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Metabolic Syndrome: The Corrective Effect of Mineral Water with an Increased Content of Organic Substances

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Abstract

The pathogenesis of MS is complex and completely not studied, which justifies the importance of conducting research in this field, including experimental ones. Recently, in order to treat MS and its complications, along with drug therapy, non-pharmacological agents are used - natural therapeutic resources, which include mineral water (MW). MW are widely used in medical practice, due to the possibility of prolonged use, the combination of general non-specific effects on the processes of body sanogenesis in general and local pathogenetic mechanisms. Objective of this study was to investigate the effect of MW with increased content of organic substances on its internal application to the metabolic rate of experimental animals with the MS. In the experiment were used 40 white male rats. For reproduction of the MS model, the animals were kept for 60 days in a standard diet, while the rats additionally received 30 grams of white bread crumbs per animal and consumed only 10% fructose solution in distilled water (as a drinking liquid). MW was injected into the esophagus of animals with a soft probe with olive, once a day for 12 consecutive days, at a dose of 1% of the body weight of the animal. At day 60 of the experiment in rats with MS, blood glucose levels were increased by 3 mmol/l ($p < 0.01$), cholesterol and triglycerides by 32 and 154% ($p < 0.01$). Against the backdrop of the development of MS, the functional state of the kidneys is impaired, the volume of daily diuresis, the excretion of urea and the excretion of chloride ions decreases by 37%, 20% and 36%, respectively ($p <0.001$). Also, the balance of the immune system parameters is disturbed: the number of lymphocytes ($p <0.001$) and active phagocytes (their absorbing and metabolic functions ($p <0.05$)) decreases reliably. At the same time, separate links of the humoral response are activated - the content of circulating immune complexes, heterophilic antibodies and antibodies to liver and kidney tissues is significantly increased (for the listed parameters, $p <0.001$). Application of MW "Lotus" leads to complete normalization of the functional state of the kidneys and partial normalization of immunological parameters. The established effects indicate a significant corrective effect of MW "Lotus" on urine forming, ion-regulating and excretory renal function.

Keywords

Metabolic Syndrome, Mineral Water, Organic Substances, Kidneys, Immune System

1. Introduction

The relevance of complex disorders of life called "metabolic syndrome" (MS), is growing due to the spread of this phenomenon. The reason for this - recently increased the prevalence of MS, which can be considered an epidemic. This illness affects about 25% of the adult population of developed countries [1, 2]. An important basis for the study of MS and the active use of therapies for its components is the high risk of developing cardiovascular diseases and their complications [3]. According to the National Cholesterol Education Program-Adult Treatment Panel III, the criteria for MS syndrome are the presence of not only three violations such as abdominal obesity, hyperglycemia on an empty stomach, hypertriglyceridemia, hypertension, low cholesterol of high density lipoprotein [4, 5]. The most widespread and commonplace is the point of view regarding the leading role of insulin resistance, as a mechanism that triggers the entire cascade of metabolic-interrelated MS violations [6]. Therefore, the search for therapeutic possibilities for the restoration of impaired target-cell sensitivity to insulin action remains the most promising in the prevention and treatment of MS complications [7, 8]. In this connection, should be indicated the possibility and efficiency of regenerative medicine, in particular the use of non-medicated means of correction of the components of the MS with the help of natural therapeutic resources, which include mineral water (MW) [9, 10].

MW is a salt solution with the characteristic content and the ratio of macro-elements (sodium, calcium, magnesium, potassium, chlorine, phosphorus, sulfur, nitrogen, carbon, oxygen, hydrogen), micro-elements and biologically active substances (BAS), such as boron, silicon, fluorine, arsenic, iron, cobalt, manganese, iodine, zinc, hydrogen sulfide, organic matter, etc. The peculiarities of this correlation determine the molarity, which is individual for each water. Microorganisms and BAS are in small quantities in the waters, but their physiological effect is very clearly manifested, which causes medical indications for the use of these MW. First of all, it applies to weakly mineralized MW, in which the sum of all substances dissolved in water (molarity) is up to 1.0 g/dm^3. Previous studies found that MW "Lotus" with increased content of organic substances has a stimulating effect on the body of healthy white rats - greatly stimulates the functional state of the kidneys: after the course of use of this MW, the volume of daily diuresis increased by 115% ($p < 0.001$) due to acceleration of glomerular filtration rate by 80% ($p < 0.001$) and reduction of tubular reabsorption by 0.15% ($p < 0.001$). The excretory function of the kidneys is also significantly stimulated, as evidenced by an increase in the daily excretion of urea and creatinine by 114% and 80% ($p < 0.001$), while the excretion of chloride ions increased by 14% ($p < 0.05$). Biliary function of the liver is also stimulated: in the blood, the level of total bilirubin is significantly reduced by 36% ($p < 0.05$) due to reduction of its fraction - indirect bilirubin (by 45% at $p < 0.01$). The action of MWon the functional activity of the central and autonomic nervous system of animals is determined by a mild soothing effect [10]. Such a stimulating effect of this MW on the healthy organism of mammals can be explained by the phenomenon of hormoses - that is, the activation of the protective and adaptive ability of the organism to the effect of the stress-inducing factor of low intensity, which is MW with high content of organic matter. That is, in general, MW "Lotus" has a beneficial effect on the metabolism of experimental animals. This provision justified the need for further studies to determine the existence of correctional effects of MW on such a common pathology as MS (in the development of which many parts of the metabolism are disturbed).

