In vivo experiments to determine the efficiency of the elementary status correction

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Abstract. The article is about the determination of the elementary factors and common elementary human statuses. To assess the effectiveness of the developed therapeutic and prophylactic preparations the analysis of biomedical research products, using weight and growth features, and hematological and biochemical parameters, is given. During the experiment the appearance and behavior of the animals, their appetite and the mass indexes change were controlled. To examine the acute toxicity the investigated product was put into stomachs of white mice and white rats males by the stomach pump. The growing dose could be lethal for most animals within 15 days. The preparation was diluted with the 0,5 cm3 distilled water for the mice and 2 cm 3 for the rats. During the experiment the animals were pithed to estimate the hematology and biochemical indicators of their collected blood. The researches helps to conclude that the developed preparation has obvious anti-anemic effects, does not affect the animal’s health, is a low-toxic chemical substance – 4-th class of toxicity, does not have allergic, embryologic, teratogenic and skin-resorptive features, the accumulation level is weak. Feeding the animals with the preparation did not show any violations of the organs, tissues and the body system. The preparation positively affects the metabolic processes and weight gain of the white rats. The developed anti-anemic preparation is biologically valued. It has high consumer qualities; mass fraction of protein and iron, vitamins and other essential nutrients. The technologies allow producing a wide range of preparation for the children, pregnant and lactating women, patients in rehabilitation period and people affected the harmful factors in accidents at the industrial enterprises, living in risk areas and working in therapeutic and preventive medicine, suffering from iron-deficiency anemia.

1. Introduction
Nutritional factors and health are closely connected. Iron-deficiency anemia is one of the most common diseases among children, adolescents and women [1, 2].

It is almost impossible to provide our organism with iron from food even by diets – only an insignificant part of this microelement is acquired, and vegetarians are under the risk: vegetable iron only 1-2% is acquired whereas from meat – ten times more [3, 4].

More attention is paid to mass prevention of iron deficiency states by enrichment of specialized
foodstuffs by biologically active natural ingredients containing a lot of digestible iron [5].

The scientists of Voronezh State University of Engineering Technology and Plekhanov Russian University of Economics have investigated the slaughter cattle biotechnological plod potential, developed a wide range of the products with the anti-anemic action.

Indicators of products biological value are one of the main criteria determining the correctness of formulation, possibilities and using limits of individual components, influence of technological effect on raw materials and formulation components. Biological value is not be reduced as a result of a change in formulation or technology compared to that of a product produced by traditional technology [6, 7].

2. Materials and methods
Biomedical research with the using of the growth and weight, hematology and biochemical indicators was conducted to estimate the efficiency of the therapeutic and prophylactic food. The appearance and behavior of the animals, their appetite and the mass indexes change. During the experiments the animals were pithed to estimate the hematology and biochemical indicators of their collected blood [8, 9].

The experiments on the anti-anemic action of the products were carried out on white rats (females) of 3 months old and with the 198±20 g weight. The animals were divided into two 30 animal groups: experimental and control. The animals were being given food with the sodium nitrate: 1000 mg per 1 kg of animal weight for 20 days. Since the 12th day the experimental animals were being given the test product for the next 20 days. The dosage was estimated according to the daily human requirement of iron (15 mg) in relation to the laboratory animal’s weight [10, 11].

During the experiment biochemical and hematology research was carried out. The liver’s amount of iron was also controlled. The results have shown that the products are definitely anti-anemic effective [12].

3. Results and Discussion
Nitrate toxicity caused the anemia in white rats (allegedly of hypo plastic nature). The hemoglobin and meth hemoglobin mass fraction (Figure 1), erythrocytes (Figure 2) and iron number of the experimental animal’s blood returned to normal on shorter notice as compared to the control ones. The iron mass fraction of the animals (Figure 3), which were eating the preparation, is increasing steadily. The weight difference of the control and experience animals’ groups is positive (Figure 4). The data shows the good iron absorbability from the developed product, creating a store of iron in the liver. It contributes to fast recovering of the animals after the nitrate poisonings.

Figure 1. Biochemical blood indicators of animals’ control group.
Figure 2. Hematological indicators of animals’ control group.

Figure 3. Macro- and minerals contents change during the experiment.
To examine the acute toxicity, the investigated product was put into the stomach of white mice and white rats males by the stomach pump. The growing dose could be lethal for most animals within 15 days. The preparation was diluted with the 0.5 cm$^3$ distilled water for the mice and 2 cm$^3$ for the rats [13 - 15].

