лабораторних щурів. Встановлено, що випоювання фітохолу щурам в дозі 0,03 г на 100 г маси тіла позитивно впливало на клінічний стан тварин, полегшувало прояв гепатоцелюлярної недостатності та розвиток деструктивних змін в гепатоцитах (знижуючи активність лужної фосфатази, аланін- і аспартатамінотрансферази в плазмі крові та оксипроліну, гексозамінів та малонового діальдегіду – в гомогенатах печінки) за гострого токсичного гепатиту. За тривалої інтоксикації організму щурів CCl_4 дія фітохолу проявлялася в покращенні функціонального стану гепатобіліарної системи та деяким відновленням білоксинтезуючої функції печінки.

Ключові слова: рослинний препарат, тетрахлорметановий токсичний гепатит, плазма крові, гомогенат печінки, білі щури

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крыс. Установлено, что выпойка фитохола крысам в дозе 0,03 г на 100 г массы тела положительно влияла на клиническое состояние животных, облегчала проявление гепатоцеллюлярной недостаточности и развития деструктивных изменений в гепатоцитах (снижая активность щелочной фосфатазы, аланин- и аспартатаминотрансферазы в плазме крови и оксипролина, гексозаминов и малонового диальдегида – в гомогенатах печени) при остром токсическом гепатите. При длительной интоксикации организма крыс CCl_4 действие фитохола проявлялось в улучшении функционального состояния гепатобилиарной системы и некоторым восстановлением белоксинтезирующей функции печени.

Ключевые слова: растительный препарат, тетрахлорметановый токсический гепатит, плазма крови, гомогенат печени, белые крысы.

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PROTECTIVE EFFECT OF QUERCETIN ON THE ORAL CAVITY TISSUES IN RATS IN THE PRESENCE OF GENOTOXICANT AND ALIMENTARY DEFICIENCY OF POLYPHENOLS

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The purpose of the study was to establish the effect of the flavonoid quercetin on the oral cavity tissues in rats in the presence of genotoxicant fluorouracil with insufficient intake of plant polyphenols. The experiment was carried out on 21 white rats of the Wistar line. Intact animals were kept on a standard vivarium diet. The control group received a 5% fluorouracil solution *per os* □g□nst th□b□kgr□und □f □fl□v□n□d d□t; fr□shl□ r□□r□d 7.5% □t□t□st□r□h g□l w□s □□□l□d t□th□□r□l mu□s□ f th□ rats. Group 3 rats were additionally treated daily with a gel containing quercetin at a dose of 0.25 mg/kg. The experiment lasted 70 days. The gums turned out to be more sensitive than the buccal mucosa to long-term exposure to the genotoxicant fluorouracil in the case of nutritional deficiency of plant polyphenols. Quercetin in the accepted experimental conditions showed a significant anti-inflammatory effect in the buccal mucosa.

Key words: fluorouracil, nutritional deficiency of plant polyphenols, quercetin, gums, buccal mucosa, protective effect, rats.

The study is a fragment of the research project "The effect of hypoxia on the processes of collagen formation and mineralization in models of dental pathology and correction of these disorders", state registration No. 0118U006963.

The occurrence of dental morbidity has recently increased due to the increased content of toxicants in the external environment. The consumption of medicinal xenobiotics has also increased significantly.

Fluorouracil is an antitumor agent used in mono- or polychemotherapy, is a structural analogue of pyrimidine, in terms of chemical structure – 2,4-dioxy-5-fluoropyrimidine. It is known that pyrimidine bases: cytosine, uracil, thymine are a part of nucleic acids. In the organism, fluorouracil can enter into a competitive relationship with uracil, being its antimetabolite, and thus turns out to be a biochemical substrate for enzymes responsible for the metabolism of uracil. Along with inhibition of DNA bases synthesis, the drug can be included in both DNA and RNA, and thus lead to inhibition of DNA synthesis and all RNA fractions, because the functioning of DNA as a DNA matrix is disrupted [12]. Considering all of the above, fluorouracil is a genotoxicant. It is classified as a highly toxic substance. When using fluorouracil, there may be pronounced changes in peripheral blood, inhibition of bone marrow hematopoiesis, hemorrhagic phenomena of various localization, stomatitis, ulceration of the mucous membrane of the oral cavity and digestive tract.

