Open Access Article

EVALUATION OF THE BIOLOGICAL ACTIVITY OF PHYLLALBIN AND RUTIN, AND APPLICATION IN WOUND DRESSINGS

Natalya Y. Tseomashko
MD, PhD, Head of the Department of Medical and Genetic Research of the Interuniversity Research Laboratory of the Tashkent Medical Academy of the Ministry of Health of the Republic of Uzbekistan. Tashkent, Uzbekistan E-mail: tsne_77@list.ru

Aziza Dj. Abidova
Junior Researcher, Interuniversity Research Laboratory Interuniversity Research Laboratory of the Tashkent Medical Academy of the Ministry of Health of the Republic of Uzbekistan. Tashkent, Uzbekistan E-mail: info@tma.uz

Sardor Dj. Makhmudov
Basic doctoral student of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan. Tashkent, Uzbekistan E-mail: info@tma.uz

Shahnoz S. Azimova
Doctor of Science, prof. Institute of the Chemistry of Plant Substances Academy of Sciences at Uzbekistan. Tashkent, Uzbekistan. E-mail: info@tma.uz

Salima F. Aripova

ABSTRACT

To accelerate the healing of wounds, ulcers, burns polyfunctional wound dressings (WD) are required. The functional properties of WD can be expanded by introducing in situ or ex situ to the structure of their biologically active components (BAC) composition. In our studies, we propose to use as a BAC the alkaloid phillalbine (Ph) which is isolated from plants of the genus Convolvulus, flavonoid quercetin (Qu) isolated from buds of Saphora japonica and glycoside of quercetin flavonoid - rutin (Ru). It is found that Ph can be used as skin fibroblast cells proliferator. Ph at a concentration of 12.5-25 μg / ml increases fibroblast proliferation by 25-79%. Ru at a concentration of 25 μg / ml exhibits antimicrobial activity against a wide range of microorganisms. Qu is a well-known antioxidant. BAC data is injected ex situ into collagen (CC) gel. Compositions of polyfunctional WD in membrane form were created: CC-Qu-Ru-Ph and CC-Qu-Ru, and were studied in comparison with commercial preparations "NeuSkin-F" (Eucare Pharmaceuticals (P) Limited, India) on experimental models of burn wounds. It was found that CC-Qu-Ru-Ph possesses high congruence and adhesion to the surface of the wound bed and accelerates wound healing 1.49 times faster compared to untreated burn wounds of the same size, and 1.27 times faster than "NeuSkin-F". CC-Qu-Ru-Ph and CC-Qu-Ru are non-toxic, do not have a local irritant effect, and are histocompatible. The safety of the obtained WD was

Received: August 12, 2021 / Revised: September 08, 2021 / Accepted: September 30, 2021 / Published: October 10, 2021
About the authors Natalya Y. Tseomashko
Corresponding author- Email: tsne_77@list.ru

confirmed by histomorphological studies of organs and tissues of rats, who took high doses of WD compositions (5000 mg / kg) orally for a month.
Keywords: phillalbin, quercetin, rutin, collagen, proliferator, antioxidant, wound dressing

抽象的
为了加速伤口、溃疡、烧伤的愈合，需要多功能伤口敷料 (WD)。 WD 的功能特性可以通过原位或异位引入其生物活性成分 (BAC) 组合物的结构来扩展。在我们的研究中，我们建议使用从旋花属植物中分离的生物碱毛蕊花碱 (Ph)、从 Saphora japonica 的芽中分离出的黄酮类槲皮素 (Qu) 和槲皮素黄酮苷的糖苷 - 芦丁 (Ru) 作为 BAC。发现Ph可用作皮肤成纤维细胞增殖剂。 Ph 在 12.5-25 μg/ml 的浓度下使成纤维细胞增殖增加 25-79%。浓度为 25 μg/ml 的 Ru 对多种微生物表现出抗菌活性。曲是一种众所周知的抗氧化剂。 BAC 数据被非原位注入胶原 (CC) 凝胶中。创建了膜形式的多功能 WD 组合物：CC-Qu-Ru-Ph 和 CC-Qu-Ru，并在实验模型上与商业制剂“NeuSkin-F”（Eucare Pharmaceuticals (P) Limited，印度）进行了比较研究的烧伤。研究发现，CC-Qu-Ru-Ph 与创面床表面具有高度的一致性和粘附性，与未处理的相同大小的烧伤创面相比，创面愈合速度快 1.49 倍，比“NeuSkin-F”快 1.27 倍. CC-Qu-Ru-Ph 和 CC-Qu-Ru 无毒，没有局部刺激作用，并且是组织相容性的。获得的WD的安全性通过大鼠器官和组织的组织形态学研究得到证实，大鼠口服高剂量WD组合物(5000mg/kg)一个月。
关键词： 畏寒素、槲皮素、芦丁、胶原蛋白、增殖物、抗氧化剂、伤口敷料

INTRODUCTION

Microbial infection and oxidative damage to cells and tissues of the wound bed often lead to prolonged and incomplete wound healing. In this regard, there is a need for wound dressings (WD) and scaffolds that would have antioxidant, antimicrobial, regenerative and protective properties. As antimicrobial components, researchers propose to introduce antibiotics into wound dressings [Daeschlein G. 2013.], plant components based on alkaloids, saponins, polyphenols [Jaya R Lakkakula, 2017.], silver nanoparticles [Mehrabani MG, 2018], etc. In addition to coatings with antimicrobial properties, WDs are required, which are capable of neutralizing excessively released free radicals in the phase of inflammation of the wound process. For this purpose, a number of scientists propose to introduce antioxidants into the composition of WD. The most commonly used compounds with antioxidant activity are tannic acid (polyphenol), quercetin (flavonoid), curcumin (polyphenol) and vitamin B6 (pyridoxine) [Ariel Vilchez, 2020]. Gufran Ajmal et al. developed a PCL-based nanofiber filled with ciprofloxacin hydrochloride (CHL) and quercetin. The effectiveness of wound healing with nanofiber was evaluated by the authors in experimental models of full-thickness wounds in rats, which showed accelerated wound healing with complete re-epithelialization and improved collagen deposition within 16 days [Gufran Ajmal, 2019]. Narges Fereydouni et al. curcumin is introduced as an antioxidant into nanofiber wound coatings [Narges Fereydouni, 2019]. Tran NQ et al. prepared an in situ gel-forming system consisting of chitosan derivatives conjugated with rutin and tyramine, horseradish peroxidase (HRP) and hydrogen peroxide, and used the gel for skin wound

healing. Rutin has been used to increase the production and accumulation of extracellular matrix during the healing process. [Tran NQ, 2011].

In connection with the above, it is relevant and interesting to conduct research to identify components of plant origin with antimicrobial, antioxidant and proliferative activities, among flavonoids isolated from Saphora japonica and phyllalbin alkaloid isolated from plants of the genus Convolvulus. Determine their most active concentrations and establish their effectiveness in the composition of collagen membranes for WD applications.

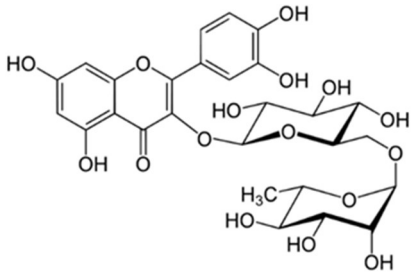
RESEARCH MATERIALS

In the research white outbred mice weighing 15-20 g, rats weighing 150-200 g, cow tails; primary cultures of normal fibroblast cells, strains of gram-positive and gram-negative bacteria and fungal strain were used. Microbial strains were obtained from the collection of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan. Chemical reagents and media used in work: culture media RPMI-1640 (Himedia, India), fetal calf serum FBS (Himedia, India), 100x antibiotic-antimycotic solution (Himedia, India), crystalline trypsin (Samson-Med, Russia), hematoxylin solution (Cyprus.diag. , Belgium), eosin solution (Cyprus. Diag., Belgium), neutral red (Himedia, India), as well as chemicals and dyes produced in the CIS countries.

