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MORPHOLOGICAL AND BIOCHEMICAL CHANGES IN THE PROCESS OF HEALING OF BURN WOUNDS WITH OINTMENTS BASED ON CARBOXYMETHYL CHITOSAN APIS MELLIFERA

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ABSTRACT

This article presents the results of obtaining biopolymers of carboxymethyl esters of chitosan from a new promising source - dry dead bees. Dried and crushed deadwood, collected during the spring renewal of the bee colony, was used. The process was carried out under general conditions according to the general method of alkylation. Provides information about thermal skin burns and emerging pathophysiological changes in integumentary tissues. The aim of studying and treat these severe processes, burn the skin of animals. A new model of thermal skin injury in rats has been created. For the occurrence of a skin burn, a soldering gun with a flat rod PP REXANT ZD-715 12-0188 with a tip heating temperature of up to 400 ^{0}C was used. The degree of the burn was determined by histological examination of skin tissues under a microscope, which was grade IIIA and IIIB. Positive results of using an ointment based on carboxymethylchitosan (CMXZ) have been recorded.

Keywords: Chitin, chitosan, bees, carboxymethyl chitosan, hidrofill, alkylation, modification, modeling, burn injury, epithelialization.

МОРФОЛОГИЧЕСКИЕ И БИОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ В ПРОЦЕССЕ ЗАЛЕЧЕНИЯ ОЖОГОВЫХ РАН МАЗЬМИ НА ОСНОВЕ КАРБОКСИМЕТИЛ-ХИТОЗАНА APIS MELLIFERA

АННОТАЦИЯ

В данной статье представлены результаты получения биополимеров карбоксиметил эфиров хитозана из нового перспективного источника – сухого подмора пчел. Использован высушенный и измельченный подмор, собранный во время весеннего обновления пчелиной семьи. Процесс проводили в общих

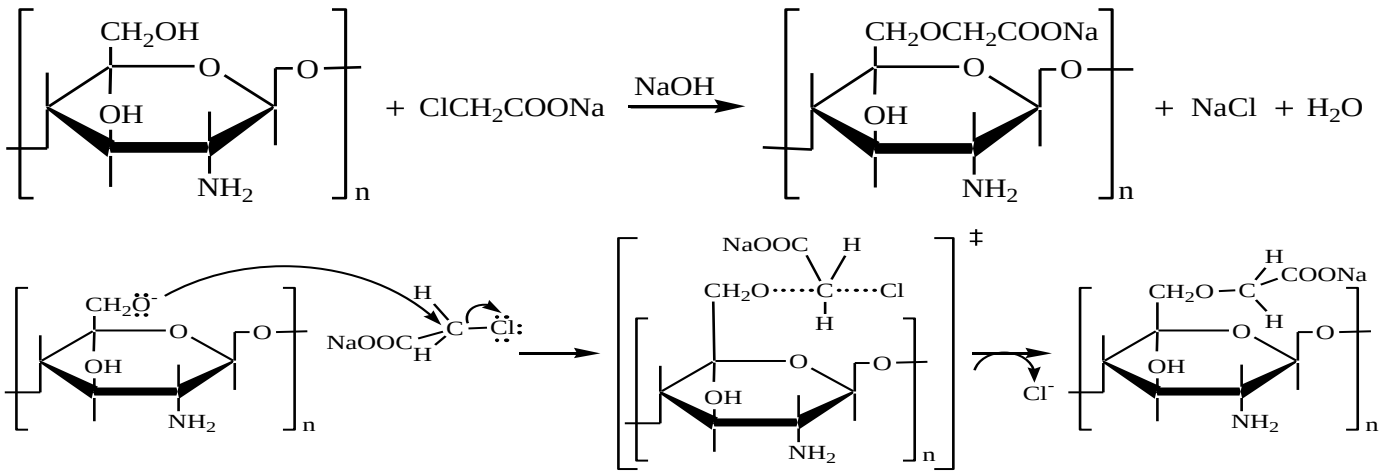
условиях по общему методу алкилирования. Приведены сведения о термических ожогах кожи и возникающих патофизиологических изменениях покровных тканей. Цель изучения и лечения этих тяжелых процессов - сжигание кожи животных. Создана новая модель термического повреждения кожи у крыс. Для возникновения ожога кожи использовали паяльник с плоским стержнем ПП REXANT ZD-715 12-0188 с температурой нагрева жала до 400 ^{0}C. Степень ожога определяли при гистологическом исследовании тканей кожи под микроскопом, которая была IIIA и IIIБ степени. Зафиксированы положительные результаты применения мази на основе карбоксиметилхитозана (КМХЗ).

