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The recovery of bone and cartilage in an experimental model of osteoporosis under the action of a new cost-effective phytotherapeutic composition (Fitokost New)

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Abstract
The problem relevance is determined by the prevalence of osteoporosis, lack of efficacy and the iatrogenic effects of drug therapy. Despite traditional medicine practices many various methods of herbal-therapy, the scientific data on its use in osteoporosis are scarce; they are presented mainly by researchers outside Russia. It was previously shown that the multicomponent Russian herbal preparation "Fitokost" corrects cartilaginous and bone tissue disorders that are caused by prednisolone in an experimental model of osteoporosis. The study objective is to determine the efficacy of a new, more economical phytocomposition "Fitokost New" in the treatment of osteoporosis in an experiment. The experiments were performed on Balb/c 3-month-old mice in an osteoporosis model. The experiment started on 15.01.2018 and completed by 28.02.2018. The animals were divided into 6 groups. Group 1, the control group, was comprised of intact animals. The animals in groups 2–6 were intramuscularly injected with prednisolone (1.0 mg/mouse) on daily basis, for 14 days. The animals in group 2 were sacrificed at the end of the last injection of prednisolone. The animals in group 3 did not receive the phyto-preparation for 30 days. The animals in group 4 received the herbal preparation "Fitokost" (50 mg / mouse) at the end of the last injection of prednisolone (that is day 15) – orally, daily, for 30 days. The animals in groups 5 and 6 received the new phyto-composition "Fitokost New" at the end of the last injection of prednisolone, at doses of 50 and 5 mg/mouse respectively, for 30 days. The experiment revealed a dose-dependent reparative effect of "Fitokost New", characterized by reduced number of chondroblasts and bone tissue regeneration, like in the case of "Fitokost" preparation. The efficacy of "Fitokost New", which has a significantly smaller number of components compared with the previously obtained "Fitokost" preparation, is not inferior to it in terms of the ability to restore cartilaginous and bone tissue in the osteoporosis model.

Keywords: osteoporosis, prednisolone, Fitokost New, cartilaginous and bone tissue

1. Introduction
The growing prevalence, insufficient methods of treatment, personal and public socio-economic burden of osteoporosis [1–3] necessitate the search for new and improvement of already known approaches to its prevention and treatment [4–6]. However, the optimistic expectations associated with the widespread introduction of many modern pharmaceuticals for its treatment into the practice are not fully justified. This is called forth by the high level of drug-induced iatrogenesis [5], high price and insufficient efficacy of drugs. That is why the search for complementary approaches to the correction of disorders observed in osteoporosis, including those in the arsenal of naturopathic remedies, remains relevant.

Interest in herbal medicine (HM) has revived in connection with drug-induced iatrogenesis. HM is recognized as one of the safest methods of treating acute and chronic diseases, provided that the treatment is done by a specialist rather than the patient. However, data on the scientific rationale and practice of using HM in osteoporosis are currently the most widely represented in foreign literature (first of all, in China [7–15]). In Russia, such data are presented to a lesser extent [16, 17]. For several patented Russian HM compositions intended for the treatment and prevention of osteoporosis [18, 19], the efficacy was evaluated for only one composition [20], which is called "Fitokost". The composition includes components from more than 60 medicinal plants, which have the most pronounced effect on individual stages of osteoporosis pathogenesis.

Using the model of osteoporosis induced in mice by the introduction of prednisolone, it was shown that the composition "Fitokost" evokes restoration of bone and cartilaginous tissues in the hip joints of animals [21]. A more pronounced effect was observed under the combined

action of "Fitokost" and cyclodextrin [22]. The analyzed Russian phyto-compositions differ from a number of foreign analogues by a significantly larger number of its components. The large number of components in "Fitokost" is reasoned by a wide range of osteoporosis pathogenetic mechanisms and the aim to cease them completely. Such an approach is appropriate for polyetiological osteoporosis, but it can also be considered as polypragmasy in other situations. Targets in the development of any therapeutic agents should include, along with ensuring their effectiveness and safety, affordability for the general population. Based on these targets, we have developed a new HM composition for the treatment of osteoporosis "Fitokost New" that contains 20 components.

The study objective: to study the impact of the new phyto-composition "Fitokost New" on regeneration of cartilaginous and bone tissue in an experimental model of osteoporosis.

