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SIMILAR AND SPECIFIC IMMUNOTROPIC EFFECTS OF SULFATE-CHLORIDE SODIUM-MAGNESIUM MINERAL WATERS "MYROSLAVA" AND "KHRYSTYNA" OF TRUSKAVETS' SPA 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 and endocrine systems. In this study, conducted in line with the concepts of neuroendocrine-immune complex and functional-metabolic continuum, data on the immunomodulatory effects of these waters on the same rats. **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 immune parameters of the thymus, spleen and blood. **Results**. The method of discriminant analysis revealed 12 parameters, according to which the animals loaded with mineral waters differed significantly from both control and intact animals. Classification accuracy is 86%. However, the difference between the immunotropic effects of mineral waters of different mineralization concerns only 9 parameters. **Conclusion**. The newly created sulfate-chloride sodium-magnesium drinking mineral waters of Truskavets' spa have both similar and specific immunomodulating effects on healthy old female rats with weekly use. This provides a basis for preclinical studies.
Keywords: sulfate-chloride sodium-magnesium mineral waters, immunity, 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 effect on the parameters of metabolism and the autonomic nervous and endocrine systems [9-11]. In this study, conducted in line with the concepts of neuroendocrine-immune complex [7,14,25,26] and functional-metabolic continuum [6], data on the immunomodulatory effects of these waters on the same rats.

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 [10].

The day after the completion of the drinking course in all rats, at first, a sample of peripheral blood (by incision of the tip of the tail) was taken for analysis of Leukocytogram (LCG), ie the relative content of lymphocytes (L), monocytes (M), eosinophils (Eo), basophils (Bas), rod-shaped (RN) and polymorphonuclear (PMN) neutrophils. Based on these data, the Entropy of the Leukocytogram (hLCG) was calculated according to the formula derived by IL Popovych [5,7,26] on the basis of the classical CE Shannon [27] formula:

$$hLCG = - [L•\log_2 L+M•\log_2 M+Eo•\log_2 Eo+Bas•\log_2 Bas+RN•\log_2 RN+PMN•\log_{2PM} N]/\log_2 6.$$

The experiment was completed by decapitation of rats in order to collect as much blood as possible.

In the blood, the parameters of immunity were determined, as described in the manual [17]: the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep by M Jondal et al [12], their theophylline-resistant (T-helper) and theophyllin-susceptible (T-cytolytic) subpopulations (by the test of sensitivity of rosette formation to theophylline by S Limatibul et al [15]; the population of B-lymphocytes by the test of complementary rosette formation with erythrocytes of sheep by C Bianco [3]. Natural killers were identified as large granules contain lymphocytes. The content of zero-lymphocytes (0L) was calculated by the balance method. For these components, as well as plasma cells (Pla), the Entropy of the Immunocytogram (hICG) was calculated;

$$hICG = - [Th•\log_2 Th+Tc•\log_2 Tc+B•\log_2 B+Pla•\log_2 Pla+NK•\log_2 NK+0L•\log_2 0L]/\log_2 6.$$

The blast transformation reaction of T-lymphocytes to phytohemagglutinin was performed separately [17].

About the condition of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytosis index, the microbial count and the killing index for Staphylococcus aureus (ATCC N25423 F49) [4].

After decapitation, the thymus and spleen were removed from the animals. Immune organs weighed and made smears-imprints for counting Thymocytogram and Splenocytogram [1-3]. The components of the thymocytogram (TCG) are lymphocytes (Lc), lymphoblasts (Lb), reticulocytes (Ret), macrophages (Mac), endotheliocytes (End), epitheliocytes (Epi) and Hassal's corpuscles (Has). The Splenocytogram (SCG) includes lymphocytes (Lc), lymphoblasts (Lb), plasma cells (Pla), reticulocytes (Ret), macrophages (Mac), fibroblasts (Fib), microphages (Mic) and eosinophils (Eos) [3,7,26].

For them Shannon's entropy was calculated too:

$$hTCG = - [Lc•\log_2 Lc+Lb•\log_2 Lb+Ret•\log_2 Ret+Mac•\log_2 Mac+En•\log_2 En+Ep•\log_2 Ep+Has•\log_2 Has]/\log_2 7$$

$$hSCG = - [Lc•\log_2 Lc+Lb•\log_2 Lb+P•\log_2 P+R•\log_2 R+Ma•\log_2 Ma+F•\log_2 F+Mi•\log_2 Mi+Eo•\log_2 Eo]/\log_2 8$$

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

RESULTS AND DISCUSION

Following the accepted algorithm, in the first stage of the analysis, both research groups were combined into the group "Salt Waters". The method of discriminant analysis [13] revealed 12 parameters, according to which the immune status of animals loaded with mineral water and tap water, as well as intact, differ significantly from each other.

Two parameters of **thymocytogram** and **splenocytogram**, 7 parameters of **leukocytogram and phagocytosis**, and also parameter of **immunocytogram** of blood were recognizable (Tables 1-2).

Table 1. Discriminant Function Analysis Summary

Step 12, N of Variables currently in the model: 12; Grouping: 3 groups

Wilks' Lambda: 0,2735; approx. $F_{(25)}$=2,74; p=0,0005

	Groups (n)			Parameters of Wilks' Statistics				
Variables currently in the model	Intact rats (10)	**Salt Waters (30)**	**Daily Water (10)**	Wilks' Λ	Partial Λ	F-remove (2,36)	p-level	Tolerancy
Microbial Count Neutrophils, Bacteria/Phagocyte	8,6 1 0	7,4 0,86 -0,65	8,2 0,95 -0,21	0,525	0,521	16,5	10^{-5}	0,104
Monocytes Blood, %	4,80 1 0	5,10 1,06 +0,10	4,20 0,88 -0,20	0,329	0,832	3,64	0,036	0,133
Phagocytic Index Neutrophils, %	69,5 1 0	69,1 0,99 -0,10	71,9 1,03 +0,56	0,406	0,674	8,69	0,001	0,259
Eosinophils Blood, %	4,60 1 0	3,63 0,79 -0,32	3,80 0,83 -0,27	0,295	0,926	1,44	0,250	0,754
Plasmocytes Thymus, %	1,80 1 0	1,97 1,09 +0,21	2,44 1,36 +0,82	0,377	0,726	6,81	0,003	0,513
Macrophages Spleen, %	7,90 1 0	8,13 1,03 +0,15	9,10 1,15 +0,75	0,379	0,721	6,96	0,003	0,604
Entropy Leukocytogram	0,596 1 0	0,571 0,96 -0,42	0,557 0,94 -0,66	0,284	0,963	0,69	0,507	0,825
Phagocytic Index Monocytes %	2,90 1 0	2,83 0,98 -0,10	2,75 0,95 -0,21	0,300	0,910	1,77	0,184	0,656
NK Lymphocytes Blood, %	15,6 1 0	16,3 1,04 +0,25	14,8 0,95 -0,30	0,299	0,915	1,67	0,203	0,124
Lymphocytes Thymus, %	70,3 1 0	68,8 0,98 -0,61	69,3 0,99 -0,43	0,311	0,880	2,45	0,101	0,587
Basophiles Blood, %	0,30 1 0	0,43 1,44 +0,28	0,30 1,00 0,00	0,306	0,893	2,15	0,131	0,561
Reticulocytes Spleen, %	14,3 1 0	15,1 1,05 +0,41	14,8 1,03 +0,26	0,303	0,903	1,93	0,160	0,653

