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Sexual dimorphism in basal and post stress parameters of neuro-endocrine-immune complex, metabolome, electrocardiogram, and gastric mucosa at rats

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Abstract
Introduction and aim. Previously, we found significant associations between sex index and a number of parameters of the neuro-endocrine-immune complex and metabolome. Therefore, the next goal was a detailed analysis of sexual dimorphism in these parameters in baseline and post stress situations.
Material and methods. The experiment conducted on the same 18 males and 20 females rats Wistar line. 10 animals remained intact and other rats after a week of tap water (n=10) or phytoadaptogen "Balm Truskavets'" (n=18) administration were exposed to water-immersion and restraint stress (WIRS). The next day after stressing, endocrine, immune and metabolic parameters as well as ECG and damage to gastric mucosa was recorded.
Results. By the method of discriminant analysis was selected 23 variables (4 endocrine, 6 immune, 9 metabolic as well as 4 markers of damage to gastric mucosa and myocardium) whose constellation is characteristic for each group**.** The distance between the centroids of the major discriminant root of intact females and males as a measure of sexual dimorphism is 16.2 units. Acute stress increases it in control rats to 23.4 units, and in pretreated with phytoadaptogen - up to 29.4 units. Acute stress increases the severity of sexual dimorphism also in relation to variables, information about which is condensed in the minor root - from 0.99 to 2.29 units, while preventive use of phytoadaptogen limits it to 1.63 units.
Conclusion. In intact rats, significant sex differences were found for a number of endocrine, immune, and metabolic variables, which increase under the influence of acute stress per se, and to an even greater extent against the background of preventive use of a phytoadaptogen.
Keywords: neuro-endocrine-immune complex, metabolome, water-immersion and restraint stress, Ukrainian phytocomposition "Balm Truskavets'", rats, sexual dimorphism.

Introduction

Previously, we found significant associations between sex index and a number of parameters of the neuro-endocrine-immune complex and metabolome in naïve and stressed rats [10,11]. Therefore, the next goal was a detailed analysis of sexual dimorphism in these parameters in baseline and post stress situations.

Material and methods

Ethics approval

All animals were kept in room having temperature 22±2ºC, and relative humidity of 44-55% under 12/12 hours light and dark cycle with standard laboratory diet and water given ad libitum. Studies have been conducted in accordance with the rules and requirements of the "General Principles for the Work on Animals" approved by the I National Congress on Bioethics (Kyïv, Ukraine, 2001) and agreed with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Council of Europe No 123, Strasbourg 1985), and the Law of Ukraine "On the Protection of Animals from Cruelty" of 26.02.2006. The removal of animals from the experiment was carried out under light inhalation (ether) anesthesia by decapitation.

Participants

The experiment is at 38 Wistar rats: 18 males (Weight Mean=227 g; SD=25 g) and 20 females (Mean=214 g; SD=27 g). 10 animals remained intact and other rats after a week of tap water (n=10) or phytoadaptogen "Balm Truskavets'" (n=18) administration [51] were exposed to water-immersion and restraint stress (WIRS).

Study design and procedure

Due to the purposeful formation of groups, the potential predictors of post-stress reactions of the neuro-endocrine-immune complex and the metabolome [32] were almost identical both in mean values and, to a lesser extent, in variance (SD). In particular, the hypoxic test (sec) was:

136±59 and 133±81; swimming test (min): 19±11 and 19±17; HRV Stress index (units) as $(AMo/2•Mo•MxDMn)^{1/3}$: 0,14±0,08 and 0,14±0,05 in intact animals and those exposed to acute stress.

Over the 10 days, one animal remained intact and 3 other rats were exposed to WIRS according to the method of Nakamura J et al. [33] in the modification of Popovych IL [40], which is to reduce the duration of stay of the rat in a fixed standing position in cold water (t^0 20-21^0 C) to the level of the xiphoid process from 8 to 4 hours. Prior to the stressing, rats were fasted for 24 h, but allowed access to tap water *ad libitum*.

The next day after stressing, a sample of peripheral blood (by incision of the tip of the tail) was taken for analysis of Leukocytogram (LCG), ie the percentage of lymphocytes (L), monocytes (M), eosinophils (E), basophils (B), rod-shaped (RN) and polymorphonucleary (PMNN) neutrophils. Based on these data, the Entropy of the Leukocytogram (hLCG) was calculated according to the equation derived by Popovych IL [16,17,36,37] on the basis of the classical Shannon's [45] equation:

$hLCG = - (L•\log_2 L+M•\log_2 M+E•\log_2 E+B•\log_2 B+RN•\log_2 RN+PMNN•\log_2 PMNN)/\log_2 6$.

Than the ECG under light ether anesthesia was re-recorded in order to assess the state of the myocardium and HRV [4], and right away the animals removed from the experiment by decapitation in order to remove the stomach, adrenal glands, thymus, spleen, and collect the maximum possible amount of blood in which was determined some endocrine, metabolic, and immune parameters.

Among endocrine parameters determined serum levels of main adaptation hormones such as corticosterone, aldosterone, testosterone, triiodothyronine, as well as parathyroid hormone and calcitonin (by ELISA, with the use of analyzer "RT-2100C" and corresponding sets of reagents from "Alkor Bio", XEMA Co, Ltd and DRG International Inc).

On lipid metabolism judged by the level of triglycerides (metaperiodate-acetylacetone colorimetric method), total cholesterol (direct method by reaction Zlatkis-Zach) and its distribution as part of α-lipoprotein (applied enzymatic method Hiller G [20]) after precipitation nonα-lipoproteins using dextransulfate/Mg^{2+}) as described in the manual [15]. State of lipid peroxidation assessed the content in the serum its products: diene conjugates (spectrophotometry of heptane phase of lipids extract [14]) and malondyaldehide (test with thiobarbituric acid [2]), as well as the activity of antioxidant enzymes: catalase of serum and erythrocytes (by the speed of decomposition hydrogen peroxide [27]) and superoxide dismutase of erythrocytes (by the degree of inhibition of nitroblue tetrazolium recovery in the presence of N-methylphenazone metasulfate and NADH [9]). On electrolytes exchange judged by the level of calcium (by the reaction with arsenazo III), phosphate (phosphate molibdate method) and chloride (mercury rodanide method) in the serum, sodium and potassium both in the serum and erythrocytes (flame photometry method) as described in the manual [15]. In addition, the activity of Na,K-ATPase of the shadows of erythrocytes was determined (by the increase of Pi in the supernatant of the incubation medium [30]).

Alanine and aspartate aminotranspherase, alkaline and acid phosphatase as well as creatine phosphokinase determined by uniform methods as described in the manual [15].

Use analyzers "Pointe-180" ("Scientific", USA) and "Reflotron" ("Boehringer Mannheim", BRD) and flame spectrophotometer "СФ-47".

The stomach was cut along the greater curvature, mounted it on gastroluminoscope and under a magnifying glass counted the amount of ulcers and their length was measured, evaluated erosive and ulcerative damage on scale by Popovych IL [40] (0÷1 points). This scale is based on the qualitative-quantitative Harrington EC [18] scale.

The parameters of immunity were determined, as described in the manual [35]. The percentage of theophylline-resistant (TR) and theophylline-susceptible (TS) T-lymphocytes, B-lymphocytes, plasma cells (Pla), and natural killers (NK) were identified. For these components the Entropy of the Immunocytogram (hICG) was calculated by Popovych IL [37] equation:

$hICG = - (TR \bullet \log_2 TR + TS \bullet \log_2 TS + B \bullet \log_2 B + Pla \bullet \log_2 Pla + NK \bullet \log_2 NK + 0L \bullet \log_2 0L)/\log_2 6.$

About the condition of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytosis index (percentage of cells, in which found microbes as activity), the microbial count (number of microbes absorbed by one phagocyte as intensity) and the killing index (percentage of dead microbes as completeness) for *Staphylococcus aureus* (ATCC N25423 F49). Based on these parameters, taking into account the absolute content of neutrophils and monocytes, their bactericidal capacity was calculated (BCC N&M) [6,37].

The Spleen and Thymus were weighed and made smears-imprints for counting Thymocytogram and Splenocytogram [5,6,21]. The components of the Thymocytogram (TCG) are lymphocytes (Lc), lymphoblastes (Lb), reticulocytes (Ret), macrophages (Mac), basophiles (B), endotheliocytes (En), epitheliocytes (Ep), and Hassal's corpuscles (H). The Splenocytogram (SCG) includes lymphocytes (Lc), lymphoblastes (Lb), plasma cells (Pla), reticulocytes (R), macrophages (Ma), fibroblasts (F), microphages (Mi), and eosinophils (Eo).

For them Shannon's entropy was calculated too [16,36]:

$hTCG = - (Lc \bullet \log_2 Lc + Lb \bullet \log_2 Lb + Ret \bullet \log_2 Ret + Mac \bullet \log_2 Mac + B \bullet \log_2 B + En \bullet \log_2 En + Ep \bullet \log_2 Ep + H \bullet \log_2 H)/\log_2 8;$

$hSCG = - (Lc \bullet \log_2 Lc + Lb \bullet \log_2 Lb + Pla \bullet \log_2 Pla + R \bullet \log_2 R + Ma \bullet \log_2 Ma + F \bullet \log_2 F + Mi \bullet \log_2 Mi + Eo \bullet \log_2 Eo)/\log_2 8.$

Statistical analysis

Statistical processing was performed using a software package "Microsoft Excell" and "Statistica 6.4 StatSoft Inc" (Tulsa, OK, USA).

Results

In order to detect sexual dimorphism, the registered actual parameters were recalculated in Z-scores separately for males and females, both intact and subjected to acute stress against the background of drinking tap water or phytocomposition.

In intact females (Table 1), by definition, testosterone levels are an order of magnitude lower than in males. However, they have a greater adrenal mass than in males and, to an even greater extent, their mass index, given their somewhat lower body mass. This is accompanied by higher serum levels of adrenal-produced corticosterone and aldosterone. In addition, females have higher levels of parathyroid hormone, but lower levels of calcitonin. No significant sex differences were found for triiodothyronine levels, as well as markers of catecholamines and sympathetic and vagal tone [4] (Fig. 1).

Table 1. Sexual dimorphism in endocrine and autonomic parameters in intact rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Body mass, g	216±11	196±9	0.45±0.44	-0.45±0.44	0,108	1,52	**>0,1**
Adrenal glands mass, mg	44±4	65±5	-0.72±0.27	0.71±0.35	0,273	-3,26	**<0,02**
Adrenal mass index, mg/100g	21±3	33±3	-0.76±0.24	0.77±0.32	0,300	-3,78	**<0,02**
Corticosterone, nM/L	290±44	406±37	-0.55±0.42	0.55±0.35	0,302	-2,03	**>0,05**
Aldosterone, pM/L	587±8	639±33	-0.45±0.14	0.45±0.58	0,093	-1,51	**>0,1**
Testosterone, nM/L	41.8±1.4	3.53±0.24	0.94±0.07	-0.94±0.01	0,895	26,5	**<0,001**
Calcitonin, ng/L	32.3±3.2	24.7±0.3	0.61±0.51	-0.62±0.05	0,217	2,39	**<0,05**
Parathyroid hormone, µg/L	154±11	185±3	-0.65±0.48	0.65±0.11	0,141	-2,63	**<0,05**
Triiodothyronine, nM/L	3.06±0.52	3.73±0.26	-0.35±0.55	0.36±0.28	0,256	-1,14	>0,2
Mode as catecholamines, msec	178±18	172±12	-0.10±0.56	0.10±0.56	0,180	-0,28	>0,5
AMo as sympathetic tone, %	54±12	43±2	0.30±0.64	-0.30±0.10	0,392	0,91	>0,2
MxDMn as vagal tone, msec	63±38	39±7	0.27±0.64	-0.27±0,15	0,878	0,82	>0,2

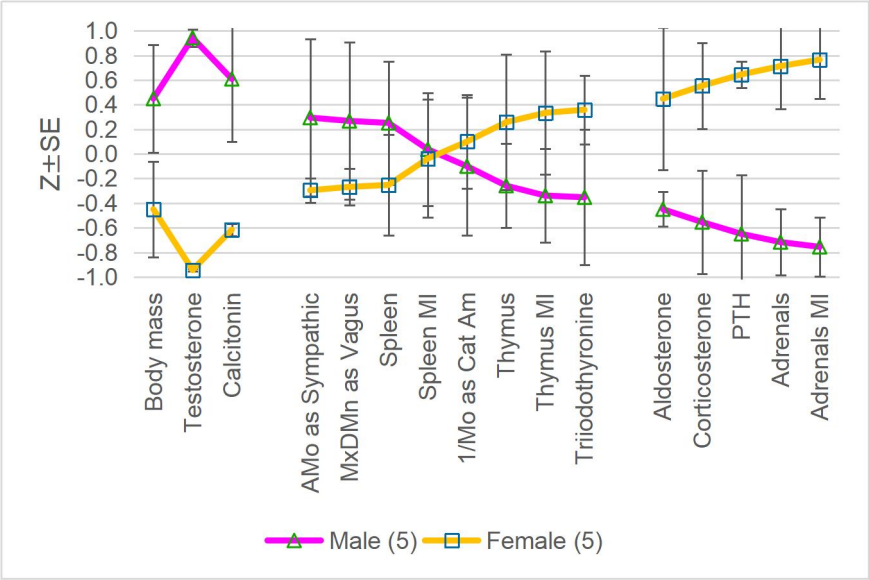

Fig. 1. Profile of mass of body, thymus, spleen, adrenal glands, and neuro-endocrine variables in intact rats

Against the background of the absence of differences in thymus mass, the thymocytogram revealed a higher percentage of lymphocytes and epithelial cells, but a lower percentage of lymphoblasts, macrophages, and reticulocytes, and as a result, a lower level of thymocytogram entropy (Table 2 and Fig. 2).