2. Materials and Methods

The experiment was conducted on white male rats of the Wistar line of outbred breeding, obtained from the private enterprise “Biomodelservice”, Kyiv, Ukraine. Experimental studies were conducted in accordance with the rules established by the Directive of the European Parliament and the Council (2010/63 / EU), by the order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 of March 1, 2012 "On Approval of the Procedure for conducting scientific experiments, experiments on animals by scientific institutions " and methodical recommendations [11, 12, 13]. During the experiment, the animals were in the experimental biological clinic (vivarium) of the UkrRIMRKB Ministry of Health of Ukraine in the conditions of free access to food and water. The animals were kept in standard laboratory conditions: photoperiod - light / darkness 12:12; air temperature - 20 ± 2 ° C; Humidity - 55 ± 10%. The rats were kept (contained) in cages of food plastic (400 × 550 × 250 mm) with soft wood chips as bedding. The experiment lasted 72 days. At the beginning of the experiment, the weight of the animals was 230.0 – 250.0 g.

. The animals were divided into three groups:

- I group - 16 intact rats (control);
- II group - 12 rats, in which the model of the metabolic syndrome was recreated;
- III group - 12 rats, which, on the background of development of metabolic syndrome (from 60 to 72 days of experiment), conducted water loading by packed and degassed MW "Lotus".

MW was injected into the animal's esophagus with a soft probe with olive, once a day for 12 consecutive days, at a dose of 1% of the body weight of the animal, in the evening (approximately at 17.00), taking into account the features of the daily biorhythm of the rats. A number of experimental models of the MS have been known [14], but we have selected and modified the model without the use of excessive vegetable or animal fats in the diet, as well as the use of less fructose concentrations than in conventional models (20 or 30% solution of fructose). For reproduction of the MS model, the animals were kept for 60 days (8 weeks) in a standard diet (full fodder, grain, vegetables), but the rats additionally received 30 g of white bread per animal and consumed only

10% fructose solution in distilled water (as a drinking liquid) in the mode of free access to drinking water.

The study of the functional state of the kidneys was carried out to determine the volume of diuresis daily, for which animals were placed in special cells to collect daily urine. The functional state of the kidneys was assessed by the state of the urine function (glomerular filtration rate, tubular reabsorption, diuresis daily), excretory function (for excretion of creatinine, urea and chlorides), acid-base reaction of daily urine was determined by hydrogen ion concentration indices. Concentration of creatinine in urine was carried out using Popper's method. Determination of urea in daily urine was carried out by urease method with a Nessler reagent. Determination of chlorides in urine was performed by the Mora method.

By biochemical methods in blood serum were determined glucose, cholesterol, triglycerides and markers of endogenous intoxication - MSM_{254} and MSM_{280}, creatinine, urea and uric acid. Determination of glucose concentration in blood was carried out by glucose oxidant method [15]. The choice of immunological tests was based on the fact that the most characteristic feature of MW action is the stimulation of factors of non-specific immune defense. In this connection, the state of the immune system was investigated in terms of changes in its cellular and humoral links. The response from the cellular link of immune defense was evaluated by the determination of the number of common T-lymphocytes. The reaction from the side of the humoral link of immune protection was evaluated by determining the level of heterophilic antibodies (HA), circulating immune complexes (CIC), the content of antibodies to the liver and kidney tissue. The activity of the phagocytic process was evaluated by determining the number of active phagocytes, their absorption function - phagocytic index (FI). The metabolic function, that is, determination of oxygen-activating ability of neutrophils was determined in the nitro blue tetrazole test (NBT) - spontaneous and stimulated [16].

