UDC 579.61

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The creation of wound dressings based on carbonized rice husk enriched with plant extracts (*Calendula officinalis, Salvia officinalis*)

Recently a great practical importance for the treatment skin wounds of different origins acquire wound dressings based on carbonized rice husk (CRH) enriched with phyto-extracts. The use of sorption-active substances, such as carbonized rice husks as sorption-applicator in the creation medical dressings possessed wide effectiveness on the wound healing process. This paper discusses the effectiveness of wound dressings based on biocomposites, including highly efficient sorbents with nanostructured surface (CRH), enriched with plant extracts (*Calendula officinalis, Salvia officinalis*). It was studied anti-inflammatory effects of wound dressings in experiments on laboratory rats. The effectiveness of the dressings was assessed by the changes of quantity of causative agents of purulent wound – *Staphylococcus aureus* on the surface of the wound after treatment with wound dressings.

Key words: wound dressing, phyto-extracts, carbonized rice husk (CRH), laboratory rats, *Staphylococcus aureus.*

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Өсімдік экстрактілерімен (*Calendula officinalis, Salvia officinalis*) байытылған карбонизделген күріш қауызы негізінде жара жабындысын жасау

Соңғы кездері фитоэкстрактілермен байытылған карбонизделген күріш қауызы негізіндегі жара жабындылары әр текті тері жараларын емдеуде үлкен практикалық маңыздылыққа ие. Жараның жазылу процесінде карбонизделген күріш қауызы сияқты сорбциялық белсенді заттарды сорбционды-аппликатор ретінде жара жабындысын жасауда пайдалану тиімділігі кең. Мақалада өсімдік экстрактілерімен (*Calendula officinalis, Salvia officinalis*) қаныққан наноқұрылымды беткейі бар жоғары эффективті сорбент (ККҚ) биокомпозитінің негізінде жара жабындыларының эффективтілігі қарастырылған. Лабораториялық егеуқұйрықтардың ірінді жараларын емдеуде жабындылардың қабынуға қарсы белсенділігі зерттелді. Жабындылардың эффективті әсерлері ірінді жараның қоздырғышы *Staphylococcus aureus* клеткасының жара жабындыларымен емдегеннен кейінгі санының ауытқуымен бағаланды.

Түйін сөздер: жара жабындысы, фитоэкстракт, карбонизделген күріш қауызы (ККҚ), лабораториялық егеуқұйрықтар, *Staphylococcus aureus.*

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Создание раневых повязок на основе карбонизованной рисовой шелухи, обогащенной растительными экстрактами (*Calendula officinalis, Salvia officinalis*)

В последнее время большую практическую значимость для лечения кожных ран различного происхождения приобретают раневые повязки на основе карбонизованной рисовой шелухи (КРШ), обогащенной фитоэкстрактами. В данной статье обсуждается эффективность раневых повязок на основе биокомпозитов, включающих высокоэффективные сорбенты с наноструктурной поверхностью (КРШ), обогащенные экстрактами растений *Calendula officinalis, Salvia officinalis.* Противовоспалительное действие раневых повязок изучалось в экспериментах на лабораторных крысах. Эффективность действия повязок оценивалось по изменению количества возбудителей гнойных ран – *Staphylococcus aureus* на поверхности раны после их обработки раневыми повязками.

Ключевые слова: раневые повязки, фитоэкстракты, карбонизованная рисовая шелуха (КРШ), лабораторные крысы, *Staphylococcus aureus.*

Purulent-inflammatory diseases of the skin tissue remain an ongoing problem of clinical medicine for centuries. One of the ways to optimize the problem of improving the quality of treatment of the wound is the use of dressings. Dressings play a major role in wound management and have developed greatly over the last 50 years from passive to more active types [1]. Special significance in the last decade paid to the construction new types of dressings with high antimicrobial and anti-inflammatory activities.

For the treatment of purulent wounds suggested a wide variety of methods and means of the local application. One of the areas in resolving these issues is the use of sorption-applicator methods [2] using sorption-active substances and their various modifications, possessing a complex effect on the wound healing process. By the results of a large number of experimentally clinical works, the synthetic sorbents positively proved themselves and used in medicine [3].

The use of wound dressings on the basis of CRH as a sorbent which has been impregnated on its surface with biologically active compounds, especially, plant extracts in wound treatment procedures have a great relevance. It is also important to study how the exudates from the wound accumulate on the surface of the material and these results in a large reduction in the material's capacity to effect sterilization [4].