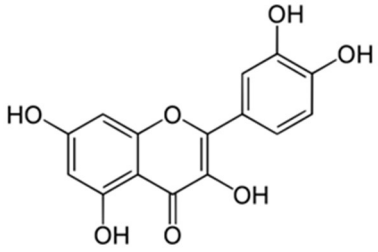
In the research the following equipment and consumables were used: Mindray MR-96A immunoassay analyzer (China), Vortex (China), Thermostat TS-1/80 SPU (Russia. JSC "Smolenskoe SKTB SPU"), Drying cabinet IC-80-01 (Russia, JSC "Smolenskoe SKTB SPU"), water purification system "Prodion" 10VS-MA (China), Tabletop centrifuge TDZ4-4WS (China, Hunan Xiangyi Laboratory Instrument Development Co., Ltd. Huna), spectrophotometer V5000 (China), automatic hematology analyzer VS-3000 (China), semi-automatic biochemical analyzer VA-88A (China, Mindray), analytical balance "SHINKO" 0.001-220 gr. (Japan), CO2-Incubator (Shel Lab, USA), Laminar box and cabinet (BBS8V1708247D, China), digital stationary pH meter (Bante210, China), portable steam sterilizer (autoclave JIBIMED, YX-280, China), automatic carousel-type histoprocessor "Thermo Fisher STP 120" (TFS, USA), rotary microtome HM 325 (TFS, USA), Axio Lab.A1 microscope (Carl Zeiss, Germany), digital video camera SDPTOP, 50 cm2 culture flasks and Petri dishes (Costar, USA), 96-well plates (Costar, USA).

The following were studied as biologically active components (BAC):

1) Quercetin flavonoid glycoside - rutin (2-(3,4-dihydroxyphenyl) -5,7-dihydroxy-3- [α-L-rhamnopyranosyl- (1 → 6) -β-D-glucopyranosyloxy] -4H-chromene- 4th) $C_{27}H_{30}O_{16}$

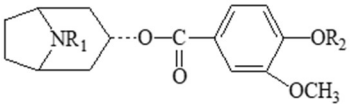


2) Flavonoid quercetin (3,3 ', 4', 5,7 - pentahydroxyflavone) C15H10O7



The flavonoids quercetin and rutin were isolated from the buds of Saphora japonica at the Pharmacology Institute of the Ministry of Health of the Republic of Uzbekistan and provided for research by S.D. Makhmudov.

3) phillalbine alkaloid



$R_1 = -CH_3$; $R_2 = -H$

Phillalbin is a dry, off-white crystalline powder with a melting point of 209-210 °. Let's well dissolve in methanol, ethanol. Phillalbin was isolated from plants of the genus Convolvulus, in the laboratory of alkaloids of the Institute of Chemical Chemistry of the Academy of Sciences of the Republic of Uzbekistan under the leadership of S.F. Aripova.

4) As a comparison, a commercial preparation "NeuSkin-F" (Eucare Pharmaceuticals (P) Limited, India) was used - a collagen-based wound dressing without additives.

RESEARCH METHODS

Obtaining fibroblast cell cultures

Fibroblasts were obtained by explant method from the skin of newborn rat pups. The cells were cultured in complete RPMI-1640 medium (Himedia, India) containing 10% FBS (Himedia, India) and 1% antibiotic-antimycotic solution (Himedia, India) containing 10,000 U / ml penicillin, 10,000 µg / ml streptomycin and 25 µg / ml amphotericin B, in a CO2 incubator (ShelLab, USA) at + 37 ° C, 5% CO2. The medium was changed every 4 days, and then the culture was maintained for work by reseeding (passages). The sieving ratio was 1: 3.

Evaluation of cell viability by the inclusion of a vital dye in a test with neutral red

For analysis, fibroblasts were plated at a density of 3x104 cells / ml in 96-well plates. At the end of 24 hours, the culture medium was replaced with a medium containing a certain amount of test substances and a control and incubated in a CO2 incubator for 24 hours. Then the plates were washed twice with a single solution of phosphate-buffered saline (1 × PBS) and incubated for 3 hours in a freshly prepared neutral red solution in RPMI-1640 medium (3.3 µg / ml) in an incubator. After removing the supernatant and thoroughly washing the wells of the 1xPBS plate, 200 µl of acidic ethanol was added to the wells and the plate was placed on a shaker for 45 min, after which spectrophotometry was performed. Absorbance was measured at 540 nm. Intact cells, the optical density (OD) of which was taken as 100% cell survival, served as a control. Cell viability was determined as the ratio (in percent) of the absorption of intact cells from non-intact cells. All experiments were performed three times for each substance and concentration.

Obtaining collagen from cow's tail tendons and membranes based on it

For these purposes, the methods of enzymatic and acid extraction were used [Kaulambaeva M.Z. 2010]. The tendons of cattle were thoroughly cleaned of muscles, ligaments and other things, crushed to pieces 1-2 mm in size, and treated with 0.25% trypsin solution at 37 ° C. After trypsinization, they were washed several times in distilled water to remove non-collagen proteins and hydrolyzed for 48-72 h in 0.1 N acetic acid solution in a ratio of 1: 100. The resulting gel was homogenized at 3000 rpm for 5-7 minutes. Then the collagen mass was filtered

through a mesh material to remove large pieces, which were sent for re-hydrolysis. The collagen solution was dialyzed prior to use. Dialyzed collagen solutions were applied 40 ml to Petri dishes (90 mm in diameter). Before polymerization, collagen was added ex situ by the physical method in different doses and combinations of BAC.

Study of the physicochemical properties of membrane wound dressings

WD membranes have been studied for their physical and chemical properties, such as adhesion strength, thickness, coefficient of swelling and polymerization. The thickness of the membranes was determined in micrometers (μm) using a micrometer in different places of the membranes (n = 6) and the average value was displayed.

The tensile strength of the membranes was determined by breaking; for this, a strip 5 mm wide and 50 mm long was cut from each membrane. The ends of the membrane were fixed with clamps and brought to rupture using a dynamometer (laboratory of mechanics at the Faculty of Physics, NUU named after M. Ulugbek). At the moment of rupture, the applied force was recorded in Newtons (N).

To determine the strength of adhesion, test specimens with an area of 1 cm were cut from each membrane. Then, on one side of the membrane, with the help of cyanoacrylic glue, fabrics of equal area with an attached silk thread were glued to one side. After that, the membranes were moistened with water and glued to wet glass, and using a dynamometer, the force in Newtons (N) required to tear the membrane off the glass surface was determined.

Study of acute toxicity of wound dressings and their components

The study of toxicity of wound dressings and their components was carried out on sexually mature white male rats weighing 150-180 g. Experimental groups were formulated 6 pieces each. The drug was administered to the animals once orally at doses: 100 - 500 - 1000 - 5000 mg / kg. 3-4 hours after the introduction of the components, the animals were given natural and briquetted feed. Observation of the animals was carried out for 14 days.

Study of chronic toxicity of wound dressings and their components

The experiments were carried out on white outbred rats. The animals were divided into groups of 6 rats per group. Experimental groups of animals were injected orally in three doses of the studied components and gel forms of wound dressings for a month. The control group of animals was injected with water under similar conditions. All experimental and control animals were kept in the same conditions and on a normal diet, under daily supervision. Then, under light ether anesthesia from animals, by one-stage decapitation, blood was collected for biochemical studies. Biochemical parameters of blood serum were determined by unified methods: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) - by the unified Reitman-Frankel method; alkaline phosphatase (ALP) - a unified method with nitrophenyl phosphate; total protein (TP) - by colorimetric biuret method; gamma-glutamyltransferase (γ GT) - by the kinetic method; cholesterol (Chol) - by the enzymatic-colorimetric method on a BA-88 A biochemical analyzer (Mindray, P.R., China) using complete reagent kits (CYPRESS Diagnostics, Belgium).

Study of the skin irritating effect of wound coverings

The study of the skin irritant effect of wound dressings was carried out in accordance with GOST (Federal Standard) ISO 10993-11-2011. On the clipped area of the skin of the back of the animals with an area of 15x10 cm, the application of the test substance in the form of a gel was applied to an area of 2x2 cm. The animals were fixed for 4 hours. The skin reaction was recorded at the end of the exposure 1 and 16 hours after the application. The skin reaction was taken into account on the scale of skin tests in points according to the indicators of the reaction of the skin.

Evaluation of antimicrobial activity by the agar diffusion method

To determine the antibiotic potential of the BAC, test bacteria from the collection of cultures of microorganisms of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan were used. A suspension of bacterial cells was prepared from a daily subculture of the corresponding strain, with 1 × 106 colonies in 1 ml. Sterile nutrient agar (Immunpräparate, Berlin, D, 25 g agar / L dis. Water) was inoculated with bacterial cells (200 µl of bacterial cells in 2 ml of 0.9% NaCl suspension and 20 ml of medium) and added to Petri dishes to obtain a solid phase. The effect of substances on non-spore test cultures was determined in the exponential growth phase (after 36-42 hours), on spore cultures - at the stage of sporulation (after 48-72 hours). Antagonistic activity was assessed on the 3rd day of incubation by the diameter of the sterile zones in the bacterial lawn formed around the wells according to the Egorov method [Egorov, 1989]. The experiments were carried out in three repetitions.

