L. I. Babaskina et al /J. Pharm. Sci. & Res. Vol. 10(5), 2018, 1238-1242

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Improving the Chemical Status of Peloids by Adding the Phytocomplex in the Rehabilitation of Patients with Osteoarthritis: Experimental Study

L. I. Babaskina, T. M. Litvinova, D. V. Babaskin, E. A. Winter, O. V. Krylova
Sechenov First Moscow State Medical University
8-2, Trubetskaya St., Moscow, Russian Federation, 119991

Abstract

The use of peloids in combination with the phytocomplex necessitates studying the compatibility and balance of their chemical compositions, which will contribute to the efficiency of peloid therapy in patients with osteoarthritis.

The goal is to study the chemical composition of the peloid and the phytocomplex for their integrated use for rehabilitation of patients with osteoarthritis.

Materials and methods. Peat peloid and phytocomplex, which is a dry extract from marsh cinquefoil, alfalfa and common hop. The mineral composition was studied using the mass-spectral and atomic-emission methods; flavonoids were separated and identified by the methods of column thin-layer liquid chromatography with the use of standard samples; the spectrophotometric method was used for quantitative determination of flavonoids; the amino acid composition was determined with an amino acid analyzer.

Results and discussion. It has been found that peloid and phytocomplex are rich in most important macro- and micronutrients that prevent lesion of the main components of the joint and, as a result, prevent emergence and development of osteoarthritis. The phytocomplex contained rather large amounts of flavonoids (7.08±0.17%) represented by flavonols and their glycosides, isoflavones, and one compound from the group of coumestans – cumestrole. The study of amino acid composition has shown that the content of some amino acids, which are the most important for preventing and treating osteoarthritis, in the complex exceeds that in peat peloid.

Conclusion. Chemical compositions of the peloid and the phytocomplex are compatible and balanced, which makes it possible to assume that it will have a positive effect on the results of rehabilitation of osteoarthritis patients with their integrated use.

Keywords: biologically active substances, flavonoids, mineral elements, amino acid composition, herbal medicine, peloid therapy.

INTRODUCTION

The therapeutic effect of peloids is determined by joint influence of thermal, mechanical, chemical and biological factors. The chemical status of therapeutic mud is largely determined by their specific composition and the quantitative content of biologically active substances [1, 2]. To enhance the pharmacological activity of peloids, extracts of medicinal plants are additionally introduced into them; for example, the phytocomplex that is a dry extract from marsh cinquefoil, alfalfa and common hop. The main active ingredients of the phytocomplex are flavonoids, polysaccharides, coumestans, phenolcarbonic acids, macro- and micronutrients, and vitamins that have the antiinflammatory, analgesic and other effects. This is especially true in rehabilitation of patients with osteoarthritis, which takes the leading place among the diseases of the locomotor apparatus, especially in persons of the senior age group. Peloid therapy is one of the most commonly used nonpharmacological approaches to osteoarthritis in many European countries, and in Japan and Israel [3-6]. The use of peloids in combination with the phytocomplex necessitates studying the compatibility and balance of their chemical compositions, which will contribute to the efficiency of peloid therapy in patients with osteoarthritis.

This work is aimed at studying the chemical composition of the peloid and the phytocomplex for their integrated use for rehabilitation of patients with osteoarthritis.

MATERIALS AND METHODS

In this work, peat peloid from the "Commune" deposit in the Tula region, Russia, was used. Some of its characteristics were as follows: humidity - 71.0±0.4%; pH value (pH) - 7.2; shear resistance – 2,430±10 dyn/cm^2; and salinity of the mud solution - 0.70±0.02 g/dm^3.

The studied phytocomplex was a dry extract from herbs and roots of marsh cinquefoil, alfalfa stems, or cones, or common hop (TU 9375-021-00003938-11 "Dry extract from cinquefoil, alfalfa and hop (phytocomplex)") [7].

