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EVALUATION OF GASTROPROTECTIVE AND REGENERATIVE PROPERTIES OF FLAVONOIDS ISOLATED FROM BUDS OF SAPHORA JAPONICA IN COLLAGEN.

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ABSTRACT
Wound dressings membranes based on rat collagen with the addition of the flavonoid quercetin (RC-Qu) and rutin (RC-Ru) are effective in the treatment of purulent wounds of the skin, since in experimental models of purulent wounds these wound dressings reduce the epithelialization time of purulent wounds by 11.1 12.2 days and 8.4-9.2 days, respectively, compared to control wounds that were not treated with anything (the wounds were completely epithelized on 28.9 ± 0.6 days), which is due to the antioxidant properties of quercetin and rutin. In addition, it has been experimentally proven that gel compositions RC-Qu and RC-Ru have gastroprotective and antiulcer properties.
Keywords: flavonoid, quercetin, rutin, antiulcer activity

抽象的
基于大鼠胶原蛋白并添加类黄酮槲皮素 (RC-Qu) 和芦丁 (RC-Ru) 的伤口敷料膜可有效治疗皮肤化脓性伤口，因为在化脓性伤口的实验模型中，这些伤口敷料可减少 化脓性伤口的上皮化时间分别为 11.1、12.2 天和 8.4-9.2 天，与未用任何治疗的对照伤口相比（伤口在 28.9 ± 0.6 天完全上皮形成），这是由于槲皮素和 芦丁。 此外，实验证明凝胶组合物 RC-Qu 和 RC-Ru 具有胃保护和抗溃疡特性。
关键词：黄酮类化合物，槲皮素，芦丁，抗溃疡活性

INTRODUCTION

The invention relates to medicine, namely to new dosage forms intended for the treatment of

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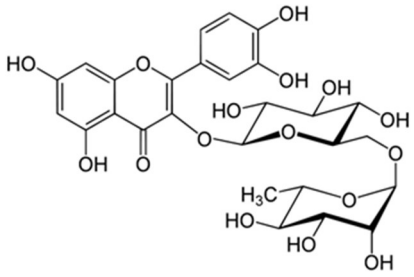
wound surfaces and methods for their preparation. It is known to use chemical-pharmaceutical and other drugs in various dosage forms for wound healing, for example, in the form of ointments, suspensions, plasters, lotions, films, gels, hydrogels, and like forms The main problem that arises during their use is the optimal combination of bactericidal and wound healing characteristics with gas permeability and high performance characteristics (coating strength, ease of separation from the wound surface, etc.).

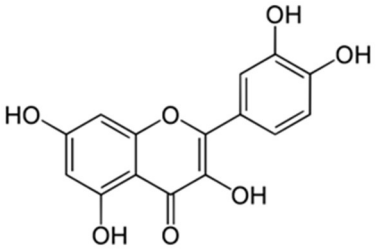
MATERIALS AND METHODS

We used rats weighing 150-200 g, rat tails. The following equipment and consumables were used in the work: Minzdray MR-96A immunoassay analyzer (China), vortex (China), thermostat TS-1/80 SPU (Russia. OJSC Smolenskoe SKTB SPU), drying cabinet IC-80-01 (Russia, OJSC "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), hematological analyzer automatic VS-3000 (China), semi-automatic biochemical analyzer VA-88A (China, Mindray), analytical balance "SHINKO" 0.001-220 gr. (Japan), Laminar box and cabinet (BBS8V1708247D, China), digital stationary pH meter (Bante210, China), portable steam sterilizer (autoclave JIBIMED, YX-280, China), automatic carousel-type "Thermo Fisher STP 120" histoprocessor (TFS, USA), rotary microtome HM 325 (TFS, USA), Axio Lab.A1 microscope (Carl Zeiss, Germany), SDPTOP digital video camera.

