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Assessment of biological activity of natural iron-containing mineral waters at their internal application

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Abstract

In experiments on rats of the Wi-star line, the influence of two iron-containing weakly mineralized mineral waters with a high content of organic matter (MW) from the sources № 13 and № 15 of Skhidnytsia deposit (Ukraine). It was determined that both MW do not have a toxic effect on their use on animals, as evidenced by the lack of significant structural and functional changes in the tissues of the stomach, kidneys, liver, and heart. It is established that MW from source No. 13 changes the activity of transamination processes in hepatocytes, increases bile formation and biliary excretion, and activates red blood counts. MW from source No. 15 has a similar effect, but more significantly affects the metabolism, red blood, and immune system. The research results justify the use of these iron-containing MW for the correction of iron deficiency conditions in laboratory animals.

Keywords: ferrous mineral waters; metabolism; red blood; structural changes of internal organs

Introduction. Mineral water (MW) is one of the most common natural healing resources of Ukraine, the biological and therapeutic effect of which is associated with the presence in their composition of macro-and micronutrients and biologically active compounds [1, 2]. Depending on the characteristics of the physicochemical composition, MW can increase the activity of the body's defense systems, which allows reasonable use of MW of different wells and sources in case of disorders in the body [3-7]. One of the most famous deposits of medical MW of Ukraine is the Skhidnytske deposit in the Lviv region, interesting for the presence of springs with weakly mineralized waters with a high content of organic substances and iron. Water springs (ws.) № 13 and № 15 contain ferrous iron in a concentration that allows them to be classified as ferrous (iron is considered to be MW with a concentration of 10.0 mg/dm^3). The content of iron ions in MV ws № 13 is 14.0 mg/dm^3, and in MW ws. № 15 - 38.0 mg/dm^3. The presence of divalent iron ions in these MWs is of particular importance because the iron in Fe^{+2} ions is well absorbed by the body. One of the most famous deposits of medical MV of Ukraine is the Skhidnytske deposit in the Lviv region, interesting for the presence of springs with weakly mineralized waters with a high content of organic substances and iron. Water springs ws № 13 and № 15 contain ferrous iron in a concentration that allows them to be classified as ferrous (iron is considered to be MW with a concentration of 10.0 mg/dm^3). The content of iron ions in MW ws. № 13 is 14.0 mg/dm^3, and in MW ws. № 15 - 38.0 mg/dm3. The presence of divalent iron ions in these MWs is of particular importance because the iron in Fe^{+2} ions is well absorbed by the body.

Iron deficiency in the human body and its extreme degree - iron deficiency anemia (IDA) causes hypoxia and biochemical, morphological and functional changes in almost all organs and systems of the body [8, 9, 10]. The main causes of iron deficiency are insufficient intake of iron from food, inadequate utilization of iron in chronic inflammatory diseases, impaired absorption or excessive loss of this trace element [11, 12, 13]. There is a significant prevalence of iron deficiency among the adult population of many countries, with anemia in women several times more common than in men [14].

According to the WHO, about a quarter of the world's population suffers from anemia [15, 16]. Although IDA is treated with iron-containing drugs, the use of iron CF is promising due to a number of advantages, the main of which are more efficient absorption of iron than with drugs or food, lack of allergic and toxic effects [17, 18].

Aim of the research. Based on the above, the purpose of the study: to assess the impact of iron MW in their internal use on the body of rats to justify their use in the correction of iron deficiency.