Among these of special interest are the carbonized sorbents with nanostructural surface which manifest not only high affinity to bacterial cells but also detoxifying activity. It means that such sorbent will act as carrier for silver ions and at the same time neutralizes various toxins of purulent wound. Several studies have shown that prospective for practical use are carbon sorbents with a nanostructured surface based on vegetable raw materials [5]. Carbon obtained by carbonization such materials retains its original thinly organized structure. By varying, the conditions of carbonization can produce complex composition of carbon, giving the final product a variety of properties that determine their practical application [6].

Moreover, for a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments.

The effects of plant extracts on wound healing have been studied by a number of researchers in different parts of the world. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines [7]. Due to the antiseptic properties, plant extracts inhibit the growth of inflammatory processes, accelerate the output of pus from the wound, and accelerate wound healing. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Many medicinal plants have been used because of their anti-microbial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils [8] as well as in tannin [9].

Consequently, it has a great importance to treat purulent wounds with CRH and plant extracts which possessed antimicrobial, antiseptic and anti-inflammatory activities. The various pores of CRH absorb toxic exudates from the wound, at the same time phyto-extracts inhibit the growth of causative agents of microorganisms. That is why, it has a great relevance to exhibit the combined effects of CRH and phyto-extracts within the dressings, and to construct medical dressings based on them in wound treatment processes.

The aim of this research was to create the wound dressings based on carbonized rice husk (CRH) enriched with phyto-extracts (*Calendula officinalis, Salvia officinalis*), moreover, to investigate the wound healing activities of dressings on the purulent wound of laboratory rat.

Materials and methods

As the materials it was taken the CRH, phyto-extracts (*Calendula officinalis, Salvia officinalis*), nonwoven materials, laboratory rat.

Carbonized rise husk

In Kazakhstan, the carbon materials with nanostructured surface for the first time obtained a high-temperature by carbonization of cheap secondary vegetable raw materials – stone fruit, walnut shells and rice husks at the Institute of Combustion Problems led by Professor Z.A.Mansurova [10]. The presence of nanostructural surface of these materials enhances their attachment ability for microbial cells. Especially, the diameter of formed pores in the carbonization process can be controlled by carbonization temperature.

Calendula officinalis is a plant in the genus *Calendula* of the family Asteraceae. Plant pharmacological studies have suggested that *Calendula* extracts may have anti-viral, anti-genotoxic, and anti-inflammatory properties in vitro. In an in vitro assay, themethanol extract of *C. officinalis* exhibited antibacterial activity and both the methanol and the ethanol extracts showed antifungal activities [11].

Salvia officinalis is a perennial, evergreen subshrub, with woody stems, grayish leaves, and blue to purplish flowers. It is a member of the family Lamiaceae. In vitro inhibitory activity of sage essential oil has been reported against Gram negative microorganisms (*Escherichia coli, Klebsiella ozanae, Shigella sonnei* and *Salmonella sp.*) and Gram positive microorganisms (*Bacillus subtilis, Micrococcus luteus*) [12].

Nonwoven materials. In this research it was used surgical robes as nonwovens. Nonwovens are the materials of choice for many wound care, surgical dressing applications. Specific functions include absorbency, liquid repellency, resilience, stretch, softness, strength, washability, filtering, bacterial barrier and sterility [13].

Laboratory rats. Rats provide an excellent model for skin wound healing by allowing the standardization of the type, size, shape, and depth of the wound injury, which facilitates comparison of data between studies of healing in all mammalian species. The Wistar rat is currently one of the most popular rats used for laboratory research. Male Wistar rats were used in experiments. The age of rats was 57-61 days and the weight was 250 ± 20 g. The rats were housed in groups of five in plastic solid-bottomed cages provided with sterile dust-free bedding and temperatures of 22 ± 2 °C, relative humidity of 45–55%, and 12 h: 12 h day/night cycles. The rats were fed with specific diet which is available for experimental animals [14].

Experimental

Preparation of phyto-extracts

As extractants used alcohol-water mixtures in a ratio of 1: 1. A flask with 100 ml of alcoholic-water solution is placed 1 g dry weight of medical plant, then maintained at a temperature 80-95°C during 15-30 minutes. Tyndallization under such condition was repeated 2 times. Then this extract is cooled at room temperature, after that filtered, then pasteurized at 70-80°C within 25-30 minutes. Obtained plant phyto-extracts were checked on microbiological purity and stored in the refrigerator.

