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Research Article

Study on the "Anti-Age" Effectiveness of Low Molecular Weight Chitosan

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Abstract

The work provides an experimental assessment of the “anti-age” effectiveness of low molecular weight chitosan (50 kDa). The study was conducted on two in vivo models: the galactose model of accelerated aging in mice (enteral administration of low molecular weight chitosan) and with multiple intradermal administrations of a low molecular weight chitosan solution to intact mice. Both models allow for the evaluation of not only the “anti-age” activity of low molecular weight chitosan but also the fundamental molecular-cellular mechanism of its action. For the quantitative assessment of the “anti-age” activity of low molecular weight chitosan, morphometric analysis of histological samples was used. As a result of the conducted study, it was shown that low molecular weight chitosan possesses pronounced “anti-age” activity on the galactose model of accelerated aging, and this effect also extends to reproductive function. When low molecular weight chitosan is administered intradermally, all morphometric parameters related to fibrillogenesis and angiogenesis are higher than in the control group. Taken together, the obtained results suggest that the “anti-age” activity of low molecular weight chitosan is mediated through the activation of tissue macrophages and the expression of tissue proteases (collagenases, elastases) as physiological factors of skin self-renewal.

Keywords: Low Molecular Weight Chitosan; Galactose Accelerated Aging Model; “Anti-Age” Activity; Skin Regeneration

Introduction

At present, chitosan is one of the most popular polysaccharides, which has found its application not only in pharmacology but also in cosmetology. Chitosan possesses a wide range of biological properties, including antimicrobial, immunostimulatory (activation of macrophages and phagocytosis), antioxidant, anti-inflammatory effects, as well as the ability to lower blood cholesterol levels and stimulate the proliferation of probiotic intestinal microflora [1]. Chitosan-based hydrogels have high biocompatibility and are used in tissue engineering for the immobilization of cells, growth factors, and regulatory peptides to stimulate cell growth and differentiation during skin regeneration, as well as for targeted drug delivery and deposition in tissues [2]. The ability of chitosan and its hydrogels to stimulate skin regeneration is widely used in the development of wound healing products. Chitosan hydrogels modified with the peptide stimulator SILVAV (Ser-Ile-Lys-Val-Ala-Val) have shown high efficacy in wound healing due to the stimulation of

keratinocyte and fibroblast growth, as well as the stimulation of angiogenesis [3]. Chitosan membranes, sponges, fibers, and hydrogels are currently among the most promising materials, which, in combination with cellular and growth factors, are used to create artificial skin and treat various wounds [4]. To stimulate skin regeneration, various nanomaterials (nanofibers, nanoparticles, nanocomposites, nanogels) based on chitosan are also used [5,6]. Membranes made from a composition of chitosan and alginic acid demonstrated high therapeutic efficacy in an experimental model of diabetic wounds in mice [7]. Chitosan coatings are among the most effective wound healing agents, which additionally stimulate nerve growth and facilitate transdermal drug delivery [8-10]. Chitosan is widely used in the creation of cosmetic compositions with “anti-age” properties [11-14]. However, when evaluating the “anti-age” effectiveness of cosmetic products, indirect evidence is often used, and there is almost no data on their effectiveness using histological research methods, including morphometric assessment of results. It is especially important to study the effects of chitosan on intact skin, as this would allow for the assessment of chitosan’s influence on the natural physiological mechanisms of skin regeneration processes. Such data would be highly valuable for modern hardware methods in cosmetology using chitosan-based compositions, including intradermal injections and the use of dermarollers.

No less interesting is the experimental evaluation of the “anti-age” effects of chitosan on the tissues of internal organs and skin in a model of accelerated aging, based on the prolonged (3-4 weeks or more) administration of large doses of galactose. Such prolonged hypergalactosemia causes changes in the internal organs of experimental animals (mice, rats), especially the liver, reproductive organs, bones, and skin, that are characteristic of aging [15-18]. Currently, there is data on the “anti-age” effectiveness of chitosan oligosaccharides (less than 1 kDa) in a galactose-induced accelerated aging model in mice, specifically confirming “anti-age” effects in the liver and kidneys [19]. Especially interesting results were obtained when assessing the impact of enteral administration of low molecular weight chitosan on age-related changes in the ovaries of mice induced by the administration of high doses of galactose. It has been shown that the therapeutic “anti-age” effect of prolonged enteral administration of low molecular weight chitosan is mediated through the activation of macrophages and phagocytosis, which have a direct impact on inflammatory factors and tissue homeostasis in the ovaries [20].

