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SULFATE-CHLORIDE SODIUM-MAGNESIUM MINERAL WATERS MODULATE NEUROENDOCRINE-IMMUNE COMPLEX AND METABOLISM IN HEALTHY FEMALE RATS

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Background. Earlier in an experiment on rats, we showed that newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets' spa has a significant modulating effect on the parameters of metabolism and the autonomic nervous, endocrine and immune systems. In this study, we combined data obtained on the same animals, in line with the concepts of neuroendocrine-immune complex and functional-metabolic continuum. **Materials and Methods**. Experiment was performed on 50 healthy female Wistar rats 230-290 g divided into 4 groups. Animals of the first group remained intact, using tap water from drinking ad libitum. Rats of the second (control) group for 6 days administered a single tap water through the tube at a dose of 1,5 mL/100 g of body mass. The rats of the main groups received the water "Myroslava" and "Khrystyna". The object of the study were the metabolic, neuro-endocrine and immune parameters. **Results**. The method of discriminant analysis revealed 31 parameters, according to which all four groups of animals differ from each other. Classification accuracy is 100%. **Conclusion**. The newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets' spa has both similar and specific effects on the neuroendocrine-immune complex and metabolism at healthy old female rats with weekly use. This provides a basis for preclinical studies.
Keywords: sulfate-chloride sodium-magnesium mineral waters, neuroendocrine-immune complex, metabolism, female rats.

INRODUCTION

Earlier in an experiment on rats, we showed that newly created sulfate-chloride sodium-magnesium drinking mineral waters "Myroslava" (5 g/L) and "Khrystyna" (10 g/L) of Truskavets' spa has a significant modulating effects on the parameters of metabolism and the autonomic nervous and endocrine systems [5,6] as well as immunity [1]. In this study, we combined data obtained on the same animals, in line with the concepts of neuroendocrine-immune complex [3,8,12-14] and functional-metabolic continuum [2].

MATERIALS AND METHODS

Experiment was performed on 50 healthy old female Wistar rats 220-300 g (M±SD=262±23 g) divided into 4 groups. Animals of the first group (10) remained intact, using tap water from drinking ad libitum. Rats of the second (control) group (10) for 6 days administered a single tap water through the tube at a dose of 1,5 mL/100 g of body mass. The rats of the main groups received the water "Myroslava" (15) and "Khrystyna" (15), prepared from the brine of the 27-*K* well of the Truskavetsian field by appropriate dilutions with fresh water [5]. The object of the study were the metabolic, neuro-endocrine [5,6] and immune [1] parameters

Digital material is statistically processed on a computer using the software package "Statistica 64".

RESULTS AND DISCUSION

Among the registered parameters, 7 neuroendocrine, 9 metabolic and 15 immune parameters (Tables 1 and 2) were identified by the method of discriminant analysis [7] (forward stepwise program), according to which the intact, control and two main groups of animals differ significantly from each other.

Table 1. Discriminant Function Analysis Summary
Step 31, N of Variables currently in the model: 31; Grouping: 4 groups
Wilks' Lambda: 0,00387; approx. $F_{(93)}$=2,83; p=0,0001

Variables currently in the model	**Groups (n)**				**Parameters of Wilks' Statistics**				
	Khrystyna (15)	**Myroslava (15)**	**Daily Water (10)**	Intact rats (10)	Wilks' Λ	Partial Λ	F-remove	p-level	Tolerancy
Calcium Plasma, mM/L	2,51 0,75 -0,83	2,91 0,87 -0,43	2,08 0,62 -1,24	3,35 1 0	0,004	0,910	0,52	0,672	0,361
Superoxide Dismutase Erythrocytes, un/mL	57,7 0,99 -0,03	49,9 0,86 -0,75	58,2 1,00 +0,02	58,0 1 0	0,005	0,814	1,22	0,335	0,263
Microbial Count Neutrophils, Bacteria/Phagocyte	7,6 0,88 -0,54	7,3 0,84 -0,70	8,2 0,95 -0,21	8,6 1 0	0,005	0,822	1,16	0,357	0,070
Sodium Excretion, µM/24h•100 g Body Mass	271 2,01 +1,62	167 1,24 +0,39	76 0,56 -0,70	135 1 0	0,005	0,782	1,49	0,255	0,057
Monocytes Blood, %	5,07 1,06 +0,09	4,87 1,01 +0,02	4,20 0,88 -0,20	4,80 1 0	0,006	0,655	2,81	0,073	0,053
Eosinophiles	4,00	3,33	3,80	4,60	0,007	0,550	4,37	0,020	0,267