Table 2. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Microbial Count Neutrophils, Bac/Phag	3,95	0,026	0,856	3,95	0,026
Monocytes Blood, %	5,07	0,010	0,701	4,46	0,002
Phagocytic Index Neutrophils, %	3,19	0,051	0,614	4,14	0,001
Eosinophiles Blood, %	2,69	0,079	0,547	3,87	0,001
Plasmocytes Thymus, %	2,32	0,111	0,494	3,64	10^{-4}
Macrophages Spleen, %	2,58	0,087	0,440	3,55	10^{-4}
Entropy Leukocytogram	1,74	0,188	0,405	3,34	10^{-4}
Phagocytic Index Monocytes, %	1,53	0,230	0,377	3,15	10^{-4}
NK Lymphocytes Blood, %	1,79	0,180	0,345	3,04	10^{-4}
Lymphocytes Thymus, %	1,25	0,297	0,324	2,88	10^{-4}
Basophiles Blood, %	1,27	0,293	0,303	2,75	0,001
Reticulocytes Spleen, %	1,93	0,160	0,274	2,74	0,001

The rest of the registered immunity parameters turned out to be outside the discriminant model, despite the fact that some of them carry identifying information (Tables 3-6).

Table 3. Immune Variables of Thymus currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	Salt Waters (30)	Daily Water (10)	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Thymus Mass Index, mg/100g Body Mass	28,5 1 0	27 0,96 -0,10	32 1,14 +0,34	0,263	0,963	0,67	0,520	0,695
Epitheliocytes Thymus, %	8,80 1 0	9,67 1,10 +0,44	8,79 1,00 -0,01	0,272	0,993	0,12	0,884	0,392
Lymphoblastes Thymus, %	7,40 1 0	6,93 0,94 -0,55	7,22 0,98 -0,21	0,261	0,953	0,87	0,430	0,763
Reticulocytes Thymus, %	4,70 1 0	4,83 1,03 +0,08	4,44 0,95 -0,15	0,273	0,997	0,04	0,956	0,674
Endotheliocytes Thymus, %	2,60 1 0	2,50 0,96 -0,10	3,00 1,15 +0,41	0,263	0,962	0,69	0,506	0,733
Macrophages Thymus, %	2,70 1 0	3,23 1,20 +0,40	3,00 1,11 +0,22	0,267	0,974	0,46	0,636	0,756
Hassal's corpuscles Thymus, %	1,70 1 0	2,02 1,19 +0,59	1,83 1,08 +0,25	0,267	0,977	0,41	0,667	0,385
Entropy Thymocytogram	0,538 1 0	0,559 1,04 +0,60	0,560 1,04 +0,61	0,269	0,985	0,26	0,769	0,043

Table 4. Immune Variables of Spleen currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	**Salt Waters (30)**	**Daily Water (10)**	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Spleen Mass Index, mg/100g Body Mass	312 1 0	289 0,93 -0,23	294 0,94 -0,18	0,263	0,961	0,71	0,497	0,647
Lymphocytes Spleen, %	48,7 1 0	48,5 1,00 -0,07	48,2 0,99 -0,18	0,270	0,988	0,22	0,804	0,576
Lymphoblastes Spleen, %	3,90 1 0	4,20 1,08 +0,25	3,80 0,97 -0,08	0,264	0,966	0,61	0,547	0,569
Plasmocytes Spleen, %	2,50 1 0	1,77 0,71 -0,46	2,00 0,80 -0,32	0,268	0,979	0,38	0,688	0,589
Fibroblastes Spleen, %	8,20 1 0	7,97 0,97 -0,11	7,90 0,96 -0,14	0,271	0,992	0,14	0,872	0,758
Microphages Spleen, %	13,0 1 0	12,9 0,99 -0,05	12,8 0,98 -0,14	0,269	0,983	0,31	0,736	0,654
Eosinophils Spleen, %	1,50 1 0	1,43 0,96 -0,06	1,40 0,93 -0,09	0,270	0,985	0,26	0,774	0,669
Entropy Splenocytogram	0,753 1 0	0,750 1,00 -0,12	0,750 1,00 -0,11	0,273	0,999	0,02	0,976	0,866

Table 5. Immune Variables of Blood currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	**Salt Waters (30)**	**Daily Water (10)**	Wilks' Λ	Partial Λ	F to enter	p-level	Tolerancy
Blast Transformation T- Lymphocytes Blood, %	78,8 1 0	75,1 0,95 -0,52	78,5 1,00 -0,04	0,269	0,982	0,32	0,727	0,636
T helper Lymphocytes Blood, %	31,5 1 0	30,6 0,97 -0,28	30,5 0,97 -0,32	0,271	0,990	0,18	0,838	0,782
T cytolytic Lymphocytes Blood, %	16,0 1 0	16,2 1,01 +0,07	15,8 0,99 -0,08	0,269	0,984	0,28	0,755	0,700
B Lymphocytes Blood, %	16,0 1 0	16,1 1,00 +0,02	16,7 1,04 +0,24	0,269	0,985	0,26	0,770	0,613
Plasmocytes Blood, %	0,47 1 0	0,85 1,82 +0,83	0,86 1,84 +0,85	0,268	0,978	0,39	0,680	0,753
0-Lymphocytes Blood, %	22,2 1 0	21,4 0,96 -0,13	23,5 1,06 +0,21	0,269	0,985	0,27	0,763	0,888
Entropy Immunocytogram	0,874 1 0	0,883 1,01 +0,51	0,887 1,02 +0,76	0,273	0,999	0,02	0,980	0,680

Table 6. Variables of Leukocytogram and Phagocytosis currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact rats (10)	Salt Waters (30)	Daily Water (10)	Wil-ks' Λ	Par-tial Λ	F to en-ter	p-level	Tole-rancy
Leukocytes Blood, 10^9/L	12,68 1 0	11,02 0,87 -0,28	12,55 0,99 -0,02	0,261	0,955	0,83	0,446	0,734
Pan Lymphocytes Blood, %	60,7 1 0	59,4 0,98 -0,14	61,1 1,01 +0,04	0,263	0,963	0,67	0,518	0,667
Rod-shaped Neutrophils Blood, %	3,60 1 0	3,23 0,90 -0,34	3,20 0,89 -0,37	0,271	0,992	0,14	0,870	0,777
Polymorphonuclear Neutrophils Blood, %	26,0 1 0	28,1 1,08 +0,31	27,4 1,05 +0,21	0,260	0,949	0,93	0,402	0,734
Killing Index Neutrophils, %	50,7 1 0	54,6 1,08 +0,62	51,9 1,02 +0,19	0,259	0,947	0,99	0,383	0,790
Microbial Count Monocytes, Bacteria/Phagocyte	5,0 1 0	4,9 0,97 -0,07	3,8 0,76 -0,64	0,271	0,992	0,14	0,866	0,345

The dividing information contained in 12 variables is condensed in 2 canonical discriminant roots (Table 7). The major root contains 75% of discriminative opportunities (r*=0,770; Wilks' Λ=0,274; $\chi^2_{(24)}$=54; p=0,0005) and the minor root 25% (r*=0,572; Wilks' Λ=0,673; $\chi^2_{(11)}$=16; p=0,125).

At the next stage, using raw coefficients and constants (Table 7), individual values of discriminant roots were calculated, which allowed to visualize each rat in the information field of these roots (Fig. 1).