Ключевые слова: Хитин, хитозан, подмор пчел, карбоксиметилхитозан (КМХЗ), гидрофил, алкилирования, модификация, моделирование, ожоговая травма, эпителизация.

Chitosan and its derivative, carboxymethylchitosan, are the most common biologically active polymers. Due to their high biological activity, these biopolymers are actively introduced into various spheres of human life.

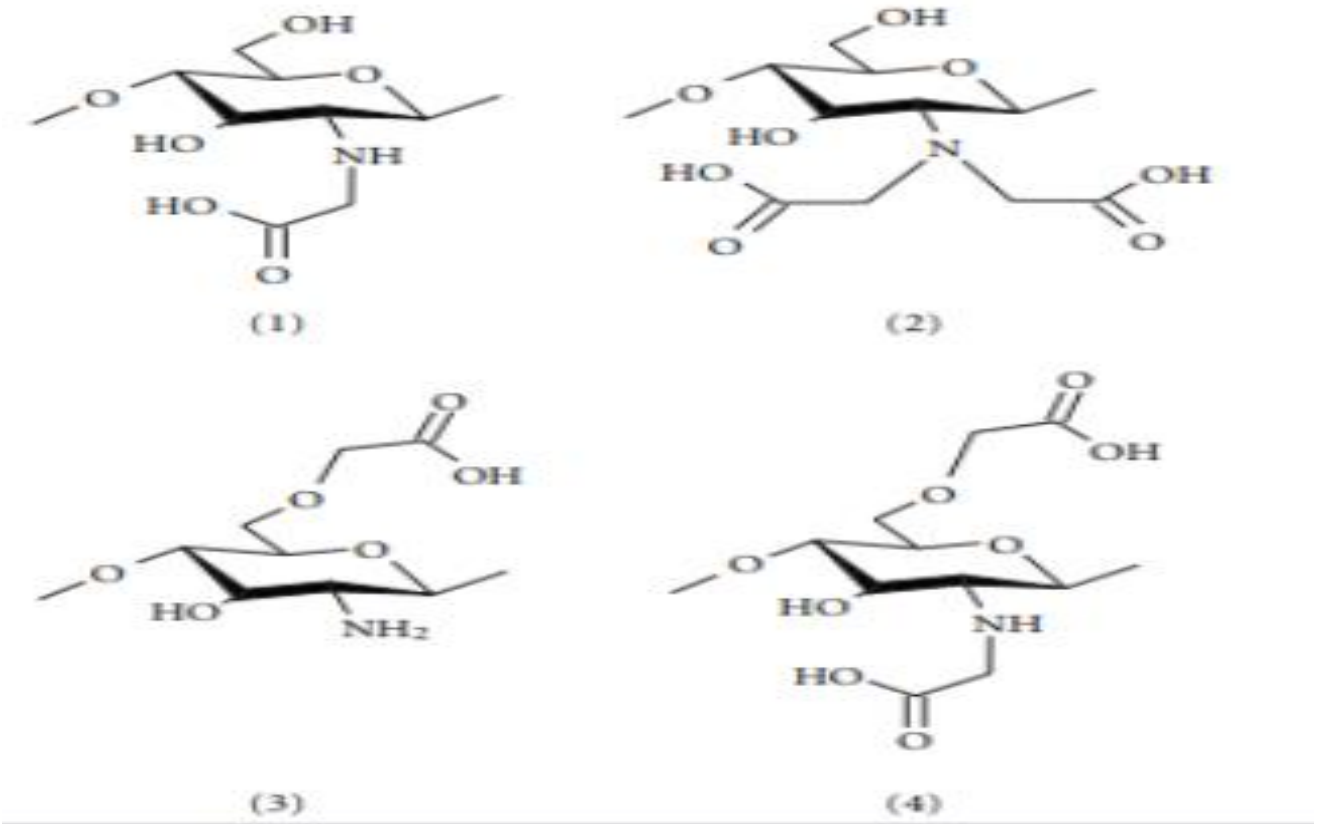
Carboxymethylchitosan KMHZ prepared the addition of a carboxymethyl group to the structure of chitosan. This modification increases its solubility in neutral and basic solutions without affecting other important characteristics [1]. CMCP is produced by carboxymethylation of the hydroxyl and amine groups of chitosan [2]. Different replacement patterns can be obtained depending on the reaction temperature used (Figure-1).