The methodical techniques and techniques used in the research were published in the manual and approved by the order of the Ministry of Health of Ukraine No. 692 of 09/28/2009 [17]. The obtained data were compared with similar indices of intact rats (control group). The statistical processing of the data obtained in the series of experiments was carried out by the method of indirect differences, while the reliable shifts were those that were within the probabilities according to the Student tables $P < 0.05$ [18]. During the experiment, each day, we recorded the weight of animals, the amount of fructose and water consumed and the amount of food consumed.

The study used mineral water «Lotus» refers to the high content of organic matter, weakly mineralization hydrocarbonate-magnesium-sodium MW. The content of hydrocarbons - 0.4453 g / l; chloride-ion content – 0.0572 g / l; sulfate content – 0.0823 g / l; the content of sodium ions and potassium ions – 0.1568 g / l; the content of calcium ions 0.0320 g / l; the content of magnesium ions – 0.0268 g / l. Total mineralization is 0.80 g / l. The content of meta-silicic acid is 16.24 mg / l. Organic matter organic carbon content – 0.016 g / l. Osmolality is - 17.8 mosm / l.

Well relates to the display of Romanov Zbruchanske deposit MW and located in the National Park "Podolski Tovtry" Khmelnytsky region of Ukraine. This water is poured and packaged and is saturated with carbon dioxide, which ensures the preservation of its biological activity for a long time (6 months and more).

3. Results and Discussion

According to the data in Table 1, MS development in rats is accompanied by a decrease in the activity of urine production processes. The volume of diuresis per day decreases by 47% due to a significant increase in the percentage of canalic reabsorption (by 0.16%) against the background of preservation of glomerular filtration velocity (GFV) at the level of control data ($p > 0.5$). A decrease in the daily excretion of nitrogen exchange products has been established - the urea excretion decreases by 20% ($p < 0.01$).

Table 1. *Functional state of kidney of rats.*

Indexes	I group	II group	III group
Daily diuresis ml / dm^2 of the body surface	100	63*	150*
Glomerular filtration velocity, ml/(dm^2×min)	100	100	166*
Tubular reabsorption, percent to filtration,%	100	100	100
Withdrawal of creatinine, mmol	100	100	166*
Urea removal, mmol	100	80*	173*
pH of daily urine, units	100	133*	99
Concentration of potassium ions in a daily urine, mmol/l	100	211*	123*
Daily excretion of potassium ions, mmol	100	114*	150*
Concentration of sodium ions in a daily urine, mmol/l	100	234*	418*
Daily excretion of sodium ions, mmol	100	138*	266*
Concentration of chloride ions in a daily urine, mmol/ l	100	88*	64*
Daily excretion of chloride ions, mmol	100	44*	90

Notes:
1. The data of the first group are taken as 100%
2. * - significant changes to the control ($p < 0.05$).

The pH of the 24-hour urine is alkalinized (its value increases by 33%). The concentration of potassium and

sodium ions increases by 111 and 134%, and their excretion by 14 and 38%, respectively. At the same time, the concentration of chloride ions is reduced by 12%, and their excretion - by 56%. It can be assumed that the delay of chloride ions in the body of rats with the model of MS contributes to the compensatory decrease in the volume of diuretic diurnal, and the kidneys withdraw some hyperosmotic urine in a small amount.

The use of MW in animals with MS leads to the restoration and stimulation of urinary and excretory renal function: the volume of diuresis per day is increased by 50% due to the acceleration of GFV by 66%, the withdrawal of creatinine and urea is increased by 66 and 73%, and potassium and sodium - at 50 and 320%. The excretion of chloride ions rises to the level of control (p> 0.5), pH of the urine is restored and does not differ from the data of the control group (p> 0.5). The excretion of potassium and sodium ions in animals with MS that were given MW increases by 50 and 166% compared to the control group (in rats with MS, the excretion of potassium and sodium ions increases by 14 and 38%). The established effects indicate a significant corrective effect of MW "Lotus" on urine forming iono-regulatory and excretory functions of the kidneys against the backdrop of the development of the pathological process. Table 2 shows data on the dynamics of changes in physiological parameters of animals with MS and animals, which was received by the MW on the background of pathology development.