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



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



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

Obtaining collagen from rat tail tendons and films based on it

For these purposes, the acid extraction method was used [Kukhareva L. V., 2010.]. To obtain collagen, the severed rat tail was placed in 70% ethanol, kept at -10 ° C for at least 15 minutes, removed from the skin and, using two clamps, broke into pieces, pulling tendon threads. They were placed in a Petri dish with sterile distilled water. Tendons from one tail were split into individual fibers using fine tweezers or chopped with scissors to a size of 1-2 mm, dipped into a flask containing 150 ml of 0.1% glacial acetic acid solution and left in the refrigerator for 48 hours. Then the contents of the flask centrifuged (6000 prm for 1 h) and received up to 80 ml of a viscous solution. To the sediment was added 30

ml of 0.1% acetic acid solution, after 24 hours it was centrifuged and up to 40 ml of collagen solution was obtained. The collagen solution was dialyzed against water prior to use. Dialyzed collagen solutions were applied 40 ml. on Petri dishes (90 mm in diameter). The collagen film was compacted with ammonia vapor. Flavonoids were added to collagen before polymerization.

Creation of models of purulent wounds

Models of purulent wounds were modeled on outbred white rats, males weighing 150-170 g. Operations were performed under ether anesthesia. On the dipillated areas of the backs of the animals, full-thickness skin wounds with a size of 2.0 x 2.0 cm were formed using a stencil. The fascial skin flap marked with a square stencil 2.0 x 2.0 cm was excised with a sharp scalpel. The muscular bottom of the wound was crushed with a Kocher forceps. The tampons were moistened with a microbial mixture and applied to the wounds at the rate of 0.2 ml per wound of a mixture containing microorganisms of the following strains: No. 9572 Candida albicans Berkhant, No. M-3-87 Staphylacoccus epidermitis, No. ATCC coli 25922 Escherihia Escherihia at a concentration of 1x105 microbial bodies/ml. Each animal was kept in a separate cage. 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 peripheral 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 histomorphology of the scarred area. To assess the rate of healing of purulent wounds, planimetric research methods were used according to the standard methodology of MP Tolstykh [Tolstykh MP. 2002.]. A sterile plate of cellophane or polyacryl 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 5, 12 and 20.

Creation of experimental models of ulcers to assess the antiulcer activity of flavonoids

An experimental model of immobilization stress in male rats weighing 155-180 g was created by immobilizing animals on special plates with their backs down for 24 hours. The experimental group of rats was injected intragastrically with rutin at a dose of 150 mg / kg for 9 days and 2 hours before the simulation of the pathology model. Control animals were injected with water. One day after immobilization, the animals were sacrificed by decapitation, blood aliquots were taken to determine the amount of MDA catalase activity. The second experimental indomethacin-induced model of gastric ulcer in male rats weighing 155-180 g was created by intragastric administration of indomethacin at a dose of 60 mg / kg, once. The experimental group of rats was injected intragastrically with quercetin at a dose of 150 mg / kg for 9 days and 2 hours before the simulation of the pathology model. Control animals were injected with water. 24 hours before the use of ulcerogen, animals were deprived of food with free access to water, so fasting due to the activation of anaerobic glycolysis helps to reduce the level of protective factors of the gastric mucosa. On the 11th day of the experiment, the animals were sacrificed by

decapitation, blood aliquots were taken to determine the amount of MDA and catalase activity.

Assessment of the amount of malondialdehyde and catalase activity in blood serum

Catalase activity was determined by decreasing H2O2 in the incubation mixture. Control and experimental samples were poured into 200 µl of serum, 2.5 ml of 10 mM H2O2. The reaction in the control tube was stopped immediately by the introduction of 50% trichloroacetic acid (TCA). The reaction in the test tube was stopped after 10 minutes. Then it was centrifuged for 10 minutes at 3000 prm and the supernatant was assessed on a spectrophotometer at a wavelength of 260 nm. The calculation took into account the degree of dilution, the volume of serum, the change in optical density for 1 minute, the coefficient of molar extinction of H2O2 is 0.071. The enzyme activity was expressed in µat/L. Malonic dialdehyde (MDA) was determined by colorimetric reaction with 2-thiobarbituric acid (TBA). For this, 3 ml of phosphoric acid solution and 1 ml of TBA solution were poured into 0.3 ml of blood serum and incubated in a water bath for an hour. Then it was cooled, 4 ml of butanol was added, mixed and centrifuged at 3000 prm for 10 minutes. The measurements were carried out on a spectrophotometer at a wavelength of 535 nm. The results were expressed in µmol / L. [Karkishchenko N. N., 2010].

