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DETERMINATION OF THE EFFECTIVE DOSE OF "SUMAKH FRUIT EXTRACT" IN CORRECTING THE GENOTOXICITY OF CHEMICALS IN AN ARTIFICIALLY INDUCED MUTATION PROCESS

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Abstract

This study explores the antimutagenic properties of sumakh fruit extract against the mutagen 4-nitroquinoline-1-oxide (4NQO) using human peripheral blood lymphocytes. The extract was tested in doses ranging from 0.001 to 10 μg/ml. Results showed that sumakh fruit extract significantly reduced sister chromatid exchanges, a marker of DNA damage, with optimal protection observed at 0.01 μg/ml. These findings highlight the extract's potential as a protective agent against mutagenic damage.

Keywords: *Sumakh fruit extract, Antimutagenic activity, Artificial mutagenesis, Natural antimutagenic substances*

The search for correctors of mutational processes of synthetic and natural origin, capable of resisting the harmful effects of mutagenic and carcinogenic substances, has been going on for almost a century. In this regard, the search for natural substances of plant origin, as well as artificial substances, is of particular interest.

In our research, to determine the effective dose as a modifier under conditions of artificial mutation, "**sumakh fruit extract**" containing a natural substance of plant origin was tested in a wide range.

To determine the effectiveness of genome protection, the maximum dose of antimutagenic activity of sumakh fruit extract was determined under the action of mutagenic substances that differ in nature, type, mechanism of interaction with hereditary substrates, as well as by the initial damage they cause in the DNA molecule.

Part of the research was conducted on "human peripheral blood lymphocytes". 4 NQO (4-nitroquinoline-1-oxide) was used as a mutagen. In this part of the experiments, test doses of the extract were administered to the subjects before the introduction of the mutagen.

As an experimental model for testing the sumakh fruit extract, a primary culture of

peripheral blood lymphocytes from healthy donors was used. For this purpose, the composition includes (1:3) heparinized plasma (0.1 ml of the ready-made Richter heparin solution + 10 ml of donor blood), blood precipitated with gelatin (1 ml of gelatin + + 10 ml of blood), a ready-made nutrient medium (medium No. 199–3 parts + lactalbumin hydrolysate – 1 part + bovine serum – – 1 part + phytohemagglutinin (PHA), a hemagglutination reaction agent (Welcome – 0.1 ml / 10 ml of the mixture). After maintaining the mixture prepared in this way at a temperature of 370 °C for 5 hours, doses from 0.001 to 10 μg/ml of the extract were added, and after 17 hours, 4NQO (2.5*10––7M) was added to the solution. The medium of the flasks with the experimental parts was added for 2 hours. An hour later, the medium of both variants was replaced with fresh standard medium. After 24 hours of culture development, 5-bromodeoxyuridine was added to all experimental and control flasks at a final dose of 10 μg/ml and placed in a thermostat. The frequency of sister chromatid exchange was analyzed in preparations prepared by differential staining of chromatids fixed at the 72 nd hour of cultivation.

In this part of the experiments, the spectrum of types of primary damage that they cause in the DNA molecule was taken into account when selecting model mutagens. Thus, the main types of damage created by 4NQO include cyclobutane-pyrimidine dimers, mutational monoadducts, and interstrand covalent crosslinks of the DNA molecule.

In experiments conducted on human peripheral blood lymphocytes obtained from a healthy donor, the extract obtained from sumakh fruits was tested in the environment of artificial mutation of 4-nitroquinoline-1-oxide (4NQO), which behaves like UV rays, for reparation, replication., as well as the ability to create artificial mutations, cell death. In quantitative analysis of alternative colored exchange in the cell, registration of sister chromatid exchange proves the antimutagenicity of the tested extract.

In experiments on the analysis of sister chromatid exchange in primary cultures of human peripheral blood lymphocytes using 4HQO, it was found that sumakh fruit extract prevented the formation of sister chromatid exchanges at doses from 0.001 to 1.0 μg/ml. The highest efficiency was demonstrated by a dose of 0.01 μg/ml.

Table 1. *Antimutagenic activity of sumakh fruit extract in primary human peripheral blood lymphocyte tissue*

Mutagens	Experience options	Extract dose, mcg/ml	Sister chromatid exchange SCE / according to the cell	td		P		AEF
				By control	By mutagen	By control	By mutagen	
	Control	0	5.17 ± 0.71	–	–	–	–	–
	4NQO	0	16.58 ± 1.97	5.46	–	<0.001		–
4NQO	Sumakh extract + 4NQO	0.001	9.16 ± 0.98	3.29	3.37	=0.001	<0.001	0.45
		0.01	8.23 ± 0.87	2.73	3.38	<0.01	<0.001	0.50
		0.1	9.34 ± 0.99	3.42	3.28	<0.05	<0.001	0.44
		1.0	11.55 ± 1.17	4.66	2.20	<0.01	<0.05	0.30
		10	12.73 ± 1.25	5.25	1.65	<0.051	<0.05	–

Calculation method:

$$M = \frac{n \cdot 100\%}{N};\ M = \sqrt{\frac{M \cdot (100 - M)}{N}};$$

$$td = \frac{M_2 - M_1}{\sqrt{m_1^{\ 2} + m_2^{\ 2}}}$$

M – the frequency of mutations; M_2 – the frequency of mutations of the experimental variant; M_1 – the frequency of mutations of the control variant; M_1^2 – error of the control variant; M_2^2 – error of the experimental variant; AEF – $\frac{i-c}{i}$. Antimutagen effectiveness factor, i – primary (previous), c – subsequent

(determined by dividing the difference between the primary and modified mutation levels by the primary indicator), n = Sister chromatid exchange, N = all stadied cells.

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