Table 7. Standardized and Raw Coefficients for Canonical Variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Microbial Count Neutrophils, Bac/Phag	-2,730	-0,734	-2,080	-0,559
Monocytes Blood, %	-1,058	-1,353	-0,437	-0,558
Phagocytic Index Neutrophils, %	1,435	-0,337	0,372	-0,087
Eosinophils Blood, %	-0,274	-0,405	-0,135	-0,199
Plasmocytes Thymus, %	0,903	-0,397	1,192	-0,525
Macrophages Spleen, %	0,735	-0,656	0,407	-0,363
Entropy Leukocytogram	-0,275	-0,019	-4,408	-0,312
Phagocytic Index Monocytes, %	0,443	-0,250	0,5118	-0,288
NK Lymphocytes Blood, %	-0,840	0,897	-0,388	0,414
Lymphocytes Thymus, %	0,529	-0,340	0,211	-0,135
Basophiles Blood, %	-0,517	0,312	-0,963	0,580
Reticulocytes Spleen, %	0,499	-0,039	0,268	-0,021
		Constants	-23,83	21,85
		Eigenvalues	1,459	0,487
		Cumulative Proportions	0,750	1

Localization in the extreme right zone of the axis of the first root of rats loaded with tap water reflects the maximum increase in immune parameters that represent the root **directly**, and the maximum decrease in **inversely** correlated with the root parameters (Table 8).

In contrast, in rats of both experimental groups, these immune parameters did not differ significantly from normal or deviated to a lesser extent.

Since the control and intact animals received the same daily fresh water, the detected changes in immune parameters, apparently due to the adversive stress from the introduction of the tube into the stomach [18-21,26]. Both mineral waters prevent or minimize the immunotropic effects of stress.

The other constellation of immune parameters was not affected at all or to a lesser extent by stress factors. Instead, they **decrease** or **increase** under the influence of mineral waters. This situation is illustrated by the top position of the rats loaded by them along the axis of the second root.

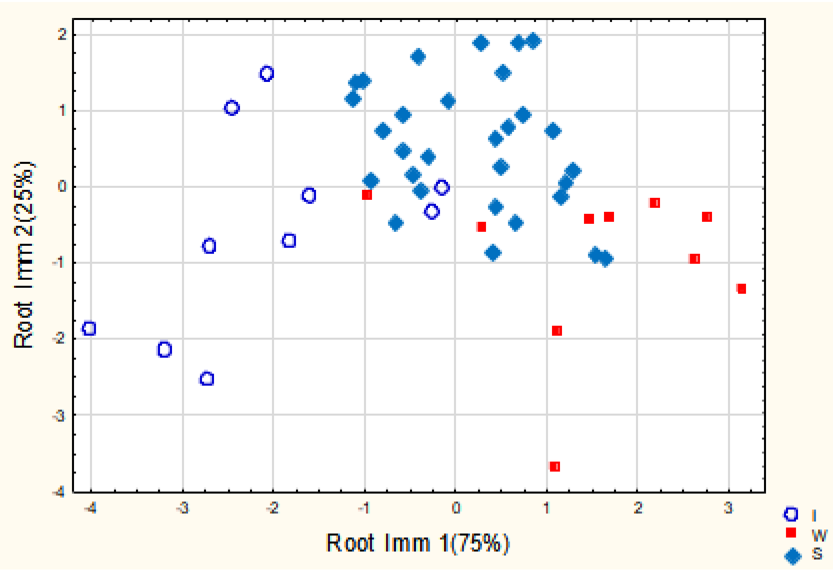


Fig. 1. Individual values of the first and second roots of the immune parameters in intact rats (I) and loaded with Daily water (W) and Salt waters (S)

Table 8. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Roots and Variables

	Correlations Variables-Roots		Intact Rats (10)	Salt Waters (30)	Daily Water (10)
Root 1 (75%)	**R1**	**R2**	-2,09	+0,18	**+1,54**
Plasmocytes Thymus, %	**0,197**	-0,197	0	+0,21	**+0,82**
Macrophages Spleen, %	**0,160**	-0,204	0	+0,15	**+0,75**
Phagocytic Index Neutrophils, %	**0,131**	-0,356	0	-0,10	**+0,56**
Endotheliocytes Thymus			0	-0,10	**+0,41**
Entropy Immunocytogram			0	+0,51	**+0,76**
Entropy Leukocytogram	**-0,170**	-0,006	0	-0,42	**-0,66**
Phagocytic Index Monocytes, %	**-0,045**	0,022	0	-0,10	**-0,21**
Microbial Count Monocytes			0	-0,07	**-0,64**
Root 2 (25%)	**R1**	**R2**	-0,61	**+0,54**	-1,02
Microbial Count Neutrophils, Bac/Phag	-0,143	**-0,533**	0	**-0,65**	-0,21
Lymphocytes Thymus, %	-0,138	**-0,229**	0	**-0,61**	-0,43
Blast Transformation T- Lymphocytes			0	**-0,52**	-0,04
Eosinophiles Blood, %	-0,126	**-0,163**	0	**-0,32**	-0,27
Plasmocytes Spleen			0	**-0,46**	-0,32

Lymphoblastes Thymus			0	**-0,55**	-0,21
NK Lymphocytes Blood, %	-0,062	**0,400**	0	**+0,25**	-0,30
Monocytes Blood, %	-0,046	**0,198**	0	**+0,10**	-0,20
Basophiles Blood, %	0,020	**0,176**	0	**+0,28**	0,00
Epitheliocytes Thymus			0	**+0,44**	-0,01
Reticulocytes Spleen, %	0,093	**0,173**	0	**+0,41**	+0,26
Killing Index Neutrophils			0	**+0,62**	+0,19
Hassal's corpuscles Thymus			0	**+0,59**	+0,25

Both mineral waters have almost the same integral modulating effect on the listed immune parameters, as evidenced by the identity of the centroids of the first immune root and the absence of significant differences between the centroids of the second root (Fig. 2).

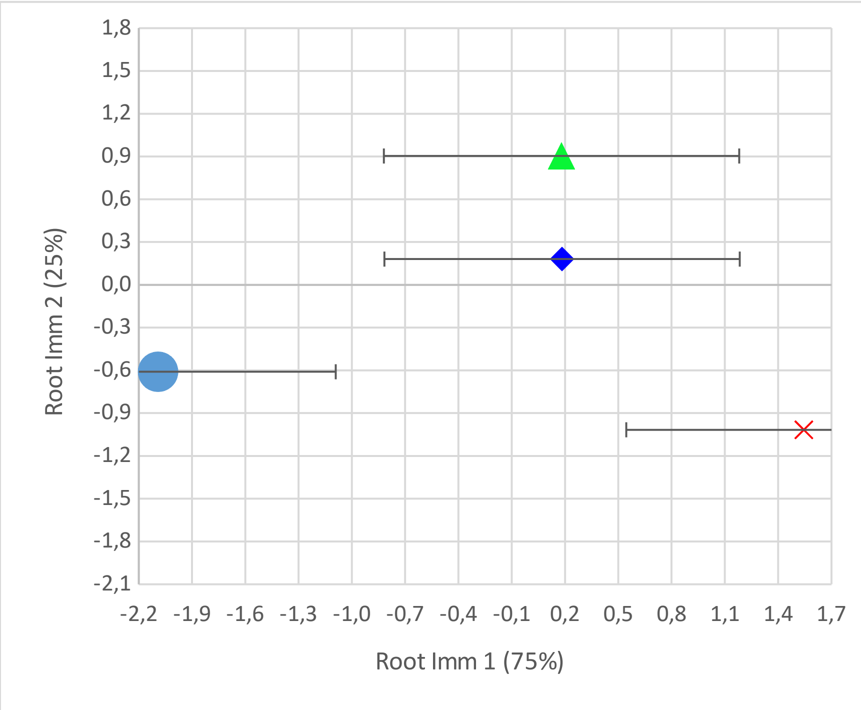


Fig. 2. Average values (Mean±SD) of the first and second roots of the immune parameters in intact rats (O) and loaded with Daily water and Salt waters Myroslava or Khrystyna

Despite the not very clear delineation of the three clusters, the differences between them are statistically significant (Table 9).