However, there is currently no data on the effect of low molecular weight chitosan (50 kDa) on the galactose model of accelerated aging in mice when assessing pathomorphological changes in the skin, liver, and testes. Such data, in conjunction with the assessment of morphological criteria during intradermal administration of low-molecular-weight chitosan, may be of interest for substantiating the molecular and cellular mechanisms of the “anti-age” efficacy of low-molecular-weight chitosan and for creating promising pharmaceutical and cosmetic compositions with confirmed “anti-age” activity.

The aim of this study is to investigate the morphological changes in the skin of mice with repeated intradermal administration of a low molecular weight chitosan solution (50 kDa) to intact mice, as well as to assess the impact of low molecular weight chitosan with enteral administration on the pathomorphological changes in the skin, liver, and testes in a galactose model of accelerated aging in mice.

Materials and Methods

Study of the effects of low molecular weight chitosan on the galactose model of accelerated aging in mice

For the study, outbred non-inbred male ICR (CD-1) mice weighing 22–24 g were used. The mice were divided into two groups: the “Experimental” group and the “Control” group, with 10 animals in each. Mice in both groups were administered high doses of galactose daily for 4 weeks: two consecutive days intraperitoneally at a dose of 500 mg per kg of body weight, followed by 1 day subcutaneously at a dose of 1000 mg per kg of body weight. Mice in the control group were given regular water to drink, while mice in the experimental group were given a 0.1% aqueous solution of low molecular weight chitosan (50 kDa) to drink. After 4 weeks of daily administration of high doses of galactose, mice from both groups were euthanized using an ether overdose. After the animals were dissected, macroscopic changes were assessed. Before euthanasia, the blood glucose levels and body weight of all the animals were measured.

The liver, skin, and testes of mice were used as the research material. The organs were fixed immediately after extraction in a 10% isotonic aqueous formalin solution, followed by standard histological processing of the samples.

The assessment of dermal thickness, volumetric density of sebaceous glands and Leydig cells, liver destruction, and the height of germinal epithelium in seminiferous tubules was conducted on sections stained with hematoxylin and eosin.

The analysis of histological samples was conducted using the AxioImager A1 microscope with the AxioCam Mrc camera (Carl Zeiss), with the help of the AxioVision software. (rel.4.7). The statistical processing of the results was conducted using the Statistica 12.0 statistical software package (StatSoft, USA). The results are presented as the mean value of the indicator and the standard error of the mean (M ± m).

Study of the effects of low molecular weight chitosan upon intradermal administration in intact mice

In the study, 20 outbred ICR (CD-1) male mice with an average body weight of 25-30 g were used. All mice had their hair shaved in the sacro-lumbar region, and the area for intradermal injections was marked 5x5 mm. All the mice were divided into 2 groups (experimental and control) with 5 animals in each. Mice in the control group received 5 intradermal injections of 10 µl of saline solution daily for 5 days in the marked 5x5 mm area. Mice in the experimental group were administered 10 µl of a 0.1% low molecular weight chitosan solution daily for 5 days in the marked 5x5 mm area. All the animals were euthanized on the 8th day after the start of the experiment by means of an ether overdose. The skin of the mice in the marked area of 5x5 mm was separated from the subcutaneous tissue to the fascia and used for histological studies. The skin of the mice was fixed immediately after extraction in a 10% isotonic aqueous formalin solution. Then followed the histological processing of samples in the STP-120 histological processing station (Thermo Scientific, USA) in isopropanol, with a final exposure in liquid paraffin. After that, the dehydrated and paraffin-impregnated tissue fragments were embedded in paraffin blocks using the EC-350 paraffin embedding station. (Thermo Scientific, USA). Histological sections with a thickness of 4-5 micrometers were prepared using a Microm microtome. (Thermo Scientific, USA). For staining using silver impregnation, Superfrost slides were used. (Thermo Scientific, USA). For staining with hematoxylin and eosin and Van Gieson, standard uncoated histological slides were used.

The volumetric density of hair follicles was assessed on sections stained with hematoxylin and eosin. The assessment of the quantity of collagen and elastin fibers in the skin was performed on sections stained with Van Gieson's picrofuchsin. The assessment of the number of vessels and reticular fibers in the skin was performed using silver impregnation.