2. Materials and method

The composition "Fitokost New" was obtained by extracting 20 phytocomponents using the previously described method [20]. It includes substances such as the Japanese pagoda tree (*Styphnolobium japonicum)* fruit, woolly-flowered astragalus (*Astragalus dasyanthus*) herb, marsh cinquefoil (*Comarum palustre*) roots, common dandelion (*Taraxacum officinale*) roots, common comfrey (*Symphytum officinale*) roots, common knotgrass (*Polygonum aviculare*) herb, gypsywort (*Lycopus europaeus*) herb, badan (*Bergenia crassifolia*) rhizomes, Aspen (*Populus tremula*) bark, Iceland moss (*Cetraria islandica*) thallus, common nettle (*Urtica dioica*) leaves, meadowsweet (*Filipendula ulmaria*) herb, sage (*Salvia officinalis*) leaves, calendula (*Calendula officinalis*) flowers, corn silk, chaga (*Inonotus obliquus*) mushroom, common balm (*Melissa officinalis)* leaves, five-seeded plume poppy (*Macleaya cordata)* herb, licorice (*Glycyrrhiza glabra*) roots, dihydroquercetin with arabinogalactan (6–10%). Dihydroquercetin is a bioflavonoid derived from the butt of Siberian larch (*Larix sibirica*), an active antioxidant, which has anti-inflammatory, analgesic and immunomodulatory effects. Arabinogalactan is a polysaccharide, which is also derived from larch wood and used to increase the absorbability of medicines characterized by low bioavailability. It has antioxidant and anti-inflammatory effects, helps to restore the structure of connective tissue [23].

According to the test report No. 19066.05.03 of 13.07.2018, "Fitokost New" complies with the technical regulations of the Customs Union TR CU 022/2011; TR CU 029/2012; TRCU 021/2011. The registration number of the declaration of conformity: EAEU No. RU D-RU.NA 30.V. 03475; the document is dated 02.08.2018.

The experiments were performed on Balb/c mice, 3-month-old females, weighing 20-23 g (Stolbovaya farm of the Russian Academy of Sciences). The experiment was started on 15.01.2018 and completed on 02.28.2018. The mice were kept under standard conditions: 5 animals per cage with controlled temperature (24ºC) and illumination (for 12 hours) and with free access to food and water.

To simulate osteoporosis, prednisolone was administered daily, intramuscularly (IM) at a dose of 1.0 mg / mouse for 14 days. The dose of administered prednisolone was selected on the basis of previously obtained data [21].

All animals were divided into 6 groups of 10 individuals. Group 1 (the control group) were intact animals. The animals in groups 2–6 were daily administered prednisolone, for 14 days. The animals in group 2 were sacrificed on the 14th day after the last injection of prednisolone. The animals in group 3 did not receive the phyto-preparation for 30 days. The animals of groups 2 and 3 formed comparison groups. At the end of the prednisolone injections, the animals in group 4 received the previously prepared herbal composition "Fitokost" at an active dose of 50 mg/mouse – orally, daily, for 30 days. The animals in groups 5 and 6 received "Fitokost New" at doses of 50 and 5 mg/mouse respectively, for 30 days.

The mice were sacrificed using cervical dislocation; the femurs were removed. The bones were preserved in 10%-formalin prepared in phosphate-saline buffer (0.02 M, pH 7.6) for 24 hours at room temperature. Then they were decalcified in 5%-trichloroacetic acid for 24–48 hours, washed in phosphate-buffered saline (0.02 M, pH 7.6) and frozen in isopentane at -40ºC. The sections were prepared with a thickness of 10 μm on a Leyca cryostat (Germany). The sections were dried at room temperature for 1 hour and stained with hematoxylin and eosin. Hematoxylin was applied for 5 seconds, then the samples were differentiated in running water for 10 minutes. Eosin was used for 15 seconds, then the sections were washed in distilled water, dried, clarified in xylol and sheathed with synthetic resin. The analysis of the specimen was performed on the microscope Olympus Vanox AH BT3 (Germany), with magnification (× 100) and (× 40). The images were photographed; the morphological analysis of the cells was performed in the field of view. The nonparametric two-way Mann-Whitney test (U-test) was used to determine the significance of differences between the experimental and control groups of the animals. All manipulations with animals were performed in accordance with the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasburg, 1986) and approved by the Ethics Committee for Animal Research of the Koltzov Institute of Developmental Biology, Russian Academy of Sciences (Registration Date: 10.01.2018, Approval No:20).