Histomoprhological studies of organs and tissues of experimental animals

Studies on histomoprhology were 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 animals were fixed in 10% buffered formalin solution for 24 hours. After fixation, the organs were run into a Thermo Fisher STP 120 automatic carousel histrocessor (TFS, USA) for dehydration, impregnation, and waxing. 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

Damage to the mucous membrane is a fairly widespread pathology, especially in patients who systematically use non-steroidal anti-inflammatory drugs. There is evidence of gastroprotective, antioxidant, hepatoprotective, capillary-protective, anti-inflammatory and antihistaminic activity of the flavonoid quercetin and its glycosides, as well as their activity is noted in the treatment of neurodegenerative diseases and oncology [Batiha GE, 2020]. According to literature data, antioxidant, anti-inflammatory and other activities of quercetin are noted when taking quercetin orally starting from

100 μg/ml and above, but there is evidence that long-term administration at doses above 1000 μg/ml causes nephrotoxic effects [Andres Susanne, 2018]. In this regard, to assess the antioxidant and gastroprotective activity of domestic flavonoids of quercetin and its glycoside - rutoside or rutin, isolated from the buds of the Japanese Saphora japonica at the Pharmaceutical Institute of the Ministry of Health of the Republic of Uzbekistan, we have chosen a dose of 150 μg/ml. Quercetin and rutin at concentrations of 150 μg / ml ex situ were introduced into a collagen gel obtained by acid extraction from rat tail tendons. Gel dosage forms of collagen and flavonoids were studied for gastro protective activity in experimental models of stomach ulcers.

We have created an experimental model of immobilization stress in male rats weighing 155-180 g, by immobilizing the animals on special plates with their backs down for 24 hours. A collagen gel with rutin (RC-Ru), 1 ml, was injected intragastrically to the experimental group of rats before the simulation of ulcers for 9 days and 2 hours before the simulation of the pathology model. Control animals were injected with water. After immobilization, the animals were sacrificed by decapitation; blood aliquots were taken to determine the amount of MDA and catalase activity. As a result of the experiment, it was found that in rats subjected to immobilization stress for 24 hours, there was a pronounced damage to the gastric mucosa, manifested in the formation of small-point, round and oval-shaped defects. The total surface of the lesion is more than 10% of the surface of the stomach. In this case, the most often noted the presence of small-point and oval forms of damage. In contrast, animals that received RC-Ru showed a significant (* P≤0.01; ** P≤0.001) decrease in the area of damage to the gastric mucosa. It is noteworthy that the latter was manifested by a shaWD decrease in the degree of ulceration (Fig. 1)

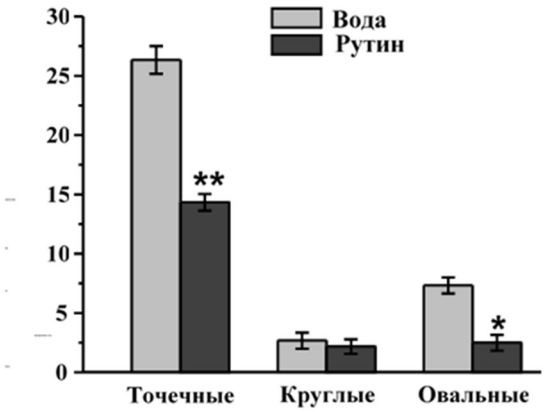


Figure: 1. The number of stomach ulcers in the experimental model of ulcers induced by immobilization stress: compared with the indicators of animals treated with water * P≤0.01; ** P≤0.001.

In addition, it was found that RC-Ru activates the enzyme catalase and significantly (P≤0.005) reduces the amount of malondialdehyde in the experimental groups of experimental animals, which also allows neutralizing the negative factors of immobilization stress (Fig. 2).

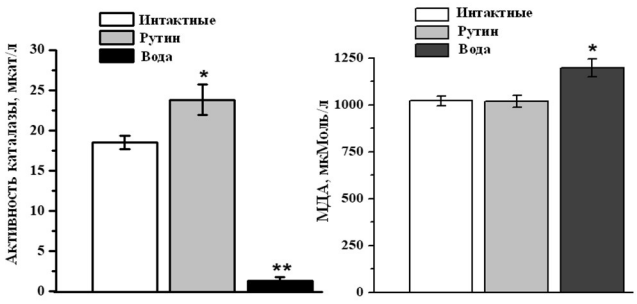


Figure: 2. Catalase activity and the amount of MDA in the blood serum of animals of the experimental model of ulcers induced by immobilization stress and treated with RC-Ru: statistical deviation of values compared

with the indices of intact animals * P≤0.005; ** P≤0.001.

