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 DISCUSSION

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 | Tolerance |
| Calcium Plasma, mM/L           | 2.51 ± 0.75  | 2.91 ± 0.87  | 2.08 ± 0.62  | 3.35 ± 1.00  | 0.004  | 0.910  | 0.52  | 0.672  | 0.361  |
| Superoxide Dismutase Erythrocytes, un/mL | 57.7 ± 0.99  | 49.9 ± 0.86  | 58.2 ± 1.00  | 58.0 ± 1.00  | 0.005  | 0.814  | 1.22  | 0.335  | 0.263  |
| Microbial Count Neutrophils, Bacteria/Phagocyte | 7.6 ± 0.88  | 7.3 ± 0.84  | 8.2 ± 0.95  | 8.6 ± 1.00  | 0.005  | 0.822  | 1.16  | 0.357  | 0.070  |
| Sodium Excretion, μM/24h•100 g Body Mass | 271 ± 2.01  | 167 ± 1.24  | 76 ± 0.56  | 135 ± 1.00  | 0.005  | 0.782  | 1.49  | 0.255  | 0.057  |
| Monocytes Blood, %             | 5.07 ± 1.06  | 4.87 ± 1.01  | 4.20 ± 0.88  | 4.80 ± 1.00  | 0.006  | 0.655  | 2.81  | 0.073  | 0.053  |
| Eosinophiles                   | 4.00 ± 3.33  | 3.80 ± 3.80  | 4.60 ± 0.00  | 4.007 ± 0.550 | 4.37  | 0.020  | 0.267 |
| Measurement                              | Value 1          | Value 2          | Value 3          | Value 4          | Value 5          |
|-----------------------------------------|------------------|------------------|------------------|------------------|------------------|
| Blood, %                                | 0.87 -0.20       | 0.72 -0.42       | 0.83 -0.27       | 1                | 0                |
| Potassium Plasma, mM/L                  | 3.33 -0.79       | 0.81 -0.72       | 3.54 -0.42       | 4.23 -0.27       | 0.006 0.647 2.91 0.067 0.344 |
| (Cap/Pp) as Parathyroid Activity        | 1.75 -0.68       | 0.75 -0.67       | 1.58 -0.62       | 2.56 -0.42       | 0.008 0.478 0.58 0.007 0.181 |
| Testosterone Plasma, nM/L               | 4.50 -1.15       | 1.27 -0.53       | 6.04 -1.54       | 3.93 -1.97       | 0.007 0.518 0.49 0.013 0.193 |
| NK Lymphocytes Blood, %                 | 16.1 -1.03       | 1.04 -0.15       | 14.8 -0.95       | 15.6 -0.30       | 0.006 0.698 2.30 0.116 0.043 |
| Malondialdehyde Urine, μM/L             | 96 -1.04         | 0.95 -0.10       | 75 -0.81         | 92 -0.40         | 0.007 0.528 0.47 0.015 0.127 |
| Leukocytes Blood, 10^9/L                | 11.76 -0.93      | 0.83 -0.15       | 12.55 -0.99      | 12.68 -0.84      | 0.004 0.920 0.47 0.710 0.510 |
| Spleen Mass Index, mg/100g Body Mass    | 312 -1.00        | 0.86 -0.44       | 294 -0.94        | 312 -0.18        | 0.004 0.902 0.58 0.635 0.365 |
| Amylase Activity Urine, g/h•L           | 204 -1.01        | 1.01 -0.04       | 217 -0.17        | 202 -0.26        | 0.009 0.437 0.86 0.003 0.092 |
| Katalase Activity Plasma, μM/h•L        | 128 -1.24        | 1.18 -0.67       | 148 -1.43        | 103 -1.58        | 0.007 0.556 0.42 0.022 0.219 |
| Chloride Excretion, μM/24h•100g Body Mass| 244 -1.69        | 1.35 -0.51       | 107 -0.74        | 144 -0.38        | 0.007 0.552 0.43 0.020 0.062 |
| Triiodothyronine Plasma, nM/L           | 2.38 -1.11       | 1.08 +0.30       | 2.11 0.99        | 2.14 -0.05       | 0.006 0.677 2.55 0.092 0.045 |
| Corticosterone Plasma, nM/L             | 460 -0.96        | 0.76 -0.17       | 383 0.80         | 482 -0.78        | 0.006 0.684 0.26 0.100 0.332 |
| Glucose Plasma, mM/L                    | 5.22 -1.05       | 1.11 +0.55       | 5.49 1.11        | 4.95 -0.49       | 0.006 0.641 0.29 0.063 0.265 |
| Phagocytic Index Monocytes %            | 2.89 -1.00       | 0.98 -0.01       | 2.75 0.95        | 2.90 -0.21       | 0.006 0.687 2.43 0.103 0.269 |
| Sodium Erythrocytes, mM/L               | 24.2 -1.10       | 0.99 +0.51       | 22.6 1.03        | 22.0 -0.13       | 0.006 0.600 0.35 0.038 0.116 |
| Amylase Activity Plasma, g/h•L          | 1.63 -1.07       | 1.02 +0.46       | 154 1.02         | 152 -0.10        | 0.005 0.717 0.20 0.140 0.266 |
| Macrophages Spleen, %                   | 8.1 -1.03        | 1.00 +0.15       | 9.1 1.15         | 7.9 -0.75        | 0.005 0.759 0.70 0.208 0.247 |
| Phagocytic Index Neutrophils, %         | 69.4 -1.00       | 0.99 0.99        | 71.9 1.03        | 69.5 1          | 0.007 0.533 0.46 0.016 0.092 |
Table 2. Summary of Stepwise Analysis