3. Results and discussion

Compared to the control group of animals that did not receive prednisolone injections (Figure 1A), a 14-days' administration of prednisolone to mice caused a significant increase in chondroblast proliferation in the head of the femoral bone (Figure 1B), which persisted for 30 days (Figure 1C). After daily intake of the "Fitokost New" phyto-composition for 30 days at a dose of 50 mg / mouse, the cellular composition of hyaline cartilage restored to its previous state, which is similar to the effect of the "Fitokost" composition administered at the same dose (Figures 1D, E). At that, in the field of view, the number of chondroblasts decreased to the control level (group 1) and the number of chondrocytes began to increase (Table). After taking Fitokost New at a dose of 5 mg/mouse, the number of chondroblasts also credibly decreased, but to a lesser extent than at a dose of 50 mg/mouse (Figure 1F).

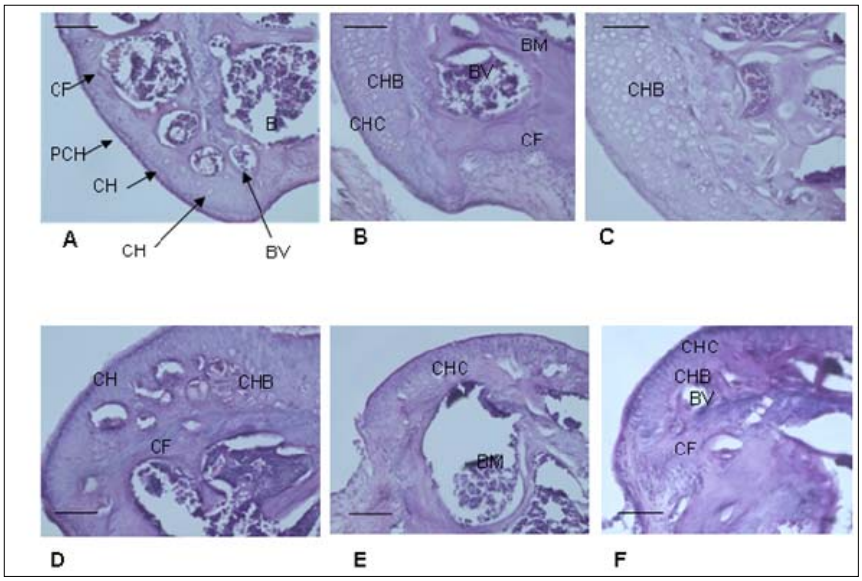

Fig 1(A): intact animals; (**B**) – after i/m administration of prednisolone at a dose of 1 mg/mouse after 14 and (**C**) 30 days; (**D**) – after daily oral administration of phytocomposition Fitokost t at a dose of 50.0 mg/mouse for 30 days; (**E**) – after daily oral administration for 30 days of phytocomposition Fitokost New at a dose of 50.0 mg/mouse and (**F**) at a dose of 5.0 mg/mouse. Coloring hematoxylin-eozin. The analysis of the preparations carried out on an Olympus Vanox AH BT3 microscope (Germany) at magnification (×100) and (×40). Number of animals in each group n = 10. Designations: BM – bone marrow, BV – blood vessels, CHB – chondroblasts, CHC – chondrocytes, CF – collagenic fibers, PCH – perichondrium. Scale bar – 50 μm

Compared with the control group 1, in the group of animals that did not receive the phyto-composition (group 3), the disorders observed in the bone tissue after 30 days were as follows: violations of its integrity, chaotic orientation of collagen fibers (Figure 2C), and changes in the cellular composition (Table). After receiving phyto-preparations at a dose of 50 mg/mouse (groups 4 and 5), the bone tissue structure was practically restored to the control level (Figures 2A, D, E). Besides, in the bone tissue of animals receiving phyto-preparations, the number of osteoblasts decreased and the number of osteocytes increased versus the comparison group 3 (Table). After taking "Fitokost New" at a dose of 5 mg/mouse, bone breaks also completely disappeared (Figure 2F) and the cellular composition changed. The effect was quite similar to the effect of the preparation taken at a high dose, although it was less pronounced (Table). The decrease in the number of chondroblasts in the cartilaginous tissue and the increase in osteocytes in the bone tissue under the action of "Fitokost New" taken at doses of 5 mg / mouse indicate a positive effect of this dose, which will probably become fully apparent later. It should also be noted that the appearance and behavior of the animals treated with the phyto-preparation favorably differed from the specimen that did not take the product.

