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Research Article

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Biologically Active Complex for the Functional Support of the Connective Tissues: Scientific Rationale, Clinical Evidence

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ABSTRACT

The prescription composition and the technology of a new type of specialized product - biologically active additive BAA have been scientifically grounded. The pharmacological properties and functional orientation of the active principles of the formulation components of the dietary supplements were studied, which made it possible to determine the quantitative and qualitative composition of the formulation which has a synergistic effect on metabolic processes in the presence of deforming osteoarthrosis: bamboo extract, glucosamine and chondroitin, aloe vera extracts, leaves and grape seeds, vitamin and mineral composition with green tea extract, and the antioxidant complex "Cifrol-5". A production technology providing high consumer performance for the developed product was approved. Clinical trials involving two groups of women aged 44-49 with deforming osteoarthritis of the knee joints confirmed the functional properties of the complex. The first group received the BAA, the second was a control group. Changes in the control indicators were registered after dietary therapy or in clinically significant periods of patient recovery. The control indicators were joint soreness, skin, hair, and nail condition, and the recovery time of postoperative sutures. The complex application of diet therapy and the basic treatment for dystrophic joint diseases and the rehabilitation measures of the postoperative period were shown to be effective. In patients, joint pain and pain index decreased, the fragility of nails and hair was reduced, the microcirculation of the nail bed improved, and the healing time of the surgical sutures decreased. The consistency of quality indicators and consumer performance was ensured by production certifications for the developed products within the requirements of international standards of the ISO 9001 and 22000 series and GMP rules.

Key words: *Biologically Active Complex - Dietary Supplements (BAA), Prescription Composition, Manufacturing Technology, Functional Properties, Efficiency, Quality, Safety, Dystrophic Joint Diseases*

INTRODUCTION

Optimizing the diet of modern people with specialized products with different specialized purposes is the most accessible and effective way to open the "windows of opportunities" for maintaining health and working capacity [1-4]. Of no less importance is the use of BAA in the prevention and treatment of common diseases [5, 6]. Solving the issues under consideration is one of the factors for improving the quality of life and life expectancy, which has been defined as an indicator of the strategic development of the state [2-4, 7].

It has been shown that nutrition can play an important role in maintaining the functional state of connective tissues, in particular the bone system, for which there is a steady increase in the number of dystrophic diseases. At the heart of this unfavorable process is a deficit of essential macro- and microelements, where the constantly-acting polyhypovitaminosis is a determining factor [8, 9].

Taking into account the biochemical and pharmacological characteristics of the acting principles, the prescription composition of the new high-tech form of BAA was developed and scientifically justified,

including: chondroitin sulfate; glucosamine sulfate; the antioxidant complex "Cifrol-5" (superoxide dismutase, hesperidin, ascorbic acid, dried hibiscus extract, tocopherol acetate, dihydroquercetin, beta-carotene, coenzyme Q_{10}); dried red wine extract; L-ornithine; para-aminobenzoic acid; calcium ascorbate; dried green tea extract; zinc citrate; dried aloe vera extract; grape seed extract; rutin; quercetin; nicotinamide; lipoic acid; copper asparaginate; bamboo extract; pyridoxine hydrochloride; thiamine mononitrate; retinol acetate; sodium selenite; biotin; and cholecalciferol.

MATERIALS AND METHODS

The objects of this study were the formulation components included in the product and in the experimental and industrial samples of the specialized product. General and special methods of researching the quality, safety, and effectiveness of dietary supplements were used in accordance with the requirements of the normative documents [10].