Total ash was determined by the gravimetric method. The mineral composition was studied by the following methods: mass spectrometry method with inductively linked plasma (MS) on spectrometer Elan-6100 (Perkin Elmer, USA), atomic emission method with inductively linked plasma (AES) on spectrometer Optima 4300 (Perkin Elmer, USA). Organic carbon was determined using the gasometric method on gas analyzer KGACh-2 (ZOMZ, Russia). The flavonoids were extracted from peat peloid using the method of circulating extraction in a Soxlet type device. The solvent was distilled in vacuum on rotary evaporator Watterbath-480 (Büchi, Switzerland). The flavonoids contained in the peloid and the phytocomplex were separated and determined using the following methods: column chromatography with the use of a flash chromatography installation (special design bureau of the Institute of Organic Chemistry) in glass columns, sorbent was silica gel (Kavalier, Czech Republic, 40x100 μm); the method of ascending paper chromatography on Whatman "Grade 3 MM Chr" paper (Aldrich) and "Type 591" paper (Aldrich); thin sorbent layer chromatography (TLC) on Silica gel 60 F_{254} (Sigma-Aldrich) and Kieselguhr F_{254} (Merck, Germany) plates; high performance liquid chromatography (HPLC) on the Agilent 1100 Series chromatograph (USA) followed by computer processing of the results in the Chemstation A. 09.03 application; qualitative reactions; spectrophotometric with the use of spectrophotometers Titertek MCC 1340 (Finland) and Specord M40 (Germany) with automatic spectra recording in the Scan Graph application. The flavonoids were identified with the use of standard samples: apigenin (Fluka 42251), apigenin-7-glycoside (44692, Fluka), biochanin A (14,563, Aldrich), genistein (G 6649, Sigma), hyperoside (00180585, Fluka), daidzein (D 7802, Sigma), isoquercitrin (00140585, Fluka), isorhamnetin (Art. 3251.98, RhitoPlan), quercetin (Q 0125, Sigma), quercitrin (00740580, Fluka), kaempferol (Art. 3240.RS RhitoPlan), cumestrole (27885, Fluka), luteolin (L 9283, Sigma), luteolin-7-glycoside (Art. 3262.RS RhitoPlan), rutin (R-5143, Sigma). The amino acid composition after hydrolysis was determined on amino acid analyzer Biotronic LC 5001 (Germany).

RESULTS AND DISCUSSION

During the peloid study, it was found that its ash content was 18.8±0.3% (in terms of dry substance). Ash composition was

dominated by calcium (Ca), iron (Fe) and sulfur (S), which was typical for calcigerous peat peloids (Figure 1).

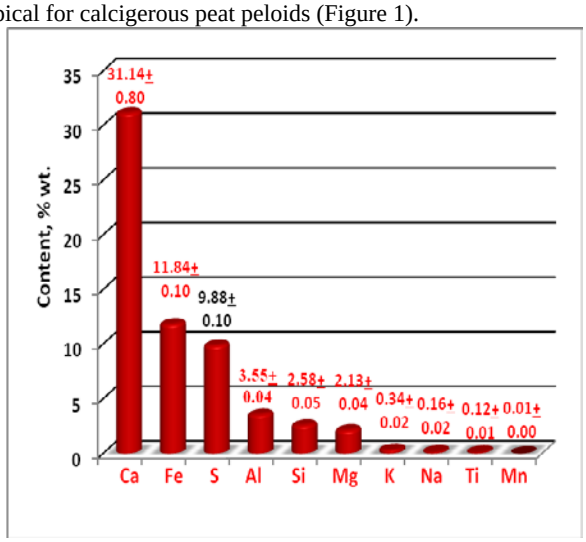

Figure 1. The content of mineral components in peat peloid (The content of Na, Mg, Al, K, Ca, Ti, Mn, Fe, and Si was determined in terms of oxides)

The complete mineral composition of peat peloid is shown in Table 1. The content of heavy metals in the tested peloid did not exceed the natural background for the soil in the location.

It is known that insufficient inflow of certain mineral elements - Ca, Mg, Cu, Zn, Mn, Fe, B, Cr, Si, K - into the organism can result in damaging cartilage, subchondral bone, periarticular tissues and, consequently, in appearance and development of osteoarthritis [8, 9].

The study of the phytocomplex mineral composition (Table 2) showed that the dry extract contained all most important macro- and microelements that prevented lesion of main joint components in case of osteoarthritis, and was particularly rich in Cu, Zn, Mn, Fe, and B. The amount of toxic elements in the phytocomplex did not exceed the maximum permissible concentrations (MPC) established in the hygienic requirements to food safety, while the content of heavy metals did not exceed 0.01%. The amount of main biogenic chemical elements such as Si, Zn, V in the phytocomplex and the peloid complemented each other in a balanced manner.

