Influence of Nano-Crystal Metals on Texture and Biological Properties of Water Soluble Polysaccharides of Medicinal Plants

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Abstract. When treating the plants seeds with nano-materials there are some quality and quantity changes of polysaccharides, the molecular mass increase and monosaccharides change that leads to the increase of physiological and pharmacological activity of carbohydrates got from medicinal plants. We have got water soluble polysaccharides and nano-metals combinations containing 0.000165- 0.000017 mg/dm³ of the metal. In a case of induced anemia the blood composition has practically restored on the 10th day of the treatment with nano-composites. The use of pectin polysaccharides (that are attributed to modifiers of biological respond) to get nano-structured materials seems to be actual relative to their physiological activity (radio nuclides persorption, heavy metals ions, bacteria cells and their toxins; lipids metabolism normalization; bowels secreting and motor functions activation and modulation of the endocrine system.

1. Introduction

Most scientific publications nowadays are devoted to studying synthesis and mechanism of protein and nucleic acids action. Carbohydrates also perform important functions in micro-organisms, plants and animals [1, 2]. They are the most important sources of chemical energy transfer to biologically available energy accumulated in the form of human macro-energetic ATP links that later transfer to mechanic, electric, chemical and osmotic work. Carbohydrates have a plastic function, participate in bony tissue and cell formation, inhibit or activate the organism ferments [3]. Some carbohydrates and their derivatives are used as pharmaceuticals prohibiting radiation damages. Their ability to influence positively the inflammatory process, strengthening nervous and muscle tissues generation has led to the appearance of different native drugs of carbohydrate nature. Carbohydrates extracted from medicinal plants are non-toxic, have mild multidimensional influence on the organism. Their getting and use play a great role in medicine [4, 5]. The irregularity of pectin polysaccharides molecules structures and the block structure of macro-molecules determine a wide spectrum of pectins immune modulating effect based on their ability to influence the growth, generation and functional activity of immune competent cells [6]. Widening the range of pectin polysaccharides use in medicine is possible.
at the expense of determining the structural determinacy of their physiological activity and synthesis of polysaccharides with definite characteristics [7].

Nano-structured derivatives based on biocompatible natural polymers and products of their modification provoke great interest. Nano-composites based on plant polysaccharides (pectin polysaccharides, galactomannans) and chitosan are polymer systems used for transfer and controlled release of biologically active substances [8]. The use of pectin polysaccharides that are attributed to biological respond modifiers for getting nano-structured materials is actual in connection with their multi-dimensional physiological activity (radio nuclides persorption, heavy metals ions, bacteria cells and their toxins; lipid metabolism normalization; bowels secreting and motor functions activation; modulation of the endocrine system, etc.) [9, 10]. Using polysaccharides that differ by molecular mass, the degree of methyletherifying, galacturonic acid debris, neutral monosaccharides, line sections length and the branched domain molecule structure one can significantly change characteristics of nano-particles and nano-structures that in its turn can lead to significant change of their functional properties [11,12, 13].

2. Methods
We have studied knotgrass, bloodline buckwheat and vetch and bloodline beans. We have treated the seeds before planting with nano-powders of ferrum (Fe), cobalt (Co) and cuprum (Cu) produced at Moscow Institute of Steel and Alloys. The drugs have had particles of free form, high specific area up to 25 m²/g and size of 20-50 nm.

When extracting and structuring water soluble polysaccharides we have used Sevaga’s method (proteins deletion), Ceyzel’s method (determining methoxy groups), chromatographic methods and dielectrolysis (estimating the polysaccharides homogeneity and monosaccharide composition) and Purdie, Hakomora & Coon’s methods of methylation. We have used the infrared spectroscopy to investigate the methylation amplitude and control the polysaccharides discharge.

We have extracted proteins using the standard methods. We have had lectins extraction by Rigas & Osgud’s method of divided ethapol fractioning and by the method of affine chromatography at sephadex G-150 (as matrix).