Creation of a model of A III-degree burn wounds

Models of burn wounds were modeled on outbred white rats weighing 180-200 g. The experiment was carried out on animals under ether anesthesia. On the depilated areas of the backs of the animals with a special electric device (a soldering iron at the end of which a copper plate 1.5x1.5 cm in size is fixed), heated to 1200C, 2 wounds were inflicted on each animal. Each animal was kept in a separate cage. On the second day, 30% salicylic acid ointment was applied for 3 hours, after which the gauze was removed scabs from wounds. The wounds were washed with sterile saline and the test WDs were applied immediately. Control over the course of the wound process was carried out on the basis of: clinical observation data, such as the presence or absence of soft tissue edema in the wound area, skin hyperemia, pain during manipulations; the presence or absence of necrotic detritus and wound exudate on the surface of the wound; noted the time of relief of the phenomena of perifocal inflammation, the appearance of granulation tissue, its maturity, the onset of epithelialization, the timing of healing; as well as an objective criterion for assessing the course of the wound process was the data of histo-morphology of the scarred area. To assess the rate of wound healing, planimetric research methods were used. To do this, a sterile cellophane plate was applied to the wound, on which the contours of the wound were applied, and then its image was transferred to graph paper and the dimensions of the contours were determined on days 3, 12 and 19.

Histomorphological studies of organs and tissues of experimental animals

Research on histo-morphology was carried out on the basis of the equipped IPSUM PATHOLOGY Laboratory (Tashkent, Uzbekistan) with the assistance of G.K. Botyralieva. The removed organs and skin areas of the animals were fixed in 10% buffered formalin solution for 24 hours. After fixation, the organs were run into a Thermo Fisher STP 120 (TFS, USA) automatic carousel histrocessor for dehydration, impregnation, and wax precipitation. Sections 3-4 μm thick obtained on a rotary microtome HM 325 (TFS, USA) were stained with hematoxylin and eosin. The resulting histological preparations were studied at different magnifications of an Axio Lab.A1 microscope (Carl Zeiss, Germany) and photographed using an SDPTOP digital video camera.

Statistical data processing

Statistical analysis was performed using special medical statistics software, SSPS v10. and STATISTICA v 6.0. The digital data obtained in the course of the study were processed using static analysis methods adopted in modern medical science. Statistical processing of digital data included the calculation of mean values (M), determination of the standard deviation and the mean mathematical error (m). The confidence interval (M + m) was determined. Differences were considered statistically significant with a probability of $P < 0.05$.

RESULTS AND DISCUSSION

The most used substrate for collagen production is mammalian skin, ligaments and tendons, especially residues from the meat industry and butcheries [Vidal Alessandra 2020]. A relatively inexpensive method is the extraction method, which can be divided into the following steps: I - mechanical treatment of the substrate (hair / wool removal, homogenization); II - extraction of non-collagen proteins; III - collagen extraction (acidic method or enzymatic method); IV - sterilization of the final product. Some of the stages have been modified over the years and are still being optimized. For example, the method of alkaline-salt extraction (stage II) has serious drawbacks, in which the tissue is treated with alkali in the presence of saturated solutions of sodium sulfate [Istrakov LP, 1969] or hydrochloric acid [Kasparyants SA, 1995], which gives a high yield at low costs of dissolved collagen with a preserved triple helix, but with deamidation of aspartic and glutamine residues, as a result of which the distribution of charges along the collagen molecule is disturbed and collagen loses its ability to form fibrils and does not form gels [Kukhareva LV, 2010]. There are difficulties at the first stage when using skin, skins - this is the removal of hair. These problems do not occur when obtaining collagen from ligaments and tendons [Terzi Alberta, 2020]. We obtained collagen from cattle tail tendons, where in stage II we used the enzymatic method (tissue trypsinization), and in stage III we used the acid method (0.1 N glacial acetic acid). Was precipitated by centrifugation (at 6000 rpm). The resulting gel-like collagen was stored in a refrigerator, and before the introduction of biologically active components (BAC) into it and the formation of film WDs, the gel was dialyzed against distilled water in order to remove acid residues. Next, the toxicity and biological activity of the BAC was evaluated in order to determine the most effective concentrations.

Assessment of cytotoxicity and proliferative activity of biologically active components

Evaluation of the cytotoxicity and proliferative activity of the BAC was carried out on primary cultures of skin cells - fibroblasts obtained from the dermis of newborn rat pups. BAC were investigated at the end of the concentration of 0.78-200 μg / ml. As a result, it was established that all BACs at a dose below 50 μg / ml stimulated fibroblasts to proliferate to one degree or another. IC50 for all BAC> 200 μg / ml (Table 1.).

Table 1.
Percentage of living fibroblast cells after 24 hours of exposure to fibroblasts of the test substances in different concentrations

Number of elements in μg / ml	Percentage of living fibroblast cells after exposure to substances		
	Phillalbin	Quercetin	Rutin
0,8	116,50±0,76	125,17±0,65*	116,0±0,37*
1,6	164,33±0,49**	121,33±0,76*	92,17±0,17
3,1	93,33±0,42	114,33±0,67	94,43±0,18
6,2	114,17±0,65	110,33±0,56	98,33±0,21
12,5	178,67±0,88**	113,17±0,70	97,67±0,21
25	124,50±0,85*	134,83±0,70*	104,67±0,21
50	77,50±0,43*	137,33±0,76*	99,0±0,37
100	62,50±0,72*	103,00±0,58*	79,33±0,84*
200	73,33±0,76*	108,50±0,67*	67,50±0,67*

Note: compared with intact cells, the viability of which is taken as 100% * P≤0.05, ** P≤0.001

As can be seen from Table 1, the daily exposure to skin cells of these BAC at concentrations from 0.78 to 200 μg / ml leads to significant changes in the proliferative activity of fibroblasts, while the alkaloid phillalbin causes the greatest bursts of proliferation of all drugs, the peak of proliferation is observed at a concentration of 1 , 5 μg / ml and the largest peak is at 12.5 - 25 μg / ml (P≤0.001). While a well-known antioxidant, the flavonoid quercetin, causes a not significant, but uniform proliferative effect at concentrations below 50 μg / ml (P≤0.05).

Assessment of acute toxicity of biologically active components

The study of acute toxicity of an individual alkaloid phillalbine isolated from plants of the genus Convolvulus was carried out on sexually mature white male rats with an initial body weight of 138-160 g. Experimental groups were formulated 6 pieces each. The alkaloid provided for testing was diluted to obtain basic dilutions of 200 mg / kg, at first 960 with ethyl alcohol, and then two-fold dilutions in saline solution and thus the following alkaloids concentrations were obtained: 200-100-50-25-12.5-6.25 -3.12 mg / kg, which were administered once intravenously (IV) and subcutaneously (SC). 3-4 hours after the introduction of doses of the studied alkaloid, the animals were given natural and briquetted feed. Observation of the experimental animals was carried out for 14 days. The introduction of the alkaloid phillalbine at a dose of 100 mg / kg caused the death of 10% of the animals and the symptoms of intoxication in the surviving animals, which disappeared a day later.

Phyllalbin doses of 50 mg / kg and below did not cause symptoms of intoxication and death of animals. As a result of the experiment, it was found that for phyllalbin LD50 = 150 mg / kg (i / v) and 200 mg / kg (s / c).

For the flavonoid quercetin and its glycoside rutin, the assessment of acute toxicity was not carried out, since it is known from the literature about their low toxicity and their LD50> 5000 mg / kg [Menshchikova E.B, 2012].

Evaluation of chronic toxicity of biologically active components

The subchronic toxicity of the individual alkaloid phyllalbine and isolated from plants of the genus Convolvulus was studied in the alkaloids laboratory of the Institute of Chemical Chemistry of the Academy of Sciences of the Republic of Uzbekistan under the guidance of prof. Aripova S.F.

The experiments were carried out on white outbred rats - females weighing 150-160 g. Solutions of alkaloids were injected into the stomachs of rats daily at doses of 5-10-50 mg / kg for one month. Each dose was tested on six rats. The control group of animals was injected with water under similar conditions. All experimental and control animals were kept in the same conditions and on the usual diet.

After the last dose of the alkaloid in all groups of animals, blood aliquots were taken from the tail vein by partial resection (0.5-1.0 cm) to determine the expanded parameters on a BC-3000 hematological analyzer (Mindray, P.R. China). Then, under light ether anesthesia from animals, by means of one-stage decapitation, blood was collected for biochemical studies, and internal organs were removed for morphological studies.

