Ivanov V. S. Study of the antimutagenic effects of plant polyphenols under the action of genotoxicants. Journal of Education, Health and Sport. 2019;9(7):799-804. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.3490241
http://ojs.ukw.edu.pl/index.php/johs/article/view/7583

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 16.07.2019. Revised: 23.07.2019. Accepted: 30.07.2019.

Study of the antimutagenic effects of plant polyphenols under the action of genotoxicants

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Abstract

In the test of dominant lethal germ cell mutations in male rats, the effect of phenobarbital and fluorouracil genotoxicants and their combined administration with the biotrit-C preparation, obtained from wheat seedlings and containing plant polyphenols, was studied. For the first time, high mutagenicity of fluorouracil and pronounced antimutagenic properties of the preparation Biotrit-C were established.

Key words: biotrit C, antimutagenic effects, phenobarbital, fluorouracil, mutagens, embryos, induced mortality.

Modern studies have identified the superfamily of nuclear receptors of aromatic carbohydrates (Ah), which are of interest in connection with the tropism of some of them (chlorobiphenyls) to the tissues of the oral mucosa. Chlorbifenels can quickly accumulate in the tissues of the oral cavity and cause hypertrophic gingivitis [1].

As a xenobiotic genomotropic ligand for the Ah receptors, we have chosen the cytostatic fluorouracil used in the treatment of malignant tumors. Fluorouracil can cause ulcerative stomatitis [2]. The reference drug was phenobarbital, a compound of pyrimidine structure. In addition to the hypnotic action, it has the properties of an inducer of cytochromes P_{450} and, as a result, activates xenobitics in liver microsomes and other tissues into active toxicants [3].

The study of the genomotropic effects of fluorouracil and phenobarbital made it possible to set the second task - to evaluate the possible antimutagenic effects of biotrit-C under these conditions.

Biotrit is a preparation containing wheat germ, developed by the State Institute of Physical Chemistry of the National Academy of Medical Sciences of Ukraine together with the Odessa Biotechnology Scientific and Production Association.

The active components of biotrit are polyphenols of plant origin: flavones, flavonoids (kaempferol, tricin), isoflavones, phytosterols; complex of vitamins of group B (B_1, B_2, B_6), antioxidant vitamins (E and C), amino acids. The addition of ascorbic acid to biotrit enhances its antioxidant and adaptogenic properties.

It is known that many bioflavones exhibit antimutagenic properties [4]. Mutagenesis was investigated, taking into account the number of dominant lethal mutations in the genital cells of male rats, leading to the death of the offspring of the first generation during the period of prenatal development [5].

The aim of the work is to study the antimutagenic properties of biotrite-C under the conditions of action of fluorouracil and phenobarbital genotoxicants.

Materials and methods

The experiments were carried out on 25 male rats and 50 females contained on the standard vivarium ration of the institute “IS CHLH NAMS of Ukraine”. Five groups of males (5 individuals weighing 176 ± 8 g) for 11 weeks (all stages of spermatogenesis) were injected with the aid of a per os probe. The grouping of experience and doses of drugs are presented in table 1.

Table 1

Grouping experience and dose of drugs

Groups of animals	Doses of drugs g / kg body weight of rats
Intact (dist.water)	1,0
Phenobarbital	0,005
Phenobarbital + biotrit-C	0,005 0,05
Fluorouracil	0,025
Fluorouracil + biotrit-C	0,025 0,05

All drugs were administered per os in a volume of 0.1 ml per rat. Due to the cytotoxicity of fluorouracil, it was administered 3 times a week, phenobarbital and biotrit-C were received by the animals 5 days a week.

After 11 weeks, 2 male females weighing 242 ± 15 g were mated to each male for a period of 1 week for mating. All animal experiments were carried out according to the European Convention for the Protection of Vertebrate Animals.

On the 19th-20th day of pregnancy, the females killed by decapitation were immediately opened and the number of live and dead embryos (LE and DE) was counted. Analysis of the results and statistical processing were carried out in accordance with the guidelines for the assessment of mutagenic activity [6]. For each study group, the following indicators were taken into account: % of pregnant females (fertility), number of LE and DE per 1 pregnant female, index of post-implantation loss:

$A = \frac{DE}{DE + LE} \times 100\ \%$, where DE and LE are the number of live and dead embryos in the study group.

To analyze the differences in mean trends between experimental and control groups, we used the index of post-implantation loss A_j, calculated for each male, using the formula:

$A_j = \frac{\Sigma_{i-1}^{n_j} A_{ji}}{n_j}$, where A_{ji} is the proportion of dead implants in a female i male j; nj is the number of pregnant females in the j male.

Wilcoxon rank test was used for the analysis [7]. The following hypotheses were tested: 1) in the study group (fluorouracil), this parameter is on average larger than in the intact group; 2) in the studied group (fluorouracil + biotrit-C), this parameter is on average less than in the control group (fluorouracil).

For each group, the average value of the post-implantation loss index was calculated for all males using the formula:

$\overline{A} = \frac{\Sigma_{j=1}^{N} A_j}{N}$, where N is the number of males in this group.