A second experimental indomethacin-induced gastric ulcer model was created in male rats weighing 155-180 g. Indomethacin was administered intragastrically at a dose of 60 mg / kg, once. The experimental group of rats, prior to the simulation of ulcers, for 9 days and 2 hours before the simulation of the pathology model, were injected intragastrically with 1 ml of collagen gel containing quercetin at a dose of 150 mg / kg (RC-Qu). Control animals were injected with water. 24 hours before the use of ulcerogen, animals were deprived of food with free access to water, so fasting due to the activation of anaerobic glycolysis helps to reduce the level of protective factors of the gastric mucosa. On the 11th day of the experiment, the animals were sacrificed by decapitation, blood aliquots were taken to determine the amount of MDA and catalase activity. The stomachs of experimental animals were removed to count the ulceration of the mucous membranes (Fig. 3).

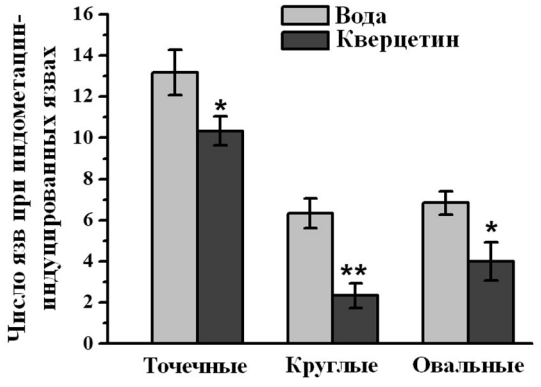


Figure: 3. The number of gastric ulcers in the experimental model of ulcers induced by indomethacin: statistical deviation of the values compared with those of animals treated with placebo (water) * P≤0.05; ** P≤0.01.

As a result, it was found that RC-Qu has gastro protective properties, since against the background of taking this composition, animals showed a statistically significant (* P≤0.05 and ** P≤0.01) manifestation of a smaller number of ulcerations of the gastric mucosa (Fig.3), an increase in catalase activity and a decrease in the amount of MDA (P≤0.001) in the blood serum (Fig. 4).

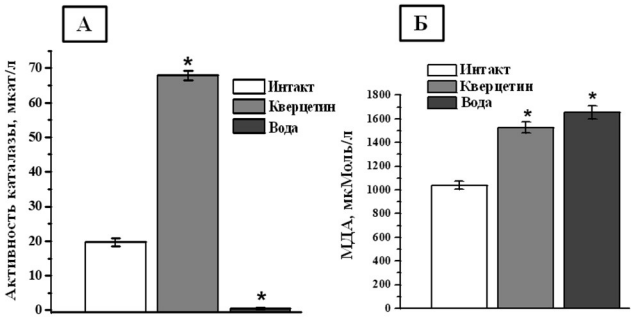


Figure: 4. A - activity of catalase and B - the amount of MDA in the blood serum of animals of the experimental model of ulcers induced by indomethacin: statistical deviation of values compared with the indices of intact animals * P≤0.001.

Thus, gel compositions RC-Qu and RC-Ru have gastro protective, antiulcer, regenerative and antioxidant properties, which is important in the treatment of wounds of various etiologies and locations.