Materials and methods. Experimental studies were performed on white female rats of the Wistar line of self-breeding breeding with a body weight of 190.0-210.0 g. The animals were divided into three groups. The first control group (comparison) consisted of intact animals. The animals of the second received MW ws. № 13, and animals of the third group received MW ws. № 15. Appropriate MW were administered to animals in the esophagus with a soft probe with olive, once a day, at a dose of 1% of the animal's body weight, in the evening (approximately 17.00), taking into account the peculiarities of the daily biorhythm of rats. Rats received MW course, which consisted of 7 daily injections. The obtained data were compared with similar indicators of the first group of rats (control group). Animal studies have been conducted according to existing legal documents and guidelines [19, 20]. Immunological studies determined the response of the peripheral blood to the development of the corresponding process, which was assessed by changes in the number of leukocytes, the ratio of the elements of the blood formula. The state of the immune system was assessed by changes in its cellular and humoral components. The response of the cellular immune defense was evaluated by determining the number of total T lymphocytes. The response of the humoral immune defense was evaluated by determining the content of heterophilic antibodies (HA) and the content of circulating immune complexes (CEC). The functional state of the liver was determined by the activity of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), the Ritis index and the level of total bilirubin and its fractions in the blood; also determined the concentration of hemoglobin and the number of erythrocytes in the peripheral blood. According to the results of morphological studies determined the state of structural and functional organization of the heart, stomach, liver and kidneys; histoenzymatic reactions established the activity of succinate dehydrogenase (SDG) and lactate dehydrogenase (LDH) in relative units of optical density (U). Methods and techniques used in the research are published in the "Guide" and guidelines [21, 22]. Statistical processing of the data obtained in the series of experiments was performed using the programs of biomedical studies Statistica and Exel. With all means of processing statistical material, those within the range of the Student's tables were considered to be significant shifts less than <0.05.

The study used MW sources № 13 and № 15 urban-type settlements (town) Skhidnytsia Drohobych district of Lviv region Ukraine. MW of this type in terms of total iron (Fe) and organic matter (Sorg.) In accordance with the requirements of the State Standard of

Ukraine (DSTU) are medicinal waters [23]. It should be noted that the stability of the chemical composition of MW ws. № 13 and № 15 were confirmed by the results of long-term observations of the State Institution "Ukrainian Research Institute of Medical Rehabilitation and Balneology of the Ministry of Health of Ukraine" during the operation of the field.

MW spring water ws. № 13 has a weakly acidic reaction (pH 6.7 units pH). Gases that affect the healing properties of water are present in concentrations below balneological norms. Hydrogen sulfide in the amount of 2.30 mg/l, carbon dioxide - 0.31 mg/l. Source water № 13 is weakly mineralized sodium-magnesium bicarbonate. MW ws. № 13 contains various components and compounds. Lead, cadmium, chromium and some others are determined in concentrations less than 0.01 mg/l. In high concentrations, water contains - total iron (Fe) in the amount of 14.0 g/l at a balneological rate of 10.0 mg/l and sorghum – in the amount of 11.42 mg/l at a balneological norm of 5.0 mg/l. Other biologically active components and compounds that are normalized in balneology and add specific properties (iodine, bromine, metasilicic acid, orthoboric acid), were found in small concentrations. The total mineralization is 0,31 g/l, bicarbonate ions was 195.2 mg/l, sulfate ions – 28.4 mg/l, chloride ions – 21.3 mg/l, magnesium ions – 36,5 mg/l, sodium and potassium ions – 20.4 mg/l, calcium ions – 10.0 mg/l.

MW ws. № 15 has a weakly acidic reaction (pH 6.2 pH units). Gases that affect the healing properties of water are present in concentrations below balneological standards. Hydrogen sulfide was determined in water – 0.54 mg/l, carbon dioxide – 1.18 mg/l. Source water № 15 is weakly mineralized sulphate-hydrocarbonate calcium-sodium. The stability of the chemical composition of water is also confirmed by the results of long-term observations during the operation of the field. MW ws. № 15 contains various components and compounds. Lead, cadmium, chromium and some others were detected in concentrations less than 0.01 mg/l. In high concentrations, water contains – total iron (Fe) in the amount of 38.0 mg/l and organic carbon ($C_{org.}$). In the amount of 10.25 mg/l.