Table 9. Squared Mahalanobis Distances between groups (over diagonal), F-values (df=12,4) and p-levels (under diagonal)

Groups	I (10)	**DW (10)**	**SW (30)**
Intact rats (I)	**0,0**	13,4	6,49
Daily Water (DW)	**4,27** **,0003**	**0,0**	4,29
Salt Waters (SW)	**3,11** **,004**	**2,05** **,048**	**0,0**

The application of the classifying functions (Table 10) enables the retrospective identification of intact rats with 3 errors, and the other two groups - with 2 errors. Total accuracy is 86% (Table 11).

Table 10. Coefficients and Constants for Classification Functions

Variables currently in the model	Intact rats	**Daily Water**	**Salt Waters**
Microbial Count Neutrophils, Bac/Phag	-40,97	-48,30	-46,34
Monocytes Blood, %	-21,68	-23,04	-23,32
Phagocytic Index Neutrophils, %	22,76	24,14	23,50
Eosinophiles Blood, %	9,189	8,780	8,654
Plasmocytes Thymus, %	68,48	73,02	70,58
Macrophages Spleen, %	17,55	19,18	18,06
Entropy Leukocytogram	363,5	347,6	353,1
Phagocytic Index Monocytes, %	15,63	17,61	16,47
NK Lymphocytes Blood, %	26,28	24,70	25,88
Lymphocytes Thymus, %	21,93	22,75	22,25
Basophiles Blood, %	-69,75	-73,49	-71,27
Reticulocytes Spleen, %	20,34	21,32	20,92
Constants	-1958	-2053	-1984

Table 11. Classification Matrix
Rows: Observed classifications; Columns: Predicted classifications

Groups	Percent correct	I p=,20	**DW** p=,20	**SW** p=,60
Intact rats (I)	70,0	**7**	0	3
Daily Water (DW)	80,0	0	**8**	2
Salt Waters (SW)	93,3	0	2	**28**
Total	86,0	7	10	33

On the second stage, the immune parameters of all four groups were subjected to discriminant analysis. The program included 15 parameters in the model: 3 parameters of **thymocytogram**, 4 parameters of **splenocytogram**, 7 parameters of **leukocytogram and phagocytosis**, and also parameter of **immunocytogram** of blood (Tables 12-13).

Table 12. Discriminant Function Analysis Summary
Step 15, N of Variables currently in the model: 15; Grouping: 4 groups
Wilks' Lambda: 0,1528; approx. $F_{(46)}$=1,88; p=0,005

Variables currently in the model	**Groups (n)**				**Parameters of Wilks' Statistics**				
	Daily Water (10)	**Myro slava (15)**	**Khry styna (15)**	Intact rats (10)	Wil ks' Λ	Par-tial Λ	F-re-move (3,3)	p-level	Tole-rancy
Microbial Count Neutrophils, Bac/Phag	8,2 0,95 -0,21	7,3 0,84 -0,70	7,5 0,87 -0,60	8,6 1 0	0,280	0,545	8,91	10^{-4}	0,119
Monocytes Blood, %	4,20 0,88 -0,20	4,87 1,01 +0,02	5,33 1,11 +0,18	4,80 1 0	0,195	0,785	2,93	0,049	0,106
Eosinophiles Blood, %	3,80 0,83 -0,27	3,33 0,72 -0,42	3,93 0,86 -0,22	4,60 1 0	0,184	0,829	2,20	0,107	0,735
Phagocytic Index	71,9	68,9	69,2	69,5	0,192	0,796	2,73	0,060	0,313

Neutrophils, %	1,03 +0,56	0,99 -0,13	1,00 -0,07	1 0					
Entropy Leukocytogram	0,557 0,94 -0,66	0,592 0,99 -0,07	0,552 0,93 -0,76	0,596 1 0	0,197	0,777	3,07	0,042	0,725
Macrophages Spleen, %	9,1 1,15 +0,75	7,9 1,00 +0,02	8,3 1,05 +0,27	7,9 1 0	0,203	0,751	3,54	0,026	0,507
Plasmocytes Thymus, %	2,44 1,36 +0,82	2,00 1,11 +0,25	1,93 1,07 +0,17	1,80 1 0	0,193	0,791	2,82	0,055	0,549
Leukocytes Blood, 10^9/L	12,55 0,99 -0,02	10,51 0,83 -0,36	11,53 0,91 -0,19	12,68 1 0	0,165	0,927	0,85	0,479	0,669
Eosinophiles Spleen, %	1,40 0,93 -0,09	1,73 1,16 +0,22	1,13 0,76 -0,34	1,50 1 0	0,169	0,903	1,15	0,343	0,747
NK Lymphocytes Blood, %	14,8 0,95 -0,30	16,3 1,04 +0,23	16,4 1,05 +0,26	15,6 1 0	0,179	0,853	1,83	0,161	0,099
Phagocytic Index Monocytes %	2,75 0,95 -0,21	2,83 0,98 -0,10	2,83 0,98 -0, 10	2,90 1 0	0,162	0,941	0,67	0,579	0,603
Spleen Mass Index, mg/100g Body Mass	294 0,94 -0,18	268 0,86 -0,44	309 0,99 -0,03	312 1 0	0,190	0,806	2,57	0,071	0,470
Lymphoblastes Spleen, %	3,80 0,97 -0,08	4,00 1,03 +0,08	4,40 1,13 +0,42	3,90 1 0	0,182	0,838	2,06	0,125	0,419
Lymphocytes Thymus, %	69,3 0,99 -0,43	68,2 0,97 -0,88	69,5 0,99 -0,33	70,3 1 0	0,188	0,813	2,45	0,081	0,417
Endotheliocytes Thymus, %	3,00 1,15 +0,41	2,47 0,95 -0,14	2,53 0,97 -0,07	2,60 1 0	0,172	0,887	1,37	0,269	0,507

Table 13. Summary of Stepwise Analysis

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Microbial Count Neutrophils, Bac/Phag	2,64	0,060	0,853	2,64	0,060
Monocytes Blood, %	4,43	0,008	0,658	3,49	0,004
Eosinophils Blood, %	2,63	0,062	0,558	3,23	0,002
Phagocytic Index Neutrophils, %	2,10	0,114	0,487	2,97	0,001
Entropy Leukocytogram	1,79	0,163	0,431	2,76	0,001
Macrophages Spleen, %	1,87	0,150	0,380	2,64	0,001
Plasmocytes Thymus, %	1,87	0,150	0,333	2,57	0,001
Leukocytes Blood, 10^9/L	1,24	0,309	0,304	2,41	0,001
Eosinophiles Spleen, %	1,07	0,372	0,280	2,26	0,002
NK Lymphocytes Blood, %	1,07	0,372	0,258	2,14	0,002
Phagocytic Index Monocytes, %	1,38	0,266	0,231	2,08	0,003
Spleen Mass Index, mg/100g Body Mass	1,09	0,367	0,211	2,00	0,003
Lymphoblastes Spleen, %	1,12	0,354	0,192	1,94	0,004
Lymphocytes Thymus, %	1,28	0,299	0,172	1,90	0,005
Endotheliocytes Thymus, %	1,37	0,269	0,153	1,88	0,005

To complete the picture, we present immune parameters not included in the model (Tables 14-17).