After staining the slides according to the selected methods, the sections were scanned using a scanner and the KFBio software (China). Then, using this program, a coordinate grid was overlaid on the section photographs, and morphometry of the studied samples was performed. For morphometry, a closed test system of 100 regularly spaced points with an area of $3.64x10^5$ μm^2 was used. The evaluation was conducted at a magnification of x200.

Results

Study of the effects of low molecular weight chitosan on the galactose model of accelerated aging in mice

- **Liver:** In the liver of animals from the control group, pronounced fatty degeneration was observed in almost 100% of hepatocytes. The cytoplasm of the cells is loose and clumpy, the nuclei of the hepatocytes are of different sizes, and there is a large number of multinucleated cells. Monocytic and lymphoid infiltrates are observed. In the animals that received chitosan, the liver was in much better condition. The number of hepatocytes in a state of pronounced hydropic degeneration was no more than 50%, the variation in cell nucleus sizes was much smaller, and infiltrates were not observed.
- **Kidneys:** In the kidneys of mice from both the control and experimental groups, hydropic degeneration of the tubular epithelium was observed, along with pronounced congestion of the capillaries in the interstitium and glomeruli. No significant changes between the groups were noted.
- **Skin:** In the control group animals, the volumetric density of individual sebocytes and sebaceous glands was higher. Also, in the control group that did not receive chitosan, thinning of the reticular dermis layer was observed.
- **Semenikins:** In mice from the control and experimental groups, the thickness of the germinal epithelium of the seminiferous tubules decreased compared to healthy animals. In the group receiving chitosan, the germinal epithelium was in better condition, and the number of spermatozoa was visually significantly higher. The number of Leydig cells in the animals from the experimental group was also higher.

The average blood glucose levels in the control group mice were 1 unit higher than those in the experimental group mice, whose glucose levels were within the physiological norm. The average body weight of the control group mice was 30-35% higher than the body weight of the experimental group animals.

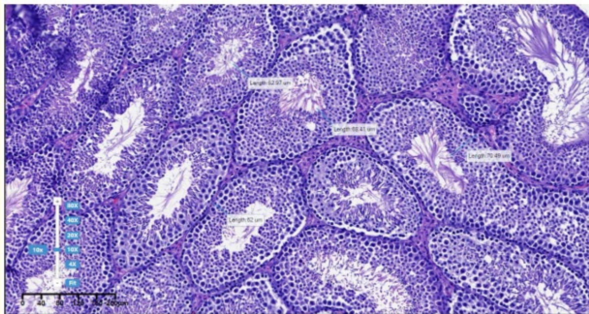

Figure 1: Mouse testis, magnification x100, experimental group. The thickness of the seminiferous tubule epithelium is preserved.

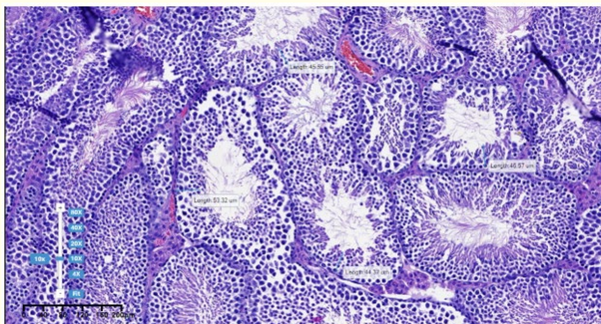

Figure 2: Mouse testis, magnification x100, control group. The thickness of the germinal epithelium of the seminiferous tubules is reduced, the lumen is enlarged, and the layer is loosened.

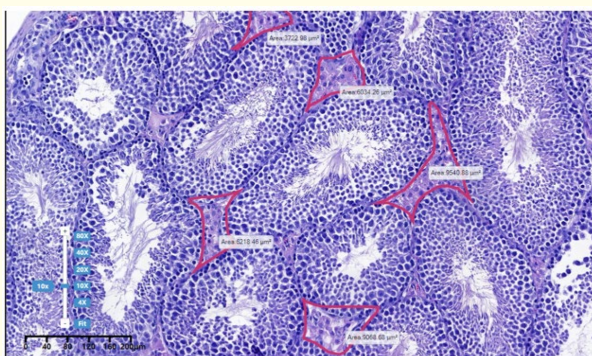

Figure 3: Mouse testis, magnification x100, experimental group. Lush, well-preserved Leydig cells.