Table 2. Sexual dimorphism in thymus and thymocytogram parameters in intact rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Thymus mass, mg	136±10	152±17	-0,26±0,34	0,26±0,55	0,210	-0,80	>0,2
Thymus mass index, mg/100g	64±8	79±11	-0,34±0,38	0,33±0,50	0,298	1,07	>0,2
Lymphocytes, %	63,8±1,6	67,8±1,7	-0,49±0,40	0,49±0,41	0,062	-1,72	**>0,1**
Lymphoblastes, %	8,80±1,74	6,20±0,58	0,42±0,57	-0,42±0,19	0,409	1,41	**>0,1**
Epitheliocytes, %	6,32±0,82	9,75±0,81	-0,69±0,33	0,69±0,32	0,310	-2,97	**<0,02**
Macrophages, %	6,38±0,76	4,40±0,24	0,63±0,48	-0,63±0,16	0,292	2,51	**<0,05**
Reticulocytes, %	5,68±1,02	2,65±0,55	0,65±0,43	-0,64±0,23	0,564	2,61	**<0,05**
Fibroblastes, %	4,86±1,02	5,80±0,86	-0,23±0,42	0,23±0,42	0,386	-0,70	>0,5
Basophiles, %	3,16±0,59	2,40±0,51	0,31±0,48	-0,31±0,42	0,441	0,98	>0,2
Hassal's corpuscles, %	1,00±0,00	1,00±0,00	0,00±0,00	0,00±0,00	0	-	-
Thymocytogram Entropy •10^3	622±14	570±24	0,52±0,28	-0,53±0,49	0,082	1,87	**>0,05**

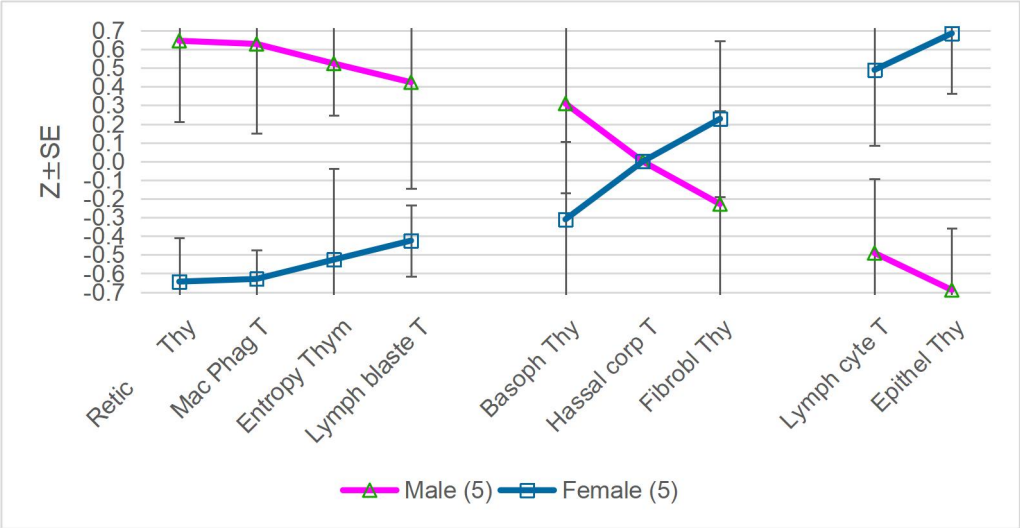

Fig. 2. Profile of Thymocytogram variables in intact rats

With the same spleen mass, the percentage of macrophages and reticulocytes was found to be higher in females, while males had a significantly higher percentage of eosinophils, and the remaining elements of the splenocytogram did not differ significantly (Table 3 and Fig. 3).

Table 3. Sexual dimorphism in spleen and splenocytogram parameters in intact rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Spleen mass, mg	819±92	726±76	0,25±0,50	-0,25±0,41	0,239	0,78	>0,2
Spleen mass index, mg/100g	378±36	372±38	0,04±0,46	-0,04±0,48	0,208	0,11	>0,9
Lymphocytes, %	70,2±2,3	67,0±2,0	0,33±0,47	-0,33±0,42	0,071	1,06	>0,2
Lymphoblastes, %	7,50±1,43	9,40±1,40	-0,30±0,45	0,30±0,44	0,373	-0,95	>0,2
Plasmocytes, %	1,75±0,19	1,60±0,40	0,10±0,29	-0,12±0,60	0,398	0,34	>0,5
Microphages, %	11,3±0,66	13,2±1,53	-0,36±0,25	0,36±0,57	0,220	-1,17	>0,2
Rod shaped neutrophils, %	1,50±0,22	2,00±0,45	-0,32±0,28	0,32±0,57	0,452	-1,00	>0,2
Macrophages, %	2,00±0,32	3,00±0,45	-0,51±0,33	0,51±0,46	0,389	-1,83	**>0,1**
Reticulocytes, %	2,25±0,19	3,00±0,32	-0,55±0,28	0,55±0,47	0,259	-2,02	**>0,05**
Eosinophiles, %	3,50±1,02	0,80±0,20	0,64±0,49	-0,64±0,09	0,981	2,59	**<0,05**
Splenocytogram Entropy •10^3	521±31	546±20	-0,22±0,55	0,22±0,35	0,106	-0,68	>0,5

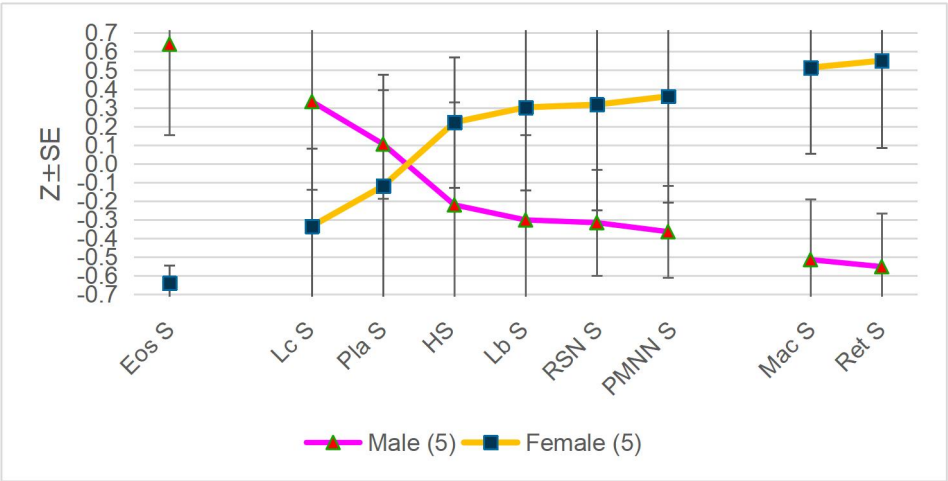

Fig. 3. Profile of Splenocytogram variables in intact rats

Regarding the absolute content of lymphocytes in the blood and the percentage of their populations, marginally significant sex differences were found only for B-Lymphocytes and plasma cells (Table 4 and Fig. 4).

Table 4. Sexual dimorphism in blood lymphocyte populations in intact rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Pan Lymphocytes, 10^9/L	5,89±1,18	8,30±1,63	-0,37±0,36	0,37±0,50	0,458	-1,20	>0,2
TR T-helper Lymphocytes, %	30,0±0,3	29,4±0,4	0,36±0,38	-0,36±0,48	0,028	1,18	>0,2
TS T-cytolytic Lymphocytes, %	15,6±1,7	15,0±1,7	0,08±0,48	-0,08±0,47	0,235	0,25	>0,5
NK Lymphocytes, %	5,52±0,48	5,06±0,55	0,21±0,43	-0,21±0,49	0,212	0,63	>0,5
B-Lymphocytes, %	14,6±1,0	12,2±1,0	0,48±0,41	-0,48±0,41	0,187	1,66	**>0,1**
Plasmocytes, %	0,00±0,00	0,79±0,49	-0,47±0,00	0,47±0,58	2,109	-1,63	**>0,1**
0-Lymphocytes, %	34,3±2,6	37,6±1,9	-0,32±0,50	0,32±0,38	0,142	-1,02	>0,2
Immunocytogram Entropy •10^3	809±11	805±14	0,07±0,42	-0,07±0,53	0,033	0,21	>0,5

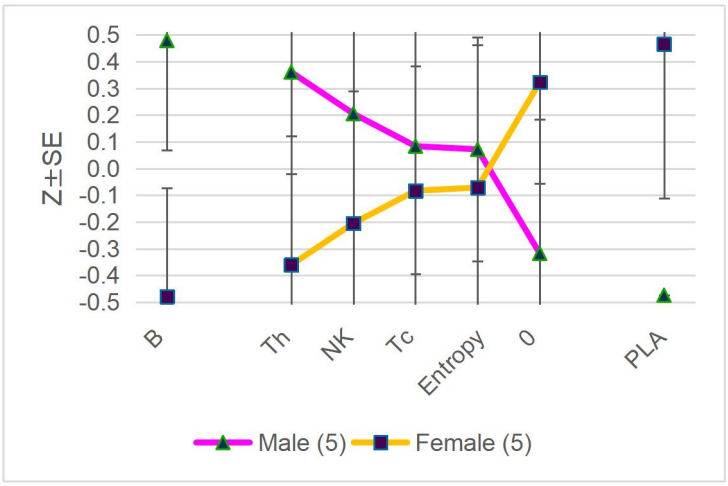

Fig. 4. Profile of Immunocytogram variables in intact rats

The blood leukocytogram revealed only a marginally significant difference between the percentage of rod-shaped neutrophils (Table 5 and Fig. 5).

Table 5. Sexual dimorphism in blood leukocyte populations in intact rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Leukocytes, 10^9/L	11,23±2,23	16,30±3,41	-0,38±0,34	0,38±0,52	0,480	-1,22	>0,2
Pan lymphocytes, %	52,2±2,6	51,4±1,8	0,08±0,56	-0,08±0,37	0,091	0,25	>0,9
PMN neutrophils, %	34,8±1,4	34,6±1,8	0,03±0,41	-0,03±0,51	0,098	0,09	>0,9
Rod-shaped neutrophils, %	2,60±0,24	1,80±0,37	0,51±0,31	-0,51±0,47	0,359	1,79	**>0,1**
Eosinophiles, %	5,00±1,34	4,80±0,73	0,04±0,59	-0,04±0,32	0,466	0,13	>0,9
Monocytes, %	5,40±1,03	7,00±1,00	-0,35±0,45	0,35±0,43	0,371	-1,11	>0,2
Leukocytogram Entropy •10^3	679±34	686±8	-0,06±0,65	0,06±0,15	0,077	-0,19	>0,9

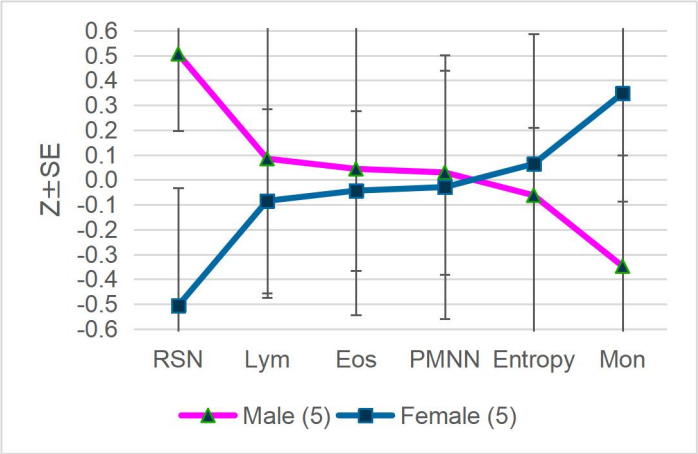

Fig. 5. Profile of Leukocytogram variables in intact rats

When comparing the parameters of phagocytosis, it was found (Table 6 Fig. 6) that males have significantly higher activity of neutrophil phagocytosis, as well as, to a lesser extent, its intensity and completeness, however, due to the lower absolute content of neutrophils in the blood, their bactericidal capacity does not differ from that of females. On the other hand, at the same levels of activity and intensity of monocyte phagocytosis, their bactericidal capacity is greater in females due to their higher absolute content in the blood.

Table 6. Sexual dimorphism in parameters of phagocytic function of blood neutrophils and monocytes in intact rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Pan neutrophils, 10^9/L	4,21±0,77	6,06±1,54	-0,34±0,28	0,34±0,56	0,536	-1,08	>0,2
Phagocytosis index neutr, %	59,6±1,8	50,8±1,4	0,77±0,31	-0,77±0,24	0,103	3,94	**<0,01**
Microbial count neutr, B/Ph	5,8±0,5	5,2±0,5	0,28±0,45	-0,28±0,45	0,196	0,87	>0,2
Killing index neutrophils, %	52,0±3,7	43,0±3,9	0,48±0,39	-0,48±0,42	0,196	1,67	**>0,1**
BC capacity neutroph, 10^9 B/L	7,70±1,78	7,38±2,35	0,04±0,41	-0,04±0,53	0,583	0,11	>0,9
Monocytes, 10^9/L	0,63±0,20	1,09±0,26	-0,42±0,37	0,42±0,45	0,610	-1,40	**>0,1**
Phagocytosis index monoc, %	5,7±0,7	6,0±0,9	-0,09±0,42	0,09±0,52	0,296	-0,26	>0,9
Microbial count monoc, B/Ph	4,5±0,3	4,4±0,4	0,07±0,42	-0,07±0,53	0,171	0,20	>0,9
Bactericidal cap mon, 10^6 B/L	144±32	273±57	-0,54±0,27	0,55±0,48	0,568	-1,98	**>0,05**

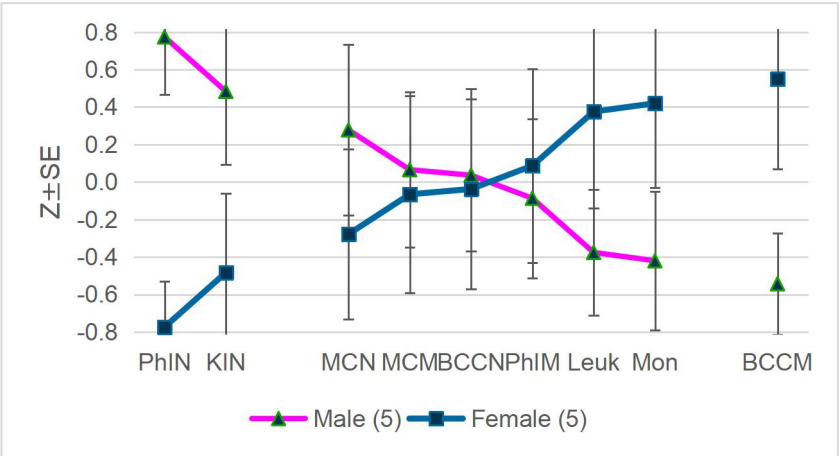

Fig. 6. Profile of Phagocytosis variables in intact rats

When comparing electrolyte metabolism parameters (Table 7 and Fig. 7), it was found that females have, to one degree or another, higher levels of calcium, phosphates, chloride,

and sodium in serum, as well as sodium in erythrocytes as a marker of natrihistia, but lower level of potassium in serum, which is combined with a tendency to decrease the activity of Na,K-ATPase in erythrocyte shadows as markers of cell membranes.

In addition, females were found to have lower serum alkaline and acid phosphatases and aspartate aminotransferase activity, but higher erythrocyte superoxide dismutase activity.