Preparation of phyto-extract/CRH biocomposite

The phyto-extract enriched CRH was synthesized by treatment with phyto-extracts of *Calendula officinalis, Salvia officinalis* at the concentrations of 1 g (CRH) to 100 ml (phyto-extracts). The flasks with phyto-extracts and CRH were shaken in Shaker for 15 minute at 200 rotations. After 24 hour CRH are filtered with filter papers.

Creation wound dressings with phyto-extract/CRH biocomposite

In the preparation of wound dressings the concentration of CRH impregnated with phyto-extracts is taken in the amount of 0.25 g per square centimeter of cloth. The dressings are prepared in size 3.5 cm × 6.5 cm. As nonwoven material it is taken surgical robes. The flasks with phyto-extracts and CRH were shaken in Shaker for 15 minute at 200 rotations. Then CRH impregnated with phyto-extracts is filtered and dried under the dry box. CRH impregnated with *Calendula officinalis, Salvia officinalis* phyto-extracts are enclosed in a nonwoven material.

Results and discussion

The laboratory rats were adapted on the condition during the experiment. Full thickness incisional wound with 23 mm diameter was made with surgical scissors and scalpel. The whole operation was taking place under lulling the rats with chloroform solution. Injured zones of skin were infected with causative agents of purulent wound *Staphylococcus aureus.* The wound of rats were infected during 1-2 min with the cotton impregnated with bacterial suspension.

After one day it was conducted healing procedures with wound dressings. Every other day rats were treated with wound dressing based on CRH and phyto-extracts of *Calendula officinalis,* *Salvia officinalis* (Fig. 1). Rats were observed for 12 days for wound healing process taken place on a skin.

Every second day the dressings were changed and it was taken smear from the wound. As illustrated in Fig. 2, the healing process of purulent wounds lasted for 12 days in control. In control variants the wounds were not treated with dressings.

However, in the case of treatment with phyto-extract impregnated CRH dressings the healing process of wound was a bit faster in comparison with control variants. The closure of wounds in the treatment with dressings on the basis of CRH/*Calendula officinalis* and CRH/*Salvia officinalis* reduced, approximately, for 2 and 4 days, respectively. The purulent wounds were healed in 7-8 days (Fig. 2).

In addition, every second days it were taken smear from wounds and inoculated on an agar media. It was counted the total bacterial count of *Staphylococcus aureus* every second day of control rats and in the treatment with phyto-extract impregnated CRH dressings.

In Table 1 was illustrated change of quantity of cells of causative agent of purulent wound infection of rat skins – *Staphylococcus aureus* after the treatment with dressings. In all variants of wounds was steady decrease of colony forming units of in 10^3 suspensions of *Staphylococcus aureus*.