Having nano-composites as the object of study we have used water soluble polysaccharides of wild chamomile (Matricária chamomilla) and knotgrass (Polygonum aviculare) got by water extraction with further ethanol settling [14]. The extracted polysaccharides are pale pink powders when saluted in water form viscous solutions. In the infrared spectrum one can see the following absorption bands: 3600 cm⁻¹ (OH⁻), 1739 cm⁻¹ — valent variations — COOCH₃, 1022 cm⁻¹, 1032 cm⁻¹ and 1082 cm⁻¹ — ring variations of the pyranoside cycle, band 840 cm⁻¹ — characterizing α-configuration of the glycosidic linkage between hexose residues of galacturonic acid and band 890 cm⁻¹ — characterizing β-linkage between sugar residues in the lateral chain. Fluctuations in band 1157 cm⁻¹ are probably connected with the maximum degree of etherification.

To the extracted powders of polysaccharides we have added 1-10 % of nano-metals (Fe, Cu, Co) having characteristics APS 25 nm, purity 99.8 %, SSA 30-50 m²/g, dissolved in water and processed in supersonic disperser UZD-1-P for 8-10 minutes and deposited nano-composites with ethanol, washed with acetone and extracted in dry matter. Nano-composites are water soluble and 5-20 % solutions contain 0.000165-0.000017 mg/dm³ of nano-metal. According to the infrared spectrography one can find in nano-composites containing the metal the weak line formation in range 1200 cm⁻¹ together with the maximum growth in range ~1250 cm⁻¹ that can be connected with type C-O-Me links formation in the probe.

3. Discussion
We have studied the increase of active substances in plants under the influence of ultra-fine ferrum, cobalt and cuprum micro-elements by the example of vetch seeds growth and development. When using low concentrations (0.01-0.08 g per hectare norm of seeds planting) there is activation of initial growing processes, and the yield increases by 25-32 %. At that we have seen protein and water soluble polysaccharides accumulation. According to the results of the vetch seeds chemical analysis it follows
that the pre-plant treatment of seeds increases protein up to 40 % depending on the metal. We have seen the maximum protein when treating the seeds with nano-cobalt (0.03-0.06 g per hectare norm of seeds planting) that influences the nitrogen and protein metabolism promoting the protein synthesis. When treating with nano-cuprum and nano-cobalt there has been an increase of polysaccharides in the vetch seeds as compared with the control by 29 % and 32 % correspondingly. At that there has been a decline of lectin in total protein for nano-cuprum by 17 % and for nano-cobalt by 24 % that has increased the vetch seeds feed value. There has been an increase of polysaccharides probably connected with the decline of lectin that links carbohydrates. Water soluble polysaccharides extracted from vetch seeds have had the rotary power of +120°…+129° and that of uronic acids up to 40 % that allows attributing them to glycoron glycans. The increase of polysaccharides has not influenced the quality of the extracted polysaccharides but changed their quantity correlations. The extracted polysaccharides have included D-galacturonic acid, galactose, glucose, mannose, arabinose aramnosa, xylose and their amount depends on the nano-metal. So treating the vetch with nano-cobalt has increased galactose by 71 % and decreased aramnose and arabinose by 43 % and 28 % correspondingly, the cuprum treatment has decreased aramnose by 8 %, arabinose by 6 %, xylose by 4 % (Figure 1). So it may be a condition for creating polysaccharides with definite properties.

![Figure 1](https://example.com/image1.png)

**Figure 1.** Percentage Composition of Monosaccharides in Vetch Seeds Polysaccharides

**Polysaccharide composition of knotgrass.** We have achieved the highest extraction of water soluble polysaccharides when treating the raw material in a boiling-water bath for 90 minutes in double replication. After fractioning from the aerial portions of knotgrass in the period of their maximum accumulation we have got homogenous fractions A, B, C, D differing in their
monosaccharide composition and physical-chemical properties. In the extracted polysaccharides there prevailed sourer fractions A and B containing 87-92 % of galacturonic acid that allows attributing them to rhamnogalacturons. Polysaccharide B has contained galactose besides rhamnose that cuts D-galacturonic acid and the bio-polymer rotary power. Polysaccharides C and D are characterized by the small amount of uronic acids and low optical activities.