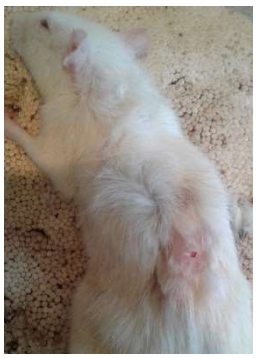
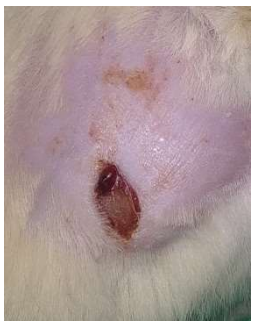
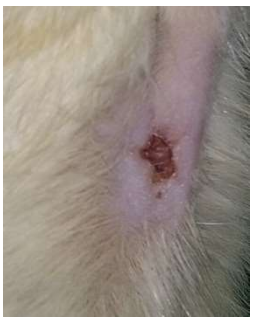
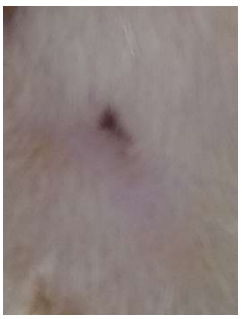
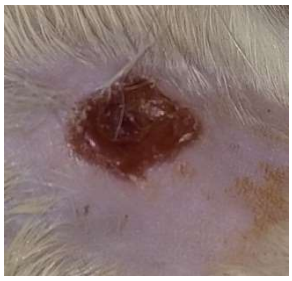
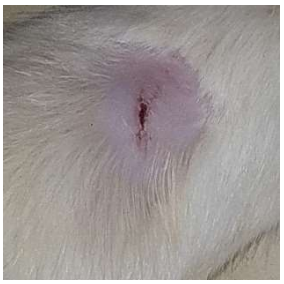
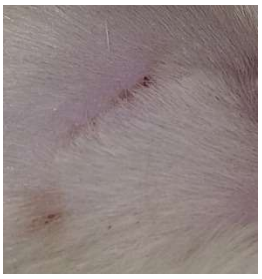
The gel forms of the RC-Qu and RC-Ru compositions were poured into Petri dishes and, under the conditions of a laminar box and ammonia vapor, film-type wound coverings were formed. The resulting films were studied for wound healing activity in experimental models of purulent wounds.

For this, a model of purulent wounds was created. The models of purulent wounds were modeled on outbred white rats, males weighing 150-200 g. The rats were divided into groups of

6 rats in each group. The operations were performed under ether anesthesia. Full-thickness skin wounds with a size of 2.0 x 2.0 cm were formed on the dipillated areas of the backs of the animals using a stencil. The muscular bottom of the wound was crushed with a Kocher forceps. Swabs were moistened with a microbial mixture and applied to the wounds based on 0.2 ml of a mixture containing microorganisms of the following strains: No. 9572 Candida albicans Berkhant, No. M-3-87 Staphylacoccus epidermitis, No. ATCC 25922 Escherihia coli at a concentration of 1x105 microbial bodies/ml. Each animal was kept in a separate cage. The therapy of wounds was performed on the third day, after the injuries of the rats, after visual determination of the presence of purulent wounds in all experimental animals. 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 histomorphology of the scarred area.

To assess the rate of healing of purulent wounds, planimetric research methods were used according to the standard method of MP Tolstykh [Tolstykh MP, 2002]. A sterile plate of cellophane or polyacryl 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. On the third day of the experiment, blood aliquots were taken from the tail vein of all groups of animals by partial resection (0.5-1.0 cm) to determine the allergenicity of WD. For histomorphology, healed or not yet completely healed parts of the skin were removed from one animal from each group on the 22nd day, after the infliction of wounds.

It has been established that collagen-based wound dressing reduces the time of wound healing by 1.5 times due to the introduction of exogenous collagen - a matrix for fibroblasts, and the addition of collagen additives such as quercetin and rutin leads to a significant reduction in the inflammation phase from 5 days to 2 3 days, due to the antimicrobial properties of rutin, as well as the antioxidant properties of quercetin and rutin. In addition, quercetin and rutin improve blood microcirculation and cleaning of wounds from non-viable tissues, which accelerates the process of migration of fibroblasts and their proliferation, wound contraction (Fig. 5 and Table 1)

		
An animal under ether anesthesia	A wound with an area of 2 cm2 was cut with a scalpel	Application of a microorganism suspension with a cotton swab
		
3rd day, after RC wound therapy	12th day, after RC wound therapy	19th day, after RC wound therapy
		
3rd day, after RC-Ru wound therapy	12th day, after RC-Ru wound therapy	19th day, after RC-Ru wound therapy