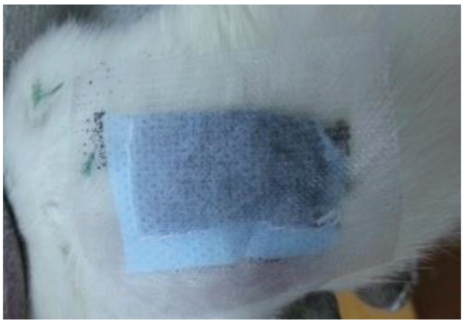

Figure 1 – Wound dressing on the rat skin

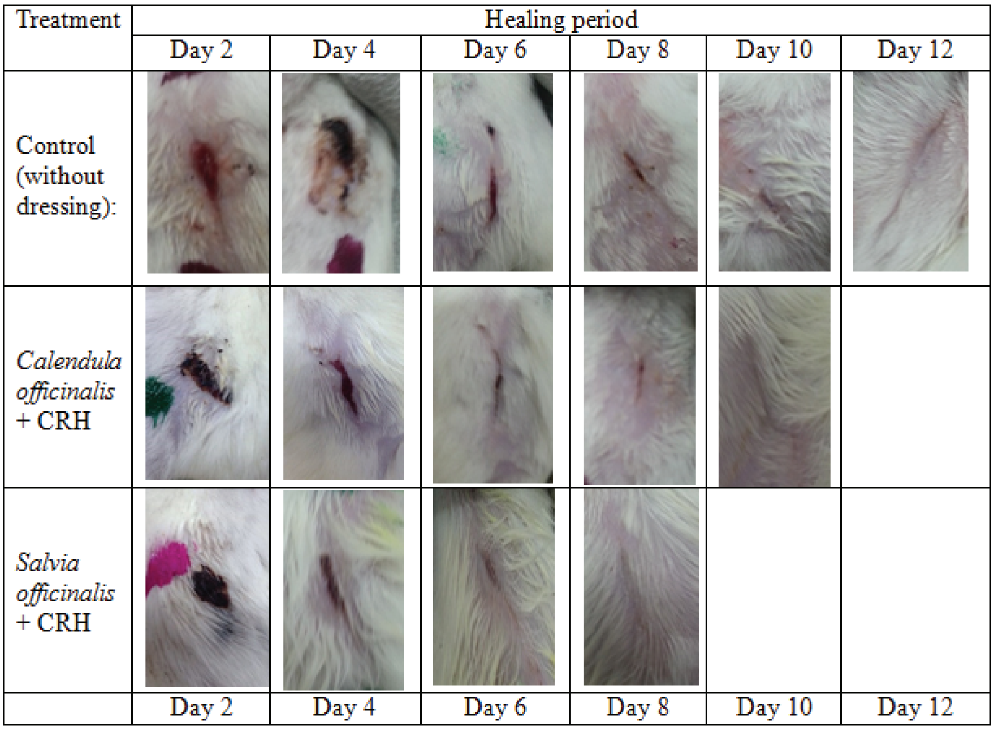

Figure 2 – Dynamics of infected wound healing in rats

Though, in the treatment with wound dressings the total bacterial counts of *Staphylococcus aureus* had sharply reduced in 6-8 days of healing. For instance, in not treated rats after 96 hour colony forming units of *Staphylococcus aureus* was $3.2*10^4$ CFU/ml, but in the presence of CRH/*Calendula officinalis* dressing colony forming units were $0.7*10^3$ CFU/ml. However, in the rat which was treated with CRH/*Salvia officinalis* based dressing colony forming units of *Staphylococcus aureus* was not found (Table 1).

The wound dressings showed antimicrobial wound-healing activities. During the wound closure had not appeared inflammatory processes of wounds. It shows anti-inflammatory, antibacterial properties of *Calendula officinalis* and *Salvia officinalis* plant extracts. But these plant extracts do not exhibit similar antimicrobial effect, the antimicrobial activities of *Salvia officinalis* a little higher than *Calendula officinalis*. Accordingly, the healing period of CRH/*Calendula officinalis* dressing showed for 2 days longer compared to dressing based on CRH/*Salvia officinalis*.

Table 1 – The change of quantity of *Staphylococcus aureus* during the wound treatment

Treatment with wound dressings	Colony forming units (CFU) of *Staphylococcus aureus* in 10^3 suspension/ml					
	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
Control	$6.9*10^6$	$4,7*10^5$	$4,9*10^4$	$3.2*10^4$	$2.3*10^5$	—
Calendula officinalis + CRH	$4,9*10^4$	$4,5*10^3$	$2.1*10^4$	$0,7*10^3$	—	—
Salvia officinalis + CRH	$4,2*10^4$	$2*10^4$	$0,3*10^2$	—	—	—

Conclusion

In this research the use of plant extracts and CRH within the dressings showed both wide effective and antimicrobial activities against a broad range of microorganisms of the purulent wound. CRH acted as carrier for phyto-extracts and at the same time neutralized various toxins of purulent wound. Moreover, CRH absorbed the exudates from the wound, and phyto-extracts *Calendula officinalis* and *Salvia officinalis* exhibited antimicrobial effects for wound healing.

The wound-healing properties of wound dressing showed high effectiveness. In the presence of CRH/ *Salvia officinalis* biocomposite the wound treatment procedures shortened for 4 days in comparison with control and for 2 days compared to CRH/*Calendula officinalis* dressing due to the anti-inflammatory and antimicrobial activities of *Salvia officinalis*. Because, according to the deviation the number of microflora of *Staphylococcus aureus,* the inhibitory effect of *Salvia officinalis* exhibited higher results than *Calendula officinalis* phyto-extracts.

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