Blood, %	0,87 -0,20	0,72 -0,42	0,83 -0,27	1 0					
Potassium Plasma, mM/L	3,33 0,79 -1,27	3,42 0,81 -1,15	3,54 0,84 -0,98	4,23 1 0	0,006	0,647	2,91	0,067	0,344
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	1,75 0,68 -0,70	1,91 0,75 -0,56	1,58 0,62 -0,84	2,56 1 0	0,008	0,478	5,82	0,007	0,181
Testosterone Plasma, nM/L	4,50 1,15 +0,53	4,97 1,27 +0,98	6,04 1,54 +1,97	3,93 1 0	0,007	0,518	4,96	0,013	0,193
NK Lymphocytes Blood, %	16,1 1,03 +0,15	16,3 1,04 +0,23	14,8 0,95 -0,30	15,6 1 0	0,006	0,698	2,30	0,116	0,043
Malondialdehyde Urine, µM/L	96 1,04 +0,09	88 0,95 -0,10	75 0,81 -0,40	92 1 0	0,007	0,528	4,77	0,015	0,127
Leukocytes Blood, 10^9/L	11,76 0,93 -0,15	10,51 0,83 -0,36	12,55 0,99 -0,02	12,68 1 0	0,004	0,920	0,47	0,710	0,510
Spleen Mass Index, mg/100g Body Mass	312 1,00 0,00	268 0,86 -0,44	294 0,94 -0,18	312 1 0	0,004	0,902	0,58	0,635	0,365
Amylase Activity Urine, g/h•L	204 1,01 +0,02	204 1,01 0,04	217 1,07 +0,26	202 1 0	0,009	0,437	6,86	0,003	0,092
Katalase Activity Plasma, µM/h•L	128 1,24 +0,88	122 1,18 +0,67	148 1,43 +1,58	103 1 0	0,007	0,556	4,25	0,022	0,219
Chloride Excretion, µM/24h•100 g Body Mass	244 1,69 +1,02	195 1,35 +0,51	107 0,74 -0,38	144 1 0	0,007	0,552	4,34	0,020	0,062
Triiodothyronine Plasma, nM/L	2,38 1,11 +0,42	2,31 1,08 +0,30	2,11 0,99 -0,05	2,14 1 0	0,006	0,677	2,55	0,092	0,045
Corticosterone Plasma, nM/L	460 0,96 -0,17	365 0,76 -0,92	383 0,80 -0,78	482 1 0	0,006	0,684	2,46	0,100	0,332
Glucose Plasma, mM/L	5,22 1,05 +0,25	5,55 1,12 +0,55	5,49 1,11 +0,49	4,95 1 0	0,006	0,641	2,98	0,063	0,265
Phagocytic Index Monocytes %	2,89 1,00 -0,01	2,83 0,98 -0,10	2,75 0,95 -0,21	2,90 1 0	0,006	0,687	2,43	0,103	0,269
Sodium Erythrocytes, mM/L	24,2 1,10 +0,51	21,8 0,99 -0,04	22,6 1,03 +0,13	22,0 1 0	0,006	0,600	3,56	0,038	0,116
Amylase Activity Plasma, g/h•L	163 1,07 +0,46	155 1,02 +0,14	154 1,02 +0,10	152 1 0	0,005	0,717	2,10	0,140	0,266
Macrophages Spleen, %	8,1 1,03 +0,15	7,9 1,00 +0,02	9,1 1,15 +0,75	7,9 1 0	0,005	0,759	1,70	0,208	0,247
Phagocytic Index Neutrophils,	69,4 1,00	68,9 0,99	71,9 1,03	69,5 1	0,007	0,533	4,67	0,016	0,092

%	-0,03	-0,13	+0,56	0					
Reticular Zone of Adrenal Cortex, µM	42 0,98 -0,12	44 1,04 +0,20	40 0,95 -0,29	43 1 0	0,006	0,609	3,42	0,043	0,219
Entropy Leukocytogram	0,551 0,93 -0,76	0,592 0,99 -0,07	0,557 0,94 -0,66	0,596 1 0	0,006	0,622	3,24	0,050	0,295
Plasmocytes Thymus, %	2,00 1,11 +0,25	2,00 1,11 +0,25	2,44 1,36 +0,82	1,80 1 0	0,006	0,690	2,40	0,106	0,300
Eosinophiles Spleen, %	1,14 0,76 -0,33	1,73 1,16 +0,22	1,40 0,93 -0,09	1,50 1 0	0,006	0,684	2,46	0,100	0,352
Glomerular Zone of Adrenal Cortex, µM	185 0,96 -0,18	182 0,94 -0,25	207 1,07 +0,29	193 1 0	0,006	0,651	2,87	0,069	0,298
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	1,42 0,99 -0,02	1,37 0,95 -0,08	2,34 1,63 +1,09	1,44 1 0	0,005	0,781	1,49	0,254	0,172
Magnesium Urine, mM/L	2,89 1,13 +0,18	2,49 0,97 -0,04	2,34 0,91 -0,12	2,56 1 0	0,005	0,818	1,19	0,346	0,118

Note. In each column, the first line is the average value, the second is the fraction of the norm, and the third is the Z-score.

