Journal of International Scientific Publications:
Materials, Methods & Technologies, Volume 7, Part 2
ISSN 1313-2539, Published at: http://www.scientific-publications.net

THE INCREASE IN BIOLOGICAL ACTIVITY OF ACTIVE SUBSTANCES IN COMPLEXES WITH A FILLER OF LICHEN THALLUS

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Abstract

The mechanochemical technology for producing of high-performance solid-state biocomplexes based on "universal active filler" which is a polymer lichen β-oligosaccharide matrix with different kind of pharmakons (known pharmaceuticals, physiologically active substances of herbs, vitamin and microelement complexes, etc.) is developed.

The application of «free solvent» processes based on natural poly-and oligosaccharides prolongates of the operation of active substance (pharmakon) and increases of its biological (incl. therapeutic) effect in a few times, while reducing the dose and toxicity.

Key words: *mechanochemistry, bio-complexes, lichens, pharmacon, physiologically active substances.*

INTRODUCTION

Searching the ways of increasing the intensity of existing pharmaceuticals and biologics is a actual task because of long-term and high cost (investment) of new drugs and biologics creating (development, making, generation) process.

It is acknowledged that much attention is being paid to developing novel methods to improve drug effectiveness by increasing their solubility and dissolution rates. Creating solid dispersion systems - composites («medicinal substance carrier») is one solution to this problem. The so-called «clathration» allows significantly increasing the bioavailability of many drugs, reducing their toxicity, and even activating new properties of the original drug (pharmacon). Sometimes, an effective dose of a pharmacon can be reduced by tens / hundreds of times. The clathration can be achieved by applying mechanochemical technologies. Mechanochemical changes are distinguishable into two groups [Dubinskaya, 1998]. The processes of the first group are associated with the rupture and the formation of intramolecular covalent bonds, while the second group includes the processes with the rupture and formation of the weaker intermolecular bonds.

The first group of mechanochemical reactions includes the decay of molecules, oxidation and hydrolysis. Such mechanical degradation of the molecules results in the formation of free radicals, which get involved in a variety of specific chemical reactions. Mechanochemical processes of the second type lead to disordering, amorphization and polymorphic transitions of the crystal lattices of the compounds, particularly conformational transitions in the components of the molecular lattice. All of these processes alter the reactivity and biological activity of the drugs and can be used to create new and more effective technologies, to obtain new active substances and a change in the properties of drug substances and dosage forms [Dubinskaya, 1998; Shakhtshneider, Boldyrev, 1999].

As very small particles are usually characterized by high activity, traditionally solid dispersions are

used for pharmaceutical purposes. These solid dispersions are composed of active substance particles dispersed in a neutral soluble matrix. Agents of different origins can serve either as the matrices or as carriers. For example, the poorly soluble non-steroidal anti-inflammatory drug ibuprofen can be dissolved by reacting either with organic or inorganic carriers [Yagodin, Boldyrev, Sysoev, 1991]. Mechanochemical treatment of sulfathiazole with polyvinylpyrrolidone translates the crystalline substance into the amorphous state. The process is accompanied by the formation of hydrogen bonds between the sulfathiazole molecules and polymer matrix. The solubility of the drug changes with the weight percent of the polymer. The weight ratio of the polymer to the drug substance equal to 1:3, gives the highest solubility [Yagodin, Boldyrev, 1989; Dushkin, Meteleva, Tolstikova, Khvostov, Dolgikh, Tolstikov, 2010].

To expand the range of biopolymers of plant origin as active solid fillers, the effect of the joint mechanical activation of dry lichen thallus with different pharmacons, in order to improve the biological activity of the latter, has been investigated in the present study.

EQUIPMENT AND MATERIALS

The object of the study was the lichen thallus of the genus Cladonia (reindeer lichen) mechanically activated and coarsely ground, as well as the solid-phase bio-complexes based on the lichen-β-oligosaccharides used as a universal matrix, up to 90-95% by weight, with different pharmacons: physiologically active compounds (PAC) of medicinal plants, vitamin-microelement complexes (VMEC).