The clinical trial included 27 women, aged 44-49, with deforming osteoarthritis of the knee joints. Of these, 10 received BAA on an outpatient basis, and 17 received inpatient treatment at a general surgery clinic. For 12 people, cosmetic sutures were healing, 3 had a stump of an amputated limb, and for 3 - secondary sutures were healing. 19 patients were examined as a control group that did not receive BAA - 8 of them with deforming osteoarthritis, 8 patients with primary healing of cosmetic sutures, and 3 patients with secondary suture healing. The degree of effectiveness of BAA was determined according to the following indices: the decrease of pain index, the reduction of nail and hair fragility, the test for microcirculation activity (reaction of the nail bed), and healing time of postoperative sutures. The difference between the comparison parameters was considered statistically dissimilar at $p \leq 0.05$. Full-scale studies were carried out in accordance with the principles of the Helsinki Declaration of the World Medical Association (in 2000, with clarifications given at the General Assembly of the WMA, Tokyo, 2004) [11, 12], the rules of the Quality Clinical Practice of the International Conference on Harmonization (ICH GCP), ethical principles set forth in Directive 2001/20/EC of the European Union, and the requirements of national Russian legislation. Each patient signed an informed consent form to participate in the research. The research was carried out at the Department of Internal Diseases of the Faculty of Advanced Studies of the Siberian State Medical University and at City Clinical Hospital No. 3 in Tomsk (Department Head, Doctor of Medical Sciences, Professor E.B. Bukreeva).

RESULTS

The biologically active complex BAA contains physiological components of connective tissues, glucosamine and chondroitin, as well as bamboo extract, which is one of the richest natural sources of silicic acid, which plays a significant role in maintaining the integrity of connective tissues. The effective concentration of bioflavonoids, antioxidants, and other bioactive substances contained in the composition of natural extracts of aloe vera and red grape leaves and seeds promotes regeneration, maintains the tonus of epithelial tissues, and has a restorative effect. The balanced vitamin and mineral composition of the complex, in combination with green tea extract, activates microcirculation, regulates the work of respiratory enzymes in cells, increases the efficiency of oxygen utilization, and tones the body. The antioxidant complex "Cifrol-5", which is a part of the framework, enhances the positive effect of the complex on neurovegetative processes, eliminates the unwanted oxidative reactions, strengthens the body's natural defense against free radicals, protects the vascular system and connective tissues, prevents the degradation of collagen and elastin, and prevents the development of chronic diseases associated with aging and impaired protective functions.

The presented acting principles' characteristics served as the basis for the scientific substantiation of the quantitative and qualitative composition of the formulation, presented in Table 1.

Table 1. Prescription composition of BAA

No.	Component name	1 tablet contains, mg
1	**Chondroitin sulfate**	**100**
2	**Glucosamine sulfate**	**100**
	Cifrol-5	**100**
	Content of active components in 100 mg of Cifrol-5	
	Superoxide dismutase	100(Units)
	Hesperidin	20,0

3	Ascorbic acid	12,5
	Hibiscus extract dry	11,5
	Tocopherol acetate	5,0
	Dihydroquercetin	5,0
	Beta carotene	1,75
	Coenzyme Q10	1,25
4	**Dried red wine extract**	**50**
5	**Raw material for the production of biologically active food additives, L-Ornithine**	**50**
6	**Paraaminobenzoic acid**	**50**
7	**Calcium ascorbate**	**30**
8	**Dried green tea extract**	**25**
9	**Zinc citrate trihydrate (food grade)**	**24,2**
10	**Dried aloe vera extract**	**15**
11	**Grape Seed Extract**	**15**
12	**Rutin**	**15**
13	**Quercetin**	**15**
14	**Nicotinamide**	**10**
15	**Lipoic acid**	**7,5**
16	**Copper asparaginate**	**4,4**
17	**Extract of bamboo**	**3,6**
18	**Pyridoxine hydrochloride**	**1**
19	**Thiamine mononitrate**	**0,85**
20	**Retinol acetate**	**0,5**
21	**Sodium selenite**	**0,11**
22	**Biotin**	**0,025**
23	**Cholecalciferol**	**0,0025**