Biochemical parameters of blood serum were determined by unified methods: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) - by the unified Reitman-Frankel method, alkaline phosphatase (ALP) - by the unified method with nitrophenyl phosphate; total protein (TP) - by colorimetric biuret method; gamma-glutamyltransferase (γ GT) - by the kinetic method; cholesterol (Chol) - enzymatic-colorimetric method using reagent kits (CYPRESS Diagnostics, Belgium) on a BA-88 A biochemical analyzer (Mindray, P.R. China).

Experimental studies were carried out in compliance with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Experiments or Other Scientific Purposes (ETS N 123), Strasbourg, 03/18/1986. The obtained results were subjected to statistical processing using the standard Statistika for Windows software package according to the well-known methods of variation statistics with an assessment of the significance of the indicators ($M \pm m$) and the differences in the samples under consideration by the Student's t-test. Differences in the compared groups were considered significant at a significance level of 95% ($P < 0.05$).

The results of the studies have shown that long-term oral administration of the alkaloid phillalbine in a low dose is well tolerated by experimental animals. All experimental animals did not differ from the control rats in general condition, behavior, and body weight gain. The hematological and biochemical parameters of the blood of rats taking the studied drugs in doses of 5-10-50 mg / kg are within the limits of generally accepted norms and indicators of the intact group of animals (Tables 2-3).

Table 2

Blood parameters of rats taking phillalbin solution for 30 days in three concentrations

Groups doses	Leukocytes 10^9/l	Absolute. number of lymphocytes,10^9/l	Absolute number of eosonophil basophil monocytes 10^9/l	Number of granulocytes, 10^9/l	Hemoglobin, g / l	Erythrocytes, g / l RBC	Hematocrit% HCT	Average concentration of hemoglobin in erythrocytes, g / l	Platelets in absolute numbers ,10^9/l	Thrombocrit %
The control	14.37±1,06	6,23±0,50	2,48±0,24	5,35±0,39	136.7±5,41	6,10±0,49	37.73±1,07	368,0±5,96	608,17±52.06	0.530±0.04
5 mg / kg	14,08±1,08	5,62±0,79	2,65±0,28	5,22±0,48	128,17±7,42	6,30±0,51	36,37±1,14	365,67±4,08	601.33±37.94	0,520±0,05
10 mg / kg	15,35±1.08	5,98±0.61	2.47±0.28	6,33±0.59	134±5.58	5,39±0.64	36.13±1.31	367±5,55	612,3±45,60	0,570±0,06
50 mg / kg	14,12±1,01	6,08±0,55	2,35±0,28	5.58±0.45	131.50±5.35	6.05±0.30	36.05±1.14	364,67±5,41	595±44,84	0,540±0,03

Note: P≥0.05 compared to control

Table 3

Biochemical parameters of the blood of rats taking phillalbin solution for 30 days in three concentrations

Groups	Alanine aminotransferase activity, ALT	Aspartate aminotransferase activity, AST	Alkaline Phosphatase Activity, ALP	Gamma Glutamyl Transferase Activity,γ GT	Cholesterol, Chol	Total protein, TP
	U / l (at 37^0C)				Mmol / l	g / dl
Control (intact)	74,27±3,16	245,50±16,39	797,17±108,98	4,83±0,76	103,90±12,02	158,93±8,94
5 mg/ kg	70,67±3,89	242,67±16,75	735,98±101,10	4,33±0,80	94,82±12,90	150,25±8,08
10mg/kg	71,63±4,48	241,67±16,94	672,88±112,35	4,17±0,79	96,82±11,55	155,62±8,16
50mg/kg	76,63±3,71	248,83±16,35	729,02±104,68	3,83±0,74	128,33±11,52	160,50±8,66

Note: P≥0.05 compared to control

As can be seen from Tables 2 and 3, the hematological and biochemical parameters of the blood of animals that took 5, 10 and 50 mg / kg of phyllalbin orally for a month do not have significant deviations from those of the blood of intact animals. Nevertheless, in animals taking phillalbin at a dose of 50 mg / kg, there is an increase in total cholesterol in the blood, which could serve as a negative sign if the enzyme activity would change. In this regard, we warn you to use in long-term therapy doses of 50 mg / kg and higher of phillalbine alkaloid.

Chronic toxicity was not evaluated for the flavonoid quercetin and its glycoside rutin, since it is known from the literature that daily oral intake of these phenolic compounds in doses of

500 mg / kg for 3 weeks in chronic toxicity experiments on dogs and rats did not cause pathological abnormalities in organs and biological fluids of experimental organisms [Menshchikova EB, 2012]. Long-term (1-2 years) feeding of rats with rutin and quercetin (1% to the diet) did not give any deviations in any parameters.

Assessment of the antimicrobial activity of biologically active components

The antimicrobial activity of phyllalbine alkaloid and quercetin and rutin flavonoids was studied. The antimicrobial activity of substances was studied by the method of holes according to Egorov [Egorov, N.S., 1989]. As indicator strains, we used Pseudomonas aeruginosa 003841/114, Staphylococcus aureus 60, Candida albicans 003592/723, Citrobacter freundii 002801/27, Serratia marcescens 367, Proteus mirabilis 002810/399, Escherichia coli BCG101 s. Substances were studied at concentrations of 12.5-200 μg / ml.

As a result, it was found that quercetin and phylalbin in the studied concentrations did not suppress the growth of microorganisms. Antimicrobial activity was noted in rutin (Fig. 1).

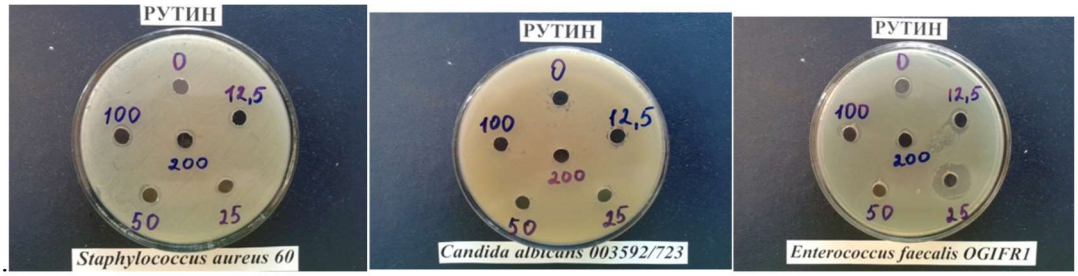


Fig. 1. Growth suppression of Staphylococcus aureus 60, Candida albicans and Enterococcus faecalis OGIFR1 solutions of rutin with different concentrations

The growth of Staphylococcu saureus 60 was inhibited by all studied concentrations of rutin, and the diameter of the growth inhibition zone was 11 mm. Escherichia coli NC101 was susceptible to solutions containing 25, 50, 100, and 200 μg / ml rutin and the diameter of the growth inhibition zone was 11 mm. Enterococcus faecalis OGIFR1 was susceptible to all rutin solutions. A rutin solution containing 25 μg / ml proved to be the most effective, the diameter of the growth inhibition zone was 19 mm. A solution containing 12.5 μg / ml rutin inhibited the growth of Klebsiella pneumoniae B-1823 with the formation of a growth inhibition zone 11 mm in diameter. Antimicrobial activity of rutin solutions was not observed against Pseudomonas aeruginosa 003841/114, Citrobacter freundii 002801/27, Serratia marcescens 367, and Proteus mirabilis 9 clinical isolate.

Thus, the flavonoid rutin exhibits antimicrobial activity and the best effect is observed when using concentrations of 25 μg / ml. This concentration does not inhibit the growth of normal fibroblast cells, and therefore can be used in WD compositions.

Obtaining compositions of wound dressings and assessment of their physicochemical, medico-biological and toxicological properties

Dialyzed collagen solutions were applied 40 ml. on Petri dishes (90 mm in diameter). Before polymerization, the collagen was added ex situ by the physical method of BAC: 25 μg / ml of rutin (Ru), a glycoside of the flavonoid quercetin, isolated from Saphora Japonica, as an

antimicrobial component; 150 μg / ml of the antioxidant quercetin (Qu), isolated from Saphora Japonica and 12.5 μg / ml of the alkaloid phillalbine (Ph), isolated from plants of the genus Convolvulus, as a fibroblast proliferator. To carry out a comparative analysis of the specific activity (wound healing) and toxicity, the following wound dressing compositions based on collagen obtained from cattle tendons were created: CC-Qu-Ru - collagen with flavonoids from Saphora Japonica; CC-Qu-Ru-Ph - collagen with flavonoids from Saphora Japonica and phillalbin alkaloid; SS - no additives.

