ-

Table 4. Influence of extracts (μg/mL) from the aboveground and underground parts of Siberian statice (*Limonium gmelinii*) on luminescence* of bacteria of the *E. coli* MG1655 (pKatG-*lux*) strain induced by hydrogen peroxide and bacteria of the *E. coli* MG1655 (pSoxS-*lux*) strain induced by paraquat

Variant of the experiment		Extract of Siberian statice, μg/mL									
		aboveground part					underground part				
		0	0.5	5.0	50.0	500.0	0	0.5	5.0	50.0	500.0
Extract (control)	*E. coli* MG1655 (pKatG-*lux*) strain	3183.03 ± 69.02	3655.56 ± 194.77	23291.50 ± 859.65	32028.06 ± 331.06	13922.38 ± 233.37	3799.23 ± 47.97	3581.94 ± 559.28	10292.13 ± 1103.88	25903.56 ± 3595.22	12747.13 ± 488.63
Hydrogen peroxide, 0.005 μg/mL + extract		37089.33 ± 1039.23	30905.75 ± 685.87	32371.42 ± 2776.918	28631.83 ± 1286.725	15146.17 ± 861.48	51378.19 ± 1008.27	35142.06 ± 1515.97	37404.44 ± 4247.03	38165.38 ± 1632.06	19912.31 ± 722.67
Extract (control)	*E. coli* MG1655 (pSoxS-*lux*) strain	7189.47 ± 153.74	5274.75 ± 61.29	5697.17 ± 156.03	4474.54 ± 132.00	3073.83 ± 225.04	8694.18 ± 186.42	7140.38 ± 291.53	7057.88 ± ±83.71	6543.92 ± 121.46	3157.75 ± 98.53
Paraquat, 5.0 μg/mL + extract		11042.65 ± 181.17	9911.21 ± 123.27	9936.50 ± 198.62	7090.67 ± 105.49	3947.38 ± 142.91	12288.02 ± 332.83	11919.13 ± 65.94	11725.38 ± 249.41	11179.79 ± 959.25	3941.67 ± 94.44

* In relative light units—RLU.

ity of BASs from the underground part of Siberian statice was 59.04% against the action of hydrogen peroxide and 64.25 ± 1.19% against the action of paraquat (Fig. 2).

A comparative analysis of the protector activity of BAS extracts from the underground and aboveground parts of the plants showed that the antigenotoxic activity against 4-NQO was higher in extracts from the aboveground part compared to extracts from the underground part. In terms of dioxidine, an opposite pattern was observed—the antigenotoxic activity of extracts from the underground part was higher than from the aboveground part. The antioxidant activity of BASs from the underground part of Siberian statice against the action of hydrogen peroxide and paraquat was higher than that from the aboveground part. However, no statistically significant differences were found.

DISCUSSION

The toxic and mutagenic potential of different chemical substances is assessed using microbiological test systems as express methods. At present, along with the Ames test, bacterial *lux* biosensors are gaining a widespread use. They represent a complex of sensor bioluminescent strains that respond to strain-specific

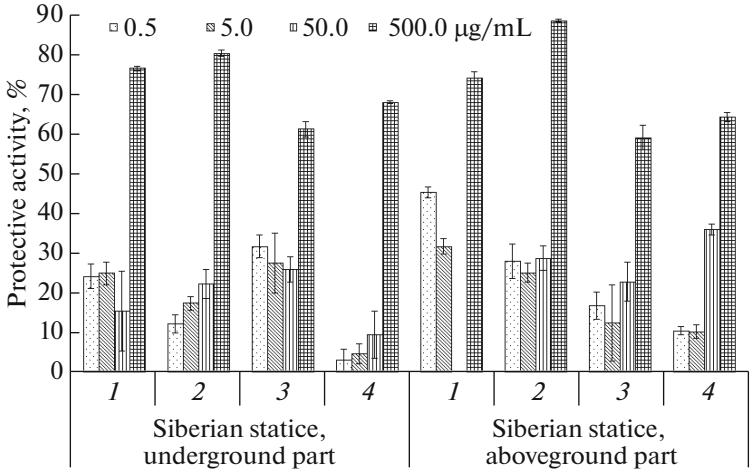

Fig. 2. Protective activity of BASs from the underground and aboveground parts of Siberian statice against the action of dioxidine (*1*), 4-NQO (*2*), hydrogen peroxide (*3*), and paraquat (*4*).

toxicants with luminescence changes and a luminometer to detect these changes.

We used a bioluminescent test to study the antigenotoxic properties of biologically active substances from *I. britannica* and *L. gmelinii* medicinal plants growing on the territory of Kazakhstan and widely used in traditional medicine.

The genotoxic and antigenotoxic activity of BASs was evaluated using the *E. coli* MG1655 (pColD-*lux*) strain, and the oxidant and antioxidant activity was evaluated using the *E. coli* MG1655 (pSoxS-*lux*) and *E. coli* MG1655 (pKatG-*lux*) strains.