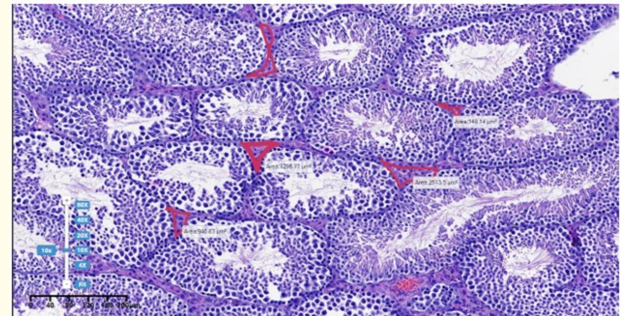

Figure 4: Mouse testis, magnification x100, control group. The volume and number of Leydig cells are reduced.

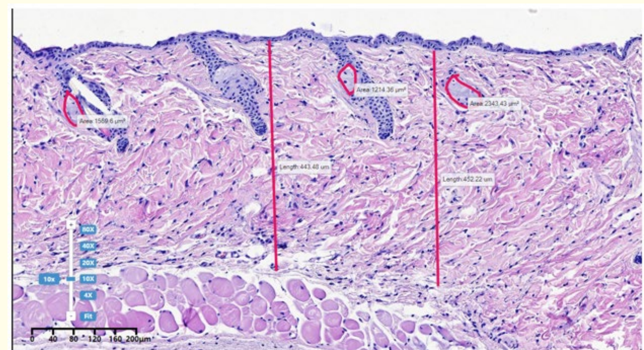

Figure 5: Mouse skin, magnification x100, experimental group. The thickness of the dermis and the condition of the sebocytes have been preserved.

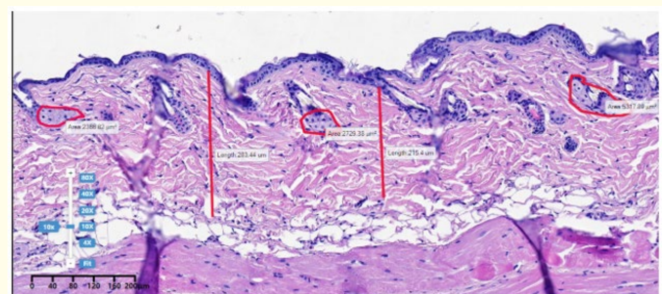

Figure 6: Mouse skin, magnification x100, control group. Increase in the volume of sebaceous gland cells, thinning of all layers of the dermis, especially the reticular layer.

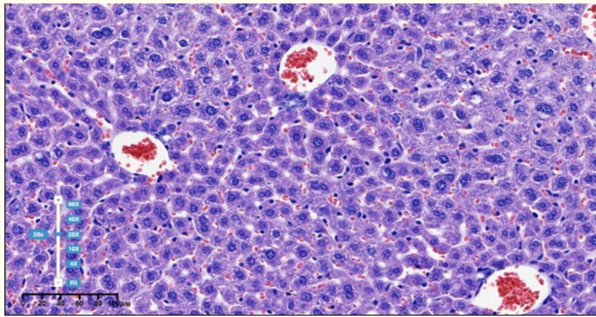

Figure 7: Mouse liver, magnification x200, experimental group. A moderate number of hepatocytes in a state of fatty degeneration.

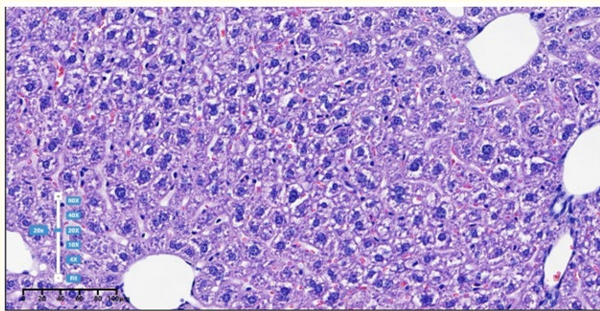

Figure 8: Mouse liver, magnification x200, control group. A significant number of hepatocytes are in a state of fatty degeneration.