Table 7. Sexual dimorphism in metabolic parameters in intact rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Na,K-ATP-ase Erythr, M/L•h	0.83±0.11	0.71±0.05	0.32±0.57	-0.32±0.28	0,249	1,02	>0,2
Sodium of Erythrocyte, mM/L	19.1±3.2	25.2±3.0	-0.42±0.44	0.42±0.41	0,328	-1,41	**>0,1**
Potassium of Erythroc, mM/L	92±9	93±3	-0.03±0.64	0.03±0.20	0,159	-0,09	>0,9
Potassium of Serum, mM/L	4.34±0.10	3.85±0.38	0.38±0.16	-0.39±0.59	0,156	1,26	>0,2
Sodium of Serum, mM/L	131.6±0.5	133.9±0.6	-0.67±0.29	0.66±0.36	0,013	-2,91	**<0,02**
Chloride of Serum, mM/L	96.1±0.7	99.6±1.0	-0.68±0.26	0.68±0.39	0,027	-2,89	**=0,02**
Calciemia, mM/L	2.56±0.37	3.80±0.07	-0.72±0.43	0.72±0.08	0,272	-3,25	**<0,02**
Phosphatemia, mM/L	1.28±0.03	1.36±0.02	-0.55±0.42	0.53±0.36	0,051	-1,97	**>0,05**
$(Ca/K)^{0,5}$ ratio of Serum	0.59±0.08	1.03±0.10	-0.73±0.26	0.73±0.34	0,374	-3,39	**<0,01**
α-LP Cholesterol, mM/L	0.87±0.06	0.80±0.08	0.24±0.40	-0.24±0.51	0,177	0,75	>0,5
nonα-LP Cholesterol, mM/L	1.00±0.15	1.07±0.05	-0.15±0.62	0.15±0.23	0.229	-0,46	>0,5
Triglycerides, mM/L	1.08±0.02	1.06±0.03	0.19±0.35	-0.20±0.56	0,052	0,60	>0,5
Diene conjugates, E^{232}/mL	1.47±0.21	1.48±0.08	-0.01±0.63	0.02±0.23	0,231	-0,04	>0,9
Malondialdehyde, µM/L	69±11	58±4	0.31±0.60	-0.31±0.22	0,279	0,97	>0,2
Superoxide dismutase, U/mL	50.7±5.5	73.0±6.1	-0.66±0.33	0.66±0.36	0,275	-2,71	**<0,05**
Catalase of Erythroc, µM/L•h	230±7	225±34	0.05±0.14	-0.05±0.65	0,232	0,14	>0,9
Catalase of Serum, µM/L•h	147±15	139±21	0.11±0.40	-0.11±0.54	0,271	0,33	>0,9
Alaninaminotranspher, µKat/L	0.58±0.08	0.48±0.05	0.32±0.55	-0.32±0.32	0,288	1,02	>0,2
Aspartataminotransph, µKat/L	0.25±0.03	0.17±0.02	0.56±0.45	-0.57±0.29	0,323	2,10	**>0,05**
Creatin Phosphokinase, IU/L	1.70±0.02	1.66±0.21	0.06±0.08	-0.06±0.67	0,184	0,18	>0,9
Acid Phosphatase, IU/L	36.0±2.4	27.8±0.9	0.72±0.41	-0.72±0.16	0,180	3,25	**<0,02**
Alkaline Phosphatase, IU/L	579±22	290±24	0.90±0.14	-0.90±0.15	0,367	8,85	**$<10^{-3}$**

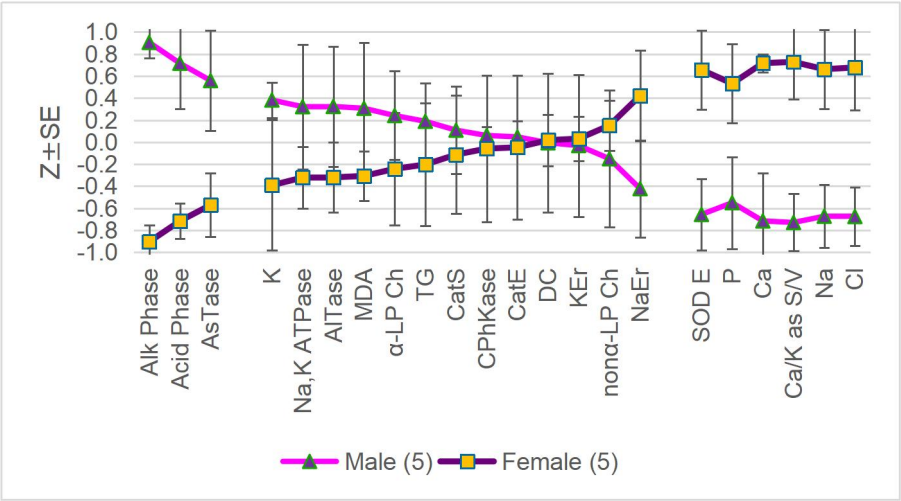

Fig. 7. Profile of Metabolic variables in intact rats

Figure 8 brings together endocrine, metabolic and immune parameters in intact rats for which significant or borderline sex differences were found, i.e. it visualizes sexual dimorphism.

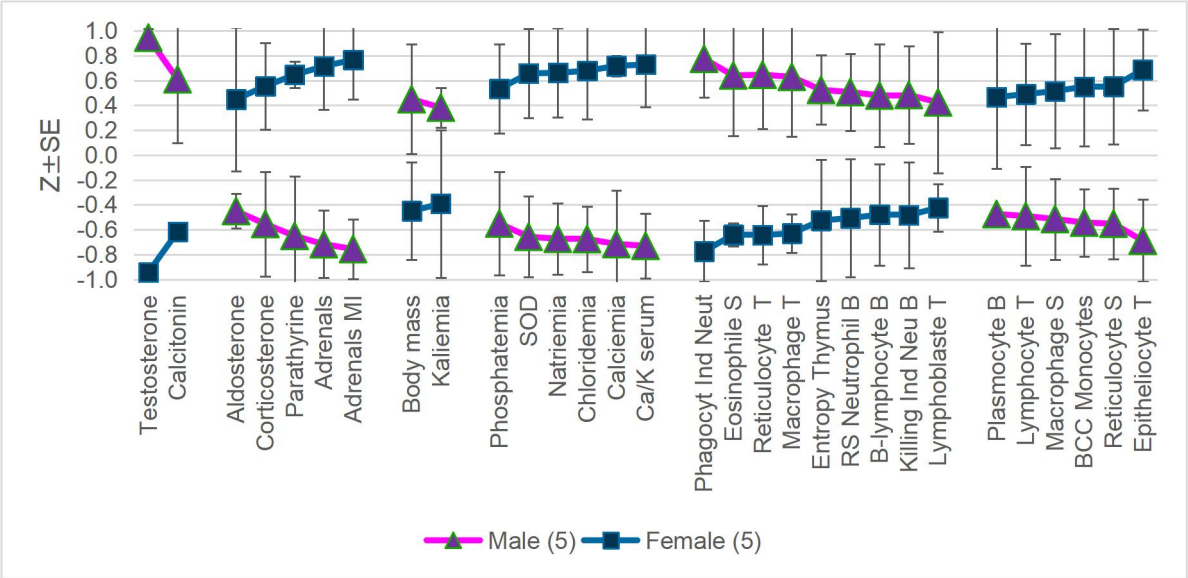

Fig. 8. Profile of endocrine, metabolic and immune parameters in intact rats

Interestingly, control rats, loaded with tap water for a week, slightly increased body weight, and to a greater extent in males, so that the sex difference became clearer. This is probably due to a greater appetite stimulated by the loading procedure as a mild aversive stress [38,39]. The next day after a stronger, but still moderate, acute stress, it was found that the sex differences between the parameters of adrenals as well as PTH levels also increased. In addition, a slight difference between the levels of catecholamines appeared in favor of males (Table 8 and Fig. 9).

Table 8. Sexual dimorphism in endocrine and autonomic parameters in control stressed rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Body mass, g	238±8	212±9	0.58±0.36	-0.58±0.39	0,099	2,21	**>0,05**
Adrenal glands mass, mg	47±1.5	76±3	-0.89±0.09	0.89±0.21	0,259	-7,85	**$<10^{-3}$**
Adrenal mass index, mg/100g	20.0±1.4	35.7±1.3	-0.90±0.16	0.90±0.15	0,313	-8,41	**$<10^{-3}$**
Corticosterone, nM/L	334±37	466±21	-0.69±0.40	0.70±0.23	0,236	-3,05	**<0,02**
Aldosterone, pM/L	580±23	695±43	-0,77±0.30	0.77±0.26	0,117	-3,86	**<0,01**
Testosterone, nM/L	38.1±3.6	3.28±0.24	0.91±0.19	-0.91±0.01	0,923	9,78	**$<10^{-3}$**
Calcitonin, ng/L	31.9±2.4	25.6±0.8	0.63±0.48	-0.63±0.16	0,174	2,51	**<0,05**
Parathyroid hormone, µg/L	151±11	205±6	-0.80±0.32	0.80±0.17	0,190	-4,38	**<0,01**
Triiodothyronine, nM/L	3.22±0.08	3.37±0.30	-0.16±0.16	0.16±0.60	0,141	-0,52	>0,5
Mode as catecholamines, msec	142±9	168±15	0.43±0.30	-0.44±0.51	0,188	1,46	**>0,1**
AMo as sympathetic tone, %	63±7	53±9	0.28±0.40	-0.28±0.50	0,302	0,87	>0,2
MxDMn as vagal tone, msec	28±8	31±12	-0.08±0.37	0.08±0.56	0,750	-0,23	>0,5

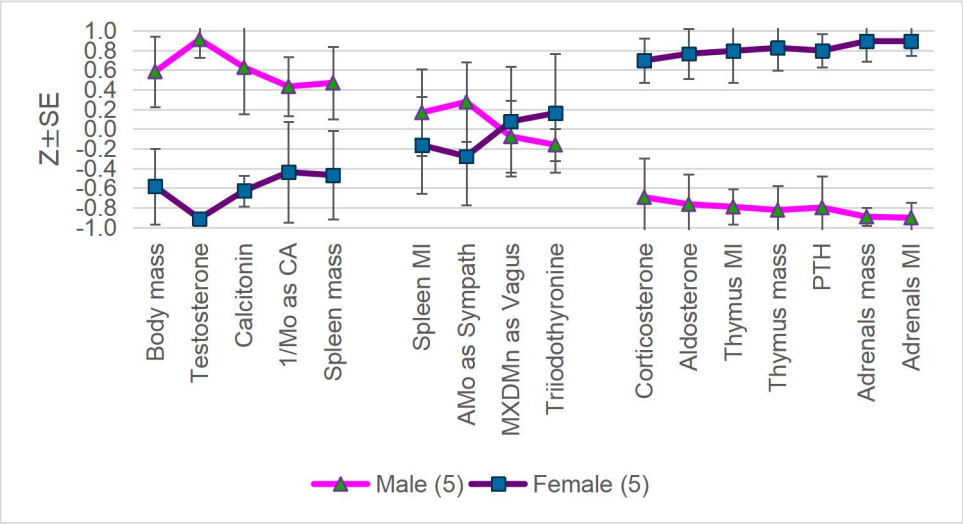

Fig. 9. Profile of body, thymus, spleen, adrenal glands mass, and neuro-endocrine variables in stressed rats

The expected post-stress decrease in thymus mass was more pronounced in males. This was accompanied by a somewhat more pronounced decrease in the percentage of lymphocytes in the thymocytogram. In contrast, in females, the percentage of lymphoblasts decreased and macrophages increased to a greater extent, so that sex differences increased in the former and decreased in the latter (Table 9 and Fig. 10).

Table 9. Sexual dimorphism in thymus and thymocytogram parameters in control stressed rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Thymus mass, mg	117±6	159±6	-0.82±0.24	0.82±0.23	0,182	-4,94	**<0,01**
Thymus mass index, mg/100g	49±3	76±5	-0.79±0.18	0.79±0.32	0,264	-4,29	**<0,01**
Lymphocytes, %	60.3±2.3	66.6±3.2	-0.46±0.34	0.46±0.47	0,108	-1,51	**>0,1**
Lymphoblastes, %	7.75±0.66	5.80±0.49	0.61±0.41	-0.61±0.31	0,236	2,15	**>0,05**
Epitheliocytes, %	6.73±0.47	8.91±0.76	-0.62±0.27	0.62±0.43	0,224	-2,34	**<0,05**
Macrophages, %	8.25±1.16	5.80±0.49	0.54±0.51	-0.54±0.22	0,324	1,72	**>0,1**
Reticulocytes, %	5.77±0.83	2.89±0.87	0.61±0.35	-0.61±0.37	0,543	2,23	**>0,05**
Fibroblastes, %	6,75±0.37	6.00±0.84	0.26±0.26	-0.27±0.59	0,223	0,80	>0,5
Basophiles, %	2.50±0.39	2.60±0.87	-0.04±0.27	0.04±0.61	0,558	-0,10	>0,9
Hassal's corpuscles, %	2.00±0.32	1.40±0.24	0.44±0.47	-0.44±0.36	0,397	1,36	>0,2
Thymocytogram Entropy •10^3	670±22	588±42	0.50±0.26	-0.50±0.51	0,132	1,68	**>0,1**

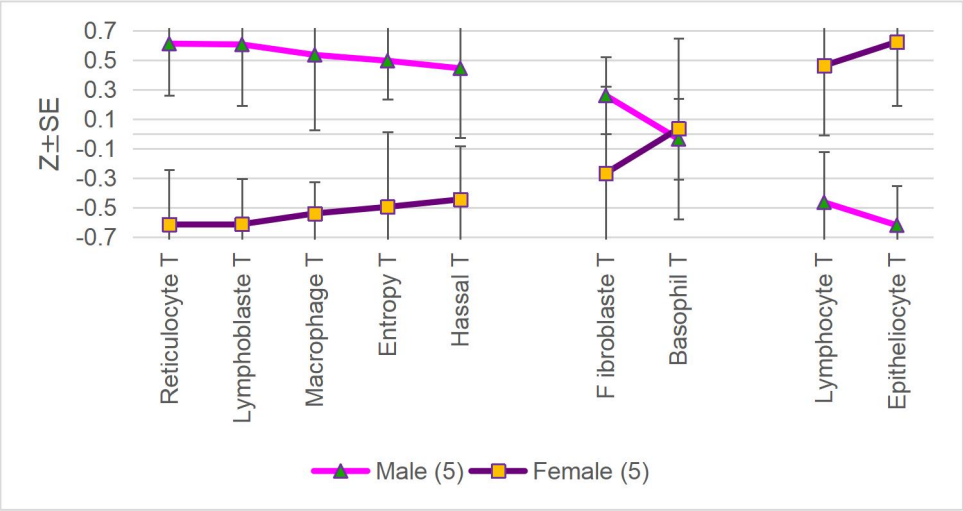

Fig. 10. Profile of Thymocytogram variables in stressed rats

The spleen mass decreased less significantly after stress than the thymus, but still somewhat more in males. This was accompanied by a decrease in the percentage of microphages in the splenocytogram of males, while there were no changes in females. On the other hand, stress eliminated the sex differences in the percentage of reticulocytes and even reversed those of macrophages: if in intact rats the percentage of reticulocytes in females significantly prevailed, and macrophages slightly, then after stress, due to the opposite changes, the differences in the former were eliminated, and in the latter there was a significant advantage of males. On the other hand, stress eliminated the advantage of males in the percentage of eosinophils in the same way (Table 10 and Fig. 11).