The pre-plant treatment of the knotgrass seeds has increased the polysaccharides extract on the average by 25 %. The comparison of the chromatography, infrared spectroscopy and ferment analysis results with the data of methylation and periodate oxidation has let determine the structural elements of homogenous A, B, C, D polysaccharides fractions extracted from the knotgrass without and after treating with nano-cobalt (Table 1). Polysaccharides A of both plants are close in their structure and composition. They consist of D-galacturonic acid blocks linked by $\alpha$-D-1,4-glycoside link. The blocks are linked together with rhamnose that is linked to the chain by 1,2-glycoside link. The amount of galacturonic acid after treating with nano-cobalt has increased in the chain. The correlation of galacturonic acid and rhamnose residues in the polymer chain has been 9:1 for the knotgrass and 8:1 with nano-cobalt. The molecular masses have been 15000 and 16000 a.e.m. correspondingly.

Table 1. Physical-Chemical Characteristics of Extracted Polysaccharides

| Plant Knotgrass | Polysaccharides Extraction | $[\alpha]^{20}_{D}$ | Uronic Acids, % | Monosaccharides, mol |
|-----------------|---------------------------|--------------------|-----------------|--------------------------|
|                 |                           |                    | Galactose       | Glucose      | Mannose      | Xylose | Arabinose | Rhamnose |
| Control         | Original                  | +116               | 46              | 14           | 1            | +      | +         | 28       | 4         |
| Nano Co         | Original                  | +126               | 42              | 29           | 3            | 2      | 1         | 2        | 22        |
| Control         | A                         | +269               | 92              | —            | —            | —      | —         | —        | +         |
| Nano Co         | A                         | +272               | 91              | —            | —            | —      | —         | —        | +         |
| Control         | B                         | +226               | 65              | 1            | —            | —      | —         | 4        |
| Nano Co         | B                         | +236               | 72              | 3            | —            | —      | —         | —        | 1         |
| Control         | C                         | +73                | +               | 2            | —            | —      | 1         | 9        |
| Nano Co         | C                         | +36                | +               | 6            | 6            | 2      | —         | 1        | 1         |
| Control         | D                         | +28                | +               | 10           | 1            | —      | —         | 3        |
| Nano Co         | D                         | +21                | +               | 9            | 10           | 1      | —         | 3        | 9         |

Nano-cobalt has influenced changing the structure of the polysaccharide from the knotgrass. It has become branchier and got a higher degree of polymerization and as a result 6.7 % higher molecular mass than the control one. Nano-cobalt has not changed the structural elements. The main chain of polysaccharides B has consisted of D-galacturonic acid and rhamnose. Rhamnose has been linked with galacturonic acid by links 1, 2 in the main chain. At that it is the point of branching lining D-galacturonic acid in position 3. D-galacturonic acid also serves the branching point linking galactose in position 3.

Polysaccharides C and D being an inconsiderable part of the plants polysaccharide complex are branchy polymers that differ by the branchy degree and molecular masses. The assumed branchy points of polysaccharides C and D can be mannose, galactose and rhamnose.

We have determined the monosaccharide composition of polysaccharides extracted in plants vegetation including galactose, glucose, xylose, arabinose, rhamnose, mannose and fucosa (Figure 2).
Under nano-cobalt effect ramnose and galactose have been decreasing as the plant develops from 37.5 % to 18.0 % and from 50.1 to 21.3 % correspondingly. Glucose has changed from 12.5 to 7.8 % for 17 days (beginning of blossom), then there has been some increase of this monosaccharide from 12.5 % for 15 days (total blossom) and finally smooth down to 6.5 % for 45 days (end of blossom). Vice versa, arabinose increases as far as the plant vegetation up to 39 %. The results of the water extract analysis show the increase of potassium by 11.9 %, calcium by 10.5 % and phosphorus by 13 % as compared with the control that must influence the properties of the extracted polysaccharides increasing their water solubility.

Monosaccharides play different roles in plants development and the fact that under nano-metals during the vegetation there has been some change of the monosaccharide composition lets suppose different directivity of some physiological and synthetic processes necessary for the plant at the concrete moment. This allows supposing that nano-metals influence the activity of ferments responsible for oxidation-reduction processes and photosynthesis activity. At that there are some changes in many sides of the plant life activity including protein and carbohydrate metabolism that leads to accumulating biologically active compounds and changing their physiological activity.