Table 2. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Calcium Plasma, mM/L	4,49	0,008	0,773	4,49	0,008
Superoxide Dismutase Erythrocytes, un/mL	4,18	0,011	0,605	4,29	0,001
Microbial Count Neutrophils, Bac/Phag	3,38	0,027	0,492	4,03	10^{-4}
Sodium Excretion, µM/24h•100 g Body Mass	3,88	0,015	0,387	4,10	10^{-4}
Monocytes Blood, %	3,07	0,038	0,317	4,00	10^{-5}
Eosinophiles Blood, %	2,49	0,074	0,268	3,83	10^{-5}
Potassium Plasma, mM/L	2,04	0,124	0,233	3,63	10^{-5}
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	2,68	0,060	0,193	3,62	10^{-5}
Testosterone Plasma, nM/L	2,07	0,121	0,166	3,51	10^{-5}
NK Lymphocytes Blood, %	2,21	0,103	0,141	3,46	10^{-6}
Malondialdehyde Urine, µM/L	2,37	0,087	0,118	3,46	10^{-6}
Leukocytes Blood, 10^9/L	1,69	0,186	0,103	3,36	10^{-6}
Spleen Mass Index, mg/100g Body Mass	1,70	0,185	0,089	3,28	10^{-6}
Amylase Activity Urine, g/h•L	1,79	0,168	0,077	3,23	10^{-6}
Katalase Activity Plasma, µM/h•L	1,25	0,307	0,069	3,12	10^{-5}
Chloride Excretion, µM/24h•100 g Body Mass	1,74	0,179	0,059	3,09	10^{-5}
Triiodothyronine Plasma, nM/L	1,57	0,217	0,051	3,04	10^{-5}
Corticosterone Plasma, nM/L	1,55	0,224	0,044	3,00	10^{-5}
Glucose Plasma, mM/L	1,31	0,292	0,038	2,93	10^{-5}
Phagocytic Index Monocytes, %	1,38	0,270	0,033	2,89	10^{-5}
Sodium Erythrocytes, mM/L	1,63	0,207	0,028	2,88	10^{-5}
Amylase Activity Plasma, g/h•L	1,59	0,217	0,024	2,87	10^{-5}
Macrophages Spleen, %	1,11	0,363	0,021	2,80	10^{-5}
Phagocytic Index Neutrophils, %	1,27	0,308	0,018	2,76	10^{-5}
Reticular Zone of Adrenal Cortex, µM	1,61	0,216	0,015	2,77	10^{-5}
Entropy Leukocytogram	1,43	0,262	0,012	2,76	10^{-4}
Plasmocytes Thymus, %	1,38	0,279	0,010	2,74	10^{-4}

Eosinophiles Spleen, %	1,36	0,285	0,008	2,73	10^{-4}
Glomerular Zone of Adrenal Cortex, μM	2,76	0,072	0,006	2,92	10^{-4}
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	1,09	0,381	0,005	2,87	10^{-4}
Magnesium Urine, mM/L	1,19	0,346	0,004	2,83	10^{-4}

The dividing information contained in 31 variables is condensed in 3 canonical discriminant roots (Tables 3 and 4). The first root contains 53,0% of discriminative opportunities (r*=0,950; Wilks' Λ=0,0039; $\chi^2_{(93)}$=175; p<10^{-6}), the second 28,9% (r*=0,914; Wilks' Λ=0,0397; $\chi^2_{(60)}$=100; p=0,0006), the third 18,1% (r*=0,871; Wilks' Λ=0,2406; $\chi^2_{(29)}$=45; p=0,030).

The calculation of the discriminant root values for each animal as the sum of the products of raw coefficients (Table 3) to the individual values of discriminant variables together with the constants enables the visualization of each rat in the information space of the roots (Fig. 1).

Table 3. Standardized and Raw Coefficients for Canonical Variables

Coefficients	**Standardized**			**Raw**		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Calcium Plasma, mM/L	0,485	-0,198	-0,058	0,593	-0,243	-0,071
Superoxide Dismutase Erythrocytes, un/mL	-0,012	0,701	-0,625	-0,0013	0,079	-0,070
Microbial Count Neutrophils, Bac/Phag	1,398	0,690	0,702	1,055	0,521	0,530
Sodium Excretion, μM/24h•100 g	1,779	-0,952	0,524	0,010	-0,0055	0,0031
Monocytes Blood, %	2,493	-0,441	-1,006	1,021	-0,180	-0,412
Eosinophiles Blood, %	1,251	0,526	-0,241	0,612	0,257	-0,118
Potassium Plasma, mM/L	1,027	0,285	-0,079	1,344	0,373	-0,104
$(Cap/Pp)^{0,5}$ as Parathyroid Activity	1,245	-1,222	0,551	1,812	-1,779	0,802
Testosterone Plasma, nM/L	1,136	-0,912	-0,913	0,549	-0,441	-0,442
NK Lymphocytes Blood, %	-1,105	1,289	2,461	-0,505	0,589	1,125
Malondialdehyde Urine, μM/L	0,765	1,886	0,528	0,023	0,058	0,016
Leukocytes Blood, 10^9/L	0,114	0,377	-0,188	0,024	0,078	-0,039
Spleen Mass Index, mg/100g Body Mass	0,181	0,436	0,327	0,0026	0,0063	0,0047
Amylase Activity Urine, g/h•L	-0,408	-2,591	0,686	-0,010	-0,065	0,017
Katalase Activity Plasma, μM/h•L	-0,670	-1,366	-0,280	-14,41	-29,38	-6,018
Chloride Excretion, μM/24h•100 g	-2,664	1,002	0,090	-0,018	0,0069	0,0006
Triiodothyronine Plasma, nM/L	-2,288	1,660	0,354	-5,598	4,062	0,866
Corticosterone Plasma, nM/L	-0,402	0,905	0,398	-0,0024	0,0055	0,0024
Glucose Plasma, mM/L	-1,140	-0,337	0,335	-1,375	-0,407	0,405
Phagocytic Index Monocytes, %	-0,764	0,094	-0,908	-0,874	0,108	-1,038
Sodium Erythrocytes, mM/L	-0,591	1,534	1,238	-0,123	0,320	0,258
Amylase Activity Plasma, g/h•L	-0,523	0,715	0,714	-0,015	0,021	0,021
Macrophages Spleen, %	-0,904	0,143	-0,541	-0,497	0,079	-0,298
Phagocytic Index Neutrophils, %	-1,157	2,080	-0,598	-0,296	0,533	-0,153
Reticular Zone of Adrenal Cortex, μM	-0,355	-1,414	-0,007	-0,033	-0,132	-0,001
Entropy Leukocytogram	0,754	-0,931	0,244	12,37	-15,26	3,994
Plasmocytes Thymus, %	-0,769	0,683	-0,384	-1,005	0,892	-0,503
Eosinophiles Spleen, %	0,112	-0,958	0,397	0,131	-1,121	0,465
Glomerular Zone of Adrenal Cortex, μM	0,273	1,032	-0,530	0,008	0,029	-0,015
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	1,076	0,519	-0,008	1,128	0,544	-0,008
Magnesium Urine, mM/L	1,208	0,111	-0,534	0,717	0,066	-0,317
			Constants	26,74	-54,47	-16,66
			Eigenvalues	9,26	5,06	3,16
			Cumulative Proportions	0,530	0,819	1