In the case of obtaining significant differences between the experimental and control groups, the index of induced mortality was determined by the formula:

$I_{ind} = \frac{\overline{A}_t - \overline{A}_c}{1 - \overline{A}_c}$, where the indices t and c mark the average values of the post-implantation loss index for the experimental and control groups, respectively. The calculated induced mortality determined the degree of mutagenic activity of the drug. The drug with induced mortality from 0 to 10% was qualified as a weak mutagen (1 point), from 10 to 25% - an average mutagen (2 points), over 25% - a strong mutagen (3 points).

Research results

In table. 2 presents the results of studying the course of pregnancy in female rats mating with males and receiving phenobarbital and fluorouracil, or their combination with biotrit-C.

Table 2

Effect of phenobarbital, fluorouracil and their combined administration with biotrit-C on fertility rates, the number of live and dead embryos

Groups of animals	Amount of females	Amount of Pregnant females	Percent fertility	Number of non-pregnant females	Amount of live embryos (LE)	Amount of dead embryos (DE)	LE for 1 Pregnant female	DE for 1 Pregnant female	$\frac{DE}{LE+DE} \cdot 100\%$
Intact (dist.water)	7	7	100	0	52	0	7,43	0	0
Phenobarbital	10	6	60	4	51	0	8,50	0	0
Phenobarbital + biotrit-C	8	7	88	1	62	1	8,86	0,14	1,6
Fluorouracil	12	7	58	5	37	23	5,29	3,29	38,3
Fluorouracil + biotrit-C	13	10	77	3	72	10	7,20	1,00	12,2

In the intact group of females, the males of which received only solvent (distilled water), 100% of pregnancies were observed.

As can be seen from the table, the introduction of phenobarbital to males caused a decrease in the number of pregnancies of females to 60%. The sedative effect of phenobarbital manifested in males could contribute to a decrease in fertility - the number of non-pregnant females in the experience increased to 4. At the same time, the use of phenobarbital practically did not cause the death of embryos. The number of live embryos per 1 pregnant female was 8.50 (table 2).

The introduction of biotrit-C moderately prevented a decrease in fertility induced by phenobarbital, i.e. The use of biotrite-C caused an increase in the fertility of rats by 28% (table 2). At the same time, the number of non-pregnant females decreased. The number of live embryos increased significantly, the number of LE per 1 pregnant female increased slightly (8.86) compared to the phenobarbital group (8.50).

The use of fluorouracil caused a decrease in the fertility of female rats almost by half - up to 58% compared with the data of the intact group (100%; table 2). The number of non-pregnant females increased to 5 individuals (table 2). The number of live embryos has sharply decreased (by 29% relative to the intact group); the number of dead embryos increased to 23. The maximum number of dead embryos per 1 pregnant female was 3.29 and the minimum number of live embryos 5.29 was noted in the fluorouracil group. The proportion of dead embryos in this group was 38.3% (table 2).

The introduction of biotrit-C against the background of fluorouracil partially leveled fertility (77%; table 2). The number of live embryos under the influence of biotrit-C increased 1.9 times (72 vs. 37 in the fluorouracil group); the number of LE increased by 1 pregnant female. At the same time, the number of dead embryos decreased 2.3 times; the number of DE per 1 pregnant female decreased to 1.0. The share of dead embryos in the fluorouracil + biotrit-C group was 12.2% (table 2).

Statistical analysis showed that in the fluorouracil group, the proportion of dead embryos, calculated per 1 male, is significantly higher ($p < 0.05$) than in the intact and higher ($p = 0.09$) than in the fluorouracil + biotrit-C group. The average values of this indicator and the index of induced mortality calculated for the groups of fluorouracil and fluorouracil + biotrit-C are presented in table.3. Under the influence of biotrit-C, the induced mortality decreased by 2.9 times and was 18.6%, while the level of mutagenicity of the drug decreased to 2 points.

Table 3

The effect of biotrita-C on post-implantation loss, induced mortality and the degree of mutagenicity of fluorouracil

Groups of animals	The average value of the index of post-implantation loss per 1 male, Ā	Induced mortality I_{ind}, %	The degree of mutagenicity, points
Intact (dist.water)	0	0	0
Fluorouracil	0,541	54,1	3
Fluorouracil + biotrit-C	0,186	18,6	2

Conclusion

The data presented indicate that in the test of dominant lethal mutations, fluorouracil showed high mutagenicity, while a decrease in the fertility of rats under the influence of phenobarbital is apparently due to its sedative properties. This is indicated by the high number

of live embryos corresponding to their level in intact animals, as well as the absence of dead embryos. The use of biotrite-C significantly reduced the negative impact of phenobarbital on male fertility. Perhaps this fact is associated with the neurotropic action of biotrit-C flavonoids.

The established high antimutagenic effect of biotrit-C requires further study of its mechanisms. The antioxidant properties of its components could play a certain role, with which biotrit-C's ability to weaken the undesirable effects of fluorouracil during chemotherapy of malignant tumors of the oral organs is consistent [8].

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