At room temperature, O-substitution is preferred, whereas at higher temperature, N-substitution is an effective pathway. Taking into account the reaction conditions and reactants, various derivatives can be produced, i.e. N-, O-, N, O- or N, N-dicarboxymethyl chitosan [3].



Recently, there has been an increase in interest in chitosan and its derivatives. In addition, they have good biological activity, radiation resistance, and the ability to form films.

These polymers fully meet the above requirements, since they are biodegradable without the formation of harmful substances, are not scarce and relatively inexpensive in relation to products for medicine.



1-Figure. The chemical structure of different types of chitosan carboxymethyl (CMHZ) is: (1) N-CMHZ, (2) N,N-CMHZ, (3) O-CMHZ, and (3) N,O-CMHZ (shows a modification in the D-glucosamine unit).

In this regard, this gives us researchers the opportunity to consider honey bees, that is, bee subpestilence, as a new promising method for producing chitin and chitosan [4]. The strength of the bee colony (the mass of worker bees in the bee colony, measured in kg) is, on average, 7.5-8 kg. In the summer during the period of active honey harvest and in the spring after wintering, the bee colony is renewed by almost 60-80% [5]. The synthesis of CMCP has been carried out on the basis of the methodology presented in [6] literature.

Special attention should be paid to the fact that the samples after the carboxymethylation reaction of chitosan are dissolved in water, while the original

chitosan is insoluble in water. This is a consequence of the introduction of hydrophilic carboxymethyl groups, which once again confirms the fact of the formation of CMHZ [7-8].

The skin is the largest organ in the body, and its destruction, especially caused by burns, can be life-threatening. Skin burns are responsible for pathophysiological changes in the body, leading to severe forms of burn disease, in which a number of complications are manifested, such as escalation of infection and high mortality, as well as prolonged hospitalization of the patient [9]. With a large area of damage, burns can turn into a systemic problem affecting a wide range of organs [10].

Burn injury is known as the most traumatic wound. In clinical practice, most patients with burn injury suffer severe pain during wound dressing; This necessitates the prompt effective treatment and use of advanced medicines. When evaluating a model for the care of burn wounds, the use of animals is considered appropriate in the study of the pathophysiology of the burn, as well as in the study of the effectiveness of the treatment strategy due to the complexity and heterogeneous nature of the burn [11].

The use of animal models with burns is crucial for burn research, especially for the study of the medicinal properties of new drugs, as it is known that new treatment strategies must be initially tested at an experimental level prior to clinical application [12]. An experimental model is necessary in the study of burns and their underlying mechanisms. Many animal models of burn injuries using mice, rats, rabbits, dogs, and pigs have been reported. They are widely used to study the pathology of burn wounds, the effect of systemic use of drugs, local therapy, and the effect of burn injury on the entire body [13-15].

In our study, we developed a new model for the development of burns on the skin of a test animal. White outbred imbreeding experimental rats with relatively similar body weights were selected. They were measured and divided into three groups. Group I - control rats with a body weight of 190-210 g. Group II - rats of 180-205 g, which received an ointment drug applied to the surface of a burn wound. The basis of the ointment preparation prepared by us was levomekol, to which powdered micro dispersed KMHZ has been added. CMHZ was synthesized by us from the bee subpestilence Apis Mellifera [16-17] and used to treat burn wounds. Group III, rats weighing 180 - 210 g. have been treated with monotherapy with the use of Levomekol ointment. The creation of the burn process was carried out in the order indicated in the literature [18].

The application of medicinal ointments and nutrition of rats (bread, cereal seeds, sunflower seeds) has been carried out once a day in the same period of time. Experimental animals have been slaughtered on days 2, 7, 14 and 22. Killing has been

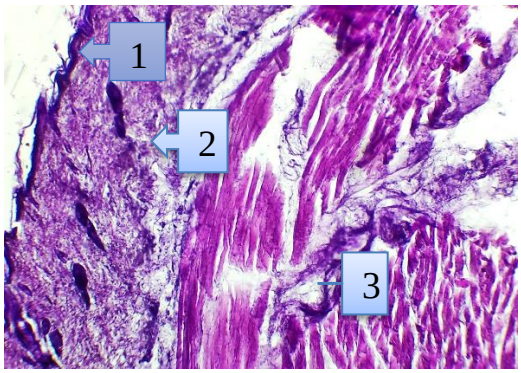
carried out after anesthesia administered intramuscularly with an anesthetic, by decapitation of animals. After that, skin areas with burn wounds have been taken from the dorsal surface of rats. After taking the skin with a burn wound, the flap was mixed into a small container with formalin with a volume of 10 ml, each container has been labeled in accordance with the group and the date of the slaughter. A container with a macropreparation in formalin has been handed over for histological examination.