Biological activity of peloids is largely determined by the degree of decomposition. This indicator in the studied peloid was 48.2±1.8%. This degree of decomposition corresponds to the norm, and gives the peloid good viscoplastic and thermal properties, contributes to its enrichment in biologically active substances. To preserve this indicator in the normal range, the phytocomplex should be introduced into the peloid at the concentration not exceeding 15%. The content of organic carbon in the studied peloid was 42.0±0.3%.

It is known that the flavonoids named quercetin and rutin are used as medications of vitamin group P, and have the ability to reduce permeability and fragility of capillaries. They show antioxidant properties, are involved in redox processes, inhibit the action of hyaluronidase, and are used for rheumatic diseases as well [10]. Quercetin is also known for its antiinflammatory activity due to blocking the lipooxygenase way of the arachidonic acid metabolism, reducing the synthesis of leukotriens, serotonin, and other mediators of inflammation. It influences the processes of bone remodeling. Isoflavonoids show antioxidant activity, have chondroprotective properties, stimulate bone formation by direct action on osteoblasts, and have an antibacterial effect [11, 12]. Isoflavonoids and coumestan are natural phytoestrogens with antiinflammatory, antidestructive, immunomodulatory effect, and are used for treating rheumatic diseases, including osteoarthritis [13]. Therefore it seemed interesting to study the phytocomplex and the peloid for the presence of the compounds in this group.

As a result of the research, fourteen flavonoids and one compound from the coumestans' group of cholestanol have been found and identified (Table 3).

Table 1. The content of trace elements in peloid

No.	Element		Content, µg/g	Method of analysis	No.	Element		Content, µg/g	Method of analysis
1	Lithium	Li	13.11±0.15	AES, MS	25	Barium	Ba	397.00±6.00	AES, MS
2	Beryllium	Be	1.33±0.03	MS	26	Lanthanum	La	12.10±0.40	MS
3	Boron	B	20.72±0.40	AES, MS	27	Cerium	Ce	26.80±0.70	MS
4	Scandium	Sc	1.07±0.02	MS	28	Praseodymium	Pr	3.22±0.03	MS
5	Vanadium	V	33.00±2.20	MS	29	Neodymium	Nd	8.70±0.10	MS
6	Chromium	Cr	12.70±0.50	AES, MS	30	Samarium	Sm	1.88±0.01	MS
7	Cobalt	Co	10.60±0.30	AES, MS	31	Europium	Eu	0.46±0.01	MS
8	Nickel	Ni	30.50±2.00	AES, MS	32	Gadolinium	Gd	1.49±0.01	MS
9	Copper	Cu	13.20±0.30	AES, MS	33	Terbium	Tb	0.28±0.00	MS
10	Zinc	Zn	24.30±2.00	AES, MS	34	Dysprosium	Dy	1.28±0.01	MS
11	Gallium	Ga	3.67±0.04	MS	35	Holmium	Ho	0.50±0.01	MS
12	Arsenic	As	11.20±0.30	MS	36	Erbium	Er	1.55±0.02	MS
13	Selenium	Se	8.60±0.20	MS	37	Thulium	Tm	0.18±0.00	MS
14	Rubidium	Rb	25.90±1.80	MS	38	Ytterbium	Yb	1.28±0.01	MS
15	Strontium	Sr	177.00±4.00	AES, MS	39	Lutetium	Lu	0.23±0.00	MS
16	Yttrium	Y	12.80±0.30	MS	40	Hafnium	Hf	1.64±0.02	MS
17	Zirconium	Zr	37.10±2.60	MS	41	Tantalum	Ta	0.78±0.01	MS
18	Niobium	Nb	4.63±0.03	MS	42	Tungsten	W	1.26±0.02	MS
19	Molybdenum	Mo	8.12±0.03	MS	43	Mercury	Hg	0.12±0.00	MS
20	Cadmium	Cd	0.53±0.01	AES, MS	44	Thallium	Tl	0.22±0.00	MS
21	Tin	Sn	0.80±0.01	MS	45	Lead	Pb	4.40±0.02	AES, MS
22	Antimony	Sb	0.33±0.01	MS	46	Bismuth	Bi	0.26±0.00	MS
23	Tellurium	Te	0.25±0.00	MS	47	Thorium	Th	2.80±0.02	MS
24	Cesium	Cs	1.15±0.01	MS	48	Uranium	U	0.92±0.01	MS

Note: results of the analysis are given in terms of dry sample.