Mechanochemical activation was carried out in air in the mill-activator flow type CEM 7-80.

Scanning electron microscopy at 5000 × magnification (TM-1000 Hitachi High Technologies, Japan) was used to study the lichen samples of different milling. This enabled the study of the plant objects in their natural state, to obtain crisp and undistorted images with no damage and pre-treatment with chemical reagents.

The content of the easily hydrolyzable carbohydrates and sorption activity against the methylene blue marker (modulating the medium and low molecular weight toxins) was measured by direct spectrophotometry according to standard methods in a spectrometer using the software LAMBDA 20. The results of adsorption were obtained using the software package Microsoft Excel 2010. The data thus obtained were processed by standard parametric statistical methods with the calculation of the mean, standard deviation, Student's t test, and 95% confidence interval (p=0.05).

The physiological activity of the biocomplexes «lichen-pharmacon» under different activations (coarsely ground and mechanochemical treatment) was measured in vivo after oral administration to mice CD-1 (Animal Breeding Center of the Filial of the Institute of Bioorganic Chemistry (FIBCH) of the Russian Academy of Sciences). The experiments were conducted based on the principles of humane treatment of animals as provided by the Guide for Care and Use of Laboratory Animals (ILAR publication, 1996, National Academy Press).

Assessment of animal behavior activity was performed using test «Swimming» and «AutoTrack» based on the standard operating procedures for 30- and 45-day administration of the drug. Animals of the control group (#1) received the solvent (water), while the other animals in the study groups received the biological product (50 mg/kg) in the stomach using oral gavage.

Statistical analysis

All of the data was processed according to the variation statistics method using the software Statistica Ver. 7.0. On analysis, the mean and standard error of the mean were deduced. The difference was

considered reliable when $p<0.05$. The Mann-Whitney (U Test) was used to compare the differences between two independent groups (for nonparametric data). For data with normal distribution, inter-group comparisons were performed using Student's t-test for multiple comparisons. Then, statistical analysis was performed for each sex separately. P value less than 0.05 was considered significant.

Behavioral tests are characterized by high volatility of the experimental object, and therefore, we carefully performed a background research of behavioral activity, which was made one day prior to the investigation, and on the basis of those results grouping was done to reduce any error.