New compositions of membranes have been studied for physical and chemical properties, such as adhesion strength, thickness and strength (Table 4.).

Table 4.

Parameters of thickness, strength and adhesion strength of WD, obtained from the calculation of 40 ml of gel-like collagen per Petri dish with a diameter of 90 mm

WD	Thickness, μm	Strength, N	Adhesion strength
CC-Qu-Ru	61,67±2,42**	51,67±1,89**	1,09±0,03*
CC-Ph-Qu-Ru	67,50±2,22**	55,67±1,71**	1,07±0,04*
CC	62,33±1,86**	58,00±2,05**	1,20±0,04*
NeuSkin-F	97,67±0,49	114,17±0,60	0,66±0,01

Note: compared to commercial WD "NeuSkin-F" * P≤0.002, ** P≤0.001

As can be seen from Table 4., the commercial preparation "NeuSkin-F" is almost 2 times (P≤0.001) thicker and stronger than our films, but also almost 2 times inferior to ours in adhesion strength. It was found that the addition of flavonoids to the collagen gel reduces the strength of the films in comparison with NeuSkin-F by almost 2.2 times (P≤0.001).

Thus, after studying the physicochemical properties of WD, the following properties of the studied WD were established, which can be listed in decreasing order of positive qualities as follows: by the rate of polymerization - CC → CC-Qu-Ru-Ph → CC-Qu-Ru; by the strength of adhesion - CC → CC-Qu-Ru → CC-Qu-Ru-Ph → "NeuSkin-F"; by strength - "NeuSkin-F" → CC → CC-Qu-Ru-Ph → CC-Qu-Ru. Nevertheless, all films were selected for further research.

Study of the hygienic and toxicological properties of wound dressing compositions

Acute toxicity of gel forms of wound dressing compositions was studied. The experiment was carried out on male rats weighing 180-200 g (6 animals per group). The animals were injected intragastrically with suspensions of the WD compositions at a dose of 5000 mg / kg (950-1000 μl of gel). As a result, due to the absence of death of animals, the maximum tolerated dose of drugs was determined at the level of 5000 mg / kg, and the LD50 values were not established. It was found that the studied WDs, according to the parameters of acute intragastric inoculation, belong to the 5th class - practically non-toxic substances.

The skin-irritating effect of all the above compositions of the WD was also studied by repeated exposure (20 skin applications) on the skin of white rats. It was found that during the entire period of the experiment, the death of animals and clinical signs of intoxication were not observed.

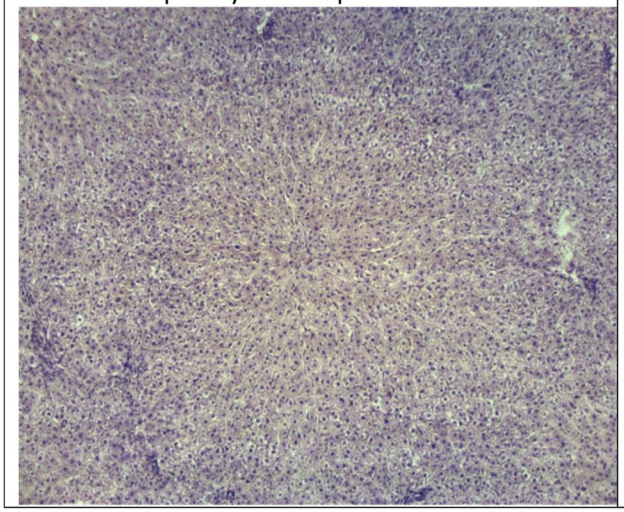
The sub chronic toxicity of WD gel compositions was studied. The experiments were carried out on white outbred rats - females weighing 150-160 g. The drugs under the abbreviated names: CC-Qu-Ru and CC-Qu-Ru-Ph were administered in 1 ml

orally for a month, which corresponded to approximately 5000 mg / kg. Each group contained six rats. The control group of animals was injected with water under similar conditions. All experimental and control animals were kept in the same conditions and on the usual diet.

Throughout the experiment, the animals were under daily observation; the general condition, behavior, food and water consumption, the condition of the hair and mucous membranes were recorded. After the last injection of drugs in all groups of animals, blood aliquots were taken from the tail vein by partial resection (0.5-1.0 cm) to determine the expanded blood parameters. Then, under light ether anesthesia from animals, by means of one-stage decapitation, blood was collected for biochemical studies, and internal organs were removed for morphological studies. The results of the studies have shown that long-term oral administration of drugs is well tolerated by experimental animals. All experimental animals did not differ from the control rats in general condition, behavior, and body weight gain. Hematological and biochemical blood parameters of experimental animals did not significantly deviate from those of intact animals.

All investigated gel compositions of WD, regardless of the attached components, did not cause significant destructive or inflammatory-necrotic changes in the organs of experimental animals during histo-morphological studies, after decapitation and dissection of animals.

In the liver, both in the control and in all groups of experimental animals, no pronounced pathological changes were found (Fig. 2). The liver capsule is not thickened, contains longitudinally oriented bundles of collagen fibers. The liver parenchyma is formed by the classic hepatic lobules, consisting of the hepatic plates or trabeculae radially oriented to the central vein. Polygonal hepatocytes, with a centrally located nucleus, the nucleolus is often determined. Binuclear hepatocytes are quite common.



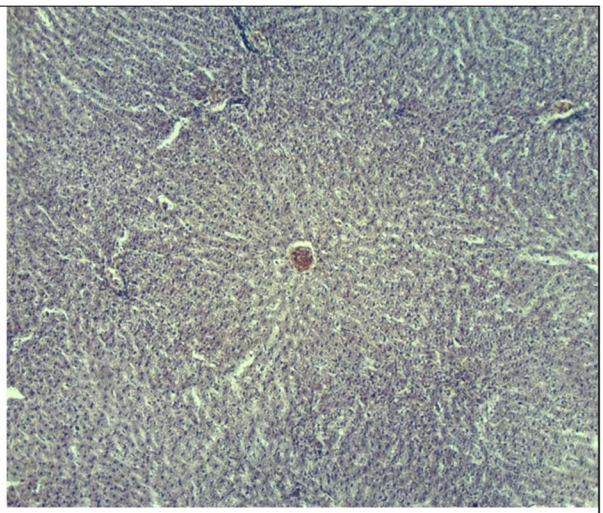


Fig. 2. A - liver of the control group. B - liver of the experimental group; HE (hematoxylin-eosin) coloring, vol. 10, circ. 10

Tinctorial properties of hepatocytes are not disturbed, hepatocytes with signs of fatty or proteinaceous degeneration were not found. Sinusoidal capillaries of normal size, single erythrocytes and leukocytes are determined in the lumen. In the wall of sinusoidal hemocapillaries and in the spaces of Disse at high magnifications, single Kupffer cells with an intact structure are revealed. In some cases, moderate expansion and blood filling of sinusoidal hemocapillaries, central and sublobular veins was noted, which is quite natural for the reactive state of the liver. The endothelial lining is without destructive changes, in places there are swollen endothelial cells with hyperchromic nuclei. The structure of cholangioli and interlobular bile ducts without pathological changes. The interlobular connective tissue is poorly developed, no signs of inflammatory infiltration and liver fibrosis were found.

All this indicates that the studied drugs at a dose of 5000 mg / kg do not have a significant negative effect on the microscopic structures of the liver.