Microscopic analysis was carried out all the animals to detect possible changes of the liver, heart, kidneys, lungs, stomach and bowel (see Figure 5). The toxicity criteria are calculated by Behrens, Litchfield and Wilcoxon method.

Feeding the rats with the blood products of the pithed animals did not affect the internal organs, what means the absence of the toxicity.

The product single oral injection (dosing from 2500 mg/kg till 20000 mg/kg) into the stomachs of white rats and mice does not have toxic effects. Daily rats feeding (for 18 days) with the anti-anemic preparation in the amount of 1000, 5000 and 20000 mg/kg also did not affect general conditions, blood indicators, behavior and the appetite of the rodents.
Experiment was carried out on the white non-pedigreed mice (males) with the weight of 138-151 g to explore chronic toxicity. The animals were kept at the isolated cages. Burettes were attached to them to control water consumption. Rats were divided into two groups: control and experimental. The animals of control group were fed with the usual menu. The experimental group got the same menu, but with the anti-anemic preparation at a rate of human daily iron requirement (15 mg). It was about 3% of the given food.

A body mass change dynamic is demonstrated in the Figure 6. We can see that the weight of animals in both control and experimental groups was growing. The higher weight gain was seen in the experimental group. In the end of the experiment the weight of experimental group was 90.5 g and the weight of the control animals was 64.3 g. It is caused by the high mass fraction of the fat and protein of the food additive.

![Figure 6. Animal weight gain changing.](image)

Therefore, the injection of the explored preparation into the menu positively effects the rats’ growth. Special differences of the eating food and the water consumption per 100 g body mass were not noticed. We explored the integral indicators (appearance, body mass, internal organs mass), biochemical indicators (the protein content of the blood serum, some enzyme activity, the lipid content of the blood serum). Exploration results may be found in Table 1.

| Group of rats  | Liver, g      | Kidney, g    | Spleen, g   | Heart, g   | Adrenalglands | Lungs, g |
|---------------|--------------|--------------|-------------|------------|---------------|----------|
| Control       | 6.80±0.6     | 1.47±0.8     | 0.94±0.3    | 0.87±0.1   | 0.02±0.002    | 1.5±0.20 |
| Experimental  | 6.69±0.4     | 1.30±0.7     | 0.67±0.1    | 0.78±0.1   | 0.016±0.001   | 1.2±0.08 |

*Note. These are data about 5 rats.

Weight gain data show that the metabolism indicators of the white rats’ organism became better because of the explored preparation effect. It correlates with biochemical research results, shown in Table 2.

The anti-anemic product does not affect the state of animals’ health. But its positive effects on the metabolic processes and weight gain of the white rats were fixed.

An allergic features of the anti-anemic preparation were explored on rabbits by the conjunctival samples and on the guinea pigs by the skin application.

Rabbits got one drop of the preparation solution under their upper eyelid. They also got one drop of saline to the left eye for the control. The results were explored in 5 minutes, 24 and 48 hours. At the same time, we took into account the state of the mucous membrane of the eyes and eyelids, vessel
injections, tear secretions. During the whole observation period we didn’t notice changes connected with the animals’ eyes.

Table 2. Biochemical blood and bone tissue indicators of white rats

| Indicators                        | Groups of animals |          |
|-----------------------------------|-------------------|----------|
|                                   | Control           | Experimental |
| Blood serum:                      |                   |           |
| Glucose, mmol/dm³                 | 2.85±0.15         | 3.1±0.11 |
| Crude protein, g/dm³              | 80.2±3.37         | 83.0±5.70|
| Lipids, g/dm³                     | 2.05±0.42         | 2.30±0.19|
| Urea, mmol/dm³                    | 6.60±0.43         | 4.83±0.35|
| Alkaline phosphatase activity, mmol/dm³ | 4.75±0.69     | 4.6±0.25 |
| Inorganic phosphorus, mmol/dm³    | 1.96±0.20         | 2.12±0.14|
| Calcium, mmol/dm³                 | 2.89±0.08         | 3.09±0.31|
| Potassium, mmol/dm³               | 8.87±0.30         | 7.28±0.54|
| Sodium, mmol/dm³                  | 160.2±3.14        | 139.4±4.70|
| Bone tissue:                      |                   |           |
| Calcium, %                        | 16.83±1.00        | 16.73±1.18|
| Inorganic phosphorous, %          | 11.75±0.28        | 10.81±0.22|
| Strontium, %                      | 0.020±0.0006      | 0.017±0.0015|
| Ftorum, %                         | 0.077±0.0087      | 0.051±0.0090|

Provocative skin samples were explored on the guinea pigs by the apicotomy applications. Before the applications the animal sensitization was carried out by the repeated covering the skin with the preparation. Daily, an aqueous solution of the preparation, diluting in the amount of 1:10, 1:100, 1:1000, was applied in the shaved skin of the three guinea pigs. On the 14th day (the incubation period) on the recently shaved skin we applied a dose of the diluted preparation.