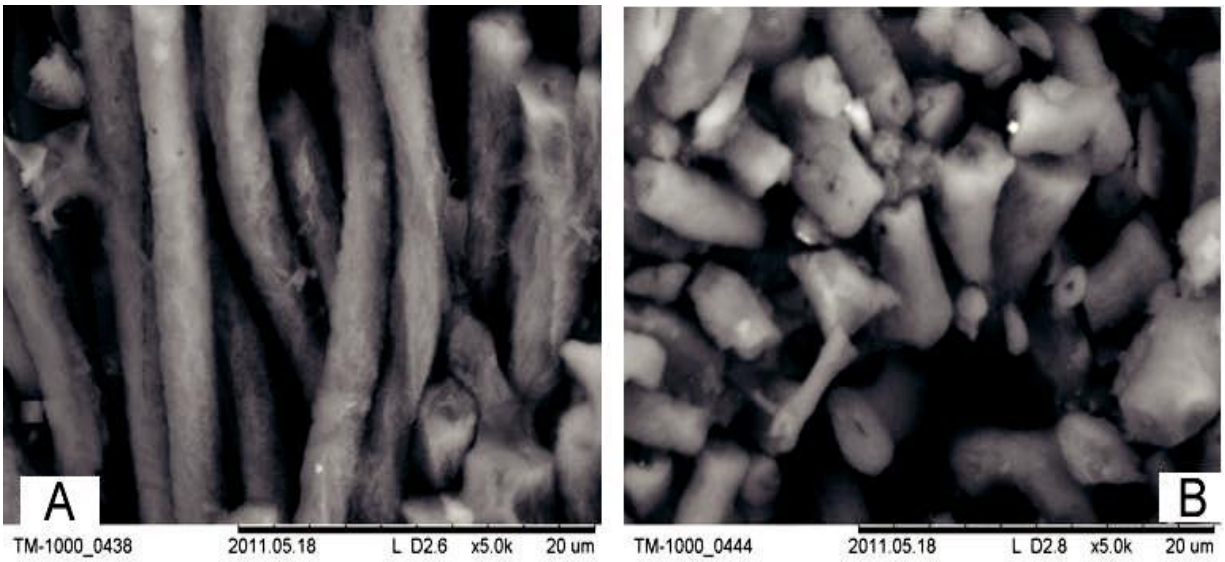
RESULTS AND DISCUSSION

Mechanochemical treatment destroys the cell walls, where the bulk of PAC is contained. The mechanochemical treatment leads to the formation of nanoscale particles in the solid phase (Fig. 1) and thus contributes to the maximizing effective output of the PAM from the cells. Along with the cell wall destruction chemical reactions occur during the mechanical activation.

The fact that partial destruction of β-glycosidic linkages occurred in the lichen β-polysaccharides with the formation of β-oligosaccharide molecules during mechanical activation was confirmed on analyzing the soluble carbohydrates using the «reducing ends» method in the extracts of the lichen thallus of the genus Cladonia after coarsely grinding or mechanical activation. The content of readily available carbohydrates in the lichen samples of genus Cladonia increased by 8 times after its mechanical activation and achieved 33.48 mg/g dry mechanically activated raw materials and a 4.61 mg/g dry sample of the coarse grinding.

Figure 1

Scanning electron photographs of the lichen structures of different grinding: coarsely ground (A), mechanically activated (B).



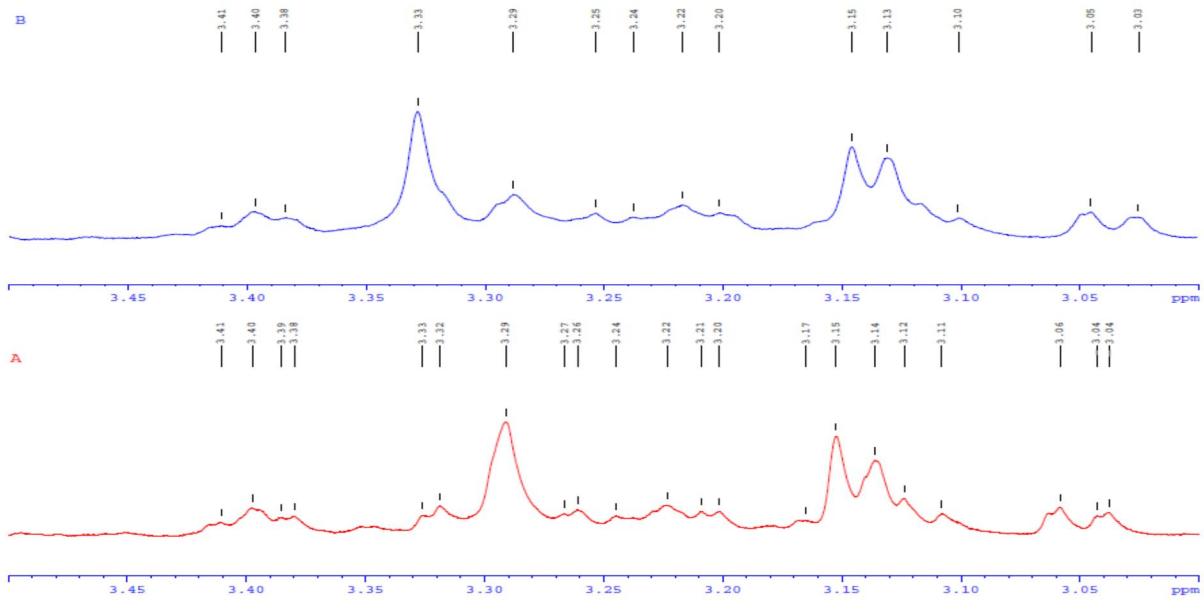
During the mechanical co-activation of the lichen mixtures with a variety pharmacons (PAM of medical plants, VMEC) a significant increase of the easily hydrolyzable carbohydrates did not occur. The lichen β-oligosaccharides formed during the process of mechanical activation was found to be able to form complexes (following the same process of mechanical activation) with the active substance due to its active carbonyl, hydroxyl, and amino groups. Herewith the carbonyl groups of the water-soluble β-oligosaccharides were found associated in the complexes with the active substance and were not subjected to titration by the «reducing ends» method. This fact confirms the assumption that the mechanical activation of mixtures of the lichen thallus and the active substance leads to the simultaneous degradation of β-polysaccharide to form β-oligosaccharides (active filler) and their complexation with pharmacon.

