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DIHYDROQUERCETIN SHOWS GENOPROTECTIVE AND RADIOPROTECTOR PROPERTIES

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The effect of dihydroquercetin (tachsifolin) on formation of polychromatophilic erythrocytes with micronuclei in the red bone marrow of males Kv:SHK mice under the action of X-rays and on the survival of mice after irradiation at a dose of 7 Gy. Various concentrations of dihydroquercetin were investigated: 30, 150 and 300 mg/kg at their intraperitoneal administration. Dihydroquercetin at a concentration of 30 mg/kg in the micronucleus test did not has a significant effect on damaged DNA with the formation of micronuclei, and in the test for survival leads to 100% death of animals, as in the control group. Determined that dihydroquercetin at concentrations of 150 and 300 mg/kg reduces the formation of radiation-induced polychromatophilic erythrocytes with micronuclei by 20% and 35% and increases the survival of animals after exposure to ionizing radiation by 20% and 60%, respectively. Thus, it was found that dihydroquercetin at concentrations of 150 and 300 mg/kg exhibits protective effect when administered to mice *in vivo.* **Key words: flavonoids, dihydroquercetin, gene protector, radioprotector, oxidative stress, reactive oxygen species.**

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By means of micronucleus test we analyzed the effect of dihydroquercetin (taxifolin) on formation of polychromatic erythrocytes with micronuclei in the bone marrow of Kv:SHK male mice the action influence of X-ray, as well as on the survival of these mice after irradiation at a dose of 7 Gy. In this study various concentrations of dihydroquercetin (30, 150 and 300 mg/kg) were investigated. All of them administered intraperitoneally. In the micronucleus test, dihydroquercetin at a concentration of 30 mg/kg does not diminish significantly DNA damage and the formation of micronuclei in the bone marrow test. Survival test with dihydroquercetin results in a 100% death of animals, the same was observed in the control. It was found that dihydroquercetin reduces the formation of radiation-induced polychromatic erythrocytes with micronuclei by 20% and 35%, and prolongs survival of animals after exposure to ionizing radiation by 20% and 60%. Thus it was established that dihydroquercetin in the concentration of 150 and 300 mg/kg shows a protective effect at administration to the mice in vivo.

Keywords: flavonoids, dihydroquercetin, genoprotector, radioprotector, oxidative stress, reactive oxygen species.

Reactive oxygen species (ROS) are constantly formed in a living cell as products of normal oxygen metabolism. Impact of environmental factors on the body, such as ionizing radiation, UV radiation, etc., also leads to ROS formation [1]. **These chemical compounds have a high reactivity ability, which allows them to enter into redox reactions with proteins, lipids, carbohydrates and nucleic acids. In sensitive DNA**

the target under the action of ROS is guanine, which, as a result of oxidative damage is converted into 8-oxoguanine [6]. The formation of 8-oxoguanine is associated with such processes such as mutagenesis, carcinogenesis, aging, etc. There is currently many different radioprotective substances that can prevent damaging effect of ionizing radiation. However, identifying the most effective of them continues [2]. Most radioprotective substances are bioantioxidants [3]. by the most promising are antioxidants of natural origin, capable of modify the damaging effect of ionizing radiation [10, 4]. Flavonoids - this is the most studied class of natural substances capable of exhibiting antioxidant, protective, immunomodulating and other properties [8]. A prominent representative of this class considered to be dihydroquercetin (DHA) [7]. DHA was first discovered in the bark of the Douglas fir, and later in Dahurian and Siberian larch. Molecular structure and function DHA is close to quercetin and rutin, but surpasses them in terms of pharmacobiological properties. activity. It was previously shown that DHA exhibits antioxidant properties [7, 9]. Target of this work was to study the gene-protective and radioprotective properties DHA in the *in vivo system.*

Materials and methods of research

Highly purified preparation dihydroquercetin was used in the work, kindly provided by A.B. Gavrilov (Pushchino, IPB RAS CJSC NPF "Flavit"), peroxide hydrogen (KhimMed, Russia), Tris(hydroxymethyl)aminomethane, 2,2'-azino-di(3-ethyl-2,3-ammonium dihydrobenzothiazoline-6-sulfonate (ABTS) (Sigma, USA), sodium chloride (AppliChem, Germany), immersion oil (Cargill, USA). We used the following salts: $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 2H_2O$ (Amresco, USA).