Table 2. The content of main mineral components in the phytocomplex

No.	Element		Content, μg/g	Method of analysis	No.	Element		Content, μg/g	Method of analysis
1	Sodium	Na	34.00±1.90	AES, MS	23	Strontium	Sr	10.20±0.20	AES, MS
2	Potassium	K	4,700.00±10.00	AES, MS	24	Yttrium	Y	0.02±0.00	MS
3	Magnesium	Mg	1,000.00±6.00	AES, MS	25	Zirconium	Zr	0.11±0.00	MS
4	Calcium	Ca	3,400.00±8.00	AES, MS	26	Niobium	Nb	< 0.01	MS
5	Sulfur	S	1,200.00±6.00	AES, MS	27	Lanthanum	La	0.04±0.00	MS
6	Iron	Fe	132.00±2.20	AES, MS	28	Molybdenum	Mo	0.06±0.00	MS
7	Aluminum	Al	65.00±1.20	AES, MS	29	Silver	Ag	0.03±0.00	MS
8	Silicon	Si	178.00±1.90	AES, MS	30	Cadmium	Cd	0.02±0.00	AES, MS
9	Manganese	Mn	16.00±0.30	AES, MS	31	Tin	Sn	0.02±0.00	MS
10	Titanium	Ti	2.33±0.02	AES, MS	32	Antimony	Sb	< 0.01	MS
11	Lithium	Li	0.06±0.00	AES, MS	33	Cesium	Cs	0.02±0.00	MS
12	Boron	B	20.10±0.40	AES, MS	34	Barium	Ba	16.70±0.30	AES, MS
13	Vanadium	V	0.18±0.00	MS	35	Cerium	Ce	0.05±0.00	MS
14	Chromium	Cr	0.77±0.00	AES, MS	36	Praseodymium	Pr	< 0.01	MS
15	Cobalt	Co	0.05±0.00	AES, MS	37	Neodymium	Nd	0.02±0.00	MS
16	Nickel	Ni	1.10±0.01	AES, MS	38	Samarium	Sm	< 0.01	MS
17	Copper	Cu	16.20±0.30	AES, MS	39	Europium	Eu	< 0.01	MS
18	Zinc	Zn	21.60±0.40	AES, MS	40	Gadolinium	Gd	< 0.01	MS
19	Gallium	Ga	0.04±0.00	MS	41	Mercury	Hg	< 0.01	MS
20	Arsenic	As	0.03±0.00	MS	42	Lead	Pb	< 0.01	AES, MS
21	Selenium	Se	0.04±0.00	MS	43	Bismuth	Bi	< 0.01	MS
22	Rubidium	Rb	2.63±0.01	MS	44	Thorium	Th	0.02±0.00	MS
Note: results of the analysis are given in terms of dry sample.									