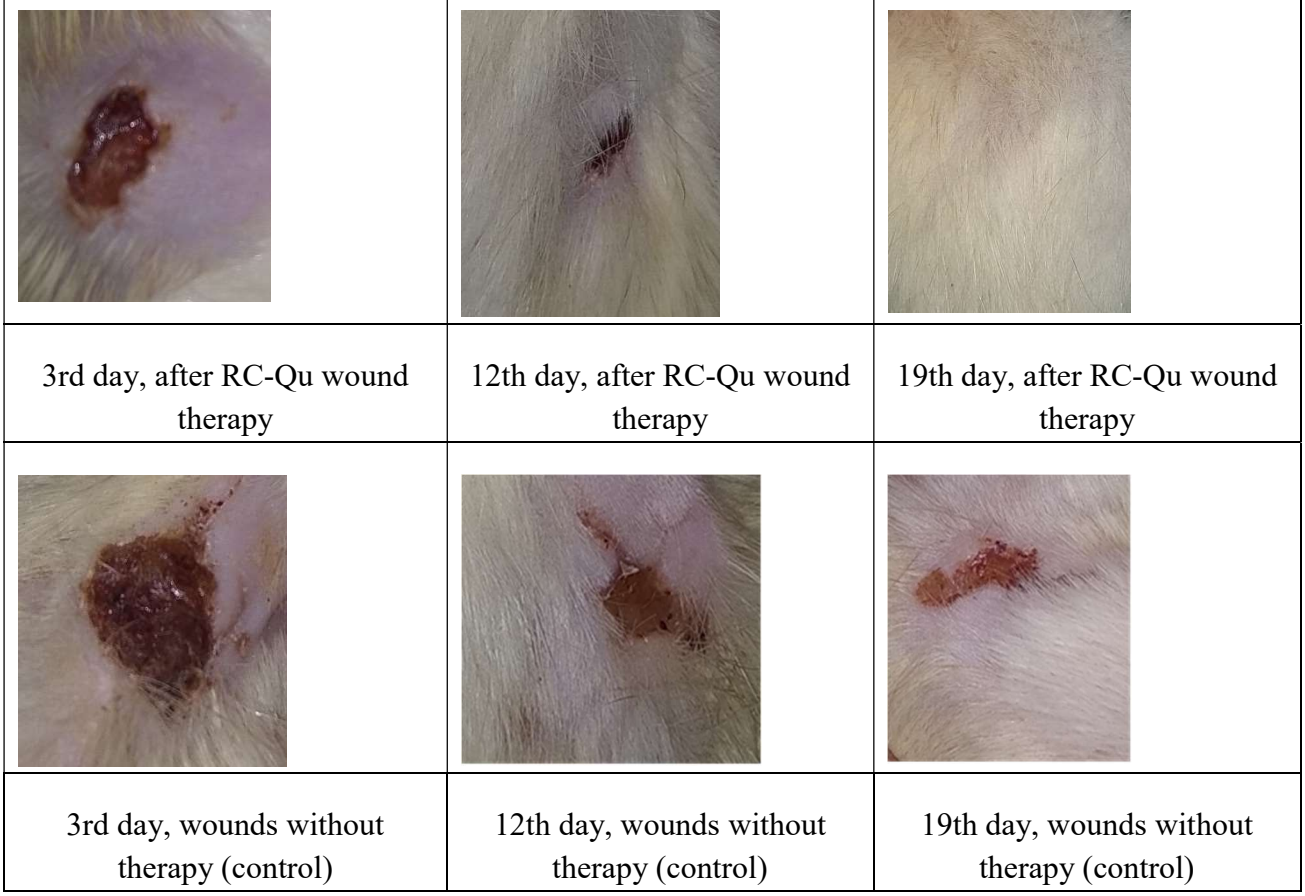


Figure: 5. Photographs of wounds on the 3rd, 12th and 19th days, after application of WD compositions to purulent wounds, purulent wounds without therapy served as control.

Table 1

Influence of wound dressing compositions on changes in wound area and healing time (M ± m, n = 10, P <0.05)

Experience conditions	Wound regeneration time			
	3rd day	12 th day	19th day	Day of complete epithelialization of wounds
RC	3,3±0,09	1,65±0,11	0,55±0,06	22,4±1,1**
RC-Ru	3,2±0,13	0,53±0,07	0,05±0,01	20,1±0,2*
RC-Qu	3,3±0,5	0,55±0,2	0	17,2±0,1*
Control	3,9±0,14	1,95±0,16	0,98±0,09	28,9±0,6

Note: * P <0.001 compared to control; ** P <0.002 compared to control

As can be seen from Table 1, for complete epithelialization of experimental purulent wounds without human intervention, it took rats 28-29.5 days, while collagen-based therapy for purulent WD wounds reduced the time for complete epithelialization of wounds to 21.3-23.5 days. and the introduction of antioxidants into the collagen coating reduces the

epithelialization time to 17.1 - 17.3 days. Collagen-based wound coverings reduce the time of wound healing by 1.5 times, due to the introduction of exogenous collagen - a matrix for fibroblasts, and the addition of collagen additives such as quercetin and rutin leads to a significant reduction in the inflammation phase from 5 days to 2-3 days. The introduction of exogenous collagen ensures the rapid elimination of the tissue defect and the strength of the forming scar, as evidenced by the data of the histological study of the formation of scar tissue. On the basis of morphological studies, depending on the severity of the regenerative-reparative elements, the state of the tissues was assessed from 0 to 5 points, where the increase in points is directly proportional to the parameters of pathological processes (Fig. 6).