Table 2. *Dynamics of changes in animal weight and fluid intake.*

Indicators	I group	II Group	III Group
Body weight, g	100	114*	106
Consumption of 10% solution of fructose, ml	—	200*	130*
The amount of food consumed:			
White bread crackers, g	100	190*	120*
Mixed feed, g	100	60*	90*
Vegetable blend, g	100	90	115*

1. The data of the first group are taken as 100%;
2. * - significant changes in relation to control (p < 0.05).

The weight of animals with MS significantly increased by 14% (<0.01), and under the influence of MW, decreased by 8% and did not differ from controls (p> 0.5). The amount of consumed white breads (sources of carbohydrates) in animals of group II increased by 90%, and the amount of mixed fodder, on the contrary, decreased by 40% against the background of appetite deterioration. Animals of the III group under the influence of MW had positive changes - the appetite of animals revived: the consumption of white bread decreased by 70%, and the use of mixed fodder and a mixture of vegetables increased by 30 and 15% compared with the indicators of the II group. The consumption of fructose solution in animals against the backdrop of MS development exceeded the control data by 100% (animals consumed almost 50 ml of fructose solution per day, and probably thirst), while in the group of rats with MS, which received dosed MW, the consumption of fructose solution decreased by 50%. It should be emphasized that the animals of the II group on the 72nd day of the experiment had a slippery appearance, the furs were dull and liquid. The animals looked stagnant, lethargic and tired, but during manipulations (drinking MW, etc.) they behaved confusedly - were frightened and annoyed, drawing attention to frequent urination.

There is a significant decrease in glucose ($p < 0.05$), but its level still exceeds the control by 1 mmol/l (table 3). The content of cholesterol, MSM_{280} and uric acid is completely restored ($p > 0.5$), but the content of triglycerides remains at the level of the rats with a pathological model, and the content of creatinine and urea exceeds the corresponding indices of rats with MS by 18 and 80% ($p < 0.01$).

Animals of group III, which received MW, at the end of the experiment did not look restrained and tired, frustration and frequent urination disappeared.

In rats with MS (Group II), a significant increase content of glucose in the blood was 3 mmol / l (58%) $p < 0.01$, an increase of the content of cholesterol and triglycerides by 32 and 154% respectively at $p < 0.01$ (Table. 3).

Also, the content of markers of endogenous intoxication - MSM_{280} increased by 41% ($p < 0.01$), creatinine and urea by 23% ($p < 0.01$) in the blood. The uric acid content increased by 66% compared with the control group ($p < 0.01$). The internal dosage introduction of MW to experimental animals with the MS model facilitated the partial normalization of disturbed metabolic parameters.

Table 3. *Dynamics of biochemical indices, M ± m.*

Blood Indicators	I group	II GROUP	III group	P_{2-1}	P_{3-1}
Glucose, mmol/l	5.11 ± 0.22	8.06 ± 0.33	6.16 ± 0.13	< 0.01	p_2
Cholesterol, mmol/l	1.63 ± 0.10	2.15 ± 0.11	1.61 ± 0.06	< 0.01	< 0.05
Triglycerides, mmol/l	1.10 ± 0.06	2.80 ± 0.27	3.08 ± 0.32	< 0.01	> 0.5
MSM_{254}, c.u.	0.34 ± 0.02	0.30 ± 0.01	0.30 ± 0.01	> 0.5	< 0.01
MSM_{280}, c.u..	0.22 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	< 0.01	> 0.5
Creatinine, mkmol/l	47.80 ± 0.63	59.04 ± 1.78	67.97 ± 1.71	< 0.05	> 0.5
Urea, mmol/l	2.80 ± 0.27	3.71 ± 0.21	6.76 ± 0.39	< 0.05	< 0.01
Uric acid, mkmol/l	292.52 ± 6.87	486.17 ± 15.32	260.27 ± 21.71	< 0.01	< 0.01

Notes:
1. P_1 - calculated between the indices of the II and the I group;
2. P_2 - calculated between the indices of the III and the I group.

The development of the MS model in rats caused a significant decrease in the total number of T-lymphocytes ($p < 0.05$) (Table 4).