Table 4. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Roots and Variables Z-scores

	Correlations Variables-Roots			Khrystyna	Myroslava	Daily Water	Intact rats
Root 1 (53,0%)	**R1**	**R2**	**R3**	-1,96	-1,59	-0,41	+5,73
$(Cap/Pp)^{0,5}$ as Parathyroid Act	**0,148**	-0,039	0,127	-0,70	-0,56	-0,84	**0**
Calcium Plasma	**0,112**	-0,084	0,212	-0,83	-0,43	-1,24	**0**
Potassium Plasma	**0,149**	-0,011	-0,003	-1,27	-1,15	-0,98	**0**
Microbial Count Neutrophils	**0,118**	0,040	-0,105	-0,54	-0,70	-0,21	**0**
Eosinophils Blood	**0,061**	0,057	0,006	-0,20	-0,42	-0,27	**0**
Glucose Plasma	**-0,070**	-0,074	-0,058	+0,25	+0,55	+0,49	**0**
Katalase Activity Plasma	**-0,068**	0,024	-0,120	+0,88	+0,67	+1,58	**0**
Amylase Activity Plasma	**-0,029**	0,038	0,027	+0,46	+0,14	+0,10	**0**
Root 2 (28,9%)	**R1**	**R2**	**R3**	+2,64	**-2,92**	+0,26	+0,15
Corticosterone Plasma	0,054	**0,104**	0,068	-0,17	**-0,92**	-0,78	0
SOD Erythrocytes	0,054	**0,166**	-0,082	-0,03	**-0,75**	+0,02	0
Sodium Erythrocytes	-0,030	**0,087**	0,021	+0,51	**-0,04**	+0,13	0
Spleen Mass Index	0,041	**0,110**	0,006	0,00	**-0,44**	-0,18	0
Leukocytes Blood	0,042	**0,044**	-0,056	-0,15	**-0,36**	-0,02	0
Eosinophils Spleen	0,015	**-0,126**	0,002	-0,33	**+0,22**	-0,09	0
Entropy Leukocytogram	0,060	**-0,114**	0,061	-0,76	**-0,07**	-0,66	0
Root 3 (18,1%)	**R1**	**R2**	**R3**	+1,06	+0,78	**-3,39**	+0,63
Testosterone Plasma	-0,059	-0,033	**-0,165**	+0,53	+0,98	**+1,97**	0
$(Ku/Nau)^{0,5}$ as MC Activity	-0,004	0,021	**-0,228**	-0,02	-0,08	**+1,09**	0
Glomerular ZAC	0,028	0,025	**-0,136**	-0,18	-0,25	**+0,29**	0
Phagocytic Index Neutrophils	0,007	0,021	**-0,164**	-0,03	-0,13	**+0,56**	0
Plasmocytes Thymus	-0,034	-0,011	**-0,144**	+0,25	+0,25	**+0,82**	0
Macrophages Spleen	-0,024	0,045	**-0,127**	+0,15	+0,02	**+0,75**	0
Amylase Activity Urine	-0,008	-0,000	**-0,068**	+0,02	+0,04	**+0,26**	0
Triiodothyronine Plasma	-0,063	0,021	**0,117**	+0,42	+0,30	**-0,05**	0
Reticular ZAC	-0,000	-0,045	**0,052**	-0,12	+0,20	**-0,29**	0
Sodium Excretion	-0,062	0,095	**0,183**	+1,62	+0,39	**-0,70**	0
Chloride Excretion	-0,064	0,049	**0,163**	+1,02	+0,51	**-0,38**	0
Malondialdehyde Urine	0,004	0,037	**0,115**	+0,09	-0,10	**-0,40**	0
Magnesium Urine	-0,010	0,040	**0,048**	+0,18	-0,04	**-0,12**	0
NK Lymphocytes Blood	-0,036	-0,002	**0,151**	+0,15	+0,23	**-0,30**	0
Monocytes Blood	-0,014	0,029	**0,084**	+0,09	+0,02	**-0,20**	0
Phagocytic Index Monocytes	0,011	-0,001	**0,026**	-0,01	-0,10	**-0,21**	0