Index	Control	Experience
Dermis thickness, μm	243,4 ± 51,1	376,7 ± 65,1
Volume density (Vv) of the sebaceous glands in the skin, %	1,5 ± 0,16	0,98 ± 1,7
Volumetric density of hepatocytes in the state of fatty degeneration (Vv), %	85,4 ± 7,1	64,8 ± 8,9
Height of germ cell epithelium of seminiferous tubules, μm	55 ± 7,6	66,2 ± 3,8
Volumetric density of Leydig cells (Vv), %	2,1 ± 0,3	2,7 ± 0,4

Table 1: Results of Morphometric Analysis of Histological Samples.

Study of the effects of low molecular weight chitosan upon intradermal administration in intact mice

Changes observed with the intradermal administration of a 0.1% low molecular weight chitosan solution, compared to the intradermal administration of a saline solution, are observed in all layers of the skin — epidermis, dermis, and hypodermis, in particular.

Epidermis

- **Thickening of the epidermis**: The thickness of the epidermis increases, making the skin thicker and less vulnerable to external factors.
- **Increase in the number of Langerhans cells**: Immune cells responsible for protection against infections and recovery after damage are increasing in number, which enhances the protective functions of the skin.

Derma

- **Significant increase in the volume and quantity of collagen and elastin fibers**: The amount of collagen and elastin in the dermis increases, leading to enhanced firmness and elasticity of the skin, smoothing out wrinkles.
- **Increase in the volume of the extracellular matrix**: The extracellular matrix, which supports the structure of the skin, increases in volume and becomes denser, making the skin firmer and less prone to the formation of folds and wrinkles.
- **Increase in the number of blood vessels**: The vascular network of the dermis becomes denser, leading to improved blood supply to the skin and, consequently, an increase in its regeneration rate.
- **Increase in the number of sebaceous and sweat glands**: The number of skin glands increases, making the skin softer and more resistant to external influences.
- **Increase in hair follicle volume**: An increase in the volume of epidermal stem cells in the hair papilla accelerates hair growth.

Hypodermis

- **Increase in the volume of subcutaneous adipose tissue**: An increase in the fat layer has been noted due to an increase in the number of adipocytes, which increases the volume of tissues that give the skin smoothness and firmness.

- **Increase in the volume of the vascular network**: Improves the nutrition of hair follicles, necessary for maintaining the overall condition of the skin.

Histological sections of the skin of experimental animals, after staining, are presented in figures 9-14. The results of morphometric studies of histological samples are presented in Table 2.

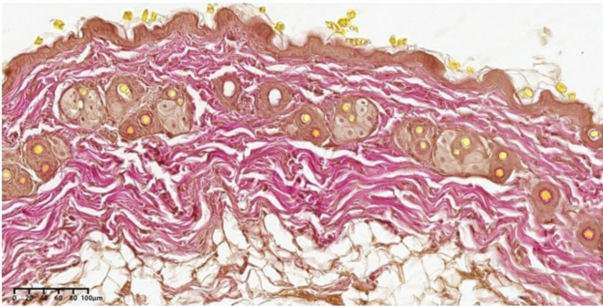

Figure 9: Mouse skin, magnification x100. Control group. Staining according to Van Gieson.

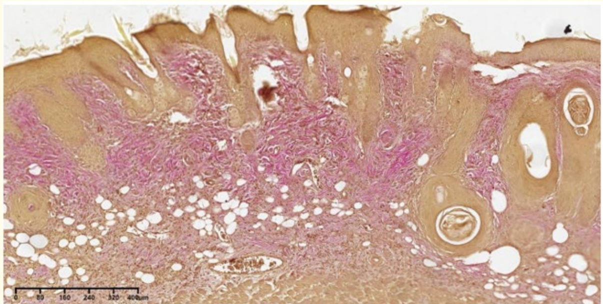

Figure 10: Mouse skin, magnification x100. Experienced group. Staining according to Van Gieson.

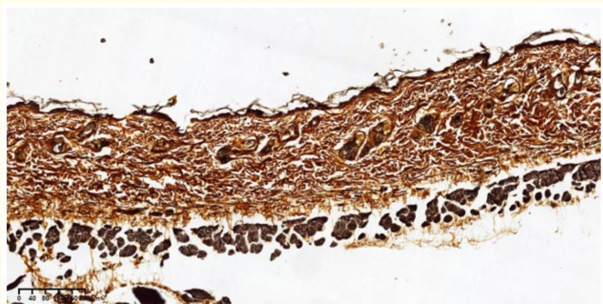

Figure 11: Mouse skin, magnification x100. Control group. Staining with silver impregnation.