At the same time of the experiment the guinea pigs were observed, their temperature was taken, the skin folds were controlled and the temperature of the body part where preparation was applied, was measured too. The changes of animals and application spots were not noticed. The reaction was appreciated as a negative one.

Skin-resorptive effects were studied on white rats by the tail immersion method. 10 heads of animals were fixed in a special machine to place 2/3 of their tails in the tube with the 50 % preparation aqueous solution. The control of the reaction was carried out in 4 hours after the appearance of the local tail changes, deaths, intoxication severity and body mass changes. In this case, experimental white rats did not have the local tail changes, deaths, intoxication severity and body mass changes.

So, it demonstrates that the preparation does not have skin-resorptive effects.

Teratogenic and embryo toxic features were studied on white rats. Anti-anemic preparation was used in doses of 1 kg/kg of the body mass. The first pregnancy day was considered the day when sperm cells were found after infusion of males to females. The control animals did not get the preparation.

Results of the preparation embryo toxicity were fixed on the 17th pregnancy day. In the uterus the number of implantation spots, live and dead animals was counted. Some of the embryos were put into the Bouin liquid for the following assets of the internal organs by the Wilson micro-anatomic technique. The other embryos were put into 96 % ethanol and prepared enlightened preparations of the embryos, which was colored with alizarin red then.

Embryo toxicity criteria were the indicators of the embryos deaths in pre- and post-implantation stages of development (embryo lethality effect), the fertility level, weight and size of the embryos and placenta, abnormal internal organs (teratogenic effect).
According to the above characteristics, significant differences among the experimental and control groups of rodents were not established.

The environmental safety assessment of the using preparation was conducted comprehensively on the basis of the pharmacology research results, health based on the chemical stability, biological activities, latitude and the intensity of the metabolic capacities using and degradation abilities.

The assessment of environmental safety was carried out on 4 classes:

1. Highly hazardous strong toxic agents (LD50 no more than 50), in the external environment very resistant (the disintegration period 1-2 years), carcinogens (developing of cancer among people, strong carcinogens in animal experiments), strong allergens (cause an allergy among people in the small doses which are found in a usual situation), ulcer teratogen (the known as people’s ugliness reproduced in animal experiments), a selective embryo toxicity (in the doses not toxic for a maternal organism), strong mutagens (100% a mutation in experiences on drosophila, substances of extreme danger by production (maximum allowable concentration of harmful substances in air of the work area less than 0.1 mg/m³).

2. Dangerous highly toxic (LD50 from 50 to 200), in the external environment resistant (the period of disintegration of 6 months), the expressed cumulating (cumulating coefficient is 1-3), carcinogens (carcinogenicity in animal experiments) the weak allergens (cause an allergy in certain individuals) suspicious on a teratogen (existence of a teratogen in animal experiments), an embryotoxicity moderate (it is shown along with other toxic effects), average mutagens (2-5% of a mutation in experiences on drosophila, substances of high danger by production (maximum allowable concentration of harmful substances in air of the work area less than 0.1-1.0 mg/m³)).

3. Low-dangerous.Middle -toxic (LD50 from 200 to 1000), in the external environment moderately resistant (the period of disintegration of 1-6 months), weak carcinogens (carcinogenicity in animal experiments up to 20%), weak mutagens (1-2 mutations at drosophila), weak cumulation (coefficient of cumulation 3-5), moderately dangerous by production (maximum allowable concentration of harmful substances in air of the work area less than 1.1-10.0 mg/m³).

4. Safe low-toxic (LD50 1000 and more), low-resistant (from the disintegration period to 1 month), suspicious on carcinogenicity (on animals yielded doubtful results), weak mutagens (0.5-1.0% of mutations at drosophila), poorly expressed cumulation (coefficient of cumulation 5).

The investigated preparation is low in toxicity (its LD50 is more than 1000), not stable external environment, the accumulation level is weak (accumulation ratio is more than 5) and it does not have allergic, embryo toxic and teratogenic features.

According to the research basis, the use of anti-anemic preparation to correct the alimentary status is safe.

4. Conclusion
The research helps to conclude that the developed preparation has obvious anti-anemic effect, it does not affect animals’ health, is a low toxic chemical substance – 4th class of toxicity, does not have allergic, embryo toxic, teratogenic and skin-resorptive features; the accumulation level is weak (more than 5). Feeding the animals with the preparation did not show any violations of the organs, tissues and the body system.