| Variables currently in the model                                      | F to enter | p-value | Λ     | F-value | p-value |
|--------------------------------------------------------------------|------------|---------|-------|---------|---------|
| 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^-4   |
| Eosinophiles Blood, %                                              | 2.49       | 0.074   | 0.268 | 3.83    | 10^-3   |
| Potassium Plasma, mM/L                                             | 2.04       | 0.124   | 0.233 | 3.63    | 10^-3   |
| (Cap/PP)^2 as Parathyroid Activity                                 | 2.68       | 0.060   | 0.193 | 3.62    | 10^-3   |
| Testosterone Plasma, nM/L                                           | 2.07       | 0.121   | 0.166 | 3.51    | 10^-3   |
| NK Lymphocytes Blood, %                                            | 2.21       | 0.103   | 0.141 | 3.46    | 10^-4   |
| Malondialdehyde Urine, μM/L                                        | 2.37       | 0.087   | 0.118 | 3.46    | 10^-4   |
| Leukocytes Blood, 10^9/L                                           | 1.69       | 0.186   | 0.103 | 3.36    | 10^-4   |
| Spleen Mass Index, mg/100g Body Mass                               | 1.70       | 0.185   | 0.089 | 3.28    | 10^-4   |
| Amylase Activity Urine, g/h•L                                      | 1.79       | 0.168   | 0.077 | 3.23    | 10^-4   |
| Katalase Activity Plasma, μM/h•L                                   | 1.25       | 0.307   | 0.069 | 3.12    | 10^-4   |
| Chloride Excretion, μM/24h•100 g Body Mass                         | 1.74       | 0.179   | 0.059 | 3.09    | 10^-4   |
| Triiodothyronine Plasma, nM/L                                      | 1.57       | 0.217   | 0.051 | 3.04    | 10^-4   |
| Corticosterone Plasma, nM/L                                       | 1.55       | 0.224   | 0.044 | 3.00    | 10^-4   |
| Glucose Plasma, m/L                                                | 1.31       | 0.292   | 0.038 | 2.93    | 10^-4   |
| Phagocytic Index Monocytes, %                                      | 1.38       | 0.270   | 0.033 | 2.89    | 10^-4   |
| Sodium Erythrocytes, mM/L                                         | 1.63       | 0.207   | 0.028 | 2.88    | 10^-4   |
| Amylase Activity Plasma, g/h•L                                     | 1.59       | 0.217   | 0.024 | 2.87    | 10^-4   |
| Macrophages Spleen, %                                             | 1.11       | 0.363   | 0.021 | 2.80    | 10^-4   |
| Phagocytic Index Neutrophils, %                                    | 1.27       | 0.308   | 0.018 | 2.76    | 10^-4   |
| Reticular Zone of Adrenal Cortex, μM                               | 1.61       | 0.216   | 0.015 | 2.77    | 10^-4   |
| Entropy Leukocytogram                                             | 1.43       | 0.262   | 0.012 | 2.76    | 10^-4   |
| Plasmocytos Thymus, %                                             | 1.38       | 0.279   | 0.010 | 2.74    | 10^-4   |

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.
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; χ²(29)=175; p<10⁻⁶), the second 28.9% (r*=0.914; Wilks' Λ=0.0397; χ²(60)=100; p=0.0006), the third 18.1% (r*=0.871; Wilks' Λ=0.2406; χ²(29)=45; p=0.030).

The calculation of the discriminant root values for each animal as the sum of the products of 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

| Variables | Coefficients | Standardized | Raw |
|-----------|--------------|--------------|-----|
|           | 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)² 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, 10⁶/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)² 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 |

The first root contains 53.0% of discriminative opportunities (r*=0.950; Wilks' Λ=0.0039; χ²(29)=175; p<10⁻⁶), the second 28.9% (r*=0.914; Wilks' Λ=0.0397; χ²(60)=100; p=0.0006), the third 18.1% (r*=0.871; Wilks' Λ=0.2406; χ²(29)=45; p=0.030).

The calculation of the discriminant root values for each animal as the sum of the products of the constants enables the visualization of each rat in the information space of the roots (Fig. 1).
Table 4. Factor Structure Matrix (Correlations Variables-Canonical Roots) and Means of Roots and Variables Z-scores

| Root 1 (53.0%) | Correlations Variables-Roots | Khrystyna | Myroslava | Daily Water | Intact rats |
|---------------|------------------------------|------------|-----------|-------------|-------------|
| Root 2 (29.9%) | R1 | R2 | R3 | +1.06 | +0.78 | -3.39 | +0.63 |

(Cap/Pp) 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 3 (18.1%) | 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 |
Testosterone Plasma | -0.059 | -0.033 | -0.165 | +0.53 | +0.98 | +1.97 | 0 |
(Ku/Nau) 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 |
Manganese 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 (○) 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 splenocytagram of eosinophils and maximum entropy of leukocytagram. 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                      | Intact rats (I) | Daily Water (DW) | Water “Myroslava” (Myr) | Water “Khrystyna” (Khr) |
|-----------------------------|----------------|------------------|-------------------------|-------------------------|
| Intact rats (I)             | 0,0            | 54               | 63                      | 65                      |
| Daily Water (DW)            | 3,03,011       | 29               | 28                      | 31                      |
| Water “Myroslava” (Myr)     | 4,25,002,080   |                  |                         |                         |
| Water “Khrystyna” (Khr)     | 4,41,001,092   | 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 | Myroslava | Khrystyna |
|-------------------------------------------|-------------|-------------|-----------|-----------|
| 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) 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       | 1,570       | 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     |
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, triiodothyroninemia, 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](image)

**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).
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