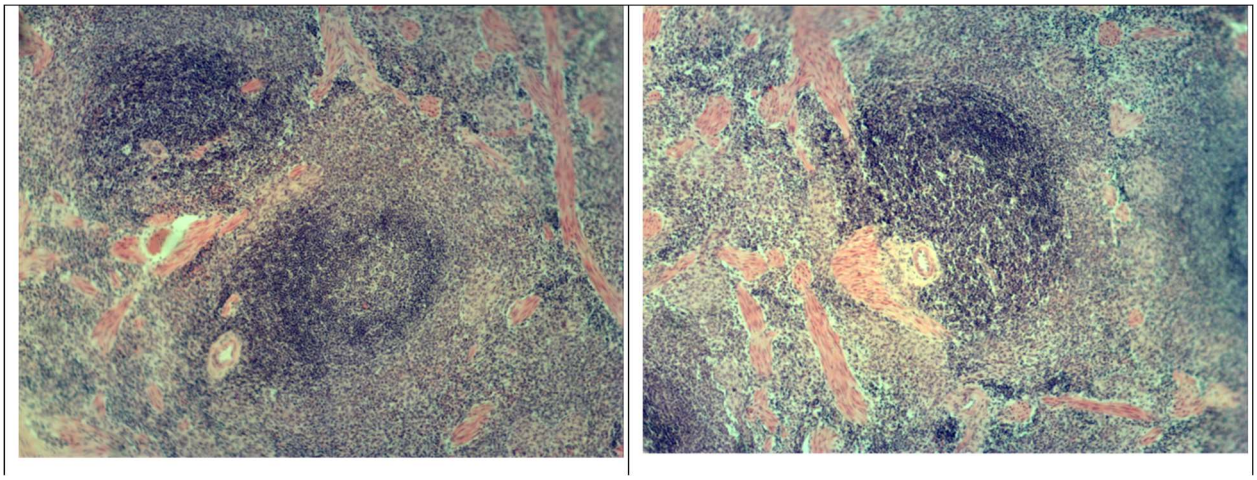


Fig. 3. A - spleen of the control group. B - spleen of the experimental group. HE (hematoxylin-eosin) coloring, vol. 10, circ. 10

In a similar way, the preparations we developed did not have a significant effect on the histological structures of the spleen (Fig. 3). In both experimental and control animals, the capsule and trabeculae are well developed, contain rather powerful bundles of smooth muscle cells. In the parenchyma, red and white pulps are clearly differentiated, which have the usual ratio characteristic of adult animals. The white pulp is represented by lymphatic follicles of various sizes, along the periphery of which the central artery is determined. The structural zones of the white pulp are quite delimited; part of the lymphatic follicles contains the germinal or

reactive center. In reactive centers, cells are often found that are at various stages of mitotic division. The red pulp is rich in erythrocytes, macrophages are also detected there, the cytoplasm of which contains a pigment - hemosiderin. Thus, long-term administration of large doses of the drugs developed by us does not have a significant negative effect on the organs of the immune system.

The histoarchitectonics of the kidneys in the experimental animals was preserved and did not have any special morphological deviations from the kidneys of the control groups of animals. The capsule is thin, without signs of edema and destruction. Numerous renal corpuscles are determined in the cortex (Fig. 4.).

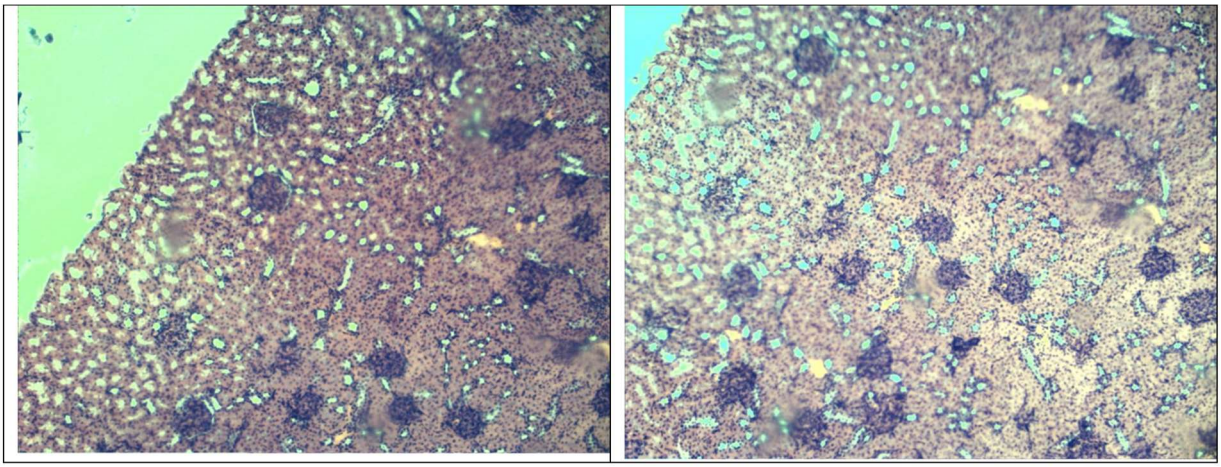
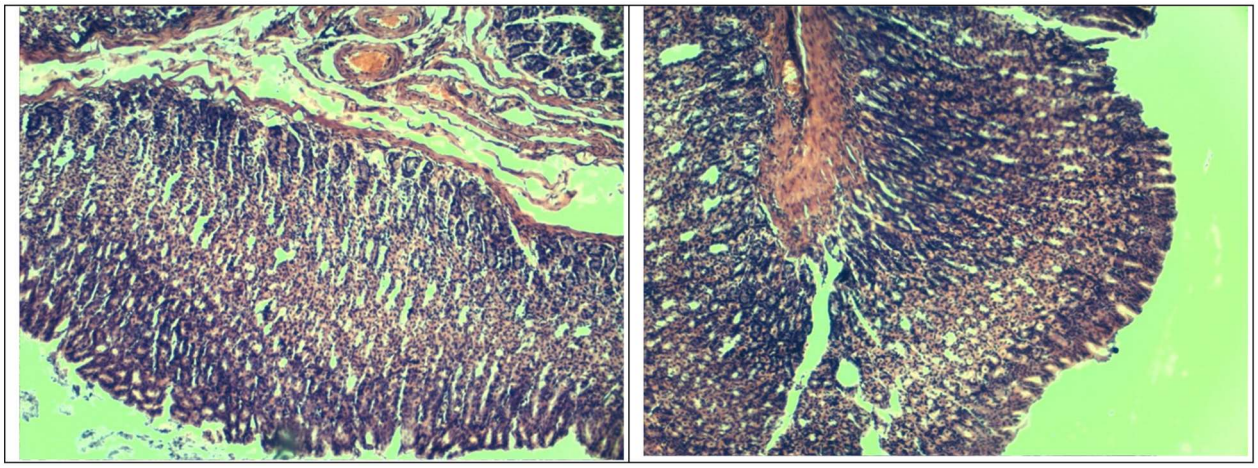


Fig. 4. A - kidney of the control group. B - kidney of the experimental group. HE (hematoxylin-eosin) coloring, vol. 10, circ. 10

Vascular glomeruli contain mainly open-type capillary loops. The cavity of the Shumlyanskiy capsule is of normal size, does not contain blood cells or any other pathological deposits. The epithelium of the proximal, thin and distal sections of the nephron has a structure characteristic of these sections, without signs of destructive changes. No precipitates or other pathological deposits were found in the lumen of the nephron tubules and collecting ducts. The connective tissue of the renal cortex and medulla is tender, without signs of edema and inflammatory infiltrates. Thus, long-term administration of our preparations to animals does not cause significant pathological changes in the kidneys compared to the control group.

Similarly, long-term administration of our drugs, regardless of the attached structural component, did not have a significant negative effect on the structural state of the "target organs", which were the stomach, small and large intestine. The gastric mucosa in the experimental animals retained its usual structure, without signs of erosions or ulcers (Fig. 5). In general, the proper glands are packed quite tightly; in some places in all groups of animals, light areas are observed, probably due to the expansion of the lymphatic vessels.

Fig. 5. A - the body of the stomach of the control group. B - the body of the stomach of the experimental group. HE (hematoxylin-eosin) coloring, vol. 10, circ. 10



Likewise, examination of the small intestine showed no significant changes in comparison with control (Fig. 6).