Recent studies have shown the diverse effects of plant polyphenols (including flavonoids) on a living organism: antioxidant, anti-inflammatory, cytoprotective, antimicrobial, antiviral, etc.

Currently, the antioxidant activity of flavonoids is paid great attention as a possible mechanism through which the biological effects of this group of compounds are realized [10]. For a long time, flavonoids have been considered as the most important antioxidants [15]. Their defining chemical property

is the tendency to easily give up protons. When oxidized, due to the conjugation of redox reactions, they promote the reduction of other biologically active substances or prevent their oxidation [11]. The antioxidant activity of flavonoids allows to normalize the processes of free radical oxidation of organic molecules and the level of peroxide radicals accumulating in the cells of the organism, which is important for the construction and renewal of structural lipids of cell membranes, for the production of a number of hormones.

The main functional groups that determine the chemical activity, biochemical and pharmacological action are phenolic hydroxyls. The more hydroxyl groups a flavonoid has, the stronger it is as an antioxidant [13]. In nature, the most common are flavonoids with 4 or 5, less often 1,2 or 6-OH groups in the molecule. The strongest antioxidant effect was found in quercetin (3,5,7,3', 4'-OH and the presence of $C_2 = C_3$), thus it contains 5 OH groups and has a wide spectrum of biological activity.

Particular attention is drawn to its antioxidant, antiradical, anti-inflammatory properties. The ability of quercetin to give up a hydrogen atom to one of the hydroxy groups and form a free phenoxy radical stabilized by an unpaired electron leads to the termination of the free radical oxidative chain reaction. Quercetin is localized near the surface of the lipid bilayer of membranes, and therefore has a stronger antioxidant effect [14].

Recently, a significant number of facts have accumulated indicating the essential role of alimentary plant components in maintaining the resistance of the oral cavity tissues to the damaging effect of factors of various nature. Despite the diverse properties of quercetin, its application in dentistry is still very limited.

The purpose of the study was to establish the effect of quercetin on the oral cavity tissues of rats under the action of genotoxicant fluorouracil under the conditions of nutritional deficiency of plant polyphenols.

Materials and methods. The study was carried out on 21 white Wistar breeding rats. Intact animals (7 animal units) were kept on a standard vivarium diet. In the second, control group, 7 rats on the background of a polyphenolic diet received pes os 5% fluorouracil solution (pyrimidine uracil antimetabolite, manufactured by the pharmaceutical company PC “Darnitsa”, Ukraine) at a dose of 12.5 mg/kg of body weight of rats. Diet without flavonoids [5] contained: wheat flour – 30%, whole milk powder – 30%, starch – 20%, sugar – 15%, sodium chloride – 1%. Alfalfa flour, which contains plant flavonoids, was excluded from the diet. Application of the gel containing freshly prepared 7.5% potato starch was carried out using a dispenser on the mucous membrane of the rats' oral cavity. Rats of the 3rd group (7 animals) were kept on a low-polyphenol diet and received *per os* a solution of fluorouracil, as well as a gel to control the effect of the carrier of the studied drug. As part of the gel, the rats of the 3rd group were daily applied quercetin (granules produced by PJSC SIC “Borshchahivskiy CPP”, Ukraine) at a dose of 0.25 mg/kg. The experiment lasted 70 days.

At the end of the experiment, the rats were sacrificed by total exsanguination from the vessels of the heart under anesthesia with thiopental (40 mg/kg). Having previously separated the gums and buccal mucosa, the upper and lower jaws were dissected out and subjected to morphometric examination [7]. The objects of biochemical studies were blood serum, supernatant of rat liver, gum and buccal mucosa homogenates. The supernatant of the homogenates was obtained by centrifugation in an OS-6 centrifuge at 3000 rpm for 15 minutes.

The lipid peroxidation level (LPL) process was assessed by the content of malondialdehyde (MDA). The state of the physiological antioxidant system was assessed by the activity of antioxidant enzymes: catalase [1], superoxide dismutase (SOD) [9], glutathione peroxidase [2]. The activity of acid phosphatase was assessed by the method [6], elastase – by the method [4].

Experimental results were processed by statistical methods with the determination of t-criteria for the reliability of differences according to Student's t-test.