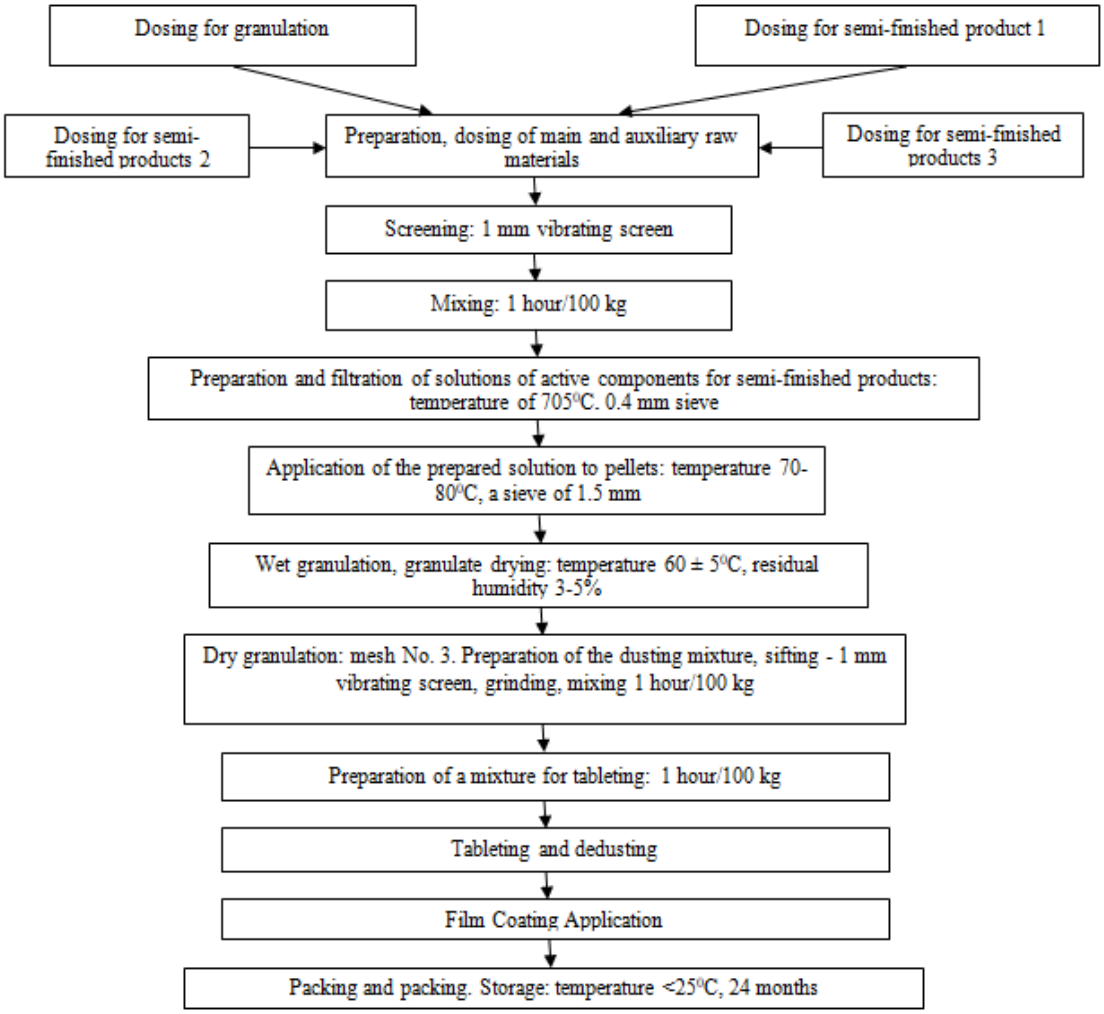

Figure 1. Technological scheme of the production of BAA

The technology producing this product includes the following main stages (Shown in Figure 1):