**Pharmacological activity of the knotgrass polysaccharide.** A polysaccharide is a mixture of polysaccharide fractions: (I) State Registration Number - 6412486 and (II) State Registration Number - 6412585.

During the experiment with guinea pigs the polysaccharide complex has not had any allergenic properties not causing the allergized animals to have any fever, eosinophilia and leukogram change in a case of repeated administration. While studying the acute toxicity of the polysaccharide extracted from the knotgrass after the seeds’ pre-plant treatment with ultra-fine Co powder it has been impossible to determine LD$\text{subscript}_50$ because of extremely low toxicity of the polysaccharide. Administering its maximum dose [1000 mg/g] a day has not caused any changes of the animals’ systemic condition and the composition of circumferential blood, therefore the ultra-fine metal powder do not cause any ghost effects of the macro-organism.
Figure 2. Percentage Composition of Monosaccharides in Knotgrass Polysaccharides in Progress

The amount of uronic acids, methoxy groups, ash content and rotary power of polysaccharides are presented in Table 2.

Table 2. Characteristics of Polysaccharides Extracted from Knotgrass Aerial Portions (average data, 2010-2011)

| Date       | Group | Yield, % | Ash, % | $[\alpha]^{20}_{D}$, degrees | % Content of Totally Dry Polysaccharide Mass | Uronic Acids | Methoxy Groups |
|------------|-------|----------|--------|-------------------------------|---------------------------------------------|--------------|----------------|
| 8-12.07    | Control | 6.2      | 18.4   | +108                          | 46.2                                        | 3.82         |                |
|            | Nano Co | 6.9      | 22.3   | +111                          | 37.2                                        | 3.80         |                |
| 15-22.07   | Control | 7.4      | 19.9   | +112                          | 45.3                                        | 3.80         |                |
|            | Nano Co | 12.2     | 24.2   | +123                          | 40.7                                        | 4.20         |                |
| 22-25.08   | Control | 12.8     | 18.2   | +118                          | 47.8                                        | 4.35         |                |
|            | Nano Co | 14.2     | 21.1   | +122                          | 42.2                                        | 4.60         |                |
| 25.08-05.09| Control | 5.8      | 18.3   | +120                          | 47.3                                        | 4.14         |                |
|            | Nano Co | 11.6     | 22.7   | +128                          | 43.8                                        | 4.70         |                |

While using water soluble polysaccharides we have not discovered any significant difference of the blood cell composition. But there has been a tendency of increasing the number of erythrocytes and hemoglobin until the 15th day of the experiment and an authentic increase of leucocytes and later gradual decrease of these parameters until the 20th day of the experiment and initial parameters reconditioning after the drugs cancellation. Probably water soluble polysaccharides have stimulating effect on the state of the blood forming organs that activate them, where polysaccharides of the knotgrass pre-plant treated with ultra-fine cobalt powder have had the most stimulating effect.

Pharmacological activity of nano-composites. To have biological investigations of nano-composites we have chosen a group of 80 white rats aged 4-14 months and weighing 140-240 g. We have divided the group in the following way: 20 animals got the normal saline solution to compare the experiment results (control), 60 animals have got anemia by being administered subcutaneously with ethanoate lead every day during 5 days in the dose of 40 mg/g (experiment). The animals of the experiment group (that got ethanoate lead during 5 days) have shown the following dynamics of blood parameters by the 5th day: the number of erythrocytes - 2.41 ± 0.6·10^{12}/l; leucocytes - 3.12 ± 0.84·10^{9}/l; hemoglobin - 43.1 ± 4.2 g/l. The animals have become slack with considerable weight loss, fall-off, being aggressive, poor appetite and hemmoroidal syndrome.

To get rid of the experimental anemia the animals got 0.5ml of 5 % solution of ferrum nano-composite and Polygonumaviculare L. (experiment group 1) and 0.5 ml of 5 % solution of ferrum nano-composite and Matricariachamomilla L. (experiment group 2) every day (Table 3).