Proton spectra (Fig. 2) of the ammonium buffer extracts of the coarsely ground samples and mechanically activated samples indicate that all the spectra in the frequency range 0-6 (m.F.) are identical in the aliphatic region of the spectrum.

The proton signals of the saccharide skeleton of the lichenin appeared in the region of 3.0-3.4 (m.F.). This saccharide skeleton consisted of sequences of 1.3 and 1.4-related glucose residues; in addition, signals of the anomeric protons of polysaccharides were found at 5.20 and 5.12 m.F. The changes in the signal intensities, 3.29 and 3.33 m.F. (reciprocal change) were marked. During mechanical activation the intensity of the 3.29 signal decreased with the increasing of the 3.33 signal, which perhaps was indicative of the change in the chemical structure in the polysaccharide cycles. In contrast to the alkaline extracts of the study samples such changes did not occur in an acid environment and the lichen spectra of the different impacts were identical.

Figure 2

The proton spectra of the coarsely ground lichen (A) and lichen after mechanical activation (B) in the ammonium buffer in the range of 3-3.5 m.F.

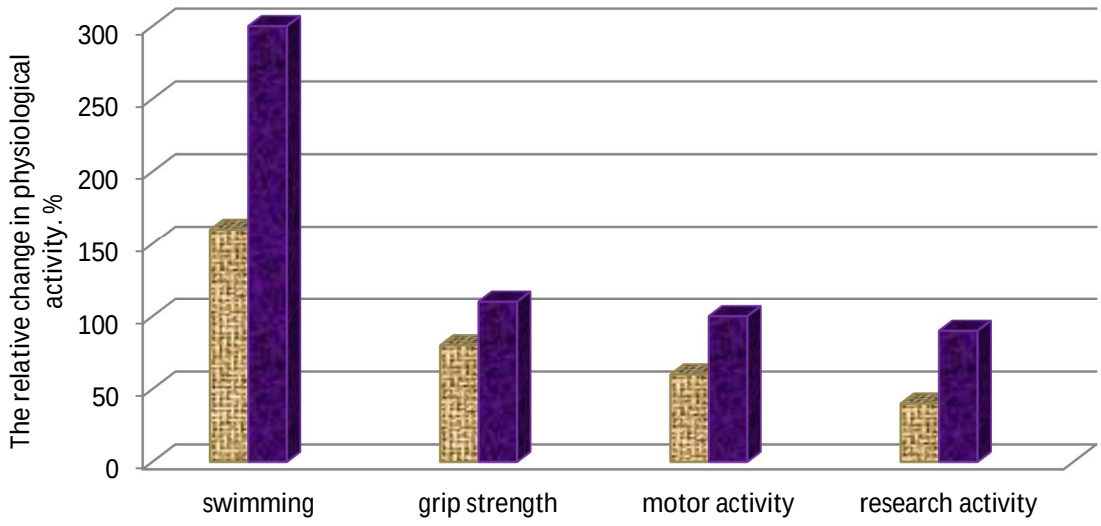


The effect of increasing the bioactivity pharmacon during its complexation with the lichen β-oligosaccharides was checked by creating the mechanochemical biological product, which was an intermolecular complex «active filler» - lichen β-oligosaccharides and VMEC in a ratio of 20:1. On analysis of the study of the physiological activity of the bio-complex, the mechanical activation of lichen β-oligosaccharides with VMEK was found to raise the resistance (endurance, motor and exploratory activity, etc.) of the CD-1 mice to the effects of the physical activity and the extreme factors of various origins by 1.7-2.0 times (Fig. 3).