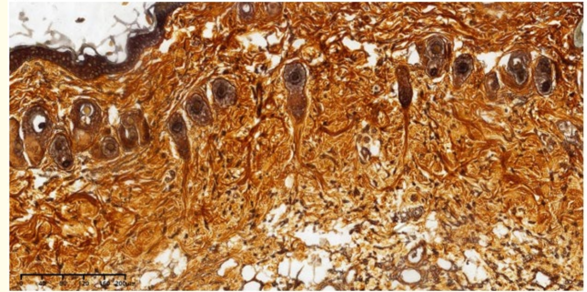

Figure 12: Mouse skin, magnification x100. Experienced group. Staining with silver impregnation.

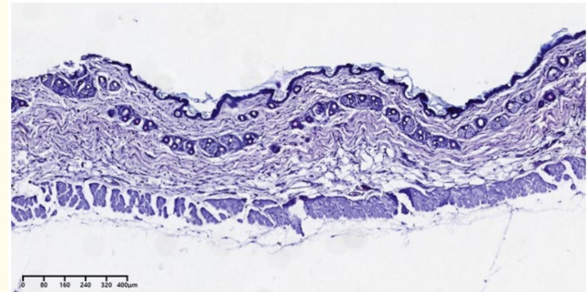

Figure 13: Mouse skin, magnification x100. Control group. Hematoxylin-eosin staining.

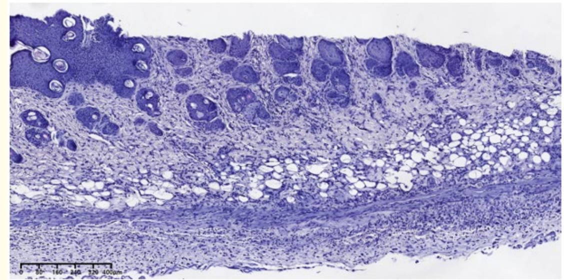

Figure 14: Mouse skin, magnification x100. Experienced group. Hematoxylin-eosin staining.

Group	Volume density of collagen fibers, Vv	Bulk density of elastin fibers, Vv	Bulk density of reticulin fibers, Vv	Bulk density of vessels, Vv	Volume density of hair follicles, Vv
Control	14,86 ± 0,48	7,88 ± 0,24	10,84 ± 0,35	44,92 ± 0,49	11,18 ± 0,36
Experience	19,02 ± 0,49	11,36 ± 0,25	13,38 ± 0,35	51,06 ± 0,36	15,08 ± 0,42

Table 2: Results of morphometric analysis of histological samples**.**

Discussion

As can be seen from the presented results, enteral administration of low molecular weight chitosan (50 kDa) to mice compensates for metabolic changes in tissues when administering high doses of galactose. At the same time, such compensation for the negative effects of hypergalactosemia is observed for all morphometric indicators: dermis thickness, volumetric density of sebaceous glands and Leydig cells, liver destruction, and the height of the germinative epithelium in the seminiferous tubules. The obtained results confirm that low molecular weight chitosan possesses pronounced “anti-age” activity. The “anti-age” effect of low molecular weight chitosan on the reproductive function of mice in a galactose model of accelerated aging is particularly interesting. In the testes of the experimental group mice, compared to the testes of the control group, the volumetric density of Leydig cells and the height of the germinal epithelium in the seminiferous tubules were higher. This may indicate compensation for the negative impact of hypergalactosemia, as a model of age-related involutional changes in the reproductive system, on hormonal balance and spermatogenesis.

With multiple intradermal administrations of a 0.1% aqueous solution of low molecular weight chitosan (50 kDa) to intact mice, compared to the control group of mice who were similarly administered saline, a positive trend and an increase in all morphometric parameters were also observed. Such an effect of intradermal injections of low-molecular-weight chitosan indicates its stimulating action on fibrillogenesis and angiogenesis. Improvement of hemodynamic indicators in the skin due to increased vascular volumetric density can positively affect the tropism of all skin cells and ensure the activation of natural physiological molecular-cellular self-renewal mechanisms of the skin and compensation for age-related changes. We find particularly interesting the effect of low molecular weight chitosan associated with the increase in the volumetric density of hair follicles, which may indicate the activation of metabolic processes that regress with age. The obtained data collectively confirm the high “anti-age” activity of low molecular weight chitosan. The most likely mechanism of such action is associated with the activation of natural physiological skin self-renewal mechanisms, which are largely related to the activity of tissue macrophages producing a complex of anti-inflammatory cytokines and tissue proteases, including elastase and collagenase.