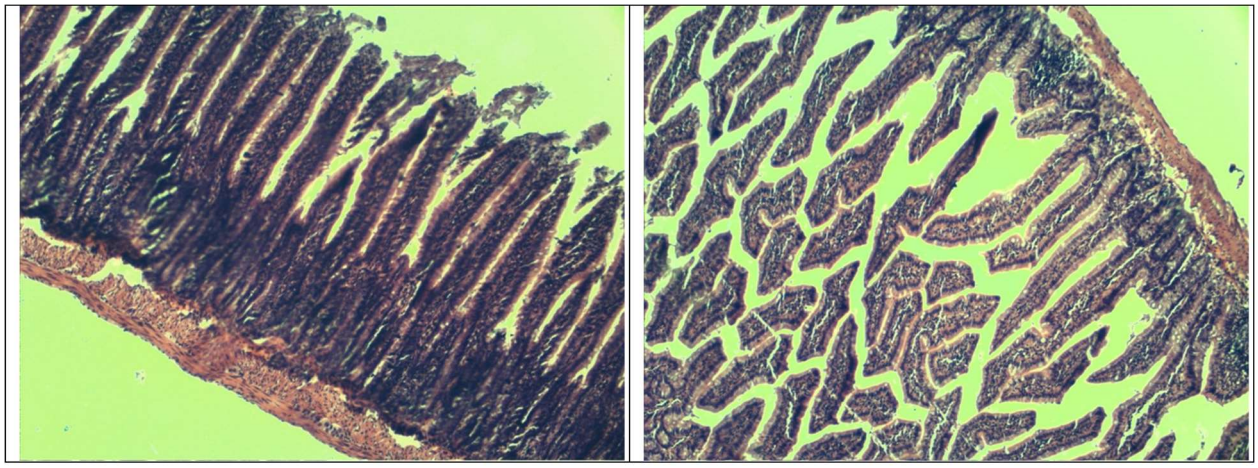
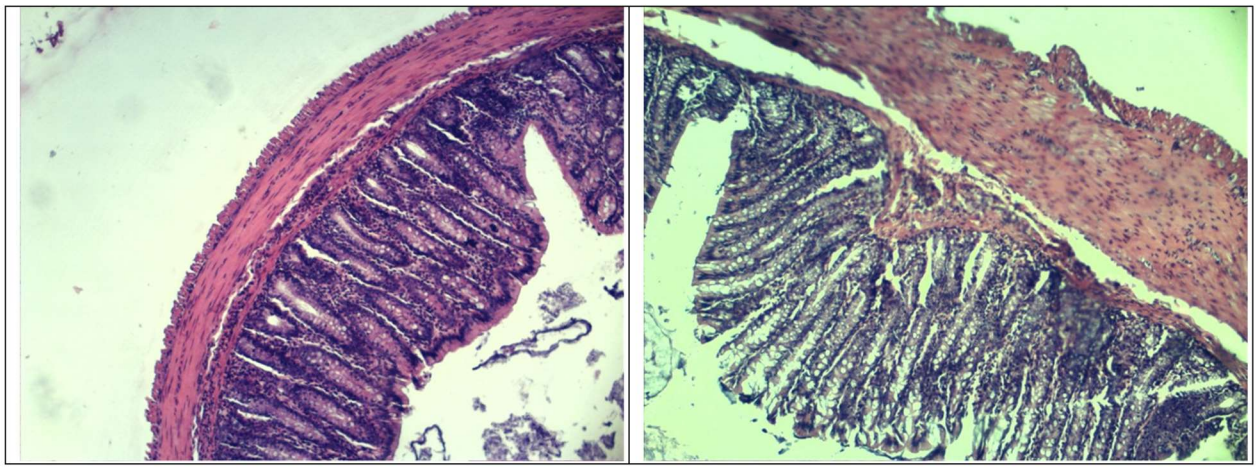


Fig. 6. A - small intestine of the control group. B - the small intestine of the experimental group. HE (hematoxylin-eosin) coloring, vol. 10, circ. 10

In all groups, the “villus-crypt” system is well preserved, the villi are covered with a single-layer cylindrical limb epithelium. Areas of intense desquamation of the epithelium with the emergence of "bare or erosive" zones were not identified. The stroma of the villi and crypts is moderately infiltrated by mononuclear cells; large infiltrates, indicating an inflammatory process, were not found.

The study of the histological structures of the large intestine of the experimental animals, which were injected with the drugs, also showed the absence of any significant deviations from the control. Only a few animals of the experimental group showed a slight increase in the number of goblet cells in the crypt epithelium (Fig. 7).

Fig. 7. A - large intestine of the control group. B-colon of the experimental group. HE (hematoxylin-eosin) coloring, vol. 10, circ. 10



This was not natural, and is most likely associated with the functional state of the large intestine, and not with the administration of the drug.

Thus, our morphological studies have shown that long-term oral administration of even large doses (5000 mg / kg) of these drugs does not cause significant destructive or inflammatory changes in the liver, kidneys, spleen, stomach, small and large intestine, which confirms their practical non-toxicity for organs and tissues of the body. These data give grounds to conclude that WD preparations in various compositions are quite safe for wide clinical use.

Evaluation of the regenerative properties of wound dressing compositions

The assessment of the wound healing activity of WD was carried out using models of IIIA degree thermal burns in rats. Wounds (2 wounds per animal) were inflicted with a copper plate 1.5x1.5 cm in size, fixed on a soldering device, which made it possible to cause burns of the same size and depth in animals. The scabs were removed with 40% salicylic acid, washed with saline solution, and wound dressings were applied. A commercial preparation "NeuSkin-F" (Eucare Pharmaceuticals (P) Limited, India) was used as a comparison. In the process of applying wound dressings, it was revealed that the commercial preparation "NeuSkin-F" has very poor adhesion and congruence with the wound surface, while our RP immediately adhered to the surface of the wound beds. It was found that wound coverings under the abbreviated name CC-Qu-Ru and CC-Qu-Ru-Ph lead to the healing of wounds of the indicated area in 17.4 - 19.0 days, while NeuSkin-F - in 22.1 22.9 days, and wounds without treatment - for 25.4-26.2 days (Table 5).

Table 5.

The effect of RP on the change in the area of wounds and the timing of healing (M ± m, n = 10,)

Experiment conditions	Average wound area, cm ^{2}				Timing of wound regeneration
	3rd day	9th day	15th day	18th day	day
CC-Qu-Ru	2,4±0,07	1,7±0,03	0,6±0,01	0,01±0,01	18,8±0,2
CC-Qu-Ru-Ph	2,3±0,08	1,5±0,02	0,4±0,01	0	17,5±0,1
NeuSkin-F	2,9±0,12	2,2±0,03	1,9±0,06	0,9±0,09	22,5±0,4
Control	2,9±0,11	2,3±0,09	1,8±0,07	1,1±0,07	25,8±0,4

Note: the timing of healing is indicated until complete epithelialization of the wounds. P <0.05

As can be seen from Table 5, it took rats 25.4 - 26.2 days to complete epithelialization of experimental burn wounds without human intervention, while therapy of wounds with RP compositions based on collagen reduced the time of complete epithelialization of wounds to 17.4 - 19.0 days. Collagen-based wound dressings with BAC reduce the wound healing time by 1.45 times due to the introduction of exogenous collagen - a matrix for fibroblasts, and the addition of collagen additives such as quercetin, rutin and phillalbin leads to a significant reduction in the inflammation phase due to antimicrobial properties rutin, the antioxidant properties of quercetin and the proliferative properties of phyllalbin stimulate the proliferative activity of fibroblasts. In addition, quercetin and rutin improve blood microcirculation and cleaning of wounds from non-viable tissues, from reactive oxygen species, which accelerates the process of migration of fibroblasts and their proliferation, wound contraction. The introduction of exogenous collagen with dietary supplements provides a quick elimination of the tissue defect and the strength of the forming scar, as evidenced by the histomorphology data of scar tissue. At the same time, the commercial comparison drug "NeuSkin-F" (Eucare Pharmaceuticals (P) Limited, India), consisting of collagen without medicinal additives, showed very low wound healing properties (complete healing only on 22.5 ± 0.4 days), but we believe that that is only because of the poor adhesiveness of this drug. It is known that when NeuSkin-F is applied to a wound bed, when used in a clinic, it is fixed with additional dressings. In this regard, our WDs, which have significantly better congruence and adhesion to the wound, show an excellent result and do not require additional fixation.

The slaughter of some of the animals for morphology was carried out on the 20th day, and therefore some of the wounds did not have time to completely heal. The morphological examination of the skin was evaluated according to a 3-point system (developed by the Ipsum Pathology company (Tashkent, Uzbekistan), where histomorphological studies were carried out), to simplify the assessment of lesions: 1 point - minor changes in the form of thinning of the epidermis, minor erosions, not thick inflammatory infiltration in the dermis, the proliferation of connective tissue elements, which characterizes the regenerative activity of the tissue; 2 points - erosive changes, moderate granulation and inflammatory infiltration with insignificant connective tissue replacement; 3 points - ulcerative skin defects, dense lymphocytic infiltration, pronounced granulomatosis (Fig. 8).