Table 10. Sexual dimorphism in spleen and splenocytogram parameters in control stressed rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Spleen mass, mg	780±49	656±60	0.47±0.37	-0.47±0.35	0,184	1,61	**>0,1**
Spleen mass index, mg/100g	330±25	311±28	0.17±0.44	-0.16±0.49	0,178	0,50	>0,5
Lymphocytes, %	72.0±2.8	70.0±3.2	0.16±0.43	-0.16±0.50	0,090	0,44	>0,5
Lymphoblastes, %	6.50±0.92	7.00±1.58	-0.09±0.34	0.09±0.58	0,406	-0,26	>0,5
Plasmocytes, %	1.75±0.37	2.60±0.75	-0.32±0.28	0.32±0.56	0,608	-0,99	>0,2
Microphages, %	9.50±0.92	13.0±1.84	-0.49±0.26	0.49±0.51	0,319	-1,64	**>0,1**
Rod-shaped neutrophils, %	2.00±0.45	1.80±0.37	0.11±0.51	-0.11±0.43	0,461	0,31	>0,5
Macrophages, %	3.00±0.32	1.60±0.40	0.66±0.30	-0.66±0.38	0,461	2,58	**<0,05**
Reticulocytes, %	2.50±0.50	2.80±0.37	-0.16±0.53	0.16±0.40	0,356	-0,44	>0,5
Eosinophils, %	2.75±0.92	1.20±0.37	0.46±0.54	-0.46±0.22	0,853	1,38	>0,2
Splenocytogram Entropy •10^3	510±34	508±37	0.01±0.45	-0.01±0.49	0,149	0,02	>0,9

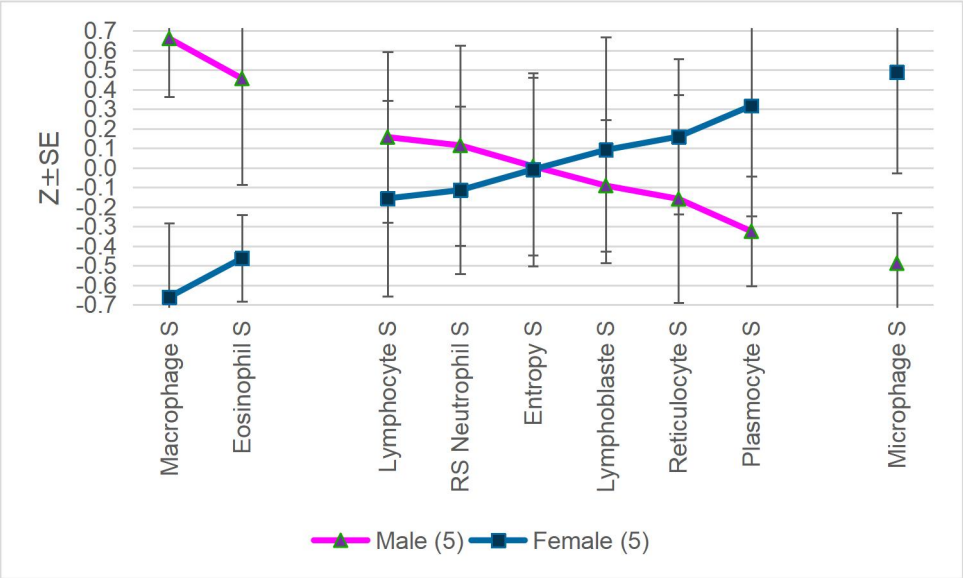

Fig. 11. Profile of Splenocytogram variables in stressed rats

The absence of significant sex differences in the content of lymphocytes in the blood and their populations in intact rats persisted after acute stress (Table 11 and Fig. 12). This is due to the same decrease in T-cytolytic and increase in NK lymphocytes for both sexes.

Table 11. Sexual dimorphism in blood lymphocyte populations in control stressed rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Pan Lymphocytes, 10^9/L	6.98±0.76	7.06±1.03	-0.02±0.40	0.02±0.54	0,270	0,07	>0,9
TR T-helper Lymphocytes, %	31.0±1.1	31.8±1.2	-0.16±0.45	0.16±0.48	0,080	-0,48	>0,5
TS T-cytolytic Lymphocytes, %	11.6±0.2	12.2±1.9	-0.11±0.09	0.11±0.66	0,239	-0,32	>0,5
NK Lymphocytes, %	6.68±0.38	5.68±0.54	0.45±0.34	-0.44±0.48	0,182	1,51	**>0,1**
B Lymphocytes, %	13.8±1.1	11.8±1.1	0.39±0.42	-0.39±0.44	0,198	1.30	>0,2
Plasmocytes, %	0.11±0.11	0.74±0.74	-0.27±0.09	0.28±0.64	2,752	-0,85	>0,5
0 Lymphocytes, %	36.8±0.5	37.8±3.3	-0.10±0.09	0.10±0.66	0,133	-0,29	>0,5
Immunocytogram Entropy •10^3	801±8	787±28	0.15±0.19	-0.15±0.63	0,055	0,46	>0,5

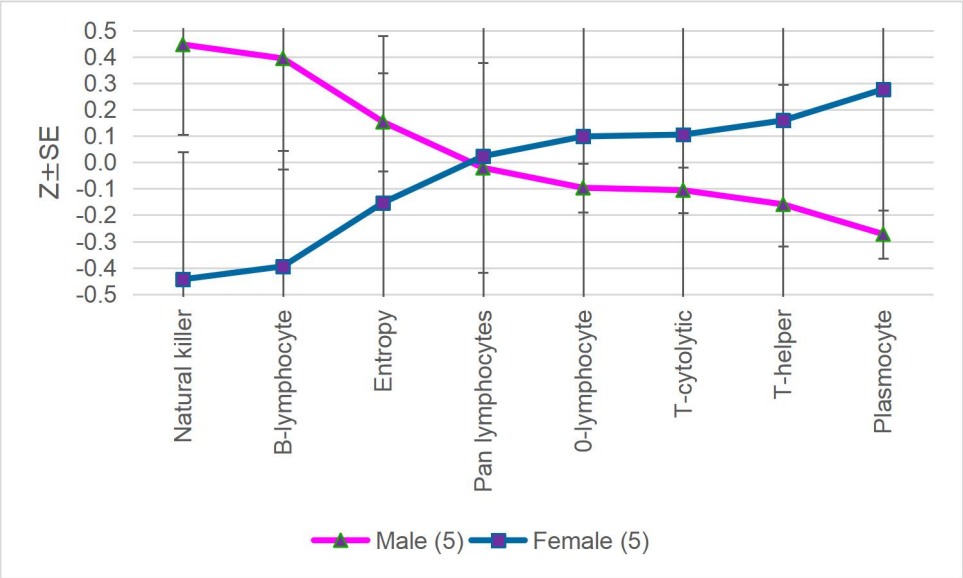

Fig. 12. Profile of Immunocytogram variables in stressed rats

The slight single sex difference in the percentage of rod-shaped neutrophils in intact rats was completely eliminated by acute stress (Table 12 and Fig. 13).

Table 12. Sexual dimorphism in blood leukocyte populations in control stressed rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Leukocytes, 10^9/L	14.9±1.7	15.2±2.2	-0.04±0.40	0.04±0.54	0,277	-0,12	>0,9
Pan lymphocytes, %	47.1±1.8	47.4±4.5	-0.02±0.24	0.02±0.62	0,153	-0,07	>0,9
PMN neutrophils, %	41.9±2.7	40.0±4.4	0.12±0.34	-0.12±0.57	0,190	0,36	>0,5
Rod-shaped neutrophils, %	2.60±0.68	2.80±0.37	-0.09±0.59	0.09±0.32	0,429	-0,26	>0,5
Eosinophils, %	2.76±0.82	3.40±0.87	-0.18±0.45	0.18±0.48	0,591	-0,53	>0,5
Monocytes, %	5.62±0.81	6.00±0.55	-0.13±0.55	0.13±0.37	0,253	-0,39	>0,5
Leukocytogram Entropy $\cdot 10^3$	658±15	671±11	-0.21±0.53	0.21±0.38	0,044	-0,64	>0,5

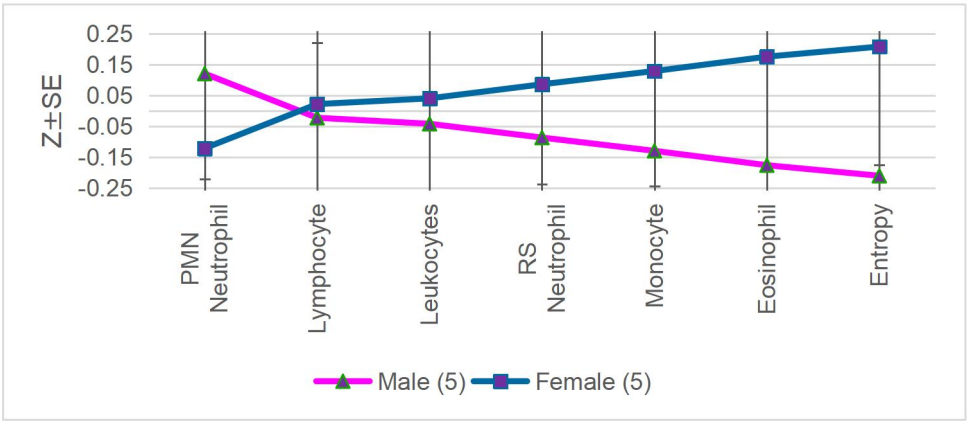

Fig. 13. Profile of Leukocytogram variables in stressed rats

The bactericidal capacity of neutrophils, the same in intact rats of both sexes, does not change after acute stress in females, and in males increases due to an increase, primarily, in their absolute content in the blood and, to a lesser extent, in the intensity of phagocytosis, which overlaps the post-stressor decrease in the completeness of phagocytosis in the absence of changes in its activity. On the other hand, the bactericidal capacity of monocytes, higher in intact females due to their absolute content in the blood, after acute stress increases in rats of both sexes, leveling the differences. In females, this is realized due to an increase in the intensity and activity of phagocytosis, and in males due to, primarily, an increase in the absolute content of monocytes and, to a lesser extent, their activity (Table 13 and Fig. 14).

Table 13. Sexual dimorphism in parameters of phagocytic function of blood neutrophils and monocytes in control stressed rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Pan neutrophils, 10^9/L	6.71±0.99	6.62±1.48	0.01±0.37	-0.02±0.56	0,399	0,04	>0,9
Phagocytosis index neutroph, %	58.4±2.6	51.0±1.8	0.60±0.43	-0.60±0.29	0,112	2,33	**<0,05**
Microbial count neutroph, B/Ph	6.2±0.5	5.2±0.2	0.53±0.52	-0.53±0.21	0,166	1,89	**>0,1**
Killing index neutrophils, %	46.6±4.3	42.8±4.5	0.20±0.45	-0.20±0.47	0,213	0,61	>0,5
BC capacity neutrophils, 10^9 B/L	11.3±2.2	7.26±1.4	0.46±0.50	-0.46±0.31	0,477	1,57	**>0,1**
Monocytes, 10^9/L	0.81±0.11	0.93±0.16	-0.20±0.37	0.20±0.54	0,339	0,61	>0,5
Phagocytosis index monocyt, %	6.2±0.7	6.8±1.3	-0.13±0.32	0.13±0.58	0,350	-0,40	>0,5
Microbial count monocyte, B/Ph	4.2±0.4	5.6±1.4	-0.31±0.17	0.31±0.61	0,456	-0,99	>0,2
Bactericidal capac mon, 10^6 B/L	207±39	460±237	-0.33±0.10	0.33±0.62	1,143	-1,05	>0,2

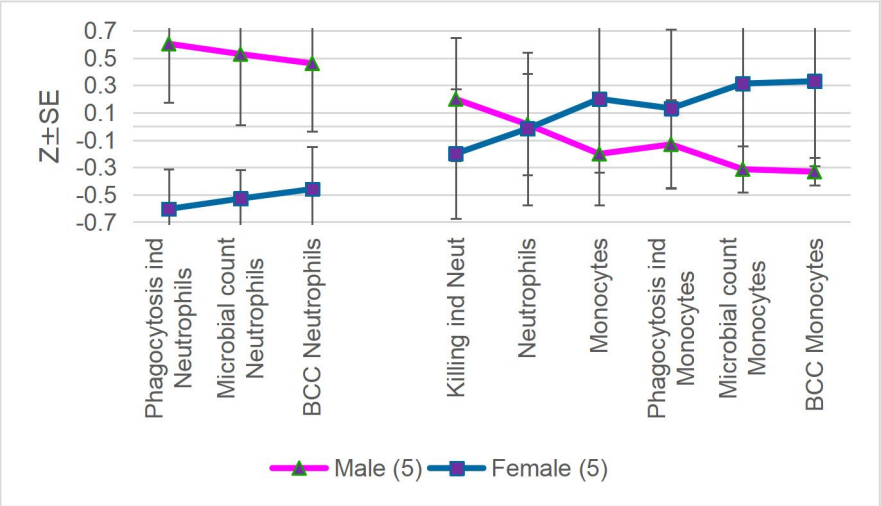

Fig. 14. Profile of Phagocytosis variables in stressed rats

The effects of acute stress on sex differences in metabolic parameters fit into seven patterns (Table 14 and Fig. 15). In particular, stress initiates a female preference for diene conjugates and increases it for SOD activity and serum calcium, while decreasing their preference for serum sodium and chloride, and reversing it for phosphate to a male preference. On the other hand, acute stress reduces the male preference for alkaline and acid phosphatase activity and eliminates it for aspartate aminotransferase, while initiating a male preference for alanine aminotransferase, α-LP cholesterol and serum potassium.