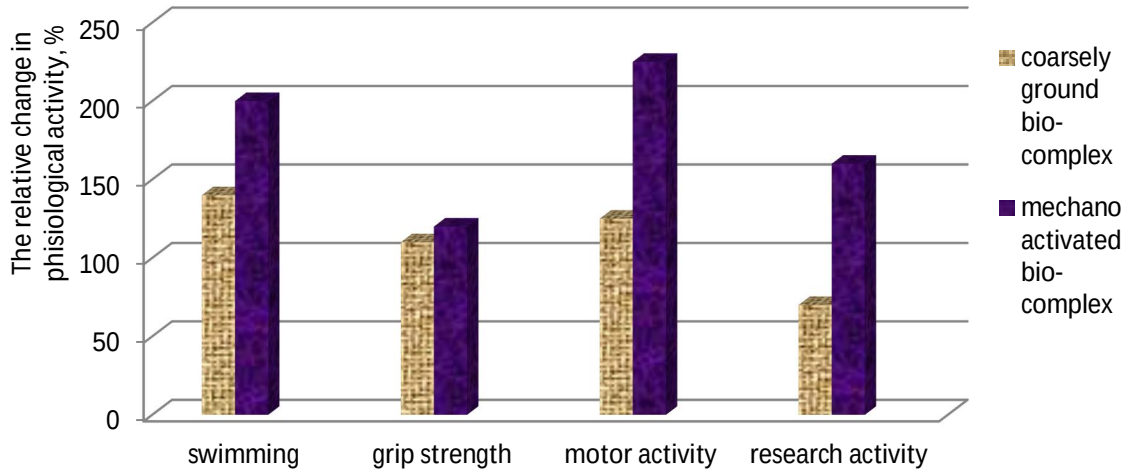
The mouse weight in the control and study groups showed no significant change, i.e. the study drugs did not contribute to any muscle mass increase; this indicates they did not possess anabolic properties. The mechanically activated lichen thallus of genera Cladonia used in this study as a complexing agent was also considered as a source for expanding the range of the sorption materials of plant origin for cleaning the internal environment of the organism from exogenous and endogenous toxins of different origins. It was noted that in vitro, the daily sorption capacity of the mechanically activated sample was 48.0 mg/g or 150.0 mmol/kg of organic matter (for example, methylene blue - the endotoxin marker with small and medium molecularweight). This was significant because this is a very high index compared with the known adsorbents such as white clay and Polyphepan whose adsorption capacity for methylene blue is 10.0 and 11.4 mg/g, respectively [Anshakova et al., 2012]. The adsorption capacity for the heavy metal salts was determined by the example of Co2+ ions from the standard solutions of cobalt chloride (II) in the concentration range 0.2-1 M. The adsorption capacity of the biomaterial of mechanoactivated reindeer lichen was 185 mmol/kg, that was about 2.5 times higher than the adsorption properties of the coarsely ground lichen powder. The increase in the sorption properties of the mechanically activated reindeer lichen is associated with a better developed surface, and with an increase in the number of functional groups [Anshakova, 2011].

Figure 3

The physiological activity of the animals: A - on the 30th, and B - on the 45th day of administration of the bio-complex reindeer lichen – VMEC.