Table 3. Blood Basic Morphological Parameters of Rats when Treating the Experiment Anemia

| Blood Parameters | Control | Group 1 | Group 2 |
|------------------|---------|---------|---------|
|                  | M±m     | %       | M±m     | %       |
| **Before Administering** |
| Erythrocytes, ∙10^{12}/l | 6.20±0.04 | 2.40±0.06 | 38.7 | 2.20±0.02 | 35.5 |
| Hemoglobin, g/l | 110±2 | 43.1±0.02 | 39.3 | 44.0±0.3 | 40.0 |
| Leucocytes, ∙10^{9}/l | 10.50±0.03 | 3.10±0.04 | 29.5 | 3.20±0.01 | 30.5 |
During the experiment the control animals have got some increase of hemoglobin during the first 5 days. Later this parameter has come back to the norm that has not changed the results of the experiment. The animals of the experiment groups 1 and 2 have got some differences in dynamics of the registered parameters. So, for instance, hemoglobin in both experiment groups of animals have initially been pretty much the same – 43.1 ± 0.02 (group 1) and 44.0 ± 0.3 (group 2), but with nano-composites hemoglobin recovery of the animals from experiment group 2 has been faster than that of the 1<sup>st</sup> experiment group. So on the 3<sup>rd</sup> day the difference between the groups has been 4 % and on the 7<sup>th</sup> day - 8.4 %. On the 10<sup>th</sup> day of the experiment the animals of both groups have had the total recovery of hemoglobin (Figure 3).
Figure 3. Dynamics of Hemoglobin Recover when Treating the Experiment Anemia

The dynamics of erythrocytes number recovery has also some differences between the groups. At the beginning of the treatment the 2nd experiment group animals have got the number of erythrocytes 3.2 % less than that of the 1st group, but erythrocytes recovery in it has been faster. So on the 3rd day the number of erythrocytes has been the same in the groups, on the 5th day the number of erythrocytes has been 11.7 % more than that of the 1st experiment group; the erythrocytes number of the 2nd experiment group animals on the 7th day has reached 95.0 % of the control; on the 10th day it has exceeded the control by 6 %; and on the 12th day it has been only 3 % higher. The animals of the 1st experiment group have had erythrocytes recovery on the 10th day of treatment and on the 12th day it has exceeded the control one by 10.8 %.

The change of leucocytes happens fast enough in a case of giving nano-composites to animals. Polysaccharides that are their compounds give the plastic material for leucocytes cells building that has the carbohydrate part presented by some monosaccharides (mannose, fukose, galaktose and others), and nano-metal gives additional energy that promotes the leucocytes number recovery. This peculiarity is presented in Figure 4.

Though at the beginning of the treatment the leucocytes number of the 1st experiment group animals has been practically as that of the 2nd experiment group animals, its recovery has been more intensive. So on the 7th day of treatment the 1st experiment group animals have had leucocytes 6.8 % more than that of the 2nd experiment group. But the 2nd experiment group animals have had the leucocytes increase even on the 12th day of getting the nano-composite whereas the leucocytes number in the control and the 1st experiment groups has been declining but remained within the physiological norm.

So on the 10th day of treating the experiment anemia the blood picture has practically regenerated and on the 12th day the number of erythrocytes and leucocytes has even exceeded the control one.

![Figure 4. Dependence of Leucocytes on the Time of Administerting the Nano-Composites when Treating the Experiment Anemia](image)

4. Conclusions
The seeds pre-plant treatment with the suspension of ferrum, cuprum and cobalt nano-powders increases accumulation of biologically active polymers by 30-40%. At that there are changes of polysaccharides structural elements and quantitative composition of monosaccharides.

The polysaccharide complex extracted from Polygonumaviculare L after treating the seeds does not have any toxicity and has high pharmacological activity.

We have got water soluble nano-composites of plants polysaccharides Matricáriachamomilla and Polygonumaviculare containing a nano-crystal metal. We have proved that they are not a mechanical mixture. In a case of the experimental anemia ferrum and polysaccharides nano-composites of different plants are a stimulating factor for blood-forming function of the bone marrow and normalization of the blood state on 10-12th days of administrating the drug.

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