Table 14. Sexual dimorphism in metabolic parameters in control stressed rats

Sex	Males (5)	Females (5)	Males (5)	Females (5)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Na,K-ATP-ase Eryth, M/L•h	0.57±0.05	0.59±0.06	-0.13±0.45	0.12±0.49	0,193	-0,37	>0,5
Sodium of Erythrocyte, mM/L	33.7±3.4	27.5±2.8	0.42±0.46	-0.42±0.38	0,242	1,39	>0,2
Potassium of Erythroc, mM/L	80±6	81±2	-0.06±0.64	0.06±0.19	0,118	-0,18	>0,9
Potassium of Serum, mM/L	4.37±0.24	3.21±0.19	0.76±0.31	-0.76±0.25	0,202	3,77	**<0,01**
Sodium of Serum, mM/L	128.1±2.8	134.8±0.6	-0.61±0.50	0.61±0.11	0,042	-2,36	**<0,05**
Chloride of Serum, mM/L	91.9±3.5	101.1±1.0	-0.63±0.48	0.63±0.14	0,076	-2,52	**<0,05**
Calciemia, mM/L	2.50±0.37	4.06±0.09	-0.78±0.37	0.78±0.09	0,303	-4,14	**<0,01**
Phosphatemia, mM/L	1.32±0.05	1.19±0.06	0.47±0.38	-0.46±0.44	0,110	1,60	**>0,1**
$(Ca/K)^{0,5}$ ratio of Serum	0.59±0.12	1.28±0.08	-0.82±0.28	0.82±0.19	0,450	-4,82	**<0,01**
α-LP Cholesterol, mM/L	0.82±0.04	0.63±0.05	0.70±0.32	-0.69±0.33	0,187	3,03	**<0,02**
nonα-LP Cholesterol, mM/L	0.79±0.13	0.77±0.16	0.03±0.41	-0.03±0.53	0,388	0,09	>0,9
Triglycerides, mM/L	1.12±0.06	1.05±0.04	0.32±0.52	-0.32±0.36	0,106	1,01	>0,2
Diene conjugates, E^{232}/mL	1.32±0.10	1.70±0.13	-0.60±0.31	0.60±0.42	0,208	-2,31	**=0,05**
Malondialdehyde, μM/L	58±7	54±4	0.16±0.57	-0.16±0.34	0,220	0,48	>0,5
Superoxide dismutase, U/mL	48.0±1.5	70.2±6.7	-0.71±0.10	0.71±0.43	0,263	-3,21	**<0,02**
Catalase of Erythroc, μM/L•h	216±19	188±20	0.32±0.44	-0.32±0.46	0,215	1,01	>0,2
Catalase of Serum, μM/L•h	121±16	111±20	0.13±0.41	-0.13±0.52	0,333	0,40	>0,5
Alaninaminotranspher, μKat/L	0.91±0.18	0.57±0.06	0.50±0.54	-0.50±0.18	0,458	1,74	**>0,1**
Aspartataminotransph, μKat/L	0.29±0.08	0.25±0.03	0.16±0.63	-0.16±0.21	0,455	0,48	>0,5
Creatin Phosphokinase, IU/L	1.80±0.05	1.85±0.13	-0.09±0.20	0.09±0.49	0,114	-0,35	>0,5
Acid Phosphatase, IU/L	36.5±2.3	28.0±2.5	0.63±0.34	-0.63±0.36	0,210	2,54	**<0,05**
Alkaline Phosphatase, IU/L	494±30	264±32	0.84±0.22	-0.84±0.23	0,363	5,27	**$<10^{-3}$**

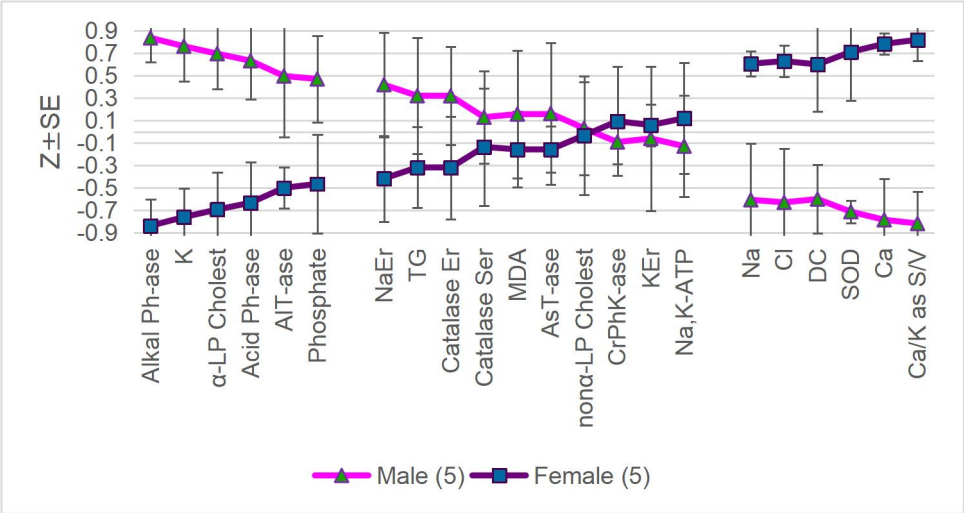

Fig. 15. Profile of Metabolic variables in stressed rats

A separate constellation is formed by indicators of stress damage to classical targets – gastric mucosa and myocardium. In intact rats, there are no sex differences in ST joint and T wave ECG voltage (see Table 22). Acute stress (S) caused their depression. It was found (Fig. 16) that the degree of depression of these markers of myocardial dystrophy in males is less, and concomitant damage to the gastric mucosa was limited to speckled erosions, while ulcers also occurred in females. Taken together, these differences indicate a higher stress tolerance of males.

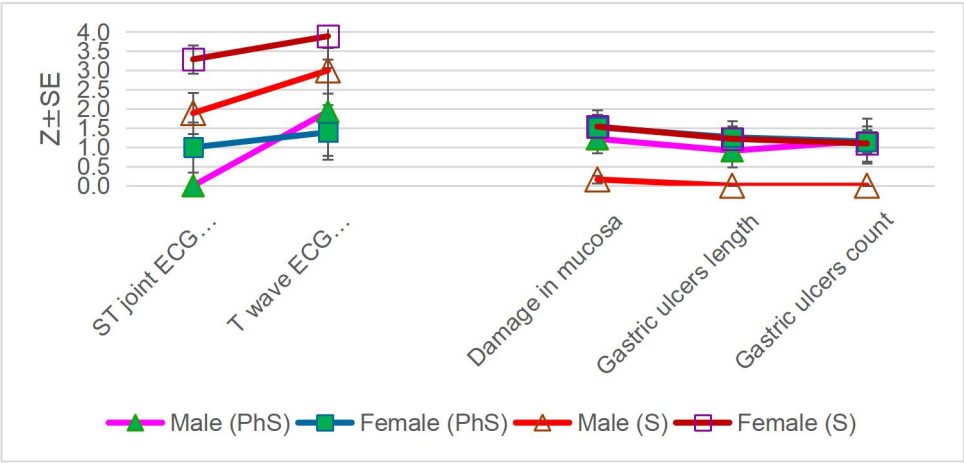

Fig. 16. Profile of post stress damage to myocardium and gastric mucosa in control and **pretreated** rats

The complete picture of sexual dimorphism in endocrine, metabolic, immune and damaged parameters in stressed rats is shown in Fig. 17. It turns out that in control stressed rats significant and borderline sex differences are recorded for 42 variables versus 30 variables in intact animals (see Fig. 8). In other words, acute stress expands sexual dimorphism.

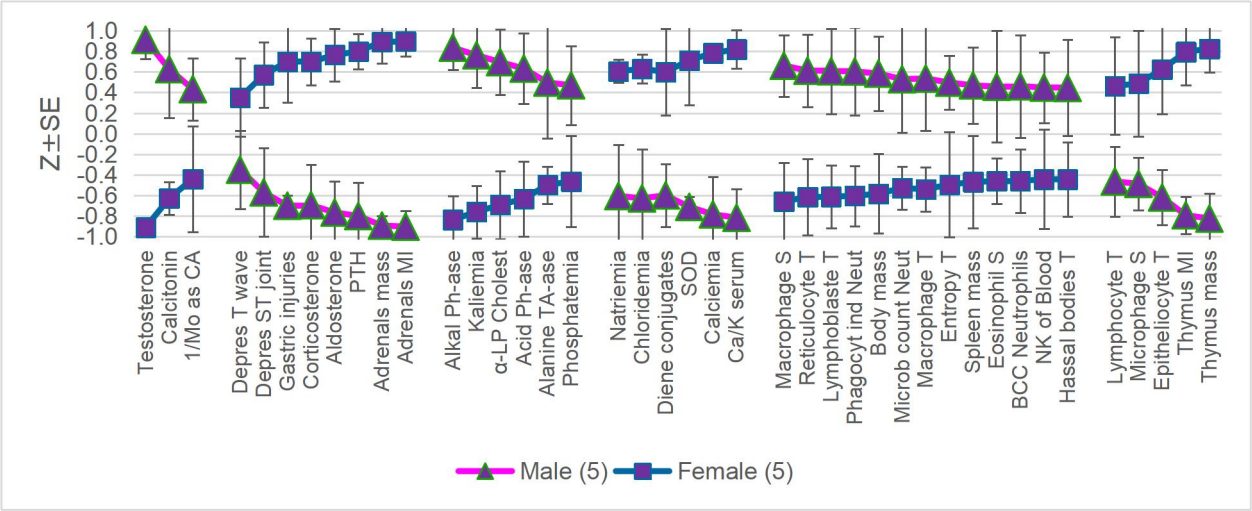

Fig. 17. Profile of endocrine, metabolic, immune and damaged parameters in stressed rats

Preventive use of the phytocomposition eliminated the difference in body weight by reducing its gain in males and increasing it in females. In contrast, the level of triiodothyronine increased slightly in males and decreased slightly in females, so that the sex differences became significant (Table 15 and Fig. 18).

Table 15. Sexual dimorphism in endocrine and autonomic parameters in pretreated stressed rats

Sex	Males (8)	Females (10)	Males (8)	Females (10)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Body mass, g	226±11	224±10	0.04±0.39	-0.04±0.35	0,123	0,16	>0,9
Adrenal glands mass, mg	48±2	70±3	-0.82±0.16	0.82±0.21	0,223	-6,20	**$<10^{-3}$**
Adrenal mass index, mg/100g	22±1	32±2	-0.76±0.11	0.75±0.28	0,257	-5,05	**$<10^{-3}$**
Corticosterone, nM/L	315±30	410±38	-0.45±0.24	0.45±0.36	0,292	-1,96	**>0,05**
Aldosterone, pM/L	591±14	677±22	-0.62±0.20	0.62±0.32	0,112	-3,28	**<0,01**
Testosterone, nM/L	37.3±1.8	3.09±0.26	0.96±0.08	-0.96±0.01	0,879	19,1	**$<10^{-3}$**
Calcitonin, ng/L	33.6±2.3	26.3±0.6	0.65±0.41	-0.65±0.11	0,195	3,06	**<0,01**
Parathyroid hormone, µg/L	168±14	198±6	-0.47±0.45	0.48±0.18	0,177	-1,94	**>0,05**
Triiodothyronine, nM/L	3.78±0.19	3.16±0.18	0.55±0.28	-0.55±0.31	0,164	2,42	**<0,05**
Mode as catecholamines, msec	154±11	170±5	0.35±0.47	-0.35±0.20	0,146	1,35	**>0,1**
AMo as sympathetic tone, %	78±7	62±5	0.45±0.39	-0.45±0.26	0,257	1,91	**>0,05**
MxDMn as vagal tone, msec	22±6	22±4	0.00±0.43	0.00±0.31	0,623	-0,02	>0,9

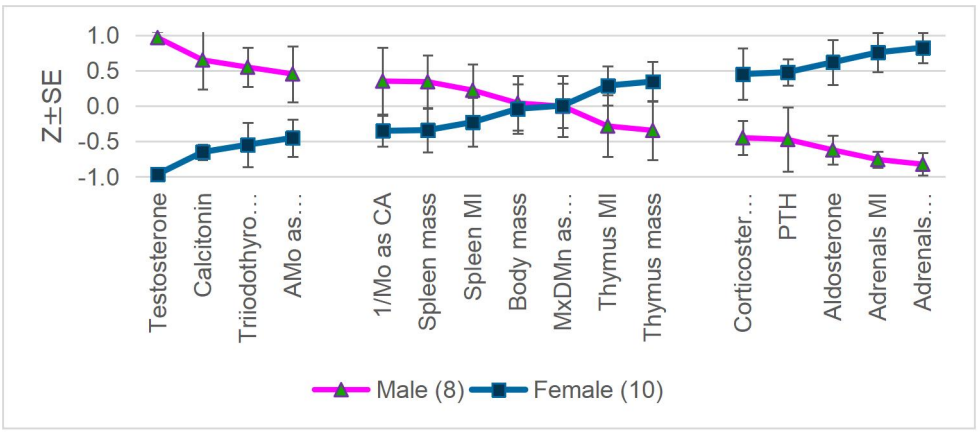

Fig. 18. Profile of body, thymus, spleen, adrenal glands mass, and neuro-endocrine variables in pretreated stressed rats

Preventive use of phytoadaptogen leveled post-stress sexual differences in thymus mass by increasing its involution in females. At the same time, in males, the post-stress percentage in the thymocytogram of lymphoblasts, macrophages and reticulocytes decreased, but epithelial cells increased, while in females the changes had the opposite direction, so that there was a reversion of sexual dimorphism of both the elements of the thymocytogram and its entropy (Table 16 and Fig. 19).

Table 16. Sexual dimorphism in thymus and thymocytogram parameters in pretreated stressed rats

Sex	Males (8)	Females (10)	Males (8)	Females (10)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Thymus mass, mg	118±16	144±10	-0.35±0.42	0.34±0.28	0,286	-1,37	**>0,1**
Thymus mass index, mg/100g	54±9	66±6	-0.28±0.44	0.29±0.27	0,336	-1,11	>0,2
Lymphocytes, %	67.0±1.2	65.2±1.0	0.30±0.35	-0.30±0.35	0.045	-1,12	>0,2
Lymphoblastes, %	5.50±0.37	6.60±0.37	-0.49±0.28	0.49±0.33	0,186	-2,10	**>0,05**
Epitheliocytes, %	8.73±1.02	7.02±0.49	0.42±0.42	-0.42±0.24	0,257	1,51	**>0,1**
Macrophages, %	6.00±0.50	6.50±0.40	-0.22±0.36	0.22±0.35	0,186	-0,78	>0,2
Reticulocytes, %	3.61±0.37	3.78±0.27	-0.11±0.39	0.11±0.34	0,218	-0,38	>0,5
Fibroblastes, %	4.33±0.73	6.00±0.30	-0.55±0.41	0.55±0.20	0,292	-2,11	**>0,05**
Basophiles, %	3.00±0.95	3.40±0.54	-0.11±0.44	0.11±0.30	0,570	-0,37	>0,5
Hassal's corpuscles, %	1.83±0.14	1.50±0.17	0.36±0.27	-0.38±0.37	0,271	1,51	**>0,1**
Thymocytogram Entropy •10^3	588±19	616±13	-0.34±0.38	0.34±0.31	0.070	-1,25	>0,2

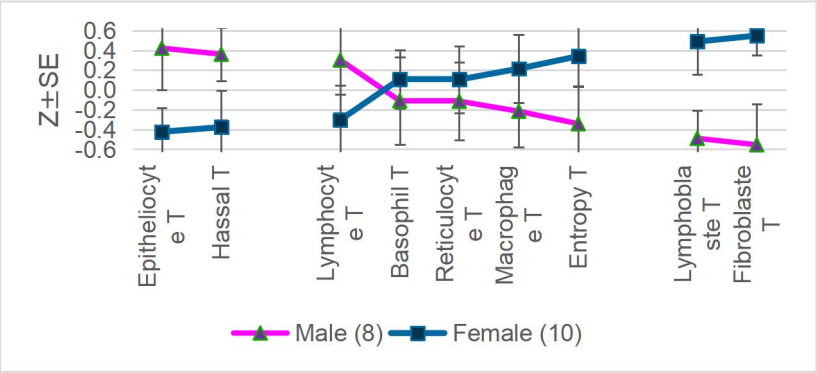

Fig. 19. Profile of Thymocytogram variables in pretreated stressed rats

The phytoadaptogen eliminated the post-stress advantage of males in terms of the percentage of macrophages in the splenocytogram, but increased it in terms of eosinophils (Table 17 and Fig. 20).