Table 14. Immune Variables of Thymus currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Daily Water (10)	Myro slava (15)	Khry styna (15)	Intact rats (10)	Wil-ks' Λ	Par-tial Λ	F to en-ter	p-level	Tole-rancy
Thymus Mass Index, mg/100g Body Mass	32,4 1,14 +0,34	27,0 0,95 -0,13	27,6 0,97 -0,08	28,5 1 0	0,149	0,976	0,25	0,860	0,677
Lymphoblastes Thymus, %	7,22 0,98 -0,21	6,93 0,94 -0,55	6,93 0,94 -0,55	7,40 1 0	0,148	0,968	0,34	0,796	0,801
Reticulocytes Thymus, %	4,44 0,95 -0,15	5,13 1,09 +0,25	4,53 0,96 -0,10	4,70 1 0	0,141	0,920	0,90	0,454	0,573
Epitheliocytes Thymus, %	8,78 1,00 -0,01	9,80 1,11 +0,50	9,53 1,08 +0,37	8,80 1 0	0,147	0,964	0,38	0,767	0,357
Macrophages Thymus, %	3,00 1,11 +0,22	3,47 1,28 +0,57	3,00 1,11 +0,22	2,70 1 0	0,143	0,936	0,71	0,555	0,632
Hassal's corpuscles Thymus, %	1,83 1,08 +0,25	2,00 1,18 +0,56	2,03 1,20 +0,62	1,70 1 0	0,144	0,941	0,65	0,588	0,578
Entropy Thymocytogram	0,560 1,04 +0,61	0,568 1,05 +0,85	0,551 1,02 +0,35	0,538 1 0	0,151	0,987	0,13	0,941	0,031

Table 15. Immune Variables of Spleen currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Daily Water (10)	Myro slava (15)	Khry styna (15)	Intact rats (10)	Wil-ks' Λ	Par-tial Λ	F to en-ter	p-level	Tole-rancy
Lymphocytes Spleen, %	48,2 0,99 -0,18	48,8 1,00 +0,04	48,2 0,99 -0,18	48,7 1 0	0,146	0,954	0,50	0,685	0,647
Plasmocytes Spleen, %	2,00 0,80 -0,32	1,73 0,69 -0,49	1,80 0,72 -0,44	2,50 1 0	0,144	0,941	0,65	0,590	0,450
Reticulocytes Spleen, %	14,8 1,03 +0,26	14,7 1,03 +0,23	15,4 1,08 +0,58	14,3 1 0	0,148	0,968	0,35	0,792	0,568
Fibroblastes Spleen, %	7,90 0,96 -0,14	8,07 0,98 -0,06	7,87 0,96 -0,16	8,20 1 0	0,152	0,993	0,08	0,973	0,746
Microphages Spleen, %	12,8 0,98 -0,14	13,0 1,00 0,00	12,9 0,99 -0,09	13,0 1 0	0,151	0,986	0,15	0,930	0,621
Entropy Splenocytogram	0,750 1,00 -0,11	0,750 1,00 -0,11	0,749 0,99 -0,14	0,753 1 0	0,149	0,972	0,29	0,831	0,494

Table 16. Immune Variables of Blood currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Daily Water (10)	Myro slava (15)	Khry styna (15)	Intact rats (10)	Wil-ks' Λ	Par-tial Λ	F to en-ter	p-level	Tole-rancy
Blast Transformation T-Lymphocytes Blood, %	78,5 1,00 -0,04	73,4 0,93 -0,75	76,8 0,97 -0,28	78,9 1 0	0,150	0,980	0,21	0,891	0,623
T helper Lymphocytes Blood, %	30,5 0,97 -0,32	30,7 0,97 -0,27	30,6 0,97 -0,29	31,5 1 0	0,152	0,997	0,03	0,991	0,711
T cytolytic Lymphocytes Blood, %	15,8 0,99 -0,08	15,6 0,98 -0,17	16,7 1,05 +0,31	16,0 1 0	0,144	0,942	0,64	0,595	0,655
B Lymphocytes Blood, %	16,7 1,04 +0,24	16,2 1,01 +0,07	15,9 1,00 -0,02	16,0 1 0	0,148	0,970	0,32	0,813	0,647
Plasmocytes Blood, %	0,86 1,84 +0,85	0,78 1,66 +0,66	0,93 1,97 +0,98	0,47 1 0	0,142	0,931	0,77	0,521	0,450
0-Lymphocytes Blood, %	23,5 1,06 +0,21	22,1 0,99 -0,02	20,7 0,93 -0,24	22,2 1 0	0,141	0,925	0,83	0,486	0,532
Entropy Immunocytogram	0,887 1,02 +0,76	0,886 1,01 +0,65	0,881 1,01 +0,37	0,887 1 0	0,145	0,951	0,53	0,665	0,516

Table 17. Variables of Leukocytogram and Phagocytosis currently not in the model

Variables	Groups (n)				Parameters of Wilks' Statistics				
	Daily Water (10)	Myro slava (15)	Khry styna (15)	Intact rats (10)	Wil-ks' Λ	Par-tial Λ	F to en-ter	p-level	Tole-rancy
Pan Lymphocytes Blood, %	61,1 1,01 +0,04	59,7 0,98 -0,10	59,1 0,97 -0,17	60,7 1 0	0,146	0,955	0,48	0,696	0,633
Basophiles Blood, %	0,30 1,00 0,00	0,40 1,33 +0,21	0,47 1,56 +0,35	0,30 1 0	0,144	0,940	0,66	0,582	0,626
Rod-shaped Neutrophils Blood, %	3,20 0,89 -0,37	3,20 0,89 -0,37	3,27 0,91 -0,31	3,60 1 0	0,152	0,993	0,08	0,972	0,697
Polymorphonuclear Neutrophils Blood, %	27,4 1,05 +0,21	28,3 1,09 +0,34	27,9 1,07 +0,28	26,0 1 0	0,145	0,950	0,54	0,657	0,709
Killing Index Neutrophils, %	51,9 1,02 +0,19	53,4 1,05 +0,42	55,9 1,10 +0,81	50,7 1 0	0,143	0,934	0,74	0,539	0,758
Microbial Count Monocytes, Bacteria/Phagocyte	3,8 0,76 -0,64	4,8 0,97 -0,08	4,9 0,98 -0,05	5,0 1 0	0,145	0,948	0,57	0,638	0,322

The dividing information contained in 15 variables is condensed in 3 canonical discriminant roots (Table 18). The first root contains 53,6% of discriminative opportunities (r*=0,774; Wilks' Λ=0,153; $\chi^2_{(45)}$=74; p=0,004), the second 34,2% (r*=0,698; Wilks' Λ=0,381; $\chi^2_{(28)}$=38; p=0,097), the third 12,2% (r*=0,506; Wilks' Λ=0,744; $\chi^2_{(13)}$=12; p=0,553). The calculation of the discriminant root values for each animal as the sum of the products of raw coefficients (Table 18) to the individual values of discriminant variables together with the constant enables the visualization of each rat in the information space of the roots (Figs. 3-4).