A biosensor with plasmid pColD detects the presence of factors in a cell that cause DNA damage. In a hybrid plasmid pColD, the transcription of luminescence genes is placed under control of the SOS promotor of the *cda* gene (generated using the pColD-CA23 plasmid containing the *colD* gene encoding colchicine synthesis). The ColD biosensor demonstrates a high sensitivity to the genotoxic effect of such substances as cisplatin, 4-NQO, and hydrogen peroxide. The *colD* gene product colchicine is necessary to cells only under stress conditions and is released into the external medium to function as a killer. The ColD biosensor can be used for primary detection of genotoxic agents in the medium and for assessing their concentration dependence [12]. Biosensors with the PkatG and PsoxS promotors sense the presence of oxidants in the medium that form hydroperoxides and superoxide anion radical in a cell. A characteristic feature of the oxidative stress in *E. coli* is the induction of antioxidant system genes and an increase in the activity of antioxidant enzymes encoded by these genes [15]. Therefore, the promotors of these genes were used in genetic constructs that make up the basis of biosensors responding to oxidative stress. The *katG* gene determines catalase synthesis; its promotor PkatG (OxyR activator protein) specifically responds to hydrogen peroxide and organic peroxides. The PsoxS promotor (SoxR activator protein) specifically responds to superoxide anion radicals.

The PcolD promotor was activated using 4-NQO and dioxidine. 4-NQO is a strong mutagen and carcinogen and an inhibitor of cell divisions, has a high level of clastogenic activity, and is widely used as a positive control in studying DNA damaging activity of different chemical substances [16]. Dioxidine is an antimicrobial agent of a wide range of action which disrupts DNA biosynthesis in a bacterial cell leading to profound changes in the nucleotide structure as early as under the action of subinhibiting concentrations [17]. Dioxidine induces mutations in bacterial cells, chromosomal aberrations and micronuclei in cells of mouse brains, DNA breaks in cells of some organs, and dominant lethal mutations in reproductive cells in mouse males [18, 19]. Under anaerobic conditions, including in an infected organism, dioxidine (as well as others derivatives of di-N-oxy quinoxaline) activates free radical processes and induces the formation of reactive oxygen species [17]. The pKatG promotor is frequently activated using a classical oxidant hydrogen peroxide. The promotor PsoxS is activated using paraquat, which is able to generate superoxide anion radical [20].

These studies showed that the response of the *E. coli* MG1655 (pColD-*lux*) strain after addition of BASs from the underground and aboveground parts of *I. britannica* and *L. gmelinii* at concentrations of 0.5, 5.0, 50.0, and 500.0 μg/mL was statistically significantly lower than the values of this parameter under the influence of dioxidine and 4-NQO ($p < 0.001$). These results point to a lack of genotoxic activity in the extracts under study. On the basis of the responses of sensors pKatG-*lux* and pSoxS-*lux*, the plant extracts of the British yellowhead and Siberian Statice do not exert an oxidant activity at the concentrations used, except for extracts from the aboveground part of Siberian statice at a concentration of 50.0 μg/mL.

Plant extracts of the British yellowhead and Siberian statice when used in combination with toxicants decreased significantly the luminescence of pColD-*lux*, pKatG-*lux*, and pSoxS-*lux* ($p < 0.05$) induced by 4-NQO and dioxidine, hydrogen peroxide, and paraquat, respectively. The degree of inhibiting the damaging action of genotoxicants and oxidants depended on the concentration of BASs. At the concentration of 500.0 μg/mL, there were strong antigenotoxic and antioxidant effects in the test system.

It was established previously that the main groups of BASs in extracts of *L. gmelinii* are tannins, polysaccharides, flavonoids, kumarines, vitamins, carotenoids, and saponins, and the extracts of *I. britannica* are dominated by saponins, tannins, vitamins, and polysaccharides. It is known that most BASs exert protective properties [9, 10]. For example, flavonoids are known antioxidants and can scavenge all types of free radicals, in particular, superoxide and hydroxyl radicals, by chelating [21, 22]. Many flavonoids exert an antimutagenic action. For example, quercetin and lutionin decreased the formation of micronuclei and chromosomal aberrations in bone marrow cells of mice treated with food mutagens [1]. The literature contains evidence that some BASs, for example, flavonoids, vitamins C and E, and carotenoids, can exert prooxidant properties along with antioxidant effects under certain conditions [23].

De Flora [24], Bouhlel et al. [25], and Wu and Yen in [26] showed that the antioxidant activity is one of the mechanisms of antigenotoxicity.

All natural biologically active substances play an enormous role in an organism despite being products of secondary metabolism. Phytocompounds influence metabolism and detoxification of foreign substances, including carcinogens and mutagens. They have an ability to bind free radicals and reactive metabolites of foreign substances and inhibit enzymes that activate

xenobiotics and activate detoxification enzymes [27]. It is necessary to perform a comprehensive study of phytocompounds as potential protectors against toxic, genotoxic, and mutagenic action of different environmental pollutants on an organism.

The antigenotoxic activity of BAS extracts from *I. britannica* and *L. gmelinii* was shown for the first time using a bioluminescent test on the *E. coli* MG1655 pColD-*lux* strain, and the antioxidant activity was shown using the *E. coli* MG1655 pSoxS-*lux* and *E. coli* MG1655 pKatG-*lux* strains.

A bioluminescent test detects the response based on bacterial luminescence and does not require any additional manipulations, such as lysis of bacteria after incubation and assay of enzymatic activity. The direct analysis of bacterial luminescence makes it possible to simultaneously detect the response both to the concentration of the tested substance and to the length of incubation in dynamics. A bioluminescent test is characterized by a high sensitivity and rapid implementation; possible automation was shown by several authors [11–13, 28]. This makes it possible to use this test not only for screening chemical substances for oxidant and genotoxic activities but also for primary screening of phytocompounds for antioxidant and antigenotoxic activities.

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