Table 17. Sexual dimorphism in spleen and splenocytogram parameters in pretreated stressed rats

Sex	Males (8)	Females (10)	Males (8)	Females (10)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Spleen mass, mg	689±45	608±37	0.34±0.38	-0.34±0.31	0,184	1,39	**>0,1**
Spleen mass index, mg/100g	309±25	278±24	0.22±0.36	-0.23±0.34	0,237	0,90	>0,2
Lymphocytes, %	68.2±1.6	67.1±2.6	0.09±0.22	-0.09±0.42	0,091	0,35	>0,5
Lymphoblastes, %	8.83±1.25	8.90±1.03	-0.01±0.37	0.01±0.36	0,325	-0,04	>0,9
Plasmocytes, %	2.50±0.58	2.00±0.39	0.20±0.40	-0.20±0.32	0,547	0,71	>0,2
Microphages, %	10.5±0.9	12.6±1.3	-0.32±0.23	0.32±0.39	0,285	-1,33	>0,2
Rod-shaped neutrophils, %	1.50±0.19	1.80±0.20	-0.28±0.30	0.28±0.37	0,328	-1,08	>0,2
Macrophages, %	2.33±0.29	2.40±0.37	-0.04±0.27	0.03±0.41	0,385	-0,14	>0,9
Reticulocytes, %	2.83±0.27	3.40±0.22	-0.42±0.33	0.41±0.32	0,219	-1,64	**>0,1**
Eosinophils, %	3.33±0.18	1.80±0.25	0.77±0.16	-0.78±0.25	0,386	4,96	**<10^{-3}**
Splenocytogram Entropy •10^3	54±17	551±28	0.02±0.22	-0.02±0.43	0,120	0,10	>0,5

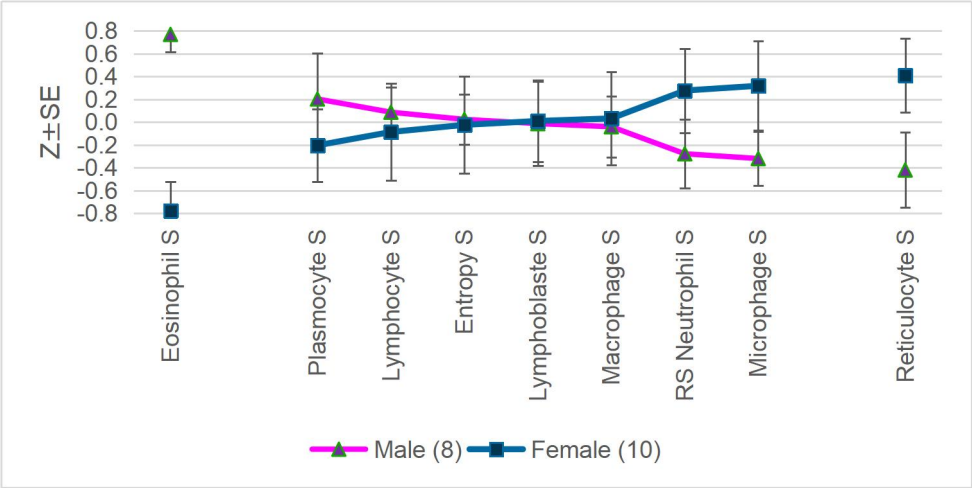

Fig. 20. Profile of Splenocytogram variables in pretreated stressed rats

Preventive use of phytoadaptogen did not affect the post-stress content of lymphocytes and their populations in the blood (Table 18 and Fig. 21).

Table 18. Sexual dimorphism in blood lymphocyte populations in pretreated stressed rats

Sex	Males (8)	Females (10)	Males (8)	Females (10)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Pan Lymphocytes, 10^9/L	6.86±0.54	7.08±0.58	-0.07±0.34	0.07±0.37	0,226	-0,18	>0,9
TR T-helper Lymphocytes, %	32.3±1.1	30.3±0.9	0.34±0.37	-0.34±0.31	0,091	1,41	**>0,1**
TS T-cytolytic Lymphocytes, %	12.8±1.1	14.0±0.9	-0.22±0.38	0.22±0.33	0,211	-0,88	>0,5
NK Lymphocytes, %	6.46±0.52	6.42±0.43	0.01±0.40	-0.02±0.33	0,202	0,06	>0,9
B-Lymphocytes, %	11.9±0.6	11.8±0.8	0.02±0.29	-0.02±0.40	0,160	0,08	>0,9
Plasmocytes, %	0.86±0.37	0.65±0.47	0.09±0.30	-0.08±0.39	1,609	0,35	>0,5
0-Lymphocytes, %	35.8±2.2	36.8±1.7	-0.09±0.41	0.09±0.32	0,149	-0,36	>0,5
Immunocytogram Entropy •10^3	807±16	806±12	0.02±0.41	-0.02±0.33	0,047	0,06	>0,9

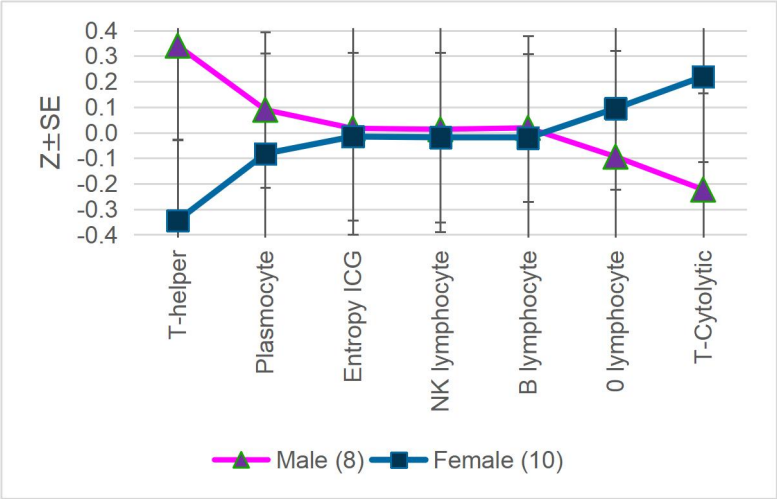

Fig. 21. Profile of Immunocytogram variables in pretreated stressed rats

No effect of the phytocomposition on the post-stress level of blood leukocytes and their populations was also found (Table 19 and Fig. 22).

Table 19. Sexual dimorphism in blood leukocyte populations in pretreated stressed rats

Sex	Males (8)	Females (10)	Males (8)	Females (10)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Leukocytes, 10^9/L	14.0±1.0	14.4±1.1	-0.08±0.35	0.08±0.37	0,205	-0,30	>0,5
Pan lymphocytes, %	49.1±1.1	49.0±1.3	0.01±0.33	-0.01±0.37	0,070	0,05	>0,9
PMN neutrophils, %	40.2±1.3	39.6±1.3	0.07±0.34	-0.08±0.37	0,092	0,31	>0,5
Rod-shaped neutrophils, %	2.94±0.33	2.50±0.27	0.26±0.39	-0.26±0.32	0,312	1,02	>0,2
Eosinophiles, %	2.84±0.47	3.20±0.29	-0.18±0.45	0.17±0.28	0,344	-0,66	>0,5
Monocytes, %	4.55±0.34	5.40±0.56	-0.30±0.24	0.29±0.39	0,286	-1,29	>0,2
Leukocytogram Entropy •10^3	654±15	663±9	-0.13±0.45	0.13±0.29	0,050	-0,50	>0,5

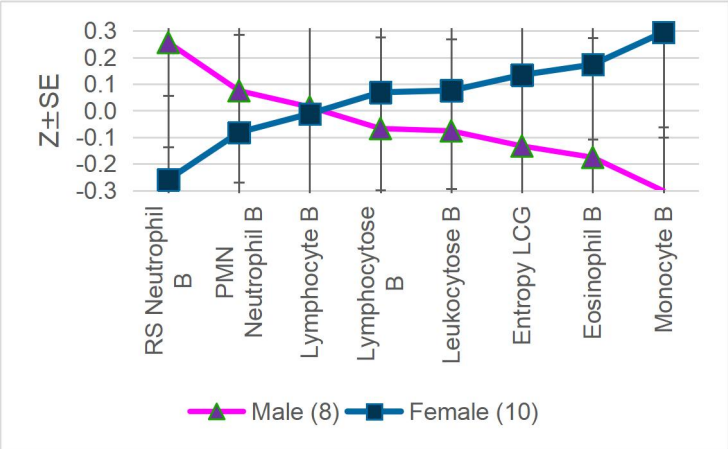

Fig. 22. Profile of Leukocytogram variables in pretreated stressed rats

Preventive use of phytoadaptogen eliminated sex differences in the activity of neutrophil phagocytosis, but instead initiated them in terms of its completion, so that the advantage of males in terms of bactericidal capacity of neutrophils increased (Table 20 and Fig. 23).

Table 20. Sexual dimorphism in parameters of phagocytic function of blood neutrophils and monocytes in pretreated stressed rats

Sex	Males (8)	Females (10)	Males (8)	Females (10)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Pan neutrophils, 10^9/L	5.99±0.42	6.07±0.51	-0.03±0.32	0.03±0.39	0,219	-0,12	>0,9
Phagocytosis index neutr, %	55.5±2.2	53.2±1.8	0.21±0.39	-0.21±0.33	0,101	0,82	>0,5
Microbial count neutr, B/Ph	6.4±0.3	6.0±0.4	0.22±0.31	-0.23±0.37	0,157	0,92	>0,5
Killing index neutrophils, %	48.0±3.3	35.7±1.0	0.70±0.38	-0.70±0.12	0,211	3,55	**<0,01**
BC capacity neutroph, 10^9 B/L	10.2±1.1	6.80±0.66	0.58±0.37	-0.58±0.23	0,342	2,70	**<0,02**
Monocytes, 10^9/L	0.64±0.08	0.76±0.08	-0.25±0.34	0.26±0.35	0,322	-1,05	>0,2
Phagocytosis index monoc, %	5.3±0.6	6.5±0.5	-0.37±0.36	0.36±0.32	0,292	-1,52	**>0,1**
Microbial count monoc, B/Ph	4.0±0.5	5.1±0.9	-0.25±0.23	0.25±0.41	0,487	-1,07	>0,2
Bactericidal cap mon, 10^6 B/L	155±50	271±68	-0.32±0.28	0.32±0.37	0,857	-1,37	**>0,1**

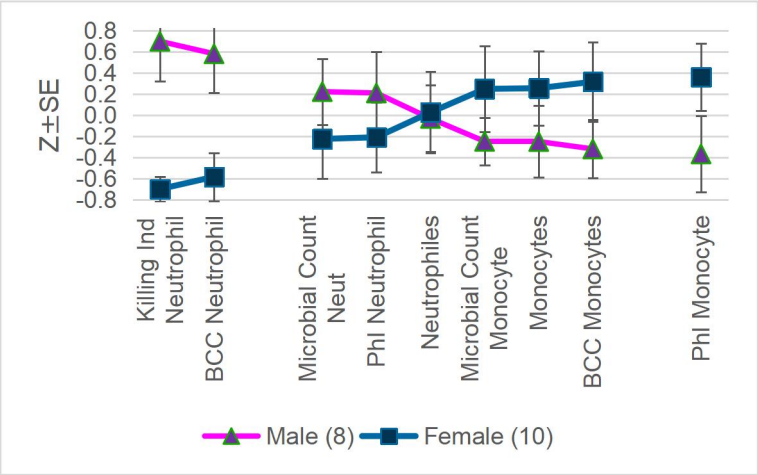

Fig. 23. Profile of Phagocytosis variables in pretreated stressed rats

Preventive use of phytoadaptogen significantly affects post stress sex differences in metabolic parameters (Table 21 and Fig. 24). In particular, phytoadaptogen reduces the male preference for α-LP cholesterol and serum potassium, eliminates it for alanine aminotransferase and phosphatemia, while initiates a male preference for aspartate aminotransferase, nonα-LP cholesterol and malondialdehyde as well as increases it for alkaline and acid phosphatase activity. On the other hand, phytoadaptogen initiates a female preference for Na,K-ATP-ase activity and potassium erythrocytes level, while decreasing their preference for serum calcium and superoxide dismutase activity.