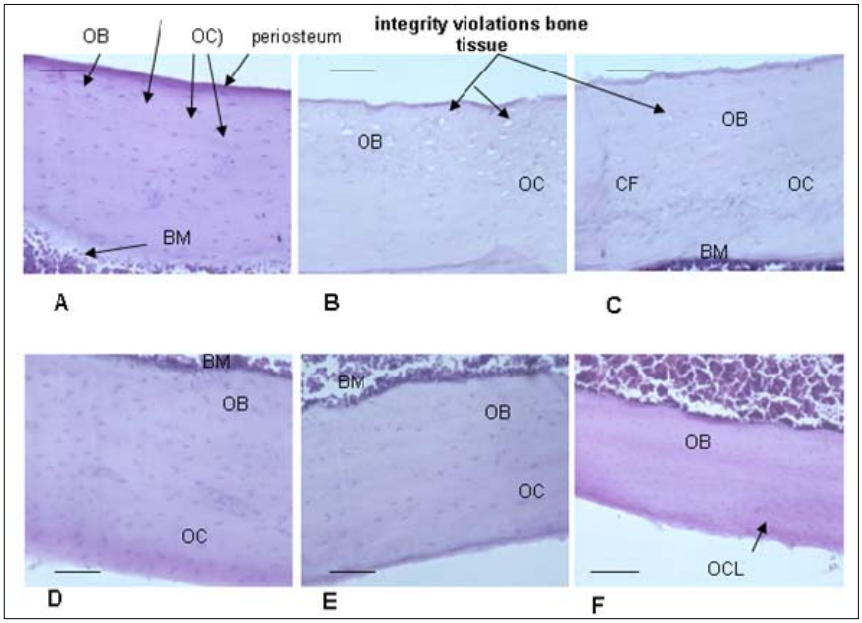

Fig 2: Histological study of the effect phytopreparations Fitokost and Fitokost New on bone tissue of the mice femur in a model of osteoporosis induced by prednisolone. (**A**) - intact animals; (**B**) - after i/m administration of prednisolone at a dose of 1 mg/mouse after 14 and (**C**) 30 days; (**D**) - after daily oral administration of phytocomposition Fitokost at a dose of 50.0 mg/mouse for 30 days; (**E**) - after daily oral administration for 30 days of phytocomposition Fitokost New at a dose of 50.0 mg/mouse and (**F**) at a dose of 5.0

Table 1: The effect of phytocompositions Fitokost and Fitokost New on the cellular structure of cartilage and bone tissues

Groups of animals, doses of phytopreparations, mg/mouse	Chondroblasts (M ± SEM) n= 10	Osteoblasts (M ± SEM) n = 10	*Osteocytes (M ± SEM) n = 10*
1 gr. intact (control)	18 ± 3	8 ± 2	*41 ± 3*
2 gr. 14 days after the administration of prednisolone	112 ± 7	26 ± 3	*8 ± 2*
3 gr. 30 days after the administration of prednisolone	83 ± 6	22 ± 3	*15 ± 3*
4 gr. Fitokost, 50.0 mg/mouse	28 ± 3*	16 ± 2*	*33 ± 3**
5 gr. Fitokost New, 50.0 mg/mouse	25 ± 3*	15 ± 3*	*34 ± 5**
6 gr. Fitokost New, 5.0 mg / mouse	37 ± 4*	18 ± 2§	21 ± 4**

Note: the significance of differences between experimental and control groups: M ± SEM; * p <0.05 compared with groups 2 and 3; ** p <0.05 compared with group 2; n –number of animals in each group. It was analyzed 20 single eyepiece fields for each case.

4. Conclusion

The new economical phyto-composition "Fitokost New" provides high efficacy in regeneration of cartilaginous and bone tissue in an experimental model of osteoporosis. The reduction of the number of components in the presented product leads to a decrease in the cost of its production.

5. Acknowledgements

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