Other biologically active components and compounds that are normalized in balneology and add specific properties (iodine, bromine, metasilicic acid, orthoboric acid), were found in concentrations below the limit. The total mineralization is 0.34 g/l, bicarbonate ions was 158.6 mg/l, sulfate ions – 66,2 mg/l, chloride ions – 21,3 mg/l, sodium and potassium ions – 68,5 mg/l, calcium ions – 20,0 mg/l, magnesium ions – 7,3 mg /l.

Results and its discussion. Under the influence of MW ws. № 13 there was a slight but significant increase in hemoglobin concentration and the number of erythrocytes in the peripheral blood (Table 1). Thus MW ws. № 15 causes a more noticeable increase in these indicators. MW ws. № 13 leads to redistribution of formed blood elements: the number of neutrophils significantly increased and the percentage of lymphocytes decreased. The number of monocytes was also significantly reduced.

Table 1. Influence of MV ws. № 13 and ws. № 15 in the course of internal use on the indicators of peripheral blood and immune system

Indicators	1 group	2 group	3 group
	($M_1 \pm m_1$)	($M_2 \pm m_2$)	($M_3 \pm m_3$)
Hemoglobin, g/l	157,05 ± 1,05	164,32 ± 0,92*	168,60 ± 0,8*0
Erythrocytes, 10^{12}/l	4,01 ± 0,09	4,41 ± 0,08*	4,63 ± 0,06*
Leukocytes, 10^9/l	5,5 ± 0,2	6,0 ± 0,2	5,8 ± 0,3
Neutrophiles, %	12,79 ± 0,64	21,60 ± 2,11*	20,20 ± 3,26*
Acidophiles, %	2,25 ± 0,23	2,20 ± 0,20	3,80 ± 0,86
Monocyte, %	3,72 ± 0,21	2,40 ± 0,51*	3,00 ± 0,32
Lymphocytes, %	81,20 ± 0,8	73,80 ± 1,98*	73,00 ± 2,92*
T-lymph. freezing, %	47,2 ± 0,6	45,1 ± 2,2	44,8 ± 2,81
CIC, mg/ml	5,7 ± 0,2	7,5 ± 0,3*	5,9 ± 0,19
HA, U	6,0 ± 0,8	5,4 ± 1,1	11,2 ± 1,96*

Notes:* - the significance of the changes calculated from the control group ($p<0.05$).

The total number of leukocytes and the number of acidophils remained within the range of intact rats. On the part of the immune system, there is a slight increase in the level of CIC while maintaining the level of control of the percentage of total T- lymphocytes and the content of heterophile antigen.

Internal use of MW ws. № 15 in rats caused a moderate response from peripheral blood and immune systems (Table 1). The redistribution of formed blood elements was determined (the number of neutrophiles increased and the percentage of lymphocytes significantly decreased). The total number of leukocytes remained within the intact rats. On the part of the immune system, there is an activation of the humoral link - a significant increase in the content of heterophilic antibodies (indicating the activation of the link of nonspecific immunity), while maintaining within control the percentage of total T lymphocytes and CIC levels.

Thus, the internal use of MW ws. № 13 and MW № 15 elicit a moderate response from peripheral blood and the immune system in rats, which is manifested in the redistribution of blood cells, a decrease in the percentage of monocytes and an increase in

CIC levels, in contrast to the more pronounced effect of MW ws. № 15, which increases the content of heterophilic antibodies. Data on the impact of MW ws. № 13 and ws. № 15 on the indicators of metabolism are given in table 1. The use of MW ws. № 13 leads to a significant decrease in the activity of enzymes reamination of AlT and AST. At the same time, the level of the Ritis index increases in comparison with the control group. The content of total, direct and indirect blood bilirubin is also significantly reduced compared to data in intact animals. Under the influence of MW ws. № 15 a significant decrease in ALT activity and an increase in the level of the AST/ALT ratio were found.