Table 3. Flavonoids and coumestans found in the phytocomplex and the peloid

No.	Empirical name	Rational name	Chemical formula	UV spectrum λ_{max}, nm	The object of the research*
1	Apigenin	5, 7, 4'-trihydroxyflavone	$C_{15}H_{10}O_5$	269, 339	F
2	Apigenin-7-glycoside	5, 4'-dihydroxyflavone-7-o-β-D-glucoside	$C_{21}H_{20}O_{10}$	u268, 337	F
3	Biochanin A	5, 7-dihydroxy-4'-methoxy-isoflavone	$C_{16}H_{12}O_5$	263, 326	F
4	Genistein	5, 7, 4'-trihydroxyisoflavone	$C_{15}H_{10}O_5$	263, 325	F
5	Hyperoside	5, 7, 3', 4'-tetrahydroxy-flavone-3-o - β-D-galactoside	$C_{21}H_{20}O_{12}$	257, 268 dens., 364	F
6	Daidzein	7, 4'-dihydroxyisoflavone	$C_{15}H_{10}O_4$	250, 260, 302	F
7	Isoquercitrin	5, 7, 3', 4'-tetrahydroxyflavone-3 - o-β-D-glycoside	$C_{21}H_{20}O_{12}$	255, 265 dens, 362	F
8	Isorhamnetin	3, 5, 7, 4'-tetrahydroxy-3'-methoxyflavone	$C_{16}H_{12}O_7$	254, 265 dens, 371	F, P
9	Quercetin	3, 5, 7, 3', 4'-pentahydroxyflavone	$C_{15}H_{10}O_7$	256, 268 dens, 370	F, P
10	Quercitrin	5, 7, 3', 4'-tetrahydroxyflavone-3- o-β-L-rhamnose	$C_{21}H_{20}O_{11}$	256, 267 dens, 363	F
11	Kaempferol	3, 5, 7, 4'-tetrahydroxyflavone	$C_{15}H_{10}O_6$	266, 368	F, P
12	Cumestrole	7, 12-dihydroxycoumestane	$C_{15}H_8O_5$	244, 304, 344	F
13	Luteolin	5, 7, 3', 4'-tetrahydroxyflavone	$C_{15}H_{10}O_6$	256, 353	F
14	Luteolin-7-glycoside	5, 3', 4'-trihydroxyflavone-7- o-β-D-glycoside	$C_{21}H_{20}O_{11}$	256, 353	F
15	Rutine	5, 7, 3', 4'-tetrahydroxyflavone-3-o-rutinoside	$C_{27}H_{30}O_{16}$	256, 362	F, P
* Objects of study: F – phytocomplex; P – peloid.					

Analysis of the flavonoid composition showed that the peloid and the phytocomplex contained phenolic compounds of the same group - the group of flavonols. The following isoflavones were additionally discovered in dry extract: biochanin A, genistein, daidzein, and a substance from the group of coumestans – cumestrole. In the peloid, the flavonoids were mainly in the form of aglycones, and in the phytocomplex – in the form of aglycones and glycosides. The prevailing flavonoid in the peloid was quercetin, and in the phytocomplex – quercetin and rutin. The quantitative content of the flavonoids in the phytocomplex and peloid was determined by spectrophotometry. The absorption spectra of the phytocomplex and the peloid were preliminarily studied. It has been shown that the extract and the peloid did not shift the maximum value of quercetin optical density (370 nm) by the intensity of which photometering was performed. As a result, the content of flavonoids in the phytocomplex – 7.08±0.17% - and in peloid – 0.22±0.01% (in terms of absolutely dry weight) - was determined. The flavonoids in the dry extract have been compatible with the chemical composition of the peloid, and one can assume that they can enhance its antiinflammatory and antioxidant activity, which is very important for rehabilitation of patients with osteoarthritis.

Efficiency of isoflavones for treating osteoarthritis is largely determined by the amino acid composition of the protein in case of their joint use [14, 15]. The research has been performed for the amino acid composition of the peloid and the phytocomplex (Figure 2).

The research identified the presence of 15 amino acids in peloid, and 14 - in the phytocomplex. The share of essential amino acids in peloid was slightly lower than that in the dry extract (36.10% and 40.02%, respectively). Arginine that participated in the formation of creatin, which played an important role in the energy processes, was very important. The content of this amino acid in peloid and in the phytocomplex was 0.67±0.03 and 0.94±0.06 g/kg (in terms of dry weight), respectively. The most pronounced effect was observed in case of the joint use of arginine and lysine: the stimulating effect on insulin production by the pancreas and increased synthesis of the growth hormone. Lysine is essential for normal formation of bone tissues; it promotes absorption of calcium. By the content of lysine, peloid

was 4.5 times inferior to the extract (0.84±0.07 and 3.85±0.16 g/kg, respectively). Histidine is an important component for tissues' growth and restoring; it is used for synthesizing histamine. The content of this amino acid in the phytocomplex exceeded its content in peloid almost 7 times (2.36±0.10 and 0.34±0.01 g/kg, respectively). Glutamic acid is involved in protein and carbohydrate metabolism; being part of the protein component, myofibril plays an important role in the activity of skeletal muscles. Dry extract contained almost 3 times more this acid than peloid (8.52±0.16 and 2.92±0.09 g/kg, respectively). Threonine helps maintain normal protein metabolism in the organism. By the content of threonine, peloid was 6 times inferior to the phytocomplex (0.51±0.03 and 3.22±0.10 g/kg, respectively). Thus, the content of some most important amino acids for the prevention and treatment of diseases of the musculoskeletal system was higher in the phytocomplex than in peloid, which could be assumed to have a positive effect on the results of rehabilitation of osteoarthritis patients.