Results of the study and their discussion. Daily oral administration of the toxicant fluorouracil to rats was carried out by keeping them on a low-polyphenol diet, which the animals tolerated well. Weight gain was the same as in the intact group. There were no differences in the behavior of the rats and their appearance.

Consider how the studied biochemical parameters in the blood serum, liver and oral mucosa of rats changed after 70-day experimental exposure.

The use of fluorouracil in low-polyphenolic insufficiency did not significantly affect the MDA content in the studied tissues (table 1). At the same time, with the specified pathogenic effect, the activity of antioxidant enzymes significantly decreased. Thus, the activity of catalase in the liver decreased by 13.4% ($p>0.05$). More significantly, by 7.1 times ($p<0.001$), the SOD activity decreased in this research object (table 1).

Table 1

MDA content and activity of antioxidant enzymes in rat tissues (M±m; p;p_1)

Studied indices	Groups of animals		
	Intact	Control (C)	C+quercetin
	Liver		
Content of MDA (µmol/g)	59.9±4.10	40.3±0.98	51.6±5.60
Activity:			
catalase (mkat/g)	920±4.70	797±18.1	904±5.7 p_1<0.001
SOD (RU)	0.64±0.017	0.090±0.024 p<0.001	0.073±0.020 p_1<0.001
gum			
Content of MDA (µmol/g)	89.9±7.02	68.2±18.7	90.0±17.7
Activity: SOD (RU)	0.55±0.090	0.33±0.069 p=0.06	0.20±0.033
Glutathione peroxidase (mkat/g)	222±24.9	93.5±13.9 p=0.006	138±22.2 p_1=0.11
buccal mucosa			
MDA content (µmol/g)	120±14.0	101±15.6	101±17.5
Activity: SOD (RU)	0.30±0.060	0.44±0.084	0.32±0.057
Glutathione peroxidase (mkat/g)	142±11.1	75.0±9.42 p=0.001	88.9±5.45

Note: In tables 1 and 2, the reliability index p was calculated in comparison with the intact group; p_1 – compared with the control.

In the gums, fluorouracil against the background of a low-polyphenolic diet caused a significant inactivation of antioxidant proteins-enzymes. Thus, SOD activity decreased by 60 % (p=0.06); glutathione peroxidase activity – by 42 % (p=0.006; table 2). In the buccal mucosa, the combination of orally administered fluorouracil against the background of a diet without polyphenols reduced the activity of glutathione peroxidase by 1.9 times (p = 0.001) and did not significantly change the SOD activity (table 1).

An increase in inflammatory phenomena in the rats' organisms was evidenced by an increase in the acid phosphatase activity in the blood serum by 1.5 times (p = 0.06) compared with the intact group (Table 2). Another enzyme that accumulates in the focus of inflammation and has a pronounced destructive effect was granulocyte elastase. The activity of blood serum elastase under the action of fluorouracil in a low-polyphenolic diet increased by 4.9 times (p <0.001): 10.0±0.91 nkat/ml versus 2.04±0.76 nkat/ml (table 2).

Table 2

Effect of quercetin on the activity of acid phosphatase and elastase in the blood serum and oral mucosa of rats (M±m; p; p_1)

Groups of animals	Activity	
	acid phosphatase (nkat/ml; nkat/g)	elastase (nkat/ml; nkat/g)
blood serum		
Intact	78.8±15.7	2.04±0.76
Control (C)	120±12.2 p=0.06	10.0±0.91 p<0.001
C+quercetin	90.5±10.7 p_1=0.09	2.07±0.16 p_1<0.001
gum		
Intact	1.30±0.27	1.78±0.13
Control (C)	0.54±0.10	2.81±0.42 p=0.04
C+quercetin	1.15±0.32	2.43±0.57
buccal mucosa		
Intact	1.27±0.28	2.22±0.28
Control (C)	0.73±0.14	1.83±0.41
C+quercetin	0.28±0.05 p=0.006 p_1=0.011	1.93±0.37

The protective effects of quercetin have been studied in the context of oral administration of fluorouracil and reproductive nutritional deficiency of flavonoids. Quercetin has shown periodontal protection properties. It reduced bone resorption of the alveolar bone of rats on the lower jaw by 9% (p_1=0.03): 32.2±1.1% vs. 35.5±0.7%, and by 11 % (p_1=0.03) in the upper jaw: 20.8±0.8 vs. 23.5±0.8% (100% in the control group).

