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## ACCUMULATION OF GLYCOSAMINOGLYCANS AND OXYPROLINE IN THE KNEE JOINT OF RATS WITH DIFFERENT METHODS OF ADMINISTRATION OF PREPARATIONS THAT CONTAIN HUMIC SUBSTANCES

**Purpose.** Identification of the most effective method of administering preparations based on humic substances in order to activate the accumulation of glycosaminoglycans and oxyproline within the structures of the knee joint in laboratory rats. **Materials, methods.** An experimental study was conducted on 30 sexually mature male laboratory rats. Animals were divided into 6 groups: group 1 – intact control; group 2 – drinking water and "Gumilid AS-10" 5 mg/kg + daily rubbing of 0.3 g "Gumipharm" ointment; group 3 – drinking water and "Gumilid AS-10" 5 mg/kg + electrophoresis with 0.3 g of "Gumipharm" ointment; group 4 – drinking water and "Gumilid AS-10" 5 mg/kg; group 5 – daily rubbing in of 0.3 g "Gumipharm" ointment; group 6 – electrophoresis and 0.3 g of "Gumipharm" ointment. Rubbing or electrophoresis with ointment was performed on the left knee joint. On day 21, the rats were taken out of the experiment under thiopental anesthesia, the left knee joints were isolated, homogenates were prepared, in which glycosaminoglycans and oxyproline were determined. **Scientific novelty.** In the experiment on laboratory animals, the effectiveness of the combined method of administering preparations containing humic substances without violating the integrity of tissues in order to activate the accumulation of glycosaminoglycans and oxyproline within the structures of the knee joint has been proven. **Results.** The conducted experimental study established the maximum accumulation of glycosaminoglycans in the knee joints of laboratory rats after oral administration of "Gumilid AS-10" in combination with local application of "Gumipharm" ointment by rubbing or electrophoresis. The level of glycosaminoglycans after using these methods of drug administration increased by 54.1 and 46.7 %, respectively.

Oral administration of only "Gumilid AS-10" to animals increased this indicator in the knee joint by 16.0 %. The use of only local administration of drugs contributed to an increase in glycosaminoglycans in the knee joint of rats by 24.8 % (rubbing) and 29.3 % (electrophoresis).

The accumulation of oxyproline in the knee joints of rats after different methods of introducing humic substances exhibited the same character. The level of oxyproline in the joint tissue increased by 60.6 % after oral administration + rubbing in the ointment, by 47.5 after oral administration + electrophoresis with ointment, by 12.1 % after oral administration, by 40.7 % after rubbing in the ointment and by 40.6 % after electrophoresis with ointment. **Conclusion.** The most appropriate way to activate the accumulation of glycosaminoglycans and oxyproline in the structures of the knee joint in laboratory rats under the influence of humic substances is the oral administration of "Gumilid AS-10" in combination with the local use of "Gumipharm" ointment by rubbing or electrophoresis. **Key words:** laboratory rats, humic substances, accumulation of glycosaminoglycans and oxyproline.

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## НАКОПИЧЕННЯ ГЛІКОЗАМІНГЛІКАНІВ ТА ОКСИПРОЛІНУ У КОЛІННОМУ СУГЛОБІ ЩУРІВ ПРИ РІЗНИХ СПОСОБАХ ВВЕДЕННЯ ПРЕПАРАТІВ З ГУМІНОВИМИ РЕЧОВИНАМИ

**Мета.** Виявлення найбільш ефективного способу введення препаратів на основі гумінових речовин для активації накопичення глікозаміногліконів та оксипроліну в структурах колінного суглоба у лабораторних щурів. **Матеріали методи.** Експериментальне дослідження провели на 30 статевозрілих лабораторних самцях щурів. Тварини було розподілено на 6 груп: 1 – інтактний контроль, 2 – з питною водою «Гумілід АС-10» 5 мг/кг + щоденне втирання мазі 0,3 г «Гуміфарм», 3 – з питною водою «Гумілід АС-10» 5 мг/кг + електрофорез із 0,3 г мазі «Гуміфарм», 4 – з питною водою «Гумілід АС-10» 5 мг/кг, 5 – щоденне втирання мазі 0,3 г «Гуміфарм», 6 – електрофорез із 0,3 г мазі «Гуміфарм». Втирання або електрофорез із маззю здійснювали на лівий колінний суглоб. На 21 день щурів виводили з експерименту під тіопенталовим нарко-

зом, виділяли ліві колінні суглоби, готували гомогенати, в яких проводили визначення глікозамінгліканів та оксипроліну. **Наукова новизна.** В експерименті на лабораторних тваринах доведена ефективність комбінованого способу введення препаратів, що містять гумінові речовини, без порушення цілісності тканин для активації накопичення глікозамінгліканів та оксипроліну в структурах колінного суглоба щурів.