Table 21. Sexual dimorphism in metabolic parameters in pretreated stressed rats

Sex	Males (8)	Females (10)	Males (8)	Females (10)	Parameters of Student's statistics		
Variables	Raw values		Z-scores		Cv	t	p
Na,K-ATP-ase Eryth, M/L•h	0.62±0.03	0.76±0.06	-0.43±0.17	0.43±0.39	0,231	-2,01	**>0,05**
Sodium of Erythrocyte, mM/L	26.2±2.0	22.9±3.9	0.17±0.21	-0.17±0.42	0,381	0,71	>0,2
Potassium of Erythroc, mM/L	72±3	86±2	-0.75±0.28	0.75±0.19	0,118	-3,88	**<0,01**
Potassium of Serum, mM/L	4.29±0.17	3.42±0.21	0.62±0.24	-0.61±0.30	0,184	3,17	**<0,01**
Sodium of Serum, mM/L	130.7±1.1	134.2±1.4	-0.44±0.27	0.44±0.36	0,030	-1,97	**>0,05**
Chloride of Serum, mM/L	94.9±1.5	100.6±2.1	-0.48±0.24	0.47±0.35	0,062	-2,23	**<0,05**
Calciemia, mM/L	2.67±0.33	3.81±0.08	-0.69±0.39	0.69±0.09	0,255	-3,42	**<0,01**
Phosphatemia, mM/L	1.17±0.08	1.21±0.06	-0.11±0.42	0.11±0.31	0,162	-0,42	>0,5
$(Ca/K)^{0,5}$ ratio of Serum	0.62±0.07	1.16±0.07	-0.75±0.19	0.76±0.25	0,401	-4,83	**$<10^{-3}$**
α-LP Cholesterol, mM/L	0.81±0.04	0.72±0.03	0.42±0.38	-0.42±0.28	0,145	1,77	**>0,05**
nonα-LP Cholesterol, mM/L	0.95±0.19	0.74±0.08	0.37±0.40	-0.37±0.29	0,342	1,43	**>0,1**
Triglycerides, mM/L	1.09±0.01	1.05±0.03	0.27±0.07	-0.28±0.44	0,069	1,26	.0,2
Diene conjugates, E^{232}/mL	1.31±0.10	1.63±0.11	-0.47±0.31	0.47±0.32	0,228	-2,13	**<0,05**
Malondialdehyde, μM/L	56.4±2.5	52.2±1.3	0.38±0.45	-0.38±0.23	0,102	1,50	**>0,1**
Superoxide dismutase, U/mL	53.8±3.8	67.0±3.7	-0.54±0.29	0.54±0.30	0,200	-2,49	**<0,02**
Catalase of Erythroc, μM/L•h	280±26	234±22	0.34±0.35	-0.34±0.33	0,262	1,37	**>0,1**
Catalase of Serum, μM/L•h	138±15	145±18	-0.08±0.31	0.08±0.39	0,327	-0,30	>0,5
Alaninaminotranspher, μKat/L	0.72±0.14	0.64±0.05	0.16±0.51	-0.15±0.21	0,374	0,52	>0,5
Aspartataminotransph, μKat/L	0.32±0.04	0.24±0.02	0.42±0.45	-0.43±0.18	0,304	1,75	**>0,05**
Creatin Phosphokinase, IU/L	1.89±0.04	1.82±0.11	0.15±0.12	-0.15±0.44	0,132	0,63	>0,5
Acid Phosphatase, IU/L	43.4±2.1	32.4±2.9	0.61±0.22	-0.61±0.32	0,239	3,08	**<0,01**
Alkaline Phosphatase, IU/L	586±42	264±29	0.85±0.21	-0.85±0.15	0,446	6,27	**$<10^{-3}$**

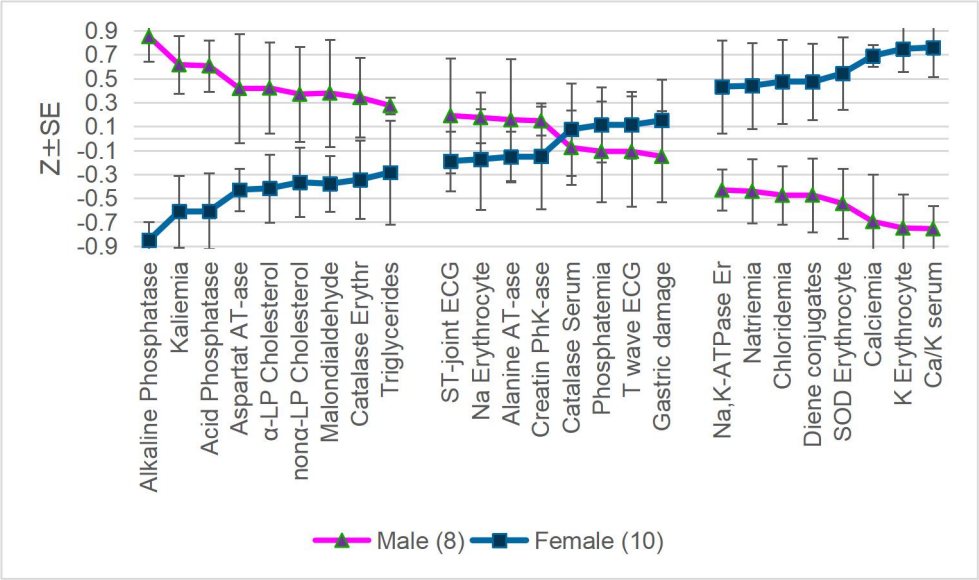

Fig. 24. Profile of Metabolic variables in pretreated stressed rats

In addition, phytoadaptogen eliminates sex difference in post stress damage to myocardium and gastric mucosa (see Fig. 16).

Fig. 25 shows that in pretreated with phytoadaptogen stressed rats significant and borderline sex differences are recorded for 35 variables versus 42 variables in control stressed rats and 30 variables in intact animals. In other words, phytoadaptogen reduces post stress sexual dimorphism.

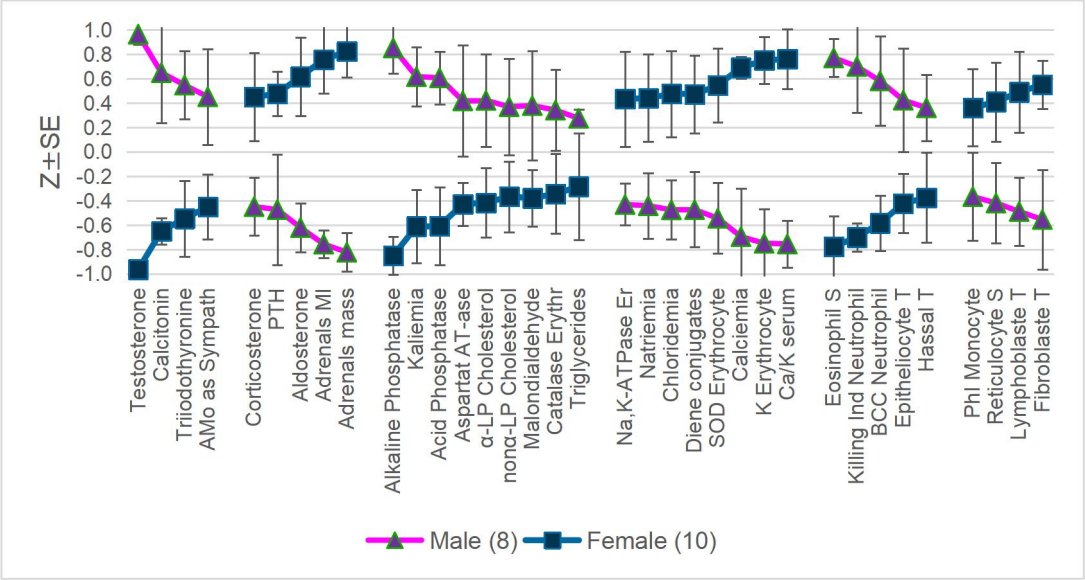

Fig. 25. Profile of endocrine, metabolic, and immune parameters in pretreated stressed rats

In order to identify exactly those parameters whose constellation is characteristic for each group, the available informational field was subjected to discriminant analysis by the method of forward stepwise [25]. To include in the model (Tables 22 and 23), the program has selected 23 variables (4 **endocrine**, 6 **immune,** 9 **metabolic**, as well as 4 markers of damage to **gastric mucosa** and **myocardium**).

Table 22. Discriminant Function Analysis Summary.
Step 23, N of vars in model: 23; Grouping: 6 grps; Wilks' Λ: 10^{-5}; approx. $F_{(115)}$=4.5; $p<10^{-6}$.
In each column, the top row is the average, the bottom is the standard error

	Intact, control and main female and male groups (n)						Parameters of Wilks' Statistics				
Variables currently in the model	F PhC Str (10)	F CW Str (5)	F Intact (5)	M CW Str (5)	M Intact (5)	M PhC Str (8)	Wilks' Λ •10^3	Partial Λ	F-remove (5.1)	p-value	Tolerancy
Testosterone, nM/L	3.09 0.26	3.28 0.27	3.53 0.24	38.1 4.1	41.8 1.7	37.3 1.8	0,018	0,457	2,38	0,114	0,273
Adrenals mass, mg	70.1 2.8	75.6 3.3	65.0 5.2	47.2 1.5	43.7 4.6	48.4 2.1	0,015	0,536	1,73	0,214	0,299
Calcitonin, ng/L	26.3 0.6	25.6 0.8	24.7 0.3	31.9 2.4	32.3 3.2	33.6 2.3	0,038	0,219	7,13	0,004	0,007
Parathyroid hormone, µg/L	198 6	205 6	185 3	151 11	154 11	168 14	0,021	0,386	3,18	0,056	0,001
Macrophages of Thymus, %	6.50 0.40	5.80 0.49	4.40 0.24	8.25 1.34	6.38 0.75	6.00 0.50	0,058	0,143	11,9	0,001	0,066
Reticulocytes of Thymus, %	3.78 0.27	2.89 0.87	2.65 0.55	5.77 0.95	5.68 1.02	3.61 0.37	0,013	0,650	1,08	0,429	0,167
Epitheliocytes of Thymus, %	7.02 0.49	8.91 0.76	9.75 0.81	6.73 0.54	6.32 0.82	8.73 1.02	0,019	0,446	2,48	0,104	0,388
Hassal's corpuscles of Thymus, %	1.50 0.17	1.40 0.24	1.00 0.00	2.00 0.37	1.00 0.00	1.83 0.14	0,016	0,501	1,99	0,166	0,229
Macrophages of Spleen, %	2.40 0.37	1.60 0.40	3.00 0.45	3.00 0.37	2.00 0.37	2,33 0.29	0,026	0,318	4,30	0,024	0,287
PMN Neutrophils of Blood, %	39.6 1.4	40.0 4.4	34.6 1.8	41.9 2.7	34.8 1.4	40.2 1.3	0,017	0,490	2,08	0,152	0,296
Damage to Gastric Mucosa, points	0.37 0.08	0.38 0.11	0	0.04 0.02	0	0.30 0.09	0,054	0,152	11,2	0,001	0,010
Gastric Ulcers Amount	2.1 0.5	2.0 0.8	0	0	0	2.1 1.1	0,022	0,371	3,39	0,048	0,018
ST joint ECG, µV	38 10	2 6	55 3	24 8	53 10	54 19	0,052	0,158	10,7	0,001	0,040
T wave ECG, µV	103 14	52 12	131 3	69 10	130 6	92 23	0,064	0,129	13,5	10^{-4}	0,019
Na,K-ATP-ase of Erythrocytes, M/L•h	0.76 0.06	0.59 0.06	0.71 0.05	0.57 0.05	0.83 0.11	0.62 0.03	0,037	0,222	7,02	0,005	0,015
Sodium of Erythrocytes, mM/L	22.9 3.9	27.5 3.3	25.2 3.0	33.7 3.4	19.1 3.2	26.2 2.4	0,055	0,150	11,3	0,001	0,010
Aspartate Aminotransspherase, nKat/L	241 15	252 25	175 20	291 90	253 31	319 42	0,026	0,322	4,21	0,025	0,123
Alkaline Phosphatase, IU/L	264 29	264 32	290 24	494 30	579 26	586 42	0,067	0,123	14,3	10^{-4}	0,040
Acid Phosphatase, IU/L	32.4 2.9	28.0 2.5	27.8 0.9	36.5 2.3	36.0 2.7	43.4 2.1	0,015	0,536	1,73	0,215	0,280
Calciemia, mM/L	3.81 0.08	4.06 0.09	3.80 0.07	2.50 0.37	2.56 0.37	2.67 0.33	0,031	0,266	5,51	0,011	0,002
Phosphatemia, mM/L	1.21 0.06	1.19 0.06	1.36 0.02	1.32 0.05	1.28 0.03	1.17 0.08	0,017	0,484	2,13	0,145	0,005
Superoxide dismutase, U/mL Erythroc.	67.0 3.7	70.2 6.7	73.0 6.1	48.0 1.5	50.7 5.5	53.8 3.8	0,031	0,267	5,49	0,011	0,166
Diene conjugates, E^{232}/mL	1.63 0.11	1.70 0.13	1.48 0.08	1.32 0.10	1.47 0.21	1.31 0.10	0,013	0,616	1,25	0,358	0,365

Table 23. Summary of Stepwise Analysis for Variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Testosterone, nM/L	25,8	10^{-6}	0,199	25,8	10^{-6}
Damage to Gastric Mucosa, points	4,62	0,003	0,114	12,2	10^{-6}
Sodium of Erythrocytes, mM/L	4,02	0,007	0,068	9,12	10^{-6}
ST joint ECG, µV	4,36	0,004	0,039	8,06	10^{-6}
Alkaline Phosphatase, IU/L	3,17	0,022	0,025	7,19	10^{-6}
Adrenals mass, mg	3,69	0,011	0,015	6,85	10^{-6}
Epitheliocytes of Thymus, %	2,50	0,057	0,010	6,35	10^{-6}
T wave ECG, µV	2,35	0,070	0,007	5,99	10^{-6}
Superoxide dismutase, U/mL Erythrocytes	2,52	0,057	0,004	5,78	10^{-6}
Gastric Ulcers Amount	1,81	0,150	0,003	5,47	10^{-6}
Macrophages of Thymus, %	1,85	0,145	0,002	5,24	10^{-6}
Na,K-ATP-ase of Erythrocytes, M/L•h	2,61	0,055	0,0010	5,23	10^{-6}
Aspartate Aminotranspherase, µKat/L	2,01	0,121	0,0009	5,13	10^{-6}
Macrophages of Spleen, %	1,72	0,178	0,0006	4,99	10^{-6}
Calciemia, mM/L	1,33	0,297	0,0005	4,78	10^{-6}
Calcitonin, ng/L	1,45	0,257	0,0003	4,63	10^{-6}
Phosphatemia, mM/L	3,68	0,021	0,0002	4,97	10^{-6}
PMN Neutrophils of Blood, %	1,70	0,196	0,0001	4,91	10^{-6}
Parathyroid hormone, µg/L	2,14	0,121	0,0001	4,98	10^{-6}
Hassal's corpuscles of Thymus, %	2,02	0,143	0,0000	5,04	10^{-6}
Acid Phosphatase, IU/L	1,52	0,256	0,0000	4,98	10^{-6}
Diene conjugates, E^{232}/mL	1,08	0,424	0,0000	4,79	10^{-6}
Reticulocytes of Thymus, %	1,07	0,429	0,0000	4,62	10^{-6}

The rest of the registered variables were left out of the model, although some of them carry discriminant (recognizable) information.

Than the 23-dimensional space of discriminant variables transforms into 5-dimensional space of a canonical roots. The canonical correlation coefficient is for Root 1 0.997 (Wilks' $\Lambda=10^{-5}$; $\chi^2_{(115)}=263$; $p<10^{-6}$), root contains 89.0% of discriminative opportunities; for Root 2 0.972 (Wilks' $\Lambda=0.002$; $\chi^2_{(88)}=144$; $p=10^{-4}$), 7.7% of discriminative opportunities; for Root 3 - 0.883 (Wilks' $\Lambda=0.029$; $\chi^2_{(63)}=80$; $p=0.076$), and only 1.6% of discriminative opportunities, therefore this and the rest of the roots will be ignored in the future.

Table 24 presents standardized (normalized) and raw (actual) coefficients for discriminant variables as well as constants. Calculating the values of discriminant roots for each rat by raw coefficients and constants allows visualization of each animal in the information space of roots.