In the experiment, the skin of rats has been burned with 3a-3b degrees. The site of the burn wound of the 1st group was left without any therapeutic measures, for the 2nd group a drug with the addition of carboxymethylchitosan has been used, and for the 3rd group, levomecol has been traditionally used to treat the burn wound of the skin surface.

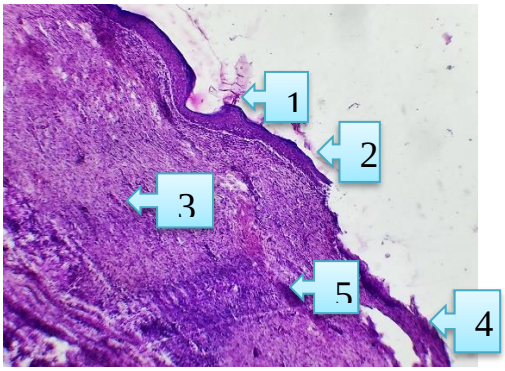
In the skin treated with carboxymethylchitosan (group 2), morphological conditions have been observed in the form of the appearance of new epithelial cells at the site of the damaged epidermis, a decrease in interstitial edema, the growth of granulation tissue in the dermal layer of the skin, rapid healing of the inflammatory process, as well as a decrease in the area of burn wounds has been observed in macroscopic preparations. In the skin tissue treated with the traditional method, some of the above morphological changes developed slowly, as evidenced by the above microscopic images.

According to the results of the experiment and histological examination of skin tissues under a microscope, it was revealed that the use of the synthesized drug has an effective effect on wound healing.

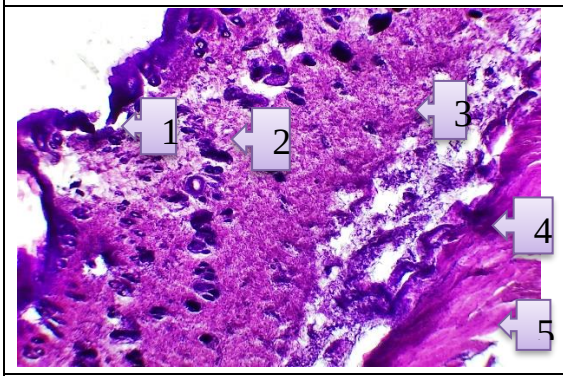
It was found that the ointment with carboxymethylchitosan has an antimicrobial effect, has pharmacological activity in the form of a specific antiseptic property, the drug does not violate the physiological functions of the skin, does not cause an allergic condition and does not cause toxicological problems.



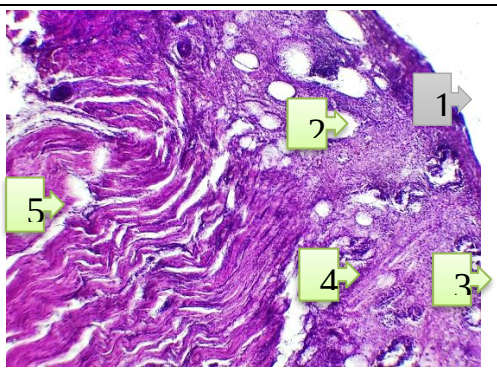
Healthy skin
Epidermis (1), granular layer (2), smooth muscle fibers (3). Hematoxylin-eosinda byalgan. About 20x40rpm



I – group.
Histological appearance of the skin. Epidermis (1), granular layer (2), inflammatory infiltration (3), incomplete wound (4), focal hemorrhage between smooth muscle tissues (5). Hematoxylin-eosin staining. About 20x40rpm



II – group.
Histological appearance of the skin. Due to epidermal regeneration (1), cell proliferation and vascular restoration (2), formation of granulation tissue (3), reduction of inflammatory infiltrate (4), edema between smooth muscle tissues (5). Hematoxylin-eosin staining. About 20x40rpm



III – group.
Histological appearance of the skin. Partial re-epithelialization of the epidermis (1), cell proliferation and vascular regeneration (2) due to tissue swelling, formation of granulation tissue (3), reduction of inflammatory infiltrate (4), swelling between smooth muscle tissues (5). Staining with hematoxylin-eosin. Approximately 20x40 vol.

Figure-2. Histological micropreparations of skin epithelialization

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