Table 1. Influence of MW ws. № 13 and ws. № 15 for course internal application for biochemical parameters

Indicators	1 group	2 group	3 GROUP
	($M_1 \pm m_1$)	($M_2 \pm m_2$)	($M_3 \pm m_3$)
Bilirubin, µmol/l total conjugated indirect	 8,44 ± 0,28 3,06 ± 0,18 5,38 ± 0,15	 5,93 ± 0,25* 2,16± 0,19* 3,77 ± 0,36*	 3,67 ± 0,18* 2,22 ± 0,16 1,76 ± 0,17*
ALT, U	113,31 ± 2,13	95,44 ± 2,34*	73,40 ± 3,61*
AST, U	289,64 ± 12,12	186,07 ± 14,06*	268,06 ± 11,19
AST/ALT ratio	2,56 ± 0,11	3,39 ± 0,30*	3,67 ± 0,18*

Notes:* - the significance of the changes calculated from the control group ($p<0.05$).

Reliable and to a greater extent than under the influence of MW ws. № 13 the content of total bilirubin decreases due to a decrease in its fractions - direct and indirect bilirubin in the blood compared with data from intact animals.

Morphological studies of target organs of healthy rats treated with MW ws № 13, set the following. Stomach: submucosal plate without changes. Mucous tubular glands, epitheliocytes with juicy stained cytoplasm, with juicy enlarged nuclei. Interstitial layers are thin. SDG activity — (7.0 ± 0.30) U; LDH activity — (6.0 ± 0.17) U. Liver: lobular organization preserved. Hepatocytes are collected in beams. Their cytoplasm is homogeneous, nuclei of medium size of moderate color. The vessels are sharply full-blooded. SDG activity — (7.0 ± 0.16) U; LDH activity — (6.0 ± 0.30) U. Heart: layered and bundle organization of the myocardium is preserved. Interstitial layers are thin. In cardiomyocytes, the nuclei are rounded, well colored. SDG activity — (7.0 ± 0.17) U; LDH activity — (7.0 ± 0.20) U.

Kidneys: structure of the nephron and its components without obvious changes. In endothelial cells of the vacuole. SDG activity — (7.0 ± 0.19) U; LDH activity — (6.0 ± 0.21) U.

Morphological studies of target organs of healthy rats treated with MW. № 15 set the following. Stomach: submucosa of densely packed fibrous fibers, glandular tubular gland, epitheliocytes with eosinophilic cytoplasm, juicy nuclei. SDG activity — (7.0 ± 0.21) U; LDH activity - (6.0 ± 0.13) U. Liver: lobular structure preserved. The vessels are sharply full-blooded. Interbeam spaces are slotted. Hepatocytes with granular eosinophilic cytoplasm. Kernels of medium size. There are areas with disordered polymorphic cells that are similar to hepatocytes. The activity of LDH — in the center of the lobe — (5.0 ± 0.11) U, on the periphery - (6.0 ± 0.19) U; LDH activity — (5.0 ± 0.71) U. Heart: layered and bundle organization of the myocardium is preserved, cardiomyocytes contain either moderately enlarged nucleus color, or nuclei elongated dark. SDG activity — (7.0 ± 0.41) U; LDH activity — (6.0 ± 0.17) U. Kidneys: the structure of the nephron and its components are normal, in the glomeruli the capillaries are stagnant. SDG activity — (6.0 ± 0.23) U; LDH activity — (6.0 ± 0.31) U. That is, significant changes in the structural and functional organization of the studied organs do not occur, except for signs of increased functional activity of the liver.

Conclusions. The research results showed that fluctuations in metabolic rates in animals under the influence of MW from both sources did not go beyond the physiological norm and did not cause harmful or toxic effects. These MW are safe when taken orally at a dose of 1% of body weight. Both MW activate the processes of bile formation and biliary excretion, provide activation of the red blood system and indicators of nonspecific immune defense, therefore, they can be considered as promising means of correcting iron deficiency states.

Prospects for further research. The data obtained allow us to continue studying of the corrective action of iron-containing mineral waters in rats with a model of iron deficiency anemia.

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