1. Preparation and filtration of solutions of the active components for the intermediate products of 1, 2, and 3: The required amount of water was dosed into the homogenizer reactor and heated to 70 °C. The active components were poured into the operating agitator and mixed until completely dissolved. The resulting solution was filtered through a sieve with a cell diameter of 0.4 mm into a special solution tank, and again loaded into the reactor. When the agitator was switched on at a particular temperature, and gum arabic was slowly added in small portions to the solution for the intermediate products of 1 and 3, and gum arabic, titanium dioxide, and a copper chlorophyll compound were added to the intermediate product of 2. The control at this stage was solution homogeneity.
2. Application of the prepared solution to pellets (intermediate products 1-3):
   The film coating unit was heated to a temperature of 70-80 ° C, the pellets were loaded, and the solution was sprayed, dried to a certain humidity, cooled to 25 °C, and unloaded. Pellets were sifted through a sieve with a hole diameter of 1.5 mm; there should be no lumps adhering to one another.
3. Granulate preparation:
   Wet granulation (extrusion) was conducted using demineralized water as a humectant. The homogeneity of granulate color was checked.
4. Drying the granulate:
   The wet granulate was dried at a temperature of 60 ± 5 ° C to a residual moisture content of 3-5%. The humidity and uniformity of drying was controlled by 10g granulate point selection from the upper, middle, and lower parts of the oven.
5. Dry granulation:
   It was conducted on a granulator-shredder with a grid number 3. There should be no extraneous inclusions in the presence of a fractional composition of the re-granulate.
6. Preparation of the dusting mixture:
   This step was carried out simultaneously with the dry granulation stage. First, "Cifrol-5", chondroitin sulfate, calcium ascorbate, quercetin, rutin, aloe vera, were dosed, then lipoic acid, pre-mixed with 3 kg of kafos was added. The next stage of dosing was adding bamboo extract, retinol acetate, and cholecalciferol, then biotin (pre-grind in a mortar, pores wiped with talc, then mixed with 0.5 kg of talc). At the final stage, talc, kafos, and impellose were subsequently dosed. The correspondence of the name, quantity, and series of raw materials was checked against the procedure sheet.
7. Screening:
   The powder mixture was sieved with a diameter of 1 mm shale holes. The screening was subjected to grinding. There should be no lumps and foreign objects.
8. Mixing:
   The powder mixture was placed in a V-mixer; the mixing process was 1 hour per 100 kg. Homogeneity was controlled, and foreign inclusions should have been absent when pressing the lumps with a pestle. If they were present, the mixture was re-sieved and mixed.
9. Preparation for tableting:
   Mixing was carried out sequentially in a V-shaped mixer for 1 hour per 100 kg: first, the intermediate products, then the dusting mixture and the regranulate were mixed.
   At this stage, the quality and safety control was carried out by determining the microbiological and physicochemical parameters, testing for radionuclides, and measuring the technological parameters of the mixture in an accredited production laboratory. At least, 5 units of the product were sampled. For each unit, three single samples were taken: from above, from below, and from the middle of the container with the intermediate product; an overall sample was created from the individual samples.
10. Tableting and dedusting:
    This was completed on a rotary tablet machine. Every 30 minutes during tableting, the average weight was checked by weighing 20 tablets together, and weighing 20 tablets individually. The deviations of the average mass and mass of individual tablets should not have exceeded ± 5%. Every 60 minutes, the appearance of 10 tablets was checked, and viewed from both sides. There should have been no chips, laminations, hillocks, pits or sticking. The tablet should have been smooth and strong. The finished tablets were transferred to the dusting agent.

11. Film coating:
    The necessary amount of water was metered into the homogenizer reactor, the prescribed amount of dry specialized product (DSP) was poured in small portions to the agitator and stirred for 15 minutes, then were homogenized for 10 minutes.
    The prepared DSP solution was pumped through a filter element (nylon filter with aperture diameter of 0.315-0.450 mm) into the flow reactor, the homogenizer was connected to the agitator in the operation to the consumable reactor, and the process of the film coating began.
    Film-coated tablets should have been smooth, without shells, chips, color and gloss - uniform.
12. Packing and storage: Packaging was done according to the requirements of the technical documentation for the developed product [13].
    Organoleptic, physical-chemical and microbiological indicators of quality and safety were investigated during the manufacturing process, and after 27 months of storage at a temperature of no higher than 25 ° C in a dry place to determine the expiration date. It was found that the test items were within the requirements of technical and regulatory documents, which made it possible to determine a shelf life of 2 years under the above conditions (with a "margin of safety" of 3 months).
13. The functional orientation and effectiveness of the dietary supplements were confirmed by conducting clinical trials in the patients with deforming osteoarthritis and the patients in the postoperative period.

BAA was taken in the amount of 2 tablets in the mornings once a day for 2 months together with the main therapy (basic treatment of deforming osteoarthritis, postoperative rehabilitation [14]. The level of the nutrient intake at the recommended daily dose has been shown in Table 2.