Conclusion

Low molecular weight chitosan can be considered not only as a pharmacological agent for stimulating regenerative and plastic processes in the skin but also as an effective “anti-age” biologically active component for cosmetic formulations. At the same time, such cosmetic compositions can be applied not only topically but also in the form of intradermal injections, including administration using dermarollers.

Bibliography

1. Guan G., *et al*. “Biological Effects and Applications of Chitosan and Chito-Oligosaccharides”. *Frontiers in Physiology* 10 (2019): 516.

2. Ahmadi F., *et al*. “Chitosan based hydrogels: characteristics and pharmaceutical applications”. *Research in Pharmaceutical Sciences* 10.1 (2015): 1-16.

3. Chen Xionglin., *et al*. “SIKVAV-Modified Chitosan Hydrogel as a Skin Substitutes for Wound Closure in Mice”. *Molecules* 23 (2018): 2611.

4. Ahmed Madni., *et al*. “Recent advancements in applications of chitosan-based biomaterials for skin tissue engineering”. *Journal of Bioresources and Bioproducts* 6.1 (2021): 11-25.

5. Milena T Pelegrino., *et al*. "Chitosan-Based Nanomaterials for Skin Regeneration". *AIMS Medical Science* 4.3 (2017): 352-381.

6. Valeria Hirschberg. "Skin and obesity in childhood: an update". *AIMS Medical Science* 8.4 (2021): 311-323.

7. JSC Breder., *et al*. "Enhancement of cellular activity in hyperglycemic mice dermal wounds dressed with chitosan-alginate membranes". *Brazilian Journal of Medical and Biological Research* 53.1 (2020): e8621.

8. Ahmed S., *et al*. "Chitosan Based Dressings for Wound Care". *Immunochemistry and Immunopathology* 1 (2015): 106.

9. Gingras M., *et al*. "Nerve regeneration in a collagen-chitosan tissue-engineered skin transplanted on nude mice". *Biomaterials* 24.9 (2023): 1653-1661.

10. Zhou Xueqin., *et al*. "Effect of Low Molecular Weight Chitosans on Drug Permeation through Mouse Skin: 1. Transdermal Delivery of Baicalin". *Journal of Pharmaceutical Sciences* 99.7 (2019): 2991-2998.

11. Rejinold NS., *et al*. "Chitosan hybrids for cosmeceutical applications in skin, hair and dental care: an update". *Emergent Material* 4 (2021): 1125–1142.

12. Sionkowska Alina., *et al*. "Preparation and characterization of collagen/chitosan/hyaluronic acid thin films for application in hair care cosmetics". *Pure and Applied Chemistry* 89.12 (2017): 1829-1839.

13. Da-In Choi., *et al*. "Development of Anti-aging Cosmetics Using Chitosan/ γ-Cyclodextrin/Fucoidan Nanoparticles (Nanogel) Based on the Drug Delivery System". *Asian Journal of Beauty and Cosmetology* 21.3 (2023): 441-452.

14. Kulka Karolina and Sionkowska Alina. "Chitosan Based Materials in Cosmetic Applications: A Review". *Molecules* 28.4 (2023): 1817.

15. Parameshwaran K., *et al*. "D-Galactose Effectiveness in Modeling Aging and Therapeutic Antioxidant Treatment in Mice". *Rejuvenation Research* 13.6 (2010): 729-735.

16. Imerb N., *et al*. "D-galactose-induced aging aggravates obesity-induced bone dyshomeostasis". *Scientific Report* 12 (2022): 8580.

17. Yang J., *et al*. "Effect of Polygonum Multiflorum Thunb on liver fatty acid content in aging mice induced by D-galactose". *Lipids Health Disease* 18 (2019): 128.

18. Ahangarpour A., *et al*. "Effects of Exendin-4 on Male Reproductive Parameters of D-Galactose Induced Aging Mouse Model". *World Journal of Mensural Health* 32.3 (2014): 176-183.

19. Kong SZ., *et al*. "Anti-Aging Effect of Chitosan Oligosaccharide on d-Galactose-Induced Subacute Aging in Mice". *Marine Drugs* 16 (2018): 181.

20. Shen HH., *et al*. "Chitosan alleviates ovarian aging by enhancing macrophage phagocyte-mediated tissue homeostasis". *Immunity Ageing* 21 (2024): 10.