A



B

The high sorption capacity of the material thus obtained was demonstrated in vivo by the example of binding of the «fatigue toxins» - lactic acid (Table 1).

Table 1

The absolute values of lactate in the blood of animals

Administered drugs	**Lactate, mmol/l**	
	30th day	**45th day**
Control	11.2±0.4	13.9±0.7
Rhodiola rosea	11.5±1.0	13.9±0.4
Coarsely ground mixture of Rhodiola rosea with the lichen (1:10)	9.2±0.5	12.6±0.7
Mechanically activated mixture of Rhodiola rosea with the lichen (1:10)	8.4±0.4	12.0±1.3

Thus, the solid-phase composition, obtained by the mechanochemical activation of the lichen thallus of genus Cladonia and VMEC in a weight ratio of 20:1 (reducing the dose of VMEC in 10 times), was shown to possess a physiological activity of about 2.5-3 times higher than the original pharmacon. This was due to the greater bioavailability and detoxification functions of the «active filler». Similar effects, but even more pronounced in quantitative measurement, were obtained by the joint mechanical activation of the lichen thallus with the tissues of certain medicinal plants: the roots and rhizomes of Rhodiola rosea, the upper leaves and shoots of Rhododendron in the ratio of 10:1 (Table 2).

Table 2

The physiological activity of animals on the 30th and 45th day of the administration bio-complex "Lichen - Rhodiola rosea" (n=15 in each group)

Day	Control	Rhodiola rosea	Coarsely ground mixture of Rhodiola rosea with the lichen thallus (1:10)	Mechanically activated mixture of Rhodiola rosea with the lichen thallus (1:10)
The absolute values of the time swimming of animals (sec)				
0	59.7±5.1	96.2±5.6	100.3±7.1	106.5±6.5
30th	86.7±11.5	96.3±16.4	306.0±24.8	449.4±57.2
45th	103.0±6.9	127.8±11.3	331.4±22.1	329.4±91.9
The absolute values of the motor activity of animals				
0	655.7±275.7	650.0±313.5	711.5±308.9	794.2±185.7
30th	867.8±250.1	762.0±311.7	605.4±63.8	673.7±171.5
45th	544.7±131.2	557.5±266.2	1463.0±422.2	1891.8±61.8
The absolute values of the behavioral activity (number of stance) animals				
0	24.5±11.1	17.3±9.5	24.8±12.0	29.8±1.5
30th	25.5±11.8	21.7±10.8	13.6±8.7	26.8±11.5
45th	18.7±10.3	12.5±9.0	22.2±9.5	36.2±12

Apparently, there could be several mechanisms of this type of efficient biological action of the complexes obtained by employing the mechanical activation of the «active filler» (the lichen β-oligosaccharides) with the pharmacon (VMEC, PAM of Rhodiola rosea):

• Lichen β-oligosaccharides bind one or the other pharmacon, transport it into the bloodstream and then pass through the cell membrane, by so doing they provide a higher digestibility (90-95%) of the pharmacon and, consequently, its bioactivity;

• The high sorption activity of the lichen β-oligosaccharides relative not only to the exo-, but also to the endotoxin, products of metabolism, reduces their levels within the cells by 1.5-2.5 times (Table 1). This too contributes to the adaptive capacity and endurance of the body.

CONCLUSIONS

The special biochemical properties of the lichen β-oligosaccharides (proximity to the structure of the oligosaccharide components of the glycocalyx of the human cell membranes) as well as capacity to form intermolecular complexes with the pharmacon promote a 5-10 fold increase in the bioavailability and bioactivity of the pharmacon. This allows a significant reduction of a clinically effective dose of an administered drug, reduces the risks of complications and side effects, which is especially

important in oncology, phthisiology, and particularly in the treatment of autoimmune and allergic diseases, etc.

Currently, the production of new biologics is being launched in the North-Eastern Federal University. The universality of the «active filler» allows a quick restructuring of the production of one product to another, changing only the pharmacon which is introduced into the mechanochemical process.

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