Table 2. Consumption of nutrients based on the recommended daily requirement (RDR)

Наименование	mg	% of RDR	Name	mg	% of RDR
vitamin A	1	100	selenium	0,1	142
vitamin E	10	100	copper	1,4	140
vitamin D	0,005	100	quercetin	30	120
Vitamin H	0,05	100	dihydroquercetin	10	40
vitamin B1	1,7	114	lipoic acid	15	50
vitamin B3	20	100	routine	30	100
vitamin B6	2,0	100	glucosamine sulfate	200	40
vitamin C	60	86	chondroitin sulfate	200	50
beta-carotene	3,5	70	proanthocyanides	6	12
zinc	5	100	-	-	-

Clinical control of the effectiveness was carried out depending on the group of patients being examined. The changes to control indicators were registered after the end of diet therapy, or in clinically significant periods of patient recovery.

In 10 patients, a positive effect was noted on 14th day of diet therapy. The subjects noted a decrease in joint soreness, which was confirmed by a reduction in the pain index (Figure 2).

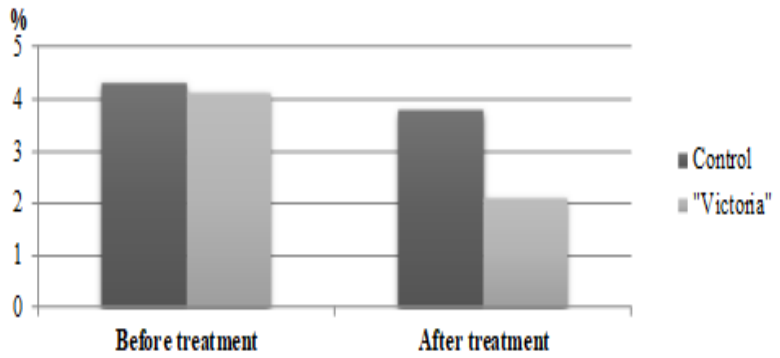

Figure 2. The reduction of joint tenderness in patients under the influence of the bioactive complex

Patients who took the product also noted the improved appearance and strengthening of hair and nails (Figure 3).

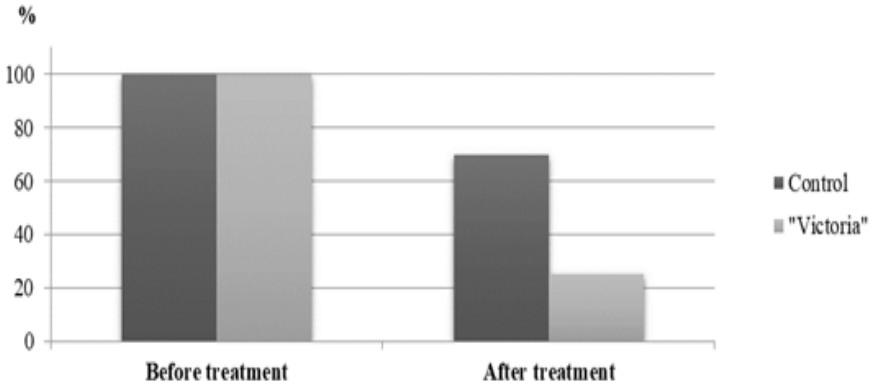

Figure 3. Reduction of nails and hair fragility under the influence of bioactive complex

The frequency of the complaints of brittle nails and hair was reduced. The observations of the doctor-cosmetologist, conducted before and after two-month intake of BAA, revealed a reduction in dryness of the skin of the face and body in 57% of the examined cases. In 64% of the clients, the complex helped to reduce swelling, and in 71% it improved the contour of the face.

A test for the microcirculation activity (nail bed reaction) showed that the subjective sensation of improvement in complexion and external attractiveness was accompanied by the activation of the microcirculatory bed. Where at the beginning of BAA intake, the same reaction of the nail bed to depression was observed in both groups, by the end of diet therapy, the speed of the microcirculatory bed increased significantly in the patients of the main group (Figure 4).