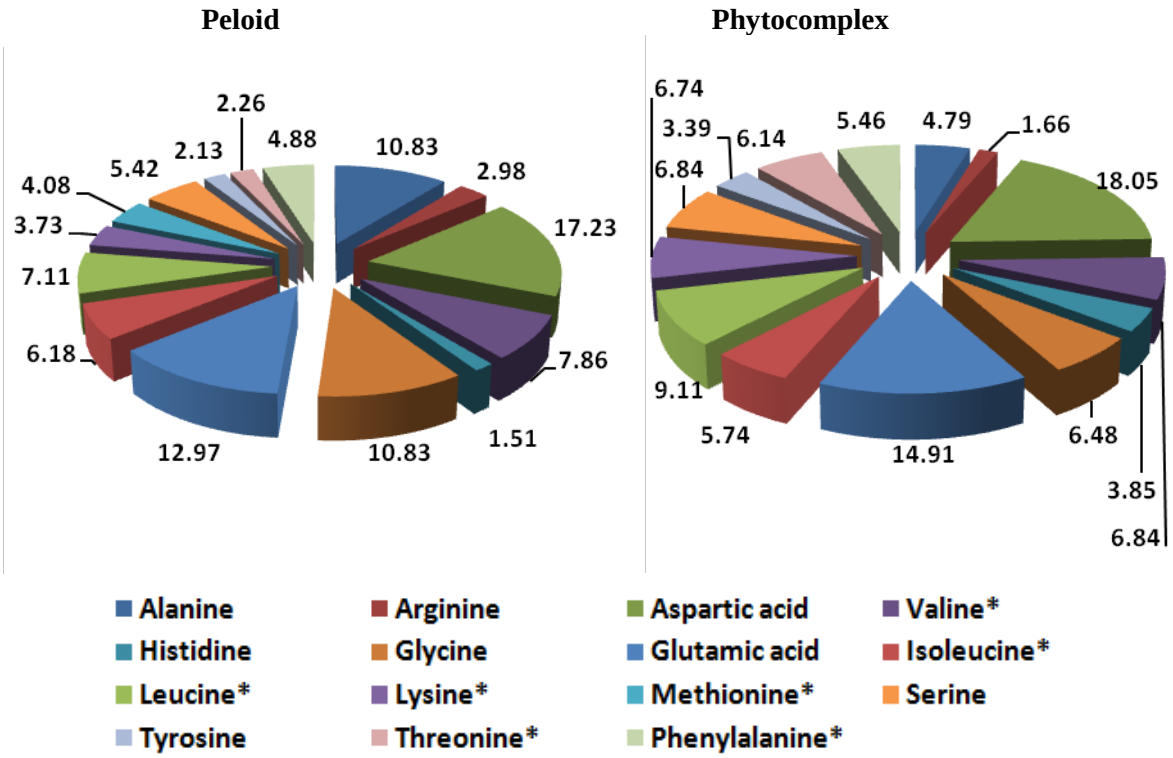

Figure 2. The content of amino acid in peloid and phytocomplex (in % of amino acids).
*** - Essential amino acids**

Conclusion

Studying the chemical composition of the peat peloid and the phytocomplex, which is a dry extract of marsh cinquefoil, alfalfa and common hop, it has been found that the peloid and the phytocomplex are rich in most important macro- and micronutrients that prevent lesion of the main components of the joint, and as a result, prevent emergence and development of osteoarthritis. The phytocomplex contained rather large amounts of flavonoids (7.08±0.17%) represented by flavonols and their glycosides, isoflavones and one compound from the group of coumestans – cumestrole. The flavonoid composition of the phytocomplex was compatible with the chemical composition of peat peloid; and it may be assumed that it can enhance its antiinflammatory and antioxidant activity, which is very important for the rehabilitation of patients with osteoarthritis. Studying the amino acid composition has shown that the content of some amino acids, which are the most important ones for preventing and treating osteoarthritis, in the complex exceeds that in the peat peloid, which can be expected to have positive effect on the rehabilitation of osteoarthritis patients in case of their joint use.