Irradiation. Irradiation of solutions was carried out on an X-ray therapeutic installation RUT-15 (15 mA, 200 kV) (MosRentgen, Russia) at a dose rate of 4 Gy/min (focal length 0.195 m) (Center for Collective Use of the IBC RAS).

micronucleus test

Cytogenetic damage to red bone marrow cells of mice was determined by the formation of polychromatophilic erythrocytes (PCE) containing micronuclei (MN). Mice were sacrificed by cervical dislocation 28 h after exposure . (X-ray and/or injection of dihydroquercetin solution). Histological preparations were prepared according to the standard method with some modifications. When counting PCEs containing NCs were used with a MikMed-2 light microscope (LOMO, St. Petersburg) with immersion objective at ×1000 magnification.

Survival test

Survival rate of male outbred mice Kv:SHK **after exposure**
X-ray radiation was determined daily for 30 **days. Dihydroquercetin in**
concentrations of 30–300 mg/kg **of live weight in saline were administered to animals**
intraperitoneally before total X-ray irradiation at a dose of 7 **Gy. control**
served a group of mice without the introduction of the drug, irradiated at a dose of 7 **Gy.**

Results and discussion

Table 1 **shows the effect of X-ray radiation at a dose** of 1 **Gy on the level**
DNA damage in mouse red bone marrow cells. It can be seen from the table that after
irradiation of animals, the percentage of PCE with MR increased by 9 **times from** 0.42±0.03% **in the absence**
exposure up to 3.93±0.04% **at a dose** of 1 **Gy. Administration of DHA to mice at concentrations of** 30 mg/kg
15 min before irradiation does not have **a significant effect on the amount of PChE with NR.**
The results of the micronucleus test for animals of this group are not statistically different.
relative to the control irradiated in the same dose. Whereas with the introduction of DHA into
concentrations of 150 **and** 300 mg/kg , **statistically significant differences are observed relative to**
control. The introduction of DHA at a concentration of 150 mg/kg **leads to a decrease in the amount of PChE**
with MN relative to the irradiated control by 20%, **the introduction of DHA at a concentration of** 300 mg/kg
leads to a decrease in the amount of PChE with MR relative to the irradiated control by almost
by 35%. **It should be noted that the administration of DHA to intact mice did not lead to a change**
DNA damage, that is, dihydroquercetin does not show genotoxic properties
even at concentrations of 300 **mg/kg.**

Table 1

The effect of DHA at concentrations of 30, 150 **and** 300 mg/kg **with its single intraperitoneal administration** 15 **minutes before exposure to X-ray irradiation at a dose** of 1 **Gy per percentage content of PCE with MR**

No.	Impact	PCE with MR, %
1	0 **Gr**	0.42±0.03
2	**DHA** 300mg /kg + **0Gy**	0.39±0.04
3	1 **Gr**	3.93±0.11
4	**DHA 30mg /**kg + 1Gy	3.73±0.12
5	**DHA** 150mg /kg + **1Gy**	3.22±0.13
6	**DHA** 300mg /kg + **1Gy**	2.60±0.24

Note. Mean values and their standard errors are indicated (n=5).

The effect of DHA on the survival of mice with its intraperitoneal a single injection 15 **minutes before total X-ray exposure at a lethal dose** of 7 **Gy (Table** 2). **The effect of DHA increases with increasing concentration. established, that with intraperitoneal administration of DHA at a concentration of** 150 **and** 300 mg / kg 15 **minutes before total irradiation at a dose** of 7 **Gy,** 20% **and** 60% **of animals** survive **for** 30 **days respectively, at** 100% **death of irradiated animals in the control group and at administration of DHA at a concentration of** 30 **mg/kg. Thus, it was found that DHA exhibits significant gene protective and radioprotective properties.**

Table 2

The effect of DHA on the survival of mice after a single intraperitoneal injection in 15 **minutes before exposure to X-ray radiation at a dose** of 7 **Gy.**

Concentration, mg/kg	**Percentage of survivors by** 30 **days after exposure (number of survivors by day** 30 / **number of animals participating in the experiment)** 0 (0/20) 20 (4/20) 60
30	(18/30) **Note. Each experimental group**
150	**consisted of** 20–30 **mice.** ***p*** - 0.05.
300	

Earlier in the laboratory of cellular engineering Zaichkina S.I. and her colleagues were it was shown that dihydroquercetin at a range of low and moderate doses showed a protective action from cytogenetic generations in the bone marrow [5]. **Thus, dihydroquercetin, when exposed to x-rays, exhibits significant gene protective and radioprotective properties.**

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