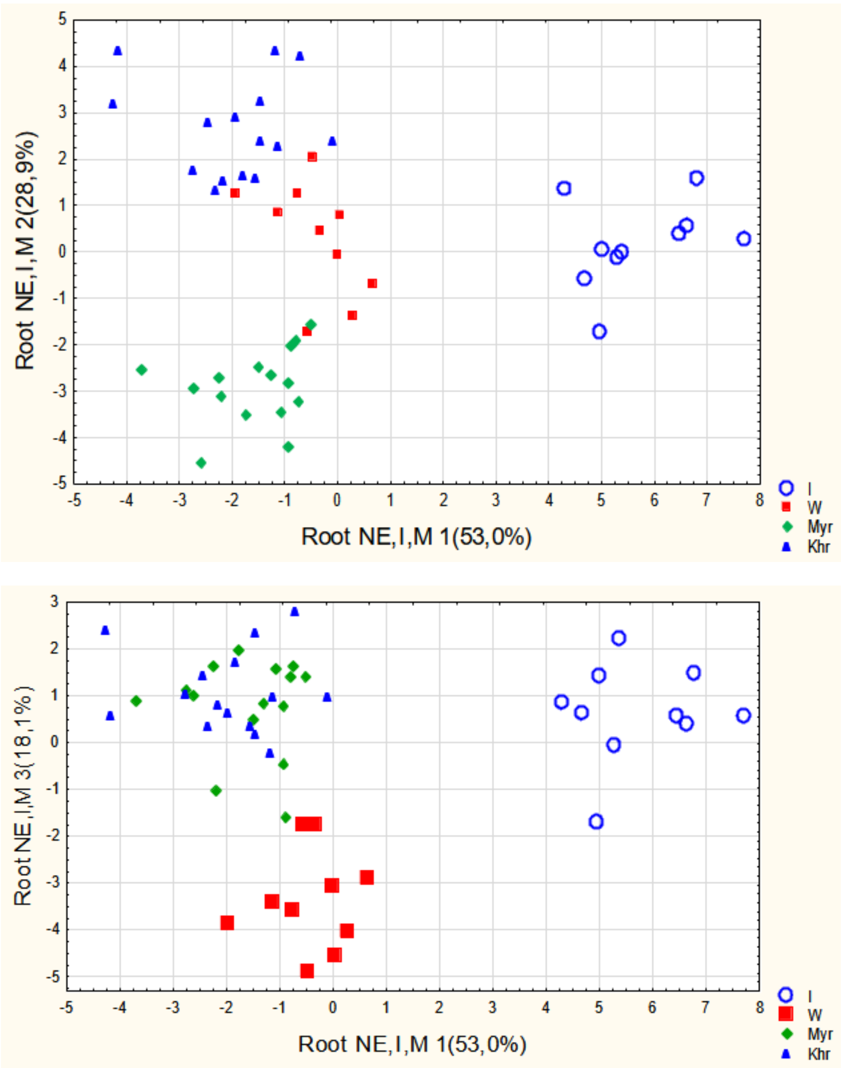

Fig. 1. Individual values of the first and second (above) and the first and third (below) roots of the endocrine and metabolic parameters in intact rats (o) and loaded with Daily water (W) and mineral waters "Myroslava" (Myr) and "Khrystyna" (Khr)

Pseudo-staining visualizes a combination of **hormonal**, **immune**, and **metabolic** parameters in the structure of each root (Table 4), consistent with previously identified neuroendocrine-immune and neuroendocrine-metabolic linkages [11,18-22].

As you can see (Fig. 1 above), along the axis of the first root of the rat, both control and both main groups, significantly distant from intact animals, while their projections on the axis are closely mixed.

This disposition reflects a decrease in parathyroid activity and plasma calcium and potassium levels, as well as eosinophils in the blood and the intensity of bacterial phagocytosis by neutrophils on the one hand, while increased plasma glucose levels and catalase and amylase activity on the other. The described changes are nonspecific and are caused, apparently, by adversarial stress [15,23].

Instead, the groups subjected to water loading are quite clearly delineated along the axis of the second root. The lowest position of "Myroslava" loaded rats showed the maximum decrease in plasma corticosterone, sodium and SOD in erythrocytes, leukocytes in blood and spleen mass in combination with the maximum content in the splenocytogram of eosinophils and maximum entropy of leukocytogram. At the opposite pole of the axis are animals loaded with "Khrystyna" water, and the rats of the control group occupy an intermediate position.