**Результати.** Проведене експериментальне дослідження встановило максимальне накопичення глікозамінгліканів у колінних суглобах лабораторних щурів після перорального введення Гумілід АС-10 у поєднанні з місцевим нанесенням мазі Гуміфарм шляхом втирання або електрофорезу. Рівень глікозамінгліканів після цих способів введення препаратів збільшився на 54,1 та 46,7 % відповідно. Тільки пероральне запровадження тваринам «Гумілід АС-10» підвищило цей показник у колінному суглобі на 16,0 %. Застосування лише місцевого введення препаратів сприяло збільшенню глікозамінгліканів у колінному суглобі щурів на 24,8 (втирання) та 29,3 % (електрофорез).

Накопичення оксипроліну в колінних суглобах щурів після різних способів введення гумінових речовин мало такий самий характер. Рівень оксипроліну збільшився у тканині суглоба на 60,6 % після перорального введення + втирання мазі, на 47,5 після перорального введення + електрофорез з маззю, на 12,1 % після перорального введення, на 40,7 % після втирання мазі та на 40,6 % після електрофорезу з маззю.

**Висновок.** Найбільш доцільним способом активації накопичення глікозамінгліканів та оксипроліну в структурах колінного суглоба у лабораторних щурів під впливом гумінових речовин є пероральне введення «Гумілід АС-10» у поєднанні з місцевим використанням мазі «Гуміфарм» шляхом втирання або електрофорезу.

**Ключові слова:** лабораторні щури, гумінові речовини, накопичення глікозамінгліканів та оксипроліну.

**Formulation of the problem.** At present, in connection with the development of new approaches to the creation of basic drugs for the treatment of diseases of the musculoskeletal system and in assessing the mechanism of action of these drugs, it is necessary to take into account the method of administration of drugs and their effect on the activation of the accumulation of glycosaminoglycans and oxyproline within the connective tissue [2; 5].

Genetic mutations that occur in animals due to inbreeding lead to connective tissue dysplasia, and in adulthood, many high-bred animals develop arthritis of various etiologies. The use of drugs that have the ability to activate the accumulation of glycosaminoglycans and oxyproline in the connective tissue is currently a relevant issue.

Dystrophic altered or crumpled areas of the articular surface of the bone, due to mechanical damage, inflammation or genetic mutations, gradually lose their luster, thin out, and become covered with star-shaped cracks. This pathological condition

of the cartilaginous tissue is called traumatic chondromalacia [1; 4]. To solve this problem, drugs with a chondroprotective effect are used.

Our attention in this regard was attracted by preparations containing humic substances. These compounds are a wide range of natural substances that have antioxidant anti-inflammatory properties, activate tissue metabolism, accelerate cell renewal, as well as the reparative and regenerative processes within damaged tissues, and also improve wound and ulcer healing [3]. On the domestic pharmaceutical market, "Gumipharm" and "Gumilid AS-10" ointments are available. The composition of the "Gumipharm" ointment includes a salt of humic acids, propolis, and alpha-tocopherol acetate. Propolis has pronounced wound healing, analgesic, anti-inflammatory and deodorizing properties. Tocopherol acetate has antioxidant properties and improves tissue regeneration processes.

As a preparation for oral administration, "Gumilid AS-10" was chosen, which is a biologically active feed additive based on humic acids isolated from environmentally friendly peat.

Taking into account the pronounced anti-inflammatory, regenerating and other biological effects of humic substances, as well as the known data on the inhibition of the synthesis of collagen and glycosaminoglycans within the matrix of the cartilage of the joints in cases of arthritis and arthrosis, we can assume certain therapeutic and prophylactic properties of preparations with humic substances when used during the development of these pathologies.

The foregoing determined the **purpose** of the experimental study – the identification of the most effective method of administering preparations based on humic substances in order to activate the accumulation of glycosaminoglycans and oxyproline within the structures of the knee joint of laboratory rats.

**Material and methods of research.** An experimental study was carried out on 30 outbred laboratory rats, male, 8 months old, with an average weight of 284 g. All animals were kept under standard light and temperature conditions of the vivarium and a standard feeding regimen. The studies were carried out on the left knee joint. Animals were divided into 6 groups, with five rats in each group:

The first group of rats served as an intact control group, kept under normal standard conditions.

The second group of animals received drinking water with the addition of humic substances in the composition of 1 % "Gumilid AS-10" preparation

(Allbiz, Ukraine) at a dose of 5 mg/kg of body weight. The calculation of the amount of administered substances was carried out taking into account the average weight of the animals and the water drunk. Additionally, the left knee joint of rats was subjected to daily rubbing of the "Gumipharm" ointment ("Farmatron", Ukraine) in the amount of 0.3 g for 1 min.