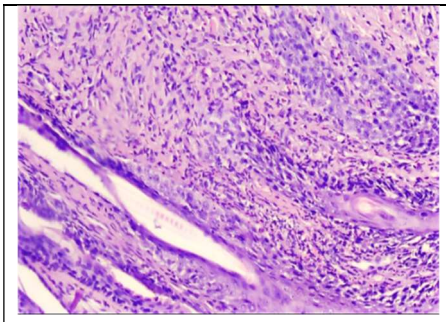
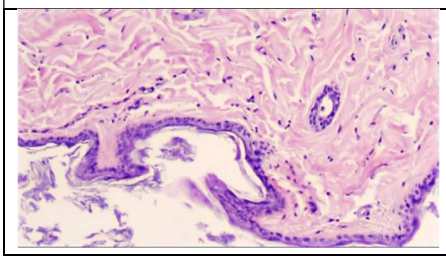
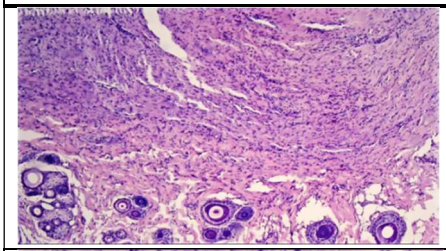
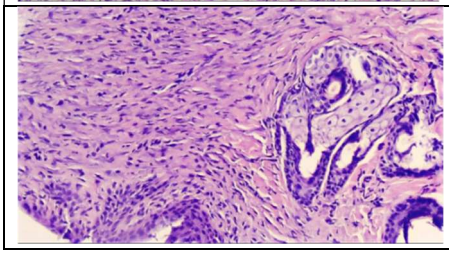
	Control: the integrity of the skin is violated, under the epidermis, pronounced mononuclear infiltration and tissue fibrosis are determined, which is characteristic of repair processes. HE coloring . Uv. 20x10. 3 points.
	**RC-Qu:** the integrity of the skin is not broken, collagen fibers of the dermis and follicles are visible under the epidermis. HE coloring Uv. 20x10. 1 point.
	**RC-Ru:** Reparative elements such as fibroblasts and lymphocytic infiltration are visible in the dermis. In this case, the skin is not disturbed. HE coloring. Uv. 20x10. 3 point
	**RC:** skin tissue with epidermal atrophy, hyperplastic in places. Elements of tissue regeneration are visible in the dermis, in the form of fibroblast proliferation. HE coloring. Uv. 20x10. 4 points.

Figure: 6. Histological evaluation of the scarred part of the skin of experimental animals.

As can be seen from Figure 6, the best effect is provided by RC-Qu wound dressings, epithelialization by the time of tissue sampling is completely completed, while in the rest of the groups the regenerative process continues, nevertheless, the skin area previously wounded and treated with RC-Ru is completely epithelized , but the regenerative processes in the dermis

continue. The histomorphology of the tissue samples from the control and RC indicates incomplete epithelization of the wounds, since they were removed on the 22nd day of the experiment, which is consistent with the data in Table 1 and photographs in Figure 5.

CONCLUSION

WD based on rat collagen with the addition of the flavonoid quercetin (RC-Qu) and rutin (RC-Ru) is effective in the treatment of purulent wounds of the skin, since in experimental models of purulent wounds these WD reduce the epithelialization time of purulent wounds by 11.1-12, 2 days and 8.4-9.2 days, respectively, compared to control wounds that were not treated with anything (the wounds were completely epithelized by 28.9 ± 0.6 days), which is due to the antioxidant properties of quercetin and rutin. In addition, it has been experimentally proven that gel compositions RC-Qu and RC-Ru have gastroprotective and antiulcer properties.

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