Table 18. Standardized and Raw Coefficients for Canonical Variables

Coefficients	Standardized			Raw		
Variables	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Microbial Count Neutrophils, Bac/Phag	2,425	-0,709	0,482	1,831	-0,535	0,364
Monocytes Blood, %	0,821	-1,821	-0,219	0,336	-0,746	-0,090
Eosinophiles Blood, %	0,453	-0,467	0,121	0,222	-0,228	0,059
Phagocytic Index Neutrophils, %	-1,017	-0,249	0,099	-0,260	-0,064	0,025
Entropy Leukocytogram	0,523	0,456	0,407	8,584	7,483	6,668
Macrophages Spleen, %	-0,715	-0,610	-0,111	-0,393	-0,335	-0,061
Plasmocytes Thymus, %	-0,744	-0,274	0,223	-0,972	-0,358	0,292
Leukocytes Blood, 10^9/L	0,343	-0,278	-0,084	0,071	-0,058	-0,017
Eosinophiles Spleen, %	-0,086	0,066	0,695	-0,101	0,078	0,814
NK Lymphocytes Blood, %	1,123	1,199	0,298	0,513	0,548	0,136
Phagocytic Index Monocytes, %	-0,328	-0,215	-0,201	-0,375	-0,246	-0,230
Spleen Mass Index, mg/100g Body Mass	0,664	0,257	-0,678	0,010	0,004	-0,010
Lymphoblastes Spleen, %	0,564	0,274	-0,788	0,407	0,198	-0,569
Lymphocytes Thymus, %	-0,615	-0,659	0,207	-0,248	-0,266	0,084
Endotheliocytes Thymus, %	-0,358	-0,519	0,253	-0,394	-0,571	0,278
			Constants	7,663	22,85	-12,10
			Eigenvalues	1,495	0,951	0,345
			Cumulative Proportions	0,536	0,878	1

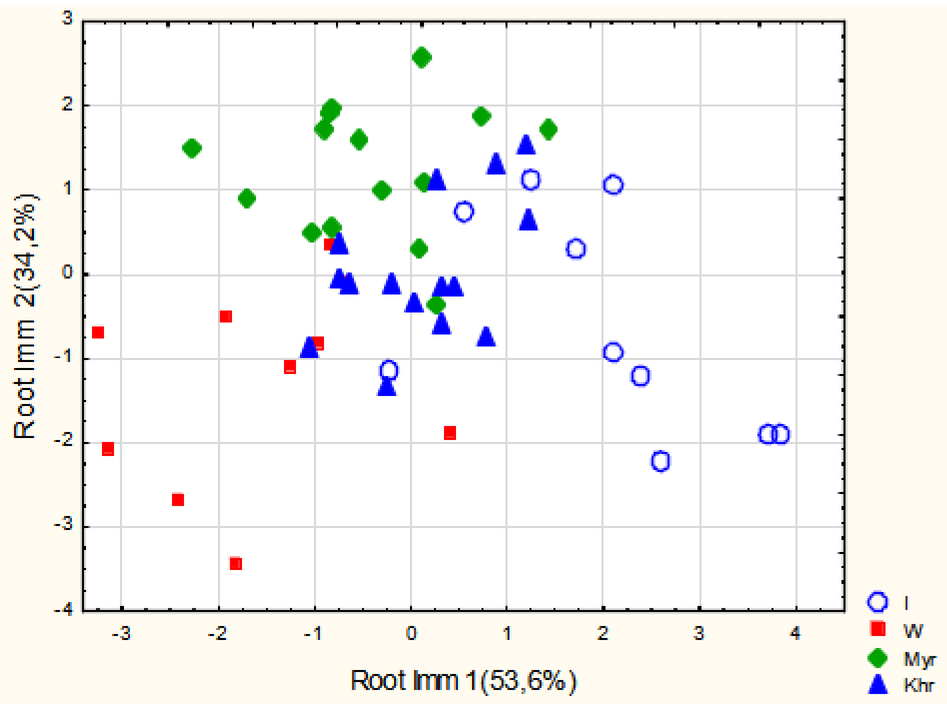
Table 19. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Roots and Variables

	Correlations Variables-Roots			Daily Water	Myro slava	Khry styna	Intact rats
Root 1 (53,6%)	R1	R2	R3	-1,59	-0,42	+0,13	+2,02
Plasmocytes Thymus	-0,201	-0,118	0,097	+0,82	+0,25	+0,25	0
Macrophages Spleen	-0,147	-0,184	-0,064	+0,75	+0,02	+0,15	0
Phagocytic Index Neutrophils	-0,131	-0,244	0,126	+0,56	-0,13	-0,03	0
Endotheliocytes Thymus	-0,080	-0,180	0,082	+0,41	-0,14	-0,10	0
Entropy Immunocytogram				+0,76	+0,65	+0,37	0
Thymus Mass Index				+0,34	-0,13	-0,08	0
B Lymphocytes Blood				+0,24	+0,07	-0,02	0
Monocytes Blood	0,061	0,086	-0,213	-0,20	+0,02	+0,09	0
Phagocytic Index Monocytes	0,044	0,017	0,003	-0,21	-0,10	-0,01	0
Microbial Count Monocytes				-0,64	-0,08	-0,05	0
Root 2 (34,0%)	R1	R2	R3	-1,29	+1,24	+0,03	-0,63
Microbial Count Neutrophils	0,137	-0,350	0,285	-0,21	-0,70	-0,54	0
Lymphocytes Thymus	0,164	-0,243	-0,113	-0,43	-0,88	-0,30	0
Spleen Mass Index	0,119	-0,195	-0,240	-0,18	-0,44	0,00	0
Leukocytes Blood	0,041	-0,187	0,030	-0,02	-0,36	-0,15	0
Eosinophils Blood	0,138	-0,153	-0,026	-0,27	-0,42	-0,20	0
Blast Transformation T-Lym				-0,04	-0,75	-0,28	0
Plasmocytes Spleen				-0,32	-0,49	-0,44	0
NK Lymphocytes Blood	0,072	0,242	-0,244	-0,30	+0,23	+0,15	0
Epitheliocytes Thymus				-0,01	+0,50	+0,37	0
Macrophages Thymus				+0,22	+0,57	+0,22	0
Reticulocytes Thymus				-0,15	+0,25	-0,10	0

Entropy Thymocytogram				+0,61	**+0,85**	+0,35	0
Root 3 (12,4%)	**R1**	**R2**	**R3**	+0,30	+0,37	**-0,86**	+0,43
Entropy Leukocytogram	0,132	0,142	0,439	-0,66	-0,07	**-0,76**	0
Eosinophils Spleen	-0,011	0,146	0,422	-0,09	+0,22	**-0,33**	0
0-Lymphocytes Blood				+0,21	-0,02	**-0,24**	0
Lymphoblastes Spleen	0,021	0,056	-0,281	-0,08	+0,08	**+0,38**	0
Killing Index Neutrophils				+0,19	+0,42	**+0,81**	0
T cytolytic Lymphocytes				-0,08	-0,17	**+0,31**	0
Reticulocytes Spleen				+0,26	+0,23	**+0,58**	0

As we can see, along the axis of the first root (Figs 3-4) immune clusters of intact and control rats are localized at opposite poles. This reflects the stress **activation**/**suppression** of 11 parameters, which is leveled or minimized to approximately the same extent by both mineral waters (Table 19).