The third group of animals received drinking water with the addition of humic substances in the composition of 1 % "Gumilid" preparation at a dose of 5 mg/kg of body weight. "Gumipharm" ointment on the knee joint was applied by electrophoresis. Electrophoresis was carried out as follows: 0.3 g of "Gumipharm" ointment was applied to a shaved knee joint; active electrodes were set up parallel to each other (this condition was mandatory) so that the active substance could penetrate into the structures of the joint as much as possible. The passive electrode was placed on the shaved part of the spino-caudal region. Electrophoresis was carried out daily for 7 minutes with current strength of 0.5 A.

The fourth group of rats received only drinking water with the addition of humic substances in the composition of 1 % "Gumilid" preparation at a dose of 5 mg/kg of body weight.

In the fifth group of animals, 0.3 g of "Gumipharm" ointment was daily rubbed into the knee joint.

In the sixth group of rats, "Gumipharm" ointment was injected into the knee joint using electrophoresis.

The maintenance and removal of animals from the experiment was carried out in accordance with the provisions established by the Directive of the European Parliament and Council (2010/63/EU) and the Order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 of March 01, 2012.

On the 21st day of the experiment, rats were withdrawn from the experiment by opening the vessels and total bloodletting from the heart under thiopental anesthesia (40 mg/kg) [8]. To assess the state of collagen, the content of total oxyproline and glycosaminoglycans was determined in the knee joints, on which therapeutic measures were carried out. For the preparation of homogenates, the prepared knee joints of rats were freed from the skin, and a 100 mg sample was ground in a mortar with 1 ml of saline. The resulting homogenates were stored in a refrigerator at +3 °C for 24 hours. After that, centrifugation was performed at 3000 rpm for 15 min. The content of glycosaminoglycans in the homogenates

of the knee joints was determined using a coloring agent according to the method [7] and expressed in mg/g of the joint tissue; the content of oxyproline was determined according to the method [6] and expressed in mmol/g of the joint tissue.

Statistical processing of the obtained results was carried out using Student's t-test.

**Results and discussion.** The results of determining the content of glycosaminoglycans in the knee joints of rats after the administration of various combos of "Gumilid AS-10" and "Gumipharm" preparations containing humic substances are shown in Fig. 1.

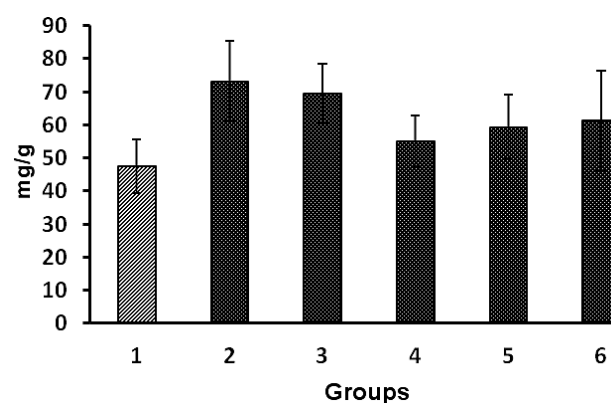


Figure 1. The content of glycosaminoglycans in the knee joints of rats after the administration of preparations containing humic substances, mg/g

Note: 1 – control; 2 – "Gumilid AS-10" with drinking water and "Gumipharm" rubbing; 3 – "Gumilid AS-10" with drinking water and "Gumipharm" electrophoresis; 4 – "Gumilid AS-10" with drinking water; 5 – "Gumipharm" rubbing, 6 – "Gumipharm" electrophoresis

As shown in Fig. 1, the content of glycosaminoglycans, which form the basis of the intercellular matrix of connective tissue, maximizes in the knee joints of rats of the 2nd and 3rd groups, which underwent combined administration of preparations of humic substances – "Gumilid AS-10" orally with local rubbing of the "Gumipharm" ointment ( $p < 0.01$ , group 2) or introducing "Gumipharm" by electrophoresis ( $p < 0.02$ , group 3). The increase in this indicator was 54.1 % in the 2nd group and 46.7 % in the 3rd group. There were no significant differences in the level of glycosaminoglycans in the joints between the 2nd and 3rd groups ( $p \geq 0.05$ ), which indicates the same effectiveness of the methods of drug administration used in these groups (Fig. 1).

Oral administration of "Gumilid AS-10" to animals caused only a tendency to increase the level of glycosaminoglycans in the knee joint of rats of the 4th group ( $0.1 < p < 0.05$ ) and increased this indicator by 16.0 % compared with the values

in the intact control group. The result obtained indicates a very low efficiency of accumulation of glycosaminoglycans in the joints of animals after oral administration of humic substances (Fig. 1).