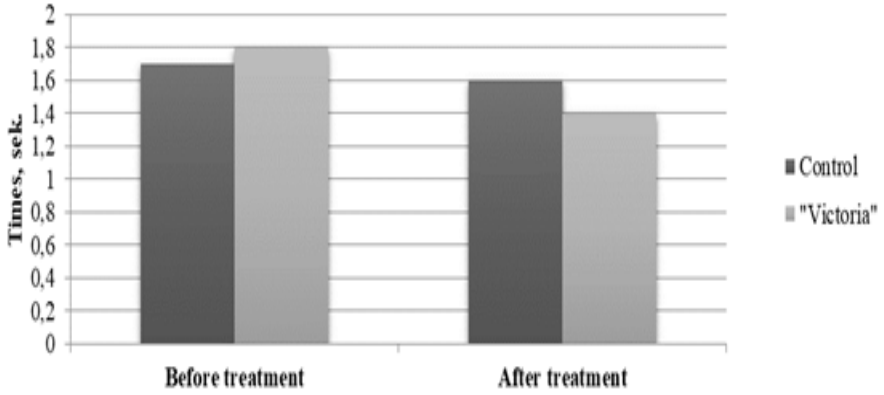

Figure 4. Improvement of microcirculation according to the response of the nail bed to depression

When assessing the state of postoperative suture dynamics, it was shown that the inclusion of the specialized product in the rehabilitation process improved healing. The scar in patients taking diet therapy was characterized by greater tenderness, less hyperemia, and infiltration. Dietary therapy and the complex made it possible to shorten the healing time of postoperative sutures both in cosmetological operations and in the presence of a secondary suture (Figure 5).

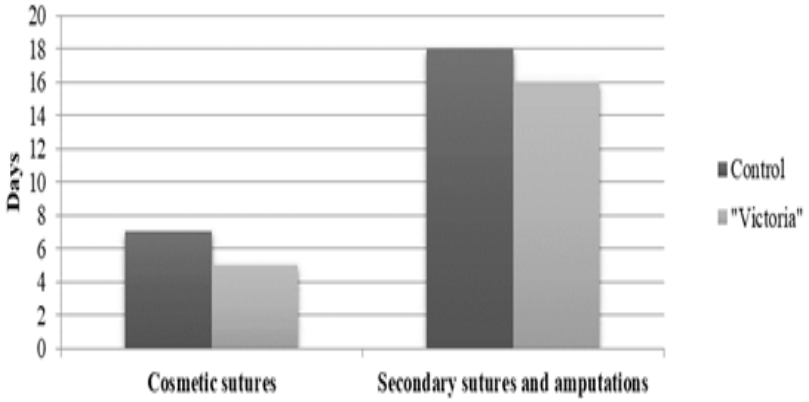

Figure 5. Healing time of postoperative sutures

DISCUSSION

The competitive advantages of the developed product were:

- directed and prolonged action, based on a scientifically based recipe;
- a single dose of the complex made the application convenient and affordable;
- the physiological dosage of active substances ensured the absence of addiction, and the other undesirable effects;
- the effective concentration of bioflavonoids, antioxidants and other bioactive substances contained in the composition of natural extracts of aloe vera, leaves and seeds of red grapes, promoted regeneration, maintained the tone of epithelial tissues, and had a tonic effect.

The study and the development of new biologically active supplements for the prevention of the described group of diseases have been topical, and there has been a lack of research on this topic [6]. The results of the present study can be used by the other authors to correctly understand the mechanism of the influence of the food factor on the correction of the metabolic processes in the disease under consideration.

CONCLUSION

Including the diet of the subjects of the biologically active complex in the amount of 2 tablets per day reduced the microcirculatory disorders, in particular, reduced the dryness of the skin of the face and body. In 64% of the cases, the complex contributed to the reduction of puffiness and improvement of the contour of the face, 71% of the women showed improvement in complexion, and the inclusion of a specialized product in the rehabilitation measures improved healing. Diet therapy using a biologically active complex has reduced the healing time of the postoperative sutures, as in the case of cosmetic surgery, and in the presence of a secondary suture.

The results obtained have been characterized by the statistical confidence ($P<0.5$) and indicated the effectiveness of dietary supplements in the treatment of the degenerative diseases of the joints and rehabilitation measures in the postoperative period. The use of this diet therapy, in conjunction with the basic treatment of deforming osteoarthrosis, contributed to the trophic support of the joints and dermis, accelerated the epithelialization processes, and had anti-inflammatory and antioxidant effects [15].

BAA was produced by Art Life (Tomsk, Russia), certified per the requirements of the international standards ISO 9001 and 22000 series and GMP rules, which ensured the stability of the quality characteristics and the competitiveness of the developed products.

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CONFLICT OF INTEREST

There was no conflict of interest between the authors.

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