Differences between the immunotropic effects of both mineral waters are visualized along the axes of the second and third roots. In particular, the top position of the “Myroslava” water cluster along the axis of the second root reflects the maximum **suppression**/**activation** of the constellation of 12 parameters, which is predominant under the influence of “Khrystyna” water. On the other hand, the lowest position of the “Khrystyna” water cluster along the axis of the third root reflects the maximum for sampling **suppression**/**activation** of another constellation of 7 parameters.



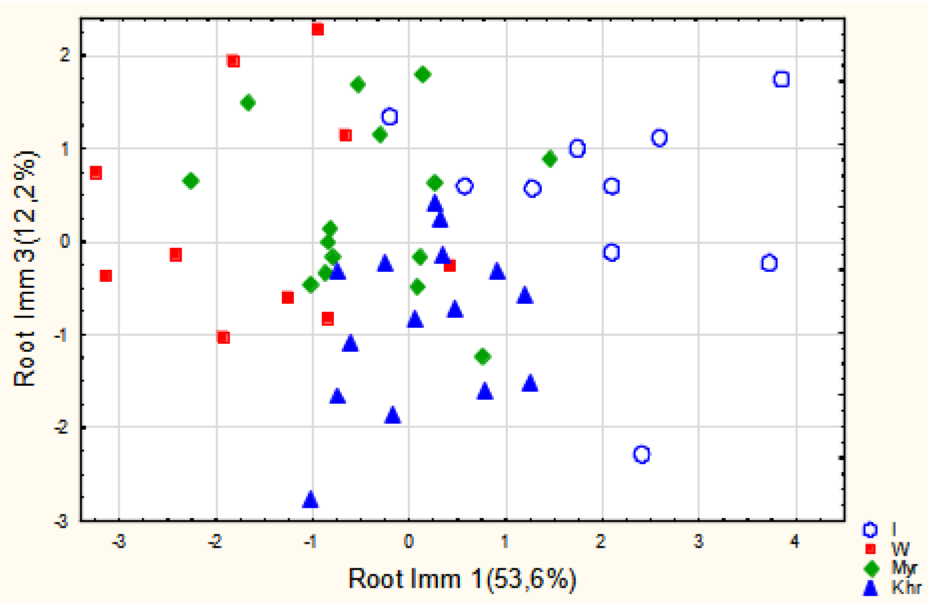
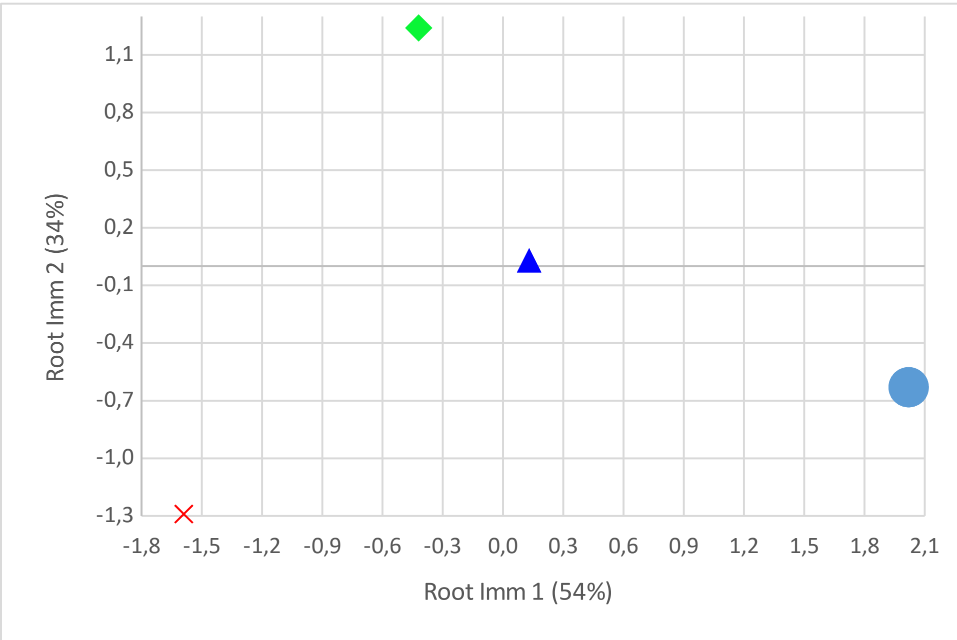


Fig. 3. Individual values of the first and second (above) and the first and third (below) roots of the immune parameters in intact rats (o) and loaded with Daily water (W) and mineral waters "Myroslava" (Myr) and "Khrystyna" (Khr)



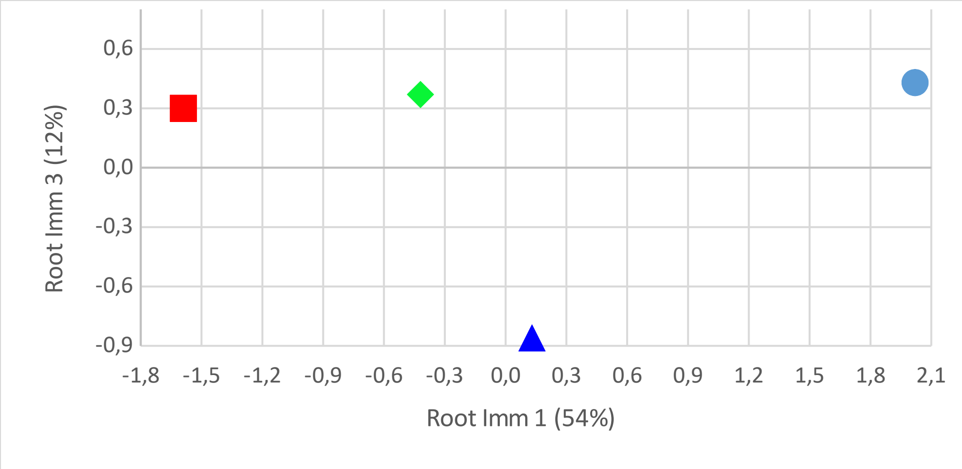


Fig. 4. Average values (Mean±SD) of the first and second (above) and the first and third (below) roots of the immune parameters in intact rats (o) and loaded with Daily water and mineral waters "Myroslava" and "Khrystyna"

However, judging by the distances of Mahalanobis (Table 20) and the accuracy of retrospective classification (Tables 21 and 22), the specificity of the immunomodulatory effects of mineral waters on the set of discriminant variables is not significant enough.

Table 20. Squared Mahalanobis Distances between groups (over diagonal), F-values (df=15) and p-levels (under diagonal)

Groups	I (10)	DW (10)	Myr (15)	Khr (15)
Intact rats (I)	**0,0**	13,4	9,44	5,64
Daily Water (DW)	**3,12** **,003**	**0,0**	7,77	6,03
Water "Myroslava" (Myr)	**2,63** **,011**	**2,16** **,033**	**0,0**	3,29
Water "Khrystyna" (Khr)	**1,57** **,139**	**1,68** **,108**	**1,14** **,361**	**0,0**