Obviously, this illustrates the specificity of the modulating effects of mineral waters with different mineralization [4].

Additional delimitation of rats of the control group occurs along the axis of the third root. Their lowest localization reflects elevated or maximal for sampling testosterone levels, mineralocorticoid activity, adrenal glomerular thickness, amylasuria, phagocytic index of blood neutrophils, as well as the content of plasma cells in the thymus and macrophages in the spleen. In contrast, this cluster is characterized by low or minimal sampling levels of triiodothyronine, adrenal reticular thickness, urinary excretion of sodium and chloride, urinary concentrations of magnesium and malonic dialdehyde, as well as phagocytic index of blood monocytes and the content of monocytes and natural killers.

Both mineral waters equally prevent changes in these parameters, which is a manifestation of their non-specific stress limiting effect.

In general, in the information field of the three roots, all four groups of animals are quite different from each other, as documented by the distances of Mahalanobis (Table 5).

Table 5. Squared Mahalanobis Distances between groups (over diagonal), F-values (df=31) and p-levels (under diagonal)

Groups	I (10)	DW (10)	Myr (15)	Khr (15)
Intact rats (I)	**0,0**	54	63	65
Daily Water (DW)	**3,03** **,011**	**0,0**	29	28
Water "Myroslava" (Myr)	**4,25** **,002**	**1,95** **,080**	**0,0**	31
Water "Khrystyna" (Khr)	**4,41** **,001**	**1,88** **,092**	**2,61** **,023**	**0,0**

The application of the classifying functions (Table 6) enables the retrospective identification of all rats without mistake (Table 7).

Table 6. Coefficients and Constants for Classification Functions

Variables currently in the model	Intact rats	Daily Water	Myro-slava	Khrys-tyna
Calcium Plasma, mM/L	-29,94	-33,33	-33,55	-35,14
Superoxide Dismutase Erythrocytes, un/mL	3,424	3,723	3,182	3,599
Microbial Count Neutrophils, Bac/Phag	62,39	53,83	53,14	55,80
Sodium Excretion, µM/24h•100 g BM	-0,818	-0,895	-0,877	-0,910
Monocytes Blood, %	-71,75	-76,39	-78,74	-80,23
Eosinophils Blood, %	2,272	-0,987	-3,021	-1,847
Potassium Plasma, mM/L	17,16	9,361	6,150	7,709
(Cap/Pp)^{0,5} as Parathyroid Activity	-124,1	-138,6	-131,8	-142,1
Testosterone Plasma, nM/L	-46,69	-48,34	-49,44	-52,21
NK Lymphocytes Blood, %	123,9	122,5	125,9	129,7
Malondialdehyde Urine, µM/L	2,913	2,710	2,567	2,883
Leukocytes Blood, 10^9/L	7,374	7,395	6,955	7,370
Spleen Mass Index, mg/100g Body Mass	0,582	0,548	0,544	0,579
Amylase Activity Urine, g/h•L	-3,227	-3,240	-2,952	-3,302
Katalase Activity Plasma, µM/h•L	-1238	-1129	-1043	-1203
Chloride Excretion, µM/24h•100 g BM	1,260	1,370	1,372	1,417
Triiodothyronine Plasma, nM/L	578,3	609,7	607,0	631,9
Corticosterone Plasma, nM/L	0,494	0,500	0,495	0,527
Glucose Plasma, mM/L	50,91	57,69	62,29	60,65
Phagocytic Index Monocytes, %	18,45	28,00	24,36	24,98

Sodium Erythrocytes, mM/L	31,46	31,22	31,42	33,32
Amylase Activity Plasma, g/h•L	2,534	2,546	2,584	2,710
Macrophages Spleen, %	12,83	17,09	16,19	16,72
Phagocytic Index Neutrophils, %	52,39	54,89	52,91	55,93
Reticular Zone of Adrenal Cortex, μM	-6,299	-6,106	-5,649	-6,372
Entropy Leukocytogram	-674,7	-768,5	-717,9	-806,0
Plasmocytes Thymus, %	113,4	121,7	118,0	123,1
Eosinophiles Spleen, %	-18,41	-21,20	-15,86	-22,00
Glomerular Zone of Adrenal Cortex, μM	1,349	1,365	1,203	1,356
$(Ku/Nau)^{0,5}$ as Mineralocorticoid Activity	-4,639	-11,47	-14,57	-11,96
Magnesium Urine, mM/L	-39,64	-42,77	-45,15	-45,13
Constants	-3953	-4046	-3974	-4291

Table 11. Classification Matrix

Rows: Observed classifications; Columns: Predicted classifications

Groups	Percent correct	I p=,20	DW p=,20	Myr p=,30	Khr p=,30
Intact rats (I)	100	**10**	0	0	0
Daily Water (DW)	100	0	**10**	0	0
Water “Myroslava” (Myr)	100	0	0	**15**	0
Water “Khrystyna” (Khr)	100	0	0	0	**15**
Total	100	10	10	15	15

Another approach to identifying the specificity of the effects is to create patterns of Z-scores parameters, both included in the discriminant model and extramodel, but carrying recognizable information. Calculating the algebraic difference between Z-scores parameters in control and experimental groups allows us to estimate the partial effects of mineral waters (Fig. 2).

