The European Journal of Technical and Natural Sciences 2024, No 3–4
ISSN 2414-2352

PREMIER Publishing
ppublishing.org

Section 2. Biology

DOI:10.29013/EJTNS-24-3.4-7-9

DETERMINATION OF THE EFFECTIVE DOSE OF "SUMAKH FRUIT EXTRACT" IN THE PROCESS OF ARTIFICIAL MUTATION CREATED USING 4-NITROQUINOLINE-1-OXIDE

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Cite: *Guliyev M., İsrafilova S., Yildirim L., Sadiqi İ. (2024). Determination of the Effective Dose of "Sumakh Fruit Extract" in the Process of Artificial Mutation Created Using 4-Nitroquinoline-1-Oxide. European Journal of Technical and Natural Sciences 2024, No 3–4.* https://doi.org/10.29013/EJTNS-24-3.4-7-9

Abstract

The study evaluated the antimutagenic efficacy of sumakh fruit extract in artificial mutagenesis caused by 4-nitroquinoline-1-oxide (4NQO). The extract was tested on peripheral blood lymphocytes of healthy donors exposed to 4NQO after administration of various doses of the extract. The frequency of chromosomal aberrations in metaphase cells was used to assess antimutagenic activity.

The results showed that the extract reduces the frequency of chromosomal changes in doses from 0.001 to 1.0 mcg/ml, with the greatest effectiveness at 0.01 mcg/ml. Thus, sumakh fruit extract has demonstrated potential as an antimutagenic agent protecting the genome from damage.
Keywords: *Sumakh fruit extract, Antimutagenic activity, 4-nitroquinoline-1-oxide (4 NQO), Artificial mutagenesis, Natural antimutagenic substances*

Introduction

Since the beginning of the last century, the search for correctors of mutation processes of synthetic and natural origin, which have the ability to resist the harmful effects of mutagenic and carcinogenic substances, has continued. In this regard, the search for natural substances of plant origin, as well as artificial substances, is of particular interest.

In the study initiated by us, "Sumakh fruit extract", containing a natural substance of plant origin, was tested in a wide range to determine the effective dose as a modifier under conditions of artificial mutagenesis.

In order to determine the value of genome protection efficiency, the highest dose of antimutagenic activity of sumakh fruit extract under the influence of mutagens differing in their nature, type, mechanism of interaction with hereditary substrates, as well as the initial damage they cause to the DNA molecule was determined.

Some of the studies were conducted on highly developed objects – human peripheral

blood lymphocytes. 4NQO (4-nitroquinoline-1-oxide) was used as a mutagen. In this part of the experiments, the subjects were given test doses of the extract before the mutagen was administered.

The primary culture of peripheral blood lymphocytes from healthy donors, which can be obtained from humans, was used as an experimental model for testing the extract. To do this, the composition includes (1: 3) heparinized plasma (0.1 ml of ready-made Richter heparin solution + 10 ml of donor blood), gelatin-precipitated blood (1 ml of gelatin + 10 ml of blood), ready-made nutrient medium (medium No. 199–3 parts + lactalbumin hydrolysate – 1 part + bovine serum – 1 part, a mixture containing phytohemagglutinin (PHA) (Welcome – 0.1 ml / 10 ml of the mixture), then doses from 0.001 to 10 mcg / ml of the extract were prepared, and after 17 hours, 4NQO (2.5 *10–7 M) was added to the medium of vials containing experimental parts for 2 hours. An hour later, the environment of both variants was replaced with a fresh standard environment. After 24 hours of culture development, 5-bromodeoxyuridine was added to all experimental and control vials at a final dose of 10 mcg/ml and placed in a thermostat. The frequency of changes (aberrations) in the chromosome structure was analyzed by analyzing metaphase cells recorded at the 72nd hour of cultivation. When selecting model mutagens in this part of the experiments, the spectrum of types of primary damage they cause in the DNA molecule was also taken into account. According to the main types of damage caused by 4NQO, cyclobutane pyrimidine dimers, mutational monoadducts and interstrand covalent bonds of the DNA molecule are distinguished.

In experiments on human peripheral blood lymphocytes obtained from a healthy donor, sumakh fruit extract was tested in an environment of artificial mutation of 4-nitroquinoline-1-oxide (4NQO), which behaves like UV rays due to its ability to reparation, replication, as well as artificial mutation, cell death. When analyzing metaphase cells, recording the frequency of changes in the chromosome structure serves as a valuable criterion for identifying the antimutagenic nature of the extract under study.

The results of experiments conducted in studying the frequency of chromosomal aberrations in primary human peripheral blood lymphocyte tissue showed that sumakh fruit extract prevented the formation of structural changes in chromosomes in doses ranging from 0.001 to 1.0 μg/ml. On the other hand, the dose of 0.01 μg/ml showed the greatest efficiency.

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

Mutagens	Experience options	Extract dose, mcg/ml	Chromosome aberration frequency M±m	td		P		AEF
				By control	By mutagen	By control	By mutagen	
Control		0	1.83 ± 0.49	–	–	–	–	–
4 NQO	4NQO	0	12.16 ± 1.32	7.33	–	< 0.001	–	–
	Sumakh extract+ 4NQO	0.001	6.11 ± 0.73	4.61	4.01	< 0.001	< 0.001	0.50
		0.01	5.38 ± 0.59	4.61	4.68	> 0.001	< 0.001	0.56
		0.1	6.79 ± 0.78	5.39	3.51	< 0.001	< 0.001	0.44
		1.0	8.15 ± 0.84	6.51	2.55	< 0.001	< 0.05	0.33
		10	9.44 ± 0.99	6.92	1.65	< 0.001	> 0.05	–

Calculation method:

$$M = \frac{n^{*}100\%}{N};\quad M = \sqrt{\frac{M^{*}\left(100-M\right)}{N}};$$

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

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;

$AEF - \frac{i-c}{i}$. $AEF -$ 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 = chromosome aberration. N = all stadied cells.

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submitted 13.06.2024;
accepted for publication 27.06.2024;
published 07.08.2024

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