Table 24. Standardized and Raw Coefficients and Constants for Canonical Variables

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
Testosterone, nM/L	-0,684	-0,924	-0,085	-0,115
Damage to Gastric Mucosa, points	4,523	8,095	22,12	39,57
Sodium of Erythrocytes, mM/L	7,793	4,039	0,901	0,467
ST joint ECG, µV	-4,442	0,360	-0,136	0,011
Alkaline Phosphatase, IU/L	-4,130	1,997	-0,044	0,021
Adrenals mass, mg	-0,588	-0,265	-0,078	-0,035
Epitheliocytes of Thymus, %	-0,795	0,006	-0,433	0,004
T wave ECG, µV	5,967	2,076	0,143	0,050
Superoxide dismutase, U/mL Erythrocytes	1,917	0,676	0,166	0,059
Gastric Ulcers Amount	-0,606	-5,785	-0,337	-3,212
Macrophages of Thymus, %	3,138	1,674	2,093	1,116
Na,K-ATP-ase of Erythrocytes, M/L•h	6,095	2,618	38,53	16,55
Aspartat Aminotranspherase, µKat/L	2,334	0,083	0,025	0,001
Macrophages of Spleen, %	-1,388	0,391	-1,501	0,423
Calciemia, mM/L	17,14	-11,34	27,78	-18,39
Calcitonin, ng/L	10,34	2,608	127,8	32,23
Phosphatemia, mM/L	0,052	8,961	0,319	54,76
PMN Neutrophils of Blood, %	0,946	-0,716	0,179	-0,136
Parathyroid hormone, µg/L	-9,673	17,12	-42,77	75,67
Hassal's corpuscles of Thymus, %	0,148	1,493	0,317	3,197
Acid Phosphatase, IU/L	-1,152	-0,025	-0,175	-0,004
Diene conjugates, E^{232}/mL	0,898	0,316	2,841	0,999
Reticulocytes of Thymus, %	-0,696	-1,243	-0,469	-0,838
		Constants	-153,1	-193,5
		Eigenvalues	195,3	16,81
		Cumulative Proportions	0,890	0,967

It can be seen (Fig. 26) that the distance between the centroids of the major discriminant root of intact females and males as a measure of sexual dimorphism is 16.2 units (2.8 + 13.4). Acute stress increases it in control rats to 23.4 units (13.2 + 10.2), and in pretreated with phytoadaptogen up to 29.4 units (15.2 + 14.2). Acute stress increases the severity of sexual dimorphism also in relation to variables, information about which is condensed in the minor root - from 0.99 (-4.63 + 5.62) to 2.29 (-0.17 + 2.46) units, while preventive use of phytoadaptogen limits it to 1.63 (4.48 – 2.85) units.

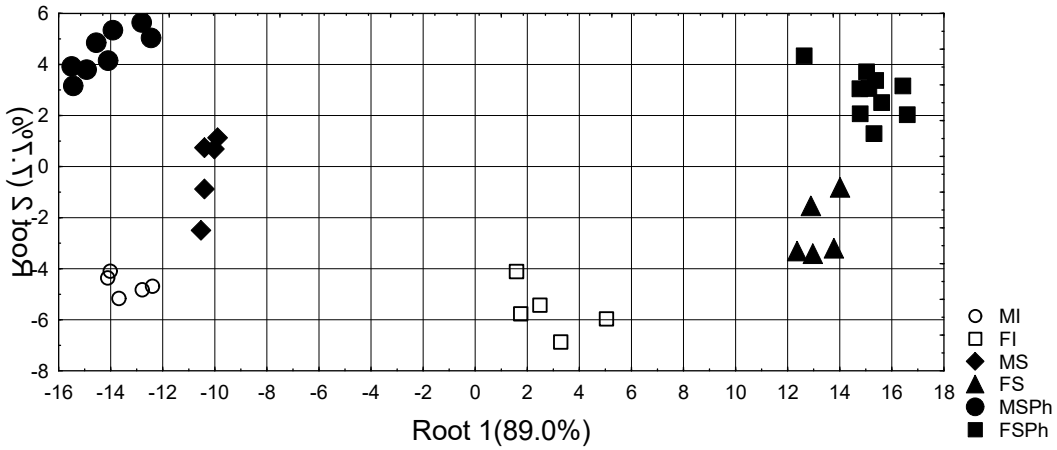

Fig. 26. Scattering of individual values of the first and second discriminant roots of males and females rats: intact (I), control stressed (S) and pretreated with Phytoadaptogen (SPh)

The apparent clear demarcation of clusters is documented by calculating Mahalanobis distances (Table 25).

Table 25. Squared Mahalanobis Distances between clusters (above the diagonal), F-values (df=23.1) and p-levels (under the diagonal)

Clusters	Male Intact (5)	Female Intact (5)	M CW Stress (5)	F CW Stress (5)	M PhC Stress (8)	F PhC Stress (10)
Male Intact (5)	0	280	52.4	743	95.5	875
Female Intact (5)	9.5 0.0004	0	229	146	397	232
M CW Stress (5)	1.8 0.172	7.8 0.0009	0	577	60.0	670
F CW Stress (5)	25.2 10^{-5}	4.9 0.006	19.6	0	811	58.4
M PhC Stress (8)	4.0 0.014	16.6 10^{-4}	2.5	33.9	0	873
F PhC Stress (10)	39.6 10^{-6}	10.5 0.0003	30.3 10^{-5}	2.6 0.056	52.7 10^{-6}	0

The same discriminant parameters can be used to retrospective identify the belonging of one or another animal to one or another cluster. This purpose of discriminant analysis is realized with the help of classifying functions (Table 26). The accuracy of classification (retrospective recognition) is **100%**.

Table 26. Coefficients and Constants for Classification Functions

Clusters	Male Intact (5)	Female Intact (5)	M CW Stress (5)	F CW Stress (5)	M PhC Stress (8)	F PhC Stress (10)
Variables	p=0.132	p=0.132	p=0.132	p=0.132	p=0.210	p=0.263
Testosterone, nM/L	-40,87	-42,51	-41,45	-43,18	-41,99	-44,25
Damage to Gastric Mucosa, points	12516	12832	12729	13172	12854	13437
Sodium of Erythrocytes, mM/L	166,1	179,9	171,8	190,1	168,9	195,5
ST joint ECG, µV	-20,56	-22,69	-20,84	-23,94	-20,23	-24,34
Alkaline Phosphatase, IU/L	-0,735	-1,438	-0,811	-1,847	-0,474	-1,835
Adrenals mass, mg	-6,655	-7,829	-7,059	-8,103	-6,571	-9,221
Epitheliocytes of Thymus, %	-31,33	-36,62	-32,28	-40,45	-29,04	-43,31
T wave ECG, µV	35,94	38,09	36,35	39,52	36,11	40,34
Superoxide dismutase, U/mL	33,91	36,58	34,56	38,20	34,23	39,10
Gastric Ulcers Amount	-935,4	-938,6	-949,6	-955,4	-966,9	-968,6
Macrophages of Thymus, %	450,4	482,2	462,9	507,1	457,6	518,6
Na,K-ATP-ase, M/L•h	6275	6873	6500	7269	6355	7510
Aspartate Aminotranspher, µKat/L	3814	4210	3895	4473	3788	4541
Macrophages of Spleen, %	-142,3	-165,3	-144,6	-183,1	-137,1	-181,2
Calciemia, mM/L	-2066	-1616	-2025	-1372	-2274	-1407
Calcitonin, ng/L	33605	35583	33996	36981	33745	37454
Phosphatemia, mM/L	22023	22001	22099	22119	22549	22416
PMN Neutrophils of Blood, %	-17,73	-14,82	-17,24	-12,96	-19,14	-13,58
Parathyroid hormone, µg/L	20960	20237	20986	19993	21739	20278
Hassal's corpuscles of Thymus, %	639,2	642,5	655,5	653,6	668,2	672,9
Acid Phosphatase, IU/L	-20,79	-23,28	-21,21	-24,98	-20,32	-25,72
Diene conjugates, E^{232}/mL	720,8	766,9	730,9	802,1	730,2	808,6
Reticulocytes of Thymus, %	-165,9	-173,4	-172,3	-180,3	-173,4	-186,0
Constants	-42891	-45118	-43752	-47248	-44559	-48651

Discussion

Sexual dimorphism across animals and human in nervous, endocrine and immune systems as well as metabolism has been the subject of a number of studies, but each of them was limited to one system [1,3,7,8,12,13,19,22-24,31,34,41-43,46-50]. The advantage of this study, in our opinion, is the **simultaneous** comparison of parameters of the autonomic, endocrine and immune systems, which closely interact with each other within the framework of the triune neuro-endocrine-immune complex, as well as with parameters of metabolism [5,10,11,17,26,28,29,37,38,40,44].

The results of this study confirm, complement, and refine the results of a previous study conducted in our laboratory with a similar design on 20 intact rats and 90 rats exposed to chronic aversive stress [38,39]. The most significant sex differences were found to be related to the morpho-functional parameters of the adrenal glands. In particular, females have higher androgenic,
glucocorticoid, and mineralocorticoid activity, estimated according to the thickness of the reticular zone of the adrenal cortex and daily excretion in the urine of 17-ketosteroids, the thickness of the fascicular corticoadrenal zone, and the plasma level of corticosterone as well as of the thickness of glomerular zone of the adrenal cortex and plasma and urine sodium and potassium. However, females have higher parathyroid and calcitonin activities, as measured by plasma and urinary levels of calcium and phosphate. In addition, females have a smaller amount of HRV mode as an inverse measure of heart rhythm, which is subject to the so-called humoral regulation channel (circulating catecholamines, glucocorticoids, electrolytes, etc.), whereas HRV-markers of sympathetic and vagal tone are not significant. Instead, the plasma triiodothyronine level was 85±15% of the male level, and the lower plasma testosterone level requires no comment. Among the reported immune rates, 12 were significantly higher in females. First of all, this is the proportion in the thymocytogram of lymphocytes and lymphoblastes, the natural killer cells and B-lymphocytes in the blood immunocytogram, as well as of fibroblasts, macrophages and microphages in the splenocytogram. In addition, females have a higher intensity of phagocytosis by monocytes of Staph. aureus, blood leukocytosis, RBTL on PhHA, as well as entropy of splenocytogram and immunocytogram. Instead, 10 indicators of immunity in females are significantly lower. This is, first of all, the phagocytosis activity of neutrophils and, to a lesser extent, monocytes, as well as thymocytogram entropy and its proportion of epitheliocytes, endothelial cells and macrophages, content of lymphocytes and lymphoblastes in the splenocytogram and 0-lymphocytes in immunocytogram as well as the completeness of phagocytosis by neutrophils of blood.

An important addition to the experimental data are our results of clinical observation, in which HRV and EEG were recorded almost simultaneously with the determination of the levels of adaptation hormones [28]. This sample is characterized (Mean±SE) by testosteronemia 3,5±0,4 nM/L vs 13,5±0,8 nM/L, and by calcitoninemia 5,7±0,4 ng/L vs 10,5±0,9 ng/L in women and men respectively. But there was no sexual dimorphism in the levels of other determined hormones: Cortisol 304±14 and 298±16 nM/L, Aldosterone 226±5 and 226±4 pM/L, Triiodothyronine 2,19±0,12 and 2,01±0,11 nM/L in women and men respectively. It was found that the Z-score (Mean±SE) for testosterone is -0,72±0,06 in women vs +0,72±0,11 in men, that is, sexual dimorphism is 1,44. The expression of sexual dimorphism of calcitoninemia is almost half as low: 0,83.

Screening of HRV and EEG parameters revealed that regardless of age, women differ significantly from men, except for drastically lower levels of testosterone and calcitonin by definition, lower levels of HRV-markers of sympathetic tone (but not heart rate), reactive anxiety, and beta-rhythm asymmetry. On the other hand, trait anxiety, levels of HRV-markers

of vagal tone, variability and amplitude of the beta-rhythm, and its PSD in 12 loci (maximum differences in T6, F3, and T3 loci), amplitude of the theta-rhythm and its PSD in 16 loci (maximum differences in F3, C3, and T3 loci), PSD of the alpha-rhythm in T3, T6, F7, and T4 loci as well as entropy of PSD in F7 and F8 loci are significantly higher in women than in men. It is also worth noting the much greater variability (SE) of neuro-endocrine (but not anxiety) parameters in women compared to men (Fig. 26).

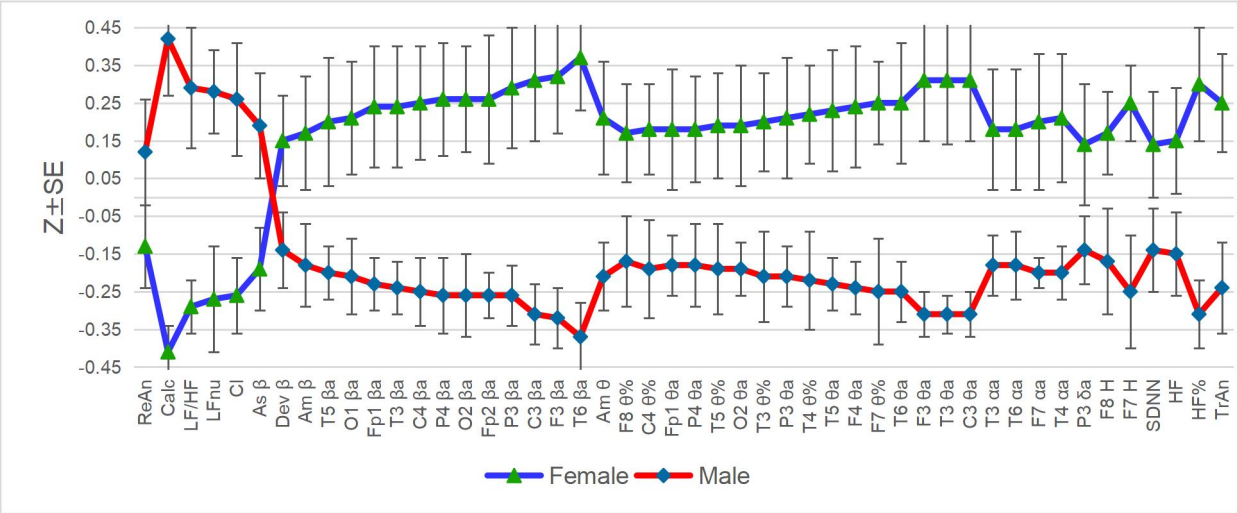

Fig. 27. Profiles of psycho-neuro-endocrine parameters (Z±SE) that differ in men and women. Testosterone (-0,72±0,06 vs +0,72±0,11) is not shown, so as not to coarsen the scale [28]

Conclusion

In intact rats, significant sex differences were found for a number of endocrine, immune, and metabolic variables, which increase under the influence of acute stress per se, and to an even greater extent against the background of preventive use of a phytoadaptogen. Sexual dimorphism should be taken into account in both experimental and clinical studies of new drugs and methods of treatment or prevention.

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