The first pattern shows how both mineral waters equally prevent the stress-induced increase in thickness of the glomerular zone of the adrenal cortex and mineralocorticoid activity, glycemia and amylasuria, thymus mass and content in the thymocytogram of endothelial cells, in the splenocytogram macrophages as well as the phagocytic index of blood neutrophils.

Significantly higher stress-induced four parameters (testosterone, plasma catalase, thymocytogram plasma cells and immunocytogram entropy) under the influence of mineral waters are reduced to the upper zone of normal.

On the other hand (third pattern), they prevent a stress-induced decrease in thickness of the reticular zone of the adrenal cortex, triiodethyroninemia, parathyroid activity, calciumemia, urinary excretion of sodium and chloride, urinary concentration of malonic dialdehyde, as well as blood monocytes count, the activity and intensity of bacterial phagocytosis by monocytes.

The following three patterns reflect the differences in the effects of mineral waters. “Myroslava” water deepens chronic stress-induced decrease in corticosterone, SOD, lymphoblast of thymocytogram content, spleen mass and plasma cell of splenocytogram content, blood content of leukocytes in general and eosinophils in particular as well as the intensity of phagocytosis of bacteria by neutrophils and the transformation of T lymphocytes into blasts. On the other hand, “Khrystyna” water does not affect this constellation of parameters in general.

The next pattern demonstrates that stress-insensitive parameters (amylasemia, natrihistia, magnesiumuria, lymphoblast and reticulocyte content in splenocytogram, T cytolytic lymphocytes content in immunocytogram, and neutrophil killing index) increase under the influence of “Khrystyna” water while “Myroslava” water is inefficient for these parameters

In contrast, “Myroslava” water, unlike “Khrystyna” water, initiates increase in the entropy of leukocytogram and thymocytogram, level in thymocytogram of epitheliocytes, macrophages and reticulocytes, as well as eosinophils in the splenocytogram, NK lymphocytes in the blood.

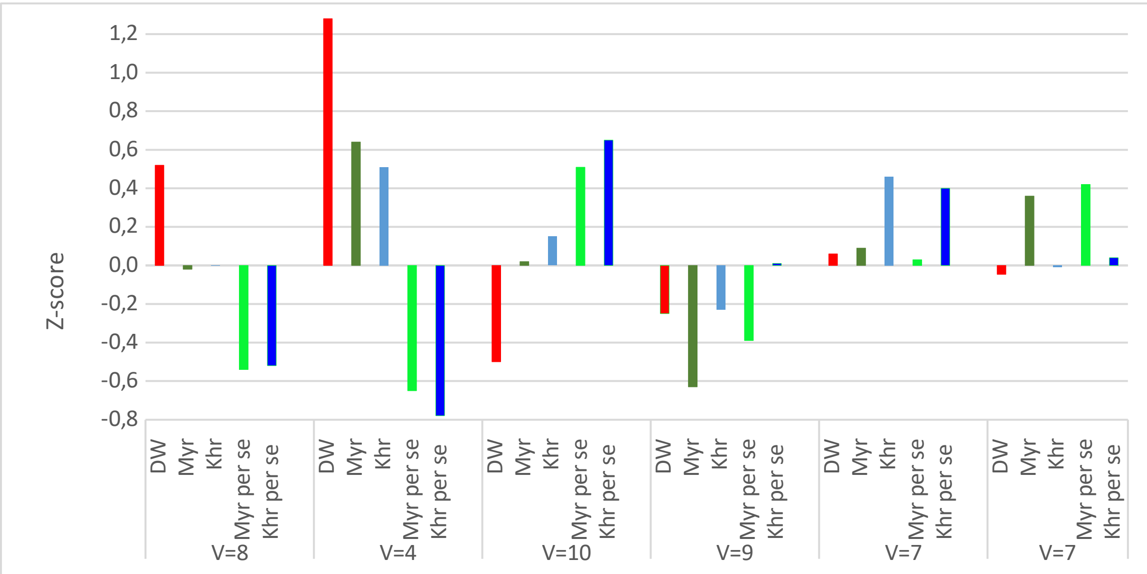

Fig. 2. Patterns (V - number of variables) of effects of daily water and mineral waters and simulated partial effects of mineral waters

CONCLUSION

The newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets’ spa has both similar and specific effects on the neuroendocrine-immune complex and metabolism at healthy old female rats with weekly use. This provides a basis for preclinical studies.

Based on preliminary data [9,10,16], it is possible to predict the modulating effect of the studied mineral waters on the parameters of the electroencephalogram in humans.

CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The conduct of experiments was approved by the Ethics Committee of the Horbachevskyi Ternopil’ National Medical University. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

REFERENCES

1. Badiuk NS, Popovych DV, Hrytsak MV, Ruzhylo SV, Zakalyak NR, Kovalchuk GY, Mel'nyk OI, Zukow X. Similar and specific immunotropic effects of sulfate-chloride sodium-magnesium mineral waters "Myroslava" and "Khrystyna" of Truskavets' spa in healthy female rats. Journal of Education, Health and Sport. 2021; 11(11): 314-353.
2. Gozhenko AI. Functional-metabolic continuum [in Russian]. J of NAMS of Ukraine. 2016; 22 (1): 3-8.
3. Gozhenko AI, Korda MM, Popadynets' OO, Popovych IL. Entropy, Harmony, Synchronization, Harmony and Their Neuro-Endocrine-Immune Correlates [in Ukrainian]. Odesa. Feniks; 2021: 232.
4. Gozhenko OA, Zavidnyuk YV, Korda MM, Mysula IR, Klishch IM, Zukow W, Popovych IL. Features of neuro-endocrine and immune reactions to various water-salt loads in female rats. Journal of Education, Health and Sport. 2018; 8(9): 11-31.
5. Hrytsak MV, Popovych DV, Badiuk NS, Hrytsan II, Zukow W. Similar neuroendocrine and metabolic effects of sulfate-chloride sodium-magnesium mineral waters "Myroslava" and "Khrystyna" of Truskavets' spa in healthy female rats. Journal of Education, Health and Sport. 2021; 11(6): 320-334.
6. Hrytsak MV, Popovych DV, Badiuk NS, Hrytsan II, Zukow W. Peculiaritias of neuroendocrine and metabolic effects of sulfate-chloride sodium-magnesium mineral waters "Myroslava" and "Khrystyna" of Truskavets' spa in healthy female rats. Journal of Education, Health and Sport. 2021; 11(9): 862-875.
7. Klecka WR. Discriminant Analysis [trans. from English in Russian] (Seventh Printing, 1986). In: Factor, Discriminant and Cluster Analysis. Moskva. Finansy i Statistika; 1989: 78-138.
8. Kozyavkina OV, Kozyavkina NV, Gozhenko OA, Gozhenko AI, Barylyak LG., Popovych IL. Bioactive Water Naftussya and Neuro-Endocrine-Immune Complex [in Ukrainian]. Kyiv: UNESCO-SOCIO; 2015: 349.
9. Kul'chyns'kyi AB**,** Kyjenko VM, Zukow W, Popovych IL. Causal neuro-immune relationships at patients with chronic pyelonephritis and cholecystitis**.** Correlations between parameters EEG, HRV and white blood cell count. Open Medicine. 2017; 12(1): 201-213.
10. Kul'chyns'kyi AB**,** Zukow W, Korolyshyn TA, Popovych IL. Interrelations between changes in parameters of HRV, EEG and humoral immunity at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2017; 7(9): 439-459.
11. Mel'nyk OI, Zukow W, Hrytsak MV, Popovych DV, Zavidnyuk YV, Bilas VR, Popovych IL. Canonical analysis of neuroendocrine-metabolic and neuroendocrine-immune relationships at female rats. Journal of Education, Health and Sport. 2021; 11(5): 356-369.
12. Polovynko IS, Zayats LM, Zukow W, Popovych IL. Neuro-endocrine-immune relationships by chronic stress at male rats. Journal of Health Sciences. 2013; 3(12): 365-374.
13. Popovych IL. Functional interactions between neuroendocrine-immune complex in males rats [in Ukrainian]. Achievements of Clinical and Experimental Medicine. 2008; 2(9): 80-87.
14. Popovych IL. The concept of neuroendocrine-immune complex (Review) [in Russian]. Medical Hydrology and Rehabilitation. 2009; 7(3): 9-18.
15. Popovych IL, Gozhenko AI, Zukow W, Polovynko IS. Variety of Immune Responses to Chronic Stress and their Neuro-Endocrine Accompaniment. Scholars' Press. Riga; 2020: 172.
16. Popovych IL, Kul'chyns'kyi AB**,** Korolyshyn TA, Zukow W**.** Interrelations between changes in parameters of HRV, EEG and cellular immunity at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2017; 7(10): 11-23.
17. Popovych IL, Vis'tak HI, Gumega MD, Ruzhylo SV. Vegetotropic Effects of Bioactive Water Naftussya and their Endocrine-Immune, Metabolic and Hemodynamic Accompaniments [in Ukrainian]. Kyiv: UNESCO-SOCIO; 2014: 163.
18. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. Nat Rev Immunol. 2006; 6(4): 318-328.
19. Thayer JF, Sternberg EM. Neural aspects of immunomodulation: Focus on the vagus nerve. Brain Behav Immun. 2010; 24(8): 1223-1228.
20. Tracey KJ. Reflex control of immunity. Nat Rev Immunol. 2009; 9(6): 418-428.

21. Uchakin PN, Uchakina ON, Tobin BV, Ershov FI. Neuroendocrine immunomodulation [in Russian]. Vestnik Ross AMN. 2007; 9: 26-32.

22. Zajats LM, Polovynko IS, Zukow W. Features neuro-endocrine support diversity of immune responses to chronic stress in male rats. Journal of Education, Health and Sport. 2017; 7(3): 97-105.

23. Zavidnyuk YV, Mysula IR, Klishch IM, Zukow W, Popovych IL, Korda MM. General non-specific metabolic, neuroendocrine and immune reactions to various water-salt loads in female rats. Journal of Education, Health and Sport. 2018; 8(3): 513-524.