Under the influence of quercetin, administered against the background of fluorouracil and the maintenance of rats on a low-polyphenolic diet for 70 days, the MDA level practically corresponded to that in intact animals in the studied liver tissues and gums (table 1).

Quercetin in the liver normalized the activity of catalase, as well as the activity of SOD in the buccal mucosa of rats in comparison with the data of intact groups (table 1). The activity of glutathione peroxidase under the action of quercetin in the gums and buccal mucosa tended to increase (by 48% and 19%, respectively) compared to the data of the control groups (table 1).

Quercetin under accepted experimental conditions reduced acid phosphatase activity by 75% (trend; $p_1 = 0.09$) in rats' blood serum. At the same time, the activity of elastase decreased by 4.8 times ($p_1<0.001$; table 2), which indicates the anti-inflammatory properties of this drug. The activity of acid phosphatase in the gums under the influence of quercetin practically corresponded to the data of the intact group (table 2). At the same time, the activity of elastase in the gums did not change significantly (table 2). In the buccal mucosa, a decrease in acid phosphatase activity was found by 2.6 times ($p_1 = 0.011$) compared with the control group and by 4.5 times ($p = 0.006$) compared with intact (table 2). The activity of elastase under the action of quercetin did not significantly change in the gums and buccal mucosa (table 2).

Studies have shown that the gum, in comparison with the buccal mucosa, turned out to be more sensitive to prolonged experimental exposure, the influence of the genotoxicant fluorouracil in conditions of nutritional deficiency of plant polyphenols. An increase in inflammation was found in the gums. There was also a significant drop in the level of protective proteins-enzymes.

Quercetin, under the accepted experimental conditions, had a significant anti-inflammatory, antioxidant effect in the oral mucosa of rats, which resulted in a decrease in the level of bone resorption processes in the bone tissue of the alveolar process.

We have previously carried out experimental studies on the protective effect of plant polyphenols, which include quercetin, under conditions of reproduction of periodontal pathology in rats using another genotoxicant – dichlorodiphenyldichloroethylene – DDE (the main metabolite of dichlorodiphenyltrichloroethane – DDT) [3]. The preparation of polyphenols (flavonoids and flavone glycosides) is a combination of concentrates of cereal seedlings and the aerial part of yarrow (Achillea millefolium) with the code name PF4. The systemic normalizing effect of the drug on transaminase activity, LPL processes, and the activity of antioxidant enzymes – catalase and glutathione exchange enzymes in rats' blood serum was demonstrated [8]. With a topical effect on the oral cavity tissues of rats, the PF4 preparation partially reduced inflammation in the oral mucosa, bone resorption of the alveolar bone in rats, and normalized the mineralization processes in a relatively short period of the experiment (35 days).

The regenerative properties of the PF preparation were studied under conditions of experimental trauma and the administration of genotoxicant DDE [15]. For this, rats under anesthesia (calypsol at a dose of 20 mg/kg, i.p.) underwent a dosed injury to the gingival margin of the incisor. The healing process was monitored for clinical signs of inflammation. The average area of the wound surface in the study group, which received the drug, already on the 2nd day of examination was significantly lower than in other groups. The average area of the wound due to epithelialization under the influence of plant polyphenols on the 7th day of examination decreased by 4.7 times compared with the group “Trauma+DDE”. The final recovery in the group of rats receiving the PF4 preparation occurred on the 10th day. In the group of rats with gum trauma and the administration of the toxicant DDE, it was only on the 13th day. On the 10th day of the experiment, only 50% of recovered animals were found in the group of rats that received the toxicant against the background of trauma [5]. Thus, the genotoxicant DDE aggravated the course of the wound process of the mucous membrane of the rats' gum in case of its dosed traumatic injuries, and the preparation of plant polyphenols under these conditions had a pronounced protective effect on the course of the wound process and the rate of healing of the mucous membrane of the rats' gums.

Experiment demonstrated the osteotropic properties of quercetin and substantiated its use as a therapeutic and prophylactic agent (quercetin granules at a dose of 100 mg/kg of body weight in rats) under conditions of reproduction of experimental periodontitis in rats, caused by their maintenance for three months on a "soft" diet with the maximum reduced load on the periodontium. Under the conditions of modeling periodontitis, the processes of LPL in periodontal tissues were significantly activated, the activity of elastase in the gum increased, and the resorption of the alveolar process bone significantly increased. Oral administration of quercetin significantly reduced the resorptive processes in the periodontal bone tissue, the activity of elastase and acid phosphatase in the gums, which indicated a high therapeutic and prophylactic efficacy of quercetin.