The developed anti-anemic preparation is biologically valued. It has high consumer qualities, mass fraction of protein and iron, vitamins and other essential nutrients. Technologies allow producing a wide range of the preparation for the children, pregnant and lactating women, patients in rehabilitation periods and people affected the harmful factors in accidents at the industrial enterprises, living in risk areas and working in therapeutic and preventive medicine, suffering from iron-deficiency anemia.

References
[1] Murawska N, Fabisiak A and Fichna J 2016 Anemia of chronic disease and iron deficiency anemia in inflammatory bowel disease: pathophysiology, diagnosis, and treatment Inflammatory Bowel Diseases 22(5)1198-1208
[2] Nairz M, Theurl I, Weiss G and Wolf D 2016 Iron deficiency or anemia of inflammation?: Differential diagnosis and mechanisms of anemia of inflammation *Wiener Medizinische Wochenschrift* **166** (13-14) 411-423

[3] Stein J, Connor S, Virgin G, Ong D E H and Pereyra L 2016 Anemia and iron deficiency in gastrointestinal and liver conditions *World Journal of Gastroenterology* **22** (35) 7908-7925.

[4] Valério de Azevedo S, Maltez C and Lopes A I 2017 Pediatric crohn's disease, iron deficiency anemia and intravenous iron treatment: a follow-up study *Scandinavian Journal of Gastroenterology* **52** (1) 29-33

[5] Friedrisch J R and Cançado R D 2015 Intravenous ferric carboxymaltose for the treatment of iron deficiency anemia *Revista Brasileira de Hematologia e Hemoterapia* **37** (6) 400-405

[6] Jimenez K, Kuhnig-Dabsch S and Gasche C 2015 Management of iron deficiency anemia *Gastroenterology and Hepatology* **11** 4 241-250

[7] Kurchaeva E E, Manzhesov V I, Maksimov I V, Parshchenko V L, Churikova S Yu and Glotova I A 2018 Biotechnological approaches in processing of secondary raw materials of meat industry *Periodico Tche Quimica* **15** (30) 717-724

[8] Suleimanov S M, Usha B V, Vatnikov Yu A, Sotnikova E D, Kulikov E V, Parshina V I, Bolshakova M V, Lyashko M U and Romanova E V 2018 Structural uterine changes in postpartum endometritis in cows *Veterinary World* **11** (10) 1473-1478

[9] Volkov A A, Staroverov S A, Kozlov S V, Kalyszhyi I I, Domnitsky I Yu, Nikulin I A and Derezina T N 2015 Study of therapeutic properties of the prototype injection of a hepatoprotective drug based on flavolignans of Silybum marianum *Biology and Medicine* **7** (2) 192-199

[10] Derezina T N, Volkov A A, Ushakova T M, Staroverov S A, Kozlov S V, Kalyszhyi I I, Domnitsky I Y and Nikulin I A 2016 Morphological characteristics of the target organs of lymphoid and digestive systems under secondary immunodeficiency condition in calves *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **7** (6) 349-354

[11] Scholz R, Knyazeva M, Porchetta D, Wegner N, Walther F, Senatov F, Salimon A and Kaloshkin S 2018 Development of biomimetic in vitro fatigue assessment for implant materials *Journal of the Mechanical Behavior of Biomedical Materials* **85** 94-101

[12] Ansari A M, Ahmed A K, Matsangos A E, Lay F, Born L J, Marti G, Harmon J W and Sun Z 2016 Cellular GPF toxicity and immunogenicity: potential confounders in in vivo cell tracking experiments *Stem Cell Reviews and Reports* **12** (5) 553-559

[13] Kurnik-Lucka M, Bugajski A, Gil K and Panula P 2018 Salsolinol: an unintelligible and double-faced molecule-lessons learned from in vitro experiments *Neurotoxicity Research* **33** (2) 485-514

[14] Ashour M L, Youssef F S, Gad H A, El-Readi M Z, Sobeh M, Wink M, Bouzabata A and Abuzeid R M 2018 Evidence for the anti-inflammatory activity of bupleurum marginatum extracts using in vitro and in vivo experiments supported by virtual screening *Journal of Pharmacy and Pharmacology* **70** (7) 952-963

[15] Motwani H V, Frostne C and Törnqvist M 2017 Parallelogram based approach for iv vivo dose estimation of genotoxic metabolites in humans with relevance to reduction of animal experiments *Scientific Report* **7** (1) 17560