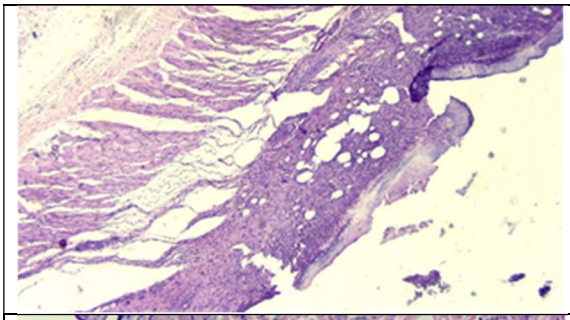
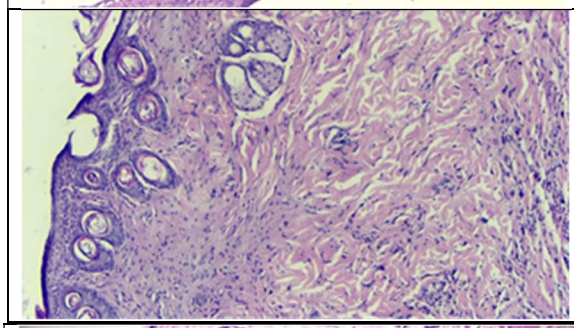
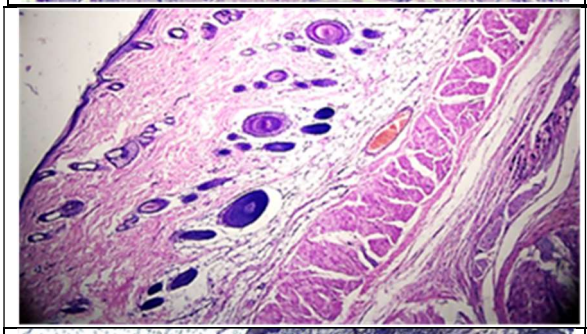
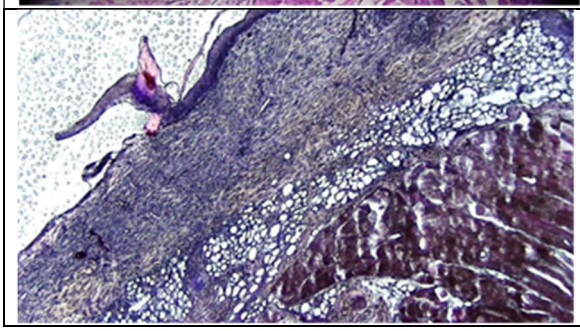
	**Control:** The integumentary epithelium is replaced by granulation tissue and is represented by an ulcerative defect. 3 points.
	**CC-K-Ru-F:** The epidermis is represented by stratified squamous keratinizing epithelium. The papillary and reticular dermis layers are well distinguishable. Sebaceous glands are noted in the dermis.
	**CC-K-Ru:** The epidermis is represented by stratified squamous keratinizing epithelium. The reticular layer of the dermis is represented by elastic fibers, the follicles of the hair follicles are visible.
	**«NeuSkin-F» - reference drug:** Detachment of the dermis and ulcerative defect of the integumentary layer. 3 points.

Fig. 8. Histomorphology of the cicatricial part of the skin of experimental animals. HE (hematoxylin-eosin) coloring, magnification 10x10.

As can be seen from Figure 8, the best effect is given by CC-Qu-Ru-Ph and CC-Qu-Ru wound dressings, epithelialization is completely completed by the time of tissue sampling, while in other groups the regenerative process continues.

CONCLUSIONS

As a result of the studies carried out, the following RP compositions have the highest specific (wound healing) activity in decreasing order of effect: CC-Qu-Ru-Ph, CC-Qu-Ru, commercial preparation NeuSkin-F.

C-Qu-Ru-Ph - RP based on collagen isolated from cattle tendons with flavonoids from Saphora japonica and phillalbin alkaloid is the most effective film for the treatment of burn wounds; possesses high congruence and adhesion to the surface of the wound bed and accelerates wound healing 1.49 times faster than untreated burn wounds of the same size, which is due to the antimicrobial, proliferative, anti-inflammatory and antioxidant properties of WD components.

The developed RP compositions contain polyfunctional plant components, are practically non-toxic, do not have a local irritating effect, and are histocompatible.

CONFLICT OF INTERESTS AND CONTRIBUTION OF AUTHORS

The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article and report on the contribution of each author.

SOURCE OF FINANCING

No funding was required for this research.

ETHICAL APPROVAL

No ethical approval is needed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

REFERENCE LIST:

1. Egorov, N.S. Industrial Microbiology - 3. A. Arkad'eva, A. M. Bezborodov, I. I. Blohina et al .; Pod red. N. S. Egorova. - M .: High. sch., 1989 .-- p. 688 (in Russ.)

2. Istrakov L.P., Bondareva L.N. The use of collagen-containing raw materials for medical and microbiological purposes.// Medicines, funds, economics, technology and prospects for obtaining. 1969, 4: 1–43

3. Kasparyants S.A., Sapozhnikova A.I., Mesropova N.V., Gordienko I.M. (1995) RF patent No. 2031597, A 23J 1/10, C 09H 1/00, C 14C 1/08. A method of producing collagen sol. // Application No. 5022810/13 of 02/12/92. Publ. 03/27/95. Bull. # 9.

4. Kukhareva L. V., Shamolina I. I., Polevaya E. V .. Method of preparation of tissue engineering and cell cultivation collagen by acid exstration of calf skin. // J. Cytology. 2010, 52 (7): -p. 597-602.

5. Terzi Alberta, Gallo Nunzia, Bettini Simona, Sibillano Teresa, Altamura Davide, Madaghiele Marta, Caro Liberato De, Valli Ludovico, Salvatore Luca, Sannino Alessandro, Giannini Cinzia. Sub- And Supramolecular X-Ray Characterization of Engineered Tissues From Equine Tendon, Bovine Dermis, and Fish Skin Type-I Collagen. // Review Macromol Biosci. 2020, 20 (5): e2000017. doi: 10.1002 / mabi.202000017.

6. Vidal Alessandra Roseline, Duarte Letícia Pereira, Schmidt Michele Mantelli, Cansian Rogério Luis, Fernandes Ilizandra Aparecida, Mello Renius de Oliveira, Demiate Ivo Mottin, Dornelles Rosa Cristina Prestes. Extraction and Characterization of Collagen From Sheep Slaughter By-Products Waste Manag. 2020, 102: 838-846. doi: 10.1016 / j.wasman.2019.12.004.

7. Kaulambaeva M.Z. Method for obtaining collagen from tendons of cattle. // (19) KZ (13) A4 (11) 23370.15.12.2010, bul. No. 2, - p. 2

8. Gufran Ajmal, Gunjan Vasant Bonde, Sathish Thokala, Pooja Mittal, Gayasuddin Khan, Juhi Singh, Vivek Kumar Pandey, Brahmeshwar Mishra. Ciprofloxacin HCl and quercetin functionalized electrospun nanofiber membrane: fabrication and its evaluation in full thickness wound healing Artif Cells Nanomed Biotechnol.

2019 Dec; 47 (1): 228-240. doi: 10.1080 / 21691401.2018.1548475.
9. Ariel Vilchez, Francisca Acevedo, Mara Cea, Michael Seeger, Rodrigo Navia Applications of Electrospun Nanofibers with Antioxidant Properties: A Review Nanomaterials (Basel). 2020 Jan 20; 10 (1): 175. doi: 10.3390 / nano10010175.
10. Nargis Fereydouni, Majid Darroudi, Jebrail Movaffagh, Azadeh Shahroodi, Alexandra E Butler, Shiva Ganjali, Amirhossein Sahebkar. Curcumin nanofibers for the purpose of wound healing. J Cell Physiol. 2019 May; 234 (5): 5537-5554. doi: 10.1002 / jcp.27362.
11. Jaya R Lakkakula, Derek Tantoh Ndinteh, Sandy F van Vuuren, Denise K Olivier, Rui W M Krause. Synthesis of silver nanoparticles from a Desmodium adscendens extract and its antibacterial evaluation on wound dressing material IET Nanobiotechnol. 2017 Dec; 11 (8): 1017-1026. doi: 10.1049 / iet-nbt.2017.0084.
12. Daeschlein G. Antimicrobial and antiseptic strategies in wound management. Int Wound J. 2013 Dec; 10 Suppl 1: 9-14. doi: 10.1111 / iwj.12175.
13. Mehrabani MG, Karimian R, Mehramouz B, Rahimi M, Kafil HS. Preparation of biocompatible and biodegradable silk fibroin / chitin / silver nanoparticles 3D scaffolds as a bandage for antimicrobial wound dressing. Int J Biol Macromol. 2018 Jul 15; 114: 961-971. doi: 10.1016 / j.ijbiomac.2018.03.128.
14. Menshchikova E.B, Lankin V.Z., Kandalintseva N.V. Phenolic antioxidants in biology and medicine. Structure, properties, mechanisms of action. - 2012: Germany, ed. LAP LAMBERT Academic Publishing. - from. 488
15. Tran NQ, Joung YK, Lih E, Park KD. In situ forming and rutin-releasing chitosan hydrogels as injectable dressings for dermal wound healing. Biomacromolecules. 2011 Aug 8; 12 (8): 2872-80. doi: 10.1021 / bm200326g.