Local administration of the "Gumipharm" ointment by rubbing in the 5th group contributed to a significant increase in the content of glycosaminoglycans in the knee joints of animals by 24.8 % ( $p < 0.05$ ). The introduction of an ointment with humic substances into the knee joint of rats using electrophoresis in the 6th group led to an increase in glycosaminoglycans in the joint by 29.3 % ( $p < 0.02$ ). There was no significant difference in this indicator between the 5th and 6th groups ( $p > 0.05$ , Fig. 1).

The picture of the accumulation of oxyproline, the main component of connective tissue collagen, under the influence of the introduction of preparations with humic substances had the same character. As can be seen from the results shown in Figure 2, the maximum content of oxyproline was recorded in the knee joints of the 2nd group of rats, which were administered the "Gumilid AS-10" humic substances with drinking water in combination with the local application of the "Gumipharm" ointment. The level of oxyproline in the knee joints of rats of this group increased by 60.6 % ( $p < 0.001$ ). Approximately the same efficiency of accumulation of oxyproline in the joints was noted after oral administration of "Gumilid AS-10" against the background of electrophoresis with "Gumipharm" in rats of the 3rd group. In this case, the level of oxyproline increased by 47.5 %. There was no significant difference in this indicator between the 2nd and 3rd groups ( $p > 0.05$ , Fig. 2).

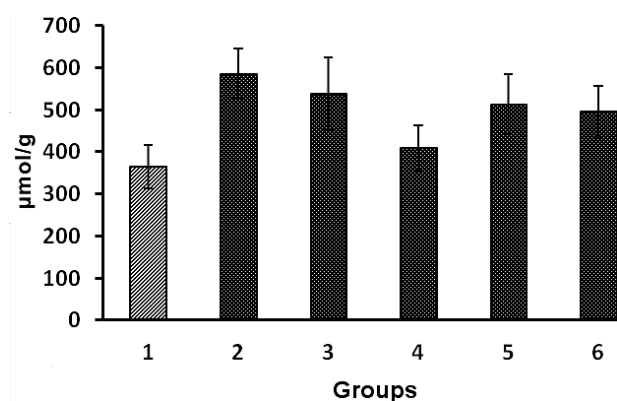


Figure 2. The content of oxyproline in the knee joints of rats after the administration of preparations containing humic substances, µmol/g

Note: 1 – control; 2 – "Gumilid AS-10" with drinking water and "Gumipharm" rubbing; 3 – "Gumilid AS-10" with drinking water and "Gumipharm" electrophoresis; 4 – "Gumilid AS-10" with drinking water; 5 – "Gumipharm" rubbing; 6 – "Gumipharm" electrophoresis

The lowest values of the content of oxyproline were established after the introduction of "Gumilid AS-10" in the composition of drinking water in the knee joints of the 4th group of rats. The increase in this indicator was 12.1 % and had no significant differences from the control values ( $p > 0.05$ ).

The efficiency of accumulation of oxyproline in the joints under the influence of rubbing in the "Gumipharm" ointment in the 5th group was higher than in the 4th group. An increase in oxyproline by 40.7 % was recorded ( $p < 0.02$ ). After electrophoresis procedures with "Gumipharm" ointment, the content of oxyproline in the knee joints of the 6th group increased by 40.6 % ( $p < 0.02$ , Fig. 2).

Thus, the conducted experimental study established the maximum accumulation of glycosaminoglycans and oxyproline in the knee joints of laboratory rats after oral administration of "Gumilid AS-10" in combination with local application of "Gumipharm" ointment by rubbing or electrophoresis. Given the known fact of a decrease in the metabolic activity of chondrocytes and a decrease in the synthesis of collagen and proteoglycans, respectively, in cases of arthritis and arthrosis, the results obtained suggest the therapeutic efficacy of the proposed method of administering the preparations containing humic substances. Further studies are needed to confirm this assumption.

**Conclusions.** 1. The maximum accumulation of glycosaminoglycans in the connective tissue of the knee joint in rats (by 54.1 and 46.7 %) was established with the simultaneous use of oral and local administration of preparations containing humic substances.

2. After oral and topical application by rubbing or electrophoresis of preparations containing humic substances, the highest content of oxyproline in the connective tissue of the knee joint of animals was also recorded. The increase was 60.6 % and 47.5 %.

**Prospects for further research.** In the experiment on laboratory animals, the effectiveness of the combined method of administration (orally and locally) of humic substances, and their effect on the activation of the accumulation of glycosaminoglycans and oxyproline in the connective tissue of the knee joint, has been proven, which allows us to recommend this technique in the future and opens up the possibility of further clinical studies on the effectiveness of the combined method of administration of the drugs in animals and humans.

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