References

[1] Babaskin D. V. The release of biologically active compounds from peat peloids. Voprosy kurortologii, fizioterapii, i lechebnoi fizicheskoi kultury. 2011 Sep-Oct; (5):52-4. (In Russ).

[2] Beer AM, Fetaj S, Lange U. Peloid therapy. An overview of the empirical status and evidence of mud therapy. Zeitschrift für Rheumatologie. 2013 Aug;72(6):581-9.

[3] Forestier R, Erol Forestier FB, Francon A. Spa therapy and knee osteoarthritis: A systematic review. Annals of Physical and Rehabilitation Medicine. 2016 Jun;59(3):216-26.

[4] Ortega E, Gálvez I, Hinchado MD, Guerrero J, Martín-Cordero L, Torres-Piles S. Anti-inflammatory effect as a mechanism of effectiveness underlying the clinical benefits of pelotherapy in osteoarthritis patients: regulation of the altered inflammatory and stress feedback response. International Journal of Biometeorology. 2017 Oct;61(10):1777-1785.

[5] Özkuk K, Gürdal H, Karagülle M, Barut Y, Eröksüz R, Karagülle MZ. Balneological outpatient treatment for patients with knee osteoarthritis; an effective non-drug therapy option in daily routine? International Journal of Biometeorology. 2017 Apr;61(4):719-728.

[6] Tenti S, Cheleschi S, Galeazzi M, Fioravanti A. Spa therapy: can be a valid option for treating knee osteoarthritis? International Journal of Biometeorology. 2015 Aug;59(8):1133-43.

[7] Babaskin DV, Babaskina LI, Babaskin VS. Sposob polucheniya fitopreparata dlya lecheniya osteoartroza i sposob reabilitatsii bol'nykh osteoartrozom. Patent RUS No.2453325. 2012;17 (I):94.

(InRuss). Date View January 10, 2017 http://www. freepatent. ru/patents/2453325

[8] Morer C, Roques CF, Françon A, Forestier R, Maraver F. The role of mineral elements and other chemical compounds used in balneology: data from double-blind randomized clinical trials. International Journal of Biometeorology. 2017 Dec;61(12):2159-2173.

[9] Roczniak W, Brodziak-Dopierała B, Cipora E, Jakóbik-Kolon A, Kluczka J, Babuśka-Roczniak M. Factors that Affect the Content of Cadmium, Nickel, Copper and Zinc in Tissues of the Knee Joint. Biological Trace Element Research. 2017 Aug;178(2):201-209.

[10] Na JY, Song K, Kim S, Kwon J. Rutin protects rat articular chondrocytes against oxidative stress induced by hydrogen peroxide through SIRT1 activation. Biochemical and Biophysical Research Communications. 2016 May 13;473(4):1301-1308.

[11] Khan NM, Ahmad I, Ansari MY, Haqqi TM. Wogonin, a natural flavonoid, intercalates with genomic DNA and exhibits protective effects in IL-1β stimulated osteoarthritis chondrocytes. Chemico-Biological Interactions. 2017 Aug 25;274:13-23.

[12] Khan NM, Haseeb A, Ansari MY, Devarapalli P, Haynie S, Haqqi TM. Wogonin, a plant derived small molecule, exerts potent anti-inflammatory and chondroprotective effects through the activation of ROS/ERK/Nrf2 signaling pathways in human Osteoarthritis chondrocytes. Free Radical Biology and Medicine. 2017 May;106:288-301.

[13] Oliviero F, Scanu A, Zamudio-Cuevas Y, Punzi L, Spinella P. Anti-inflammatory effects of polyphenols in arthritis. Journal of the Science of Food and Agriculture. 2018 Mar;98(5):1653-1659.

[14] Arjmandi BH, Lucas EA, Khalil DA, Devareddy L, Smith BJ, McDonald J, Arquitt AB, Payton ME, Mason C. One year soy protein supplementation has positive effects on bone formation markers but not bone density in postmenopausal women. Nutrition Journal. 2005 Feb 23;4:8.

[15] Kreijkamp-Kaspers S, Kok L, Grobbee DE, de Haan EH, Aleman A, Lampe JW, van der Schouw YT. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. JAMA. 2004 Jul 7; 292(1):65-74.