Table 21. Coefficients and Constants for Classification Functions

Variables currently in the model	**Intact rats**	**Daily Water**	**Myro slava**	**Khry styna**
Microbial Count Neutrophils, Bac/Phag	-5,543	-11,84	-11,03	-9,811
Monocytes Blood, %	-14,03	-14,73	-16,23	-15,03
Eosinophiles Blood, %	7,771	7,116	6,800	7,128
Phagocytic Index Neutrophils, %	15,53	16,51	16,05	15,95
Entropy Leukocytogram	94,33	57,53	86,99	74,49
Macrophages Spleen, %	9,139	10,79	9,474	9,738
Plasmocytes Thymus, %	46,53	50,23	48,21	47,75
Leukocytes Blood, 10^9/L	1,045	0,829	0,765	0,896
Eosinophils Spleen, %	26,64	26,84	26,98	25,83
NK Lymphocytes Blood, %	31,69	29,46	31,46	30,91
Phagocytic Index Monocytes, %	2,796	4,343	3,265	3,638
Spleen Mass Index, mg/100g Body Mass	-0,063	-0,098	-0,078	-0,066
Lymphoblastes Spleen, %	-15,83	-17,35	-16,42	-15,73
Lymphocytes Thymus, %	23,84	24,89	23,94	24,02
Endotheliocytes Thymus, %	23,92	25,68	23,79	23,93
Constants	-1714	-1755	-1688	-1696

Table 22. 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)	70,0	**7**	1	2	
Daily Water (DW)	70,0	0	**7**	1	2
Myroslava (Myr)	80,0	0	0	**12**	3
Khrystyna (Khr)	86,7	0	0	2	**13**
Total	78,0	7	10	33	

Therefore, a different approach was used [24]. It consists in creating 6 patterns of Z-scores immune parameters (Table 19 and Fig. 4).

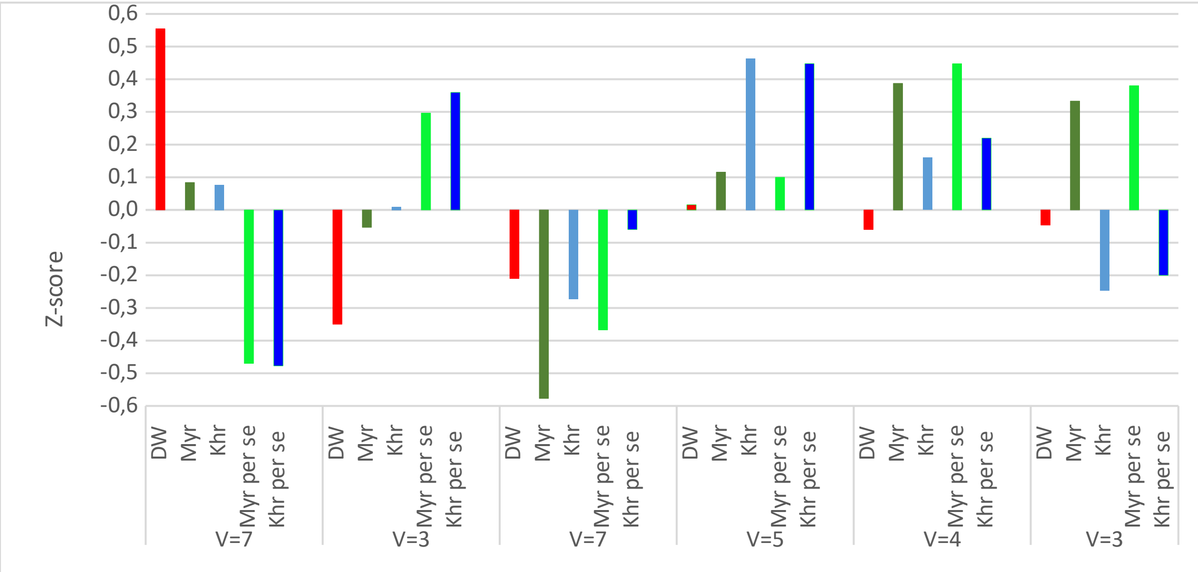


Fig. 5. Patterns (V - number of variables) of effects of daily water and mineral waters and simulated partial effects of mineral waters

The first pattern shows how both mineral waters equally prevent the stress-induced increase in thymus mass and content in the thymocytogram of plasma cells and endothelial cells, in the splenocytogram macrophages, in the immunocytogram B-lymphocytes and its entropy, as well as the phagocytic index of blood neutrophils.

On the other hand (second pattern), they prevent a stress-induced decrease in blood cell counts and the activity and intensity of bacterial phagocytosis by monocytes.

The third pattern shows how “Myroslava” water significantly exacerbates the stress-induced decrease in 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 hardly potentiates the effect of stress on these parameters.

The fourth pattern demonstrates that stress-insensitive immune parameters (lymphoblast and reticulocyte content in splenocytogram, T cytolytic lymphocytes content in immunocytogram, and neutrophil killing index) increase (the content of 0-lymphocytes decreases) under the influence of mineral waters, and “Khrystyna” water is much more active than “Myroslava” water.

In contrast, “Myroslava” water is much more active than “Khrystyna” water in increasing the level in thymocytogram of epitheliocytes, macrophages and reticulocytes, as well as NK lymphocytes in the blood.

In addition, on the entropy of the leukocytogram and thymocytogram, as well as the content of eosinophils in the splenocytogram "Khrystyna" water has the opposite effect.

Calculating the algebraic difference between Z-scores of immune parameters in control and experimental groups allows us to estimate the partial immunotropic effects of mineral waters (Figs. 5 and 6).

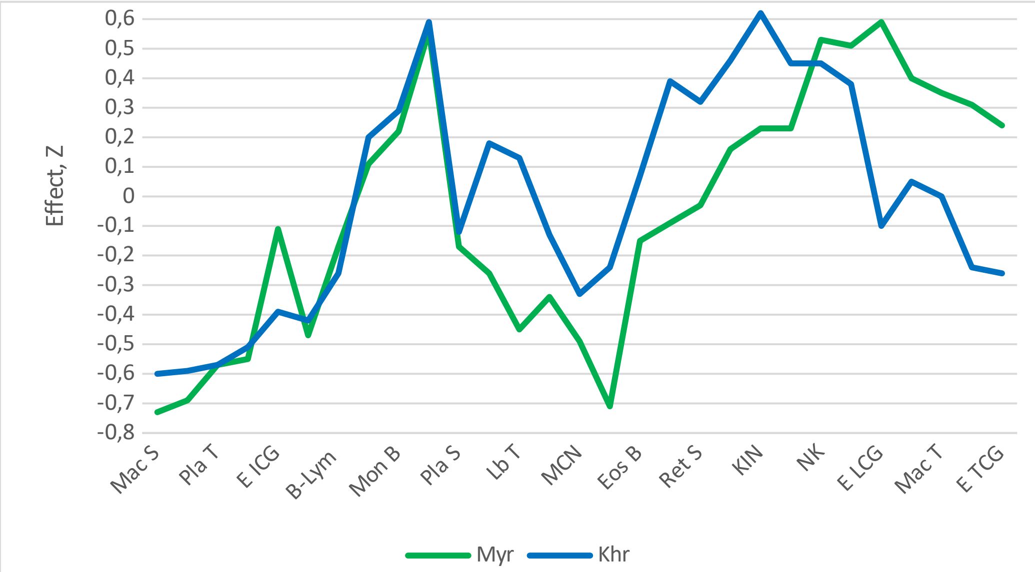


Fig. 6. Profiles of simulated immune Z-scores in rats after consumption of sulphate-chloride sodium-magnesium mineral waters Myroslava and Khrystyna

CONCLUSION

Thus, sulphate-chloride sodium-magnesium mineral waters Myroslava and Khrystyna have both common and specific modulating effects on the immune system of healthy female rats. The data obtained earlier on the same animals on the modulating neuroendocrine effects of these mineral waters [8,16,28] give grounds to associate the identified immunotropic effects with them.

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).

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