***Table 4. I**ndicators of the immune system, (M ± m).*

Indicators	I group	II Group	III Group	P_{2-1}	P_{3-1}	P_{3-2}
Common T-lymphocytes,%	47.2 ± 0.6	35.8 ± 1.16	38.2 ± 0.7	< 0.001	< 0.001	> 0.5
Phagocytosis, the number of active phagocytes,%	39.9 ± 0.5	38.0 ± 0.3	39.4 ± 0.7	< 0.05	> 0.5	> 0.5
Phagocytic index, c.u.	2.10 ± 0.04	1.80 ± 0.04	1.98 ± 0.03	< 0.001	< 0.05	< 0.001
Nitrosin tetrazole test, mg / ml:						
Spontaneous	0.039 ± 0.001	0.032 ± 0.001	0.035 ± 0.001	< 0.005	< 0.05	< 0.05
Stimulated	0.090 ± 0.002	0.082 ± 0.001	0.085 ± 0.002	< 0.05	> 0.5	> 0.5
Circulating Immune Complexes, mg / ml	5.7 ± 0.2	6.7 ± 0.1	6.0 ± 0.2	< 0.001	> 0.5	< 0.005
Heterophilic antibodies, c.u.	6.00 ± 0.80	8.15 ± 0.21	6.54 ± 0.57	< 0.05	> 0.5	< 0.01
Antibodies to the liver tissue, c.u.	3.3 ± 0.7	16.0 ± 2.5	10.0 ± 3.2	< 0.001	> 0.5	> 0.5
Antibodies to the kidney tissue, c.u.	2.2 ± 0.3	10.0 ± 3.2	8.0 ± 3.7	< 0.05	> 0.5	> 0.5

Notes:
1. P_{3-1} - Reliability of the differences between the indicators of III and I groups
2. P_{3-2} - Reliability of the differences between the indicators of II and I groups
3. P_{2-1} - Reliability of the differences between the indicators of II and I groups

On the part of the indicators of phagocytic process, a significant decrease in the number of active phagocytes of their absorption and metabolic functions was established ($p < 0.05$). In rats with MS, activation of individual links of humoral response has been established. The level of CIC and GA was significantly higher than that of intact animals ($p < 0.05$). Animals with the MS model have antibodies to the liver and kidney tissues (their content is significantly increased in a significant amount, $p < 0.001$), which is probably due to metabolic disorders.

The internal use of MW "Lotus" in rats with the MS model is accompanied by the normalization of most indicators of the immune system (Table 4). On the part of indicators of cellular immunity there is a partial normalization of indicators phagocytic process, suppressed in rats with the model of MS. The number of active phagocytes of peripheral blood and the metabolic function (the index of stimulated NBT test) reach the level of indices of intact rats and do not differ from them ($p > 0.5$).

On the part of indicators of humoral immunity level, the restoration of the content of CIC and GA to the level of intact animals ($p > 0.5$) and the end of the inflammatory process (the disappearance of antibodies to the liver and kidney tissue) was established.

Thus, the internal application of MW "Lotus" in rats with the model of MS restricts the manifestations of the pathological process, as evidenced by the normalization of the number of leukocytes. On the part of immunological parameters have been established the partial normalization of the process of phagocytosis, reduction level of GA and CIC to the level of intact animals, and limitation of inflammatory process from the liver and kidneys.

4. Conclusion

The use of MW with increased content of organic substances on the background of development of MS significantly reduces the level of glucose, completely restores the content of cholesterol and uric acid ($p > 0.5$), which indicates the effect of the organic matter of this MW on the restoration of lipid management and to a lesser extent Carbohydrate metabolism.

Under the influence of MB "Lotus" there is established the complete restoration of impaired functional state of the kidneys and stimulation of urine forming, ion-regulating and excretory functions. The level of tubal reabsorption, pH of daily urine and removal of chlorides is restored to the control group ($p > 0.5$). The volume of daily diuresis, the velocity of glomerular filtration, the withdrawal of creatinine and urea significantly exceeds the control group indices by 50%, 66% and 73% respectively ($p < 0.001$). The excretion of potassium and sodium ions, respectively, exceeds control data by 50% and 166% ($p < 0.001$).

The use of MW "Lotus" in rats with the model of MS leads to the normalization of most indicators of the immune system: a partial normalization of phagocytic process parameters is established; the number of active phagocytes of the peripheral blood and the metabolic function (the index of stimulated NBT test) reach the level of indices of intact rats and do not differ from them ($p > 0.5$). On the part of indicators of humoral immunity level was established the restoration of the content of CIC and GA to the level of intact animals ($p > 0.5$) and the end of the inflammatory process (the disappearance of antibodies to the liver and kidney tissue).

Conflict of Interest Statement

All the authors do not have any possible conflicts of interest.

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