Thus, bioflavonoid quercetin, which has a variety of biological effects, can be recommended as an effective therapeutic and prophylactic agent for dental diseases: gingivitis, periodontitis, etc.

Conclusions

1. Genotoxicant fluorouracil in combination with a diet without plant polyphenols caused an increase in inflammation in the gums of rats and a significant drop in the level of protective enzymes-proteins – SOD and glutathione peroxidase.

2. The flavonoid quercetin has a protective and anti-inflammatory effect. It normalized the activity of the pro-inflammatory enzyme of acid phosphatase in the gums and significantly reduced its activity in the buccal mucosa of rats.

3. Quercetin showed osteotropic action. It reduced the level of resorption processes in the alveolar bone in rats.

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Реферати

ЗАХИСНИЙ ВПЛИВ ФЛАВОНОЇДУ КВЕРЦЕТИНУ НА СТАН ТКАНИН РОТОВОЇ ПОРОЖНИНИ ЩУРІВ В УМОВАХ ДІЇ ГЕНОТОКСІКАНТА І АЛІМЕНТАРНОЇ НЕДОСТАТНОСТІ ПОЛІФЕНОЛІВ

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Метою дослідження було вивчення впливу флавоноїду кверцетину на тканини ротової порожнини щурів при дії генотоксіканта фторурацилу при недостатньому надходженні рослинних поліфенолів. У дослідження було взято 21 білого щура лінії Вістар. Інтактні тварини утримувалися на стандартному раціоні віварію. Контрольна група на тлі бесфлавоноїдного раціону отримувала per os 5% розчин фторурацилу, на слизову оболонку порожнини рота щурів наносили гель – свіжоприготований 7,5%-ий картопляний крохмаль. Щурам 3-ої групи додатково щодня наносили гель, до складу якого входив кверцетин в дозі 0,25 мг/кг. Тривалість дослідження склала 70 днів. Десна виявилися більш чутливими, ніж слизова оболонка щоки до тривалого впливу генотоксиканта фторурацилу при аліментарній недостатності рослинних поліфенолів. Кверцетин в прийнятих експериментальних умовах проявив значну протизапальну дію в слизовій оболонці щоки.

Ключові слова: фторурацил, аліментарна недостатність рослинних поліфенолів, кверцетин, ясна, слизова оболонка щоки, захисна дія, щури.

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ЗАЩИТНОЕ ВЛИЯНИЕ ФЛАВОНОИДА КВЕРЦЕТИНА НА СОСТОЯНИЕ ТКАНЕЙ РОТОВОЙ ПОЛОСТИ КРЫС В УСЛОВИЯХ ДЕЙСТВИЯ ГЕНОТОКСИКАНТА И АЛИМЕНТАРНОЙ НЕДОСТАТОЧНОСТИ ПОЛИФЕНОЛОВ

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Целью исследования явилось изучение влияния флавоноида кверцетина на ткани ротовой полости крыс при действии генотоксиканта фторурацила при недостаточном поступлении растительных полифенолов. В опыт была взята 21 белая крыса линии Вистар. Интактные животные содержались на стандартном рационе вивария. Контрольная группа на фоне бесфлавоноидного рациона получала *per os* 5% раствор фторурацила, на слизистую оболочку полости рта крыс наносили гель – свежеприготовленный 7,5%-ный картофельный крахмал. Крысам 3-ей группы дополнительно ежедневно наносили гель, в состав которого входил кверцетин в дозе 0,25 мг/кг. Длительность опыта составила 70 дней. Десна оказалась более чувствительной, чем слизистая оболочка щеки к длительному воздействию генотоксиканта фторурацила при алиментарной недостаточности растительных полифенолов. Кверцетин в принятых экспериментальных условиях проявил значительное противовоспалительное действие в слизистой оболочке щеки.

Ключевые слова: фторурацил, алиментарная недостаточность растительных полифенолов, кверцетин, десна, слизистая оболочка щеки, защитное действие, крысы.

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