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MODULATING EFFECTS OF BIOACTIVE WATER NAFTUSSYA FROM LAYERS TRUSKAVETS' AND POMYARKY ON NEURO-ENDOCRINE-IMMUNE COMPLEX AND METABOLISM AT RATS EXPOSED TO ACUTE STRESS

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Abstracts

Background. Previously we have been carry out integrated quantitative estimation of neuroendocrine, immune and metabolic responses to acute stress at male and female rats. The **aim** of this study is to compare the modulating effects of Bioactive Water Naftussya (BAWN) from layers Truskavets' and Pomyarky on neuro-endocrine-immune complex and metabolism at rats exposed to acute stress. **Materials and methods.** The experiment is at white rats Wistar line. Rats were divided into intact and 5 test groups treated for seven days with tap (control) water, distilled water as well as table water "Truskavets'ka" and BAWN from Truskavets' and Pomyarky layers. A day after the end of course animals test groups subjected to water immersion restraint stress. A day after stress determined HRV, endocrine, immune and metabolic parameters as well as gastric mucosa injuries and comparing them with parameters of intact animals. **Results.** BAWN both Truskavets' and Pomyarky layers minimizes or obviates abnormal 15 parameters caused by stress, but does not affect the poststressory deviation of 7 parameters. On the other hand, BAWN causes deviation of 15 other parameters which little or do not change under stress. Distilled water and water "Truskavets'ka" affect some parameters the opposite way, other less favorable, but some more favorably than BAWN. **Conclusion.** BAWN both Truskavets' and Pomyarky layers limit pathogenic effects of stress, and initiate or enhance its compensatory and sanogenic effects.
Keywords: acute stress, HRV, hormones, immunity, metabolism, rats, bioactive water Naftussya.

INTRODUCTION

Previously we have been carry out integrated quantitative estimation of neuroendocrine, immune and metabolic responses to acute stress at male and female rats. Integrated quantitative measure manifestations of Acute Stress as mean of modules of Z-Scores makes for 10 metabolic parameters 0,75±0,10 σ and for 8 neuro-endocrine parameters 0,40±0,07 σ. Among immune parameters some proved resistant to acute stress factors, while 10 significant suppressed and 12 activated. Integrated quantitative measure poststressory changes makes 0,73±0,08 σ. Found significant differences integrated status intact males and females, whereas after stress differences are insignificant [45]. Previously we have been shown that at men with moderate disfunction of neuroendocrine-immune complex (disadaptation) Bioactive Water Naftussya from layers Truskavets', Pomyarky and Skhidnyts'a causes approximately equal immediate effects on 29 parameters of neuro-endocrine-immune complex different from effects of Control (distillated, filtered, well) Waters [44]. It is known about bouth immediate and course stresslimiting effects of Bioactive Water Naftussya from layer Truskavets' at rats and humans caused by its organic matter and microflora [6,25,30,34-36,39]. The aim of this study is to compare the modulating effects of Bioactive Water Naftussya from layers Truskavets' and Pomyarky on neuro-endocrine-immune complex and metabolism at rats exposed to acute stress.

MATERIAL AND RESEARCH METHODS

The experiment is at 58 (28 male and 30 female) white rats Wistar line weighing 170-280 g (Mean=220 g; SD=28 g). It was created by 6 groups that were equivalent to about sex and body weight of both averages and their dispersion. 10 animals of the first group received tap water through a tube at a dose 2% of body weight once daily for seven days, that remained relatively intact. 48 rats were divided into 5 test groups and treated with tap (control) water, distilled water as well as table water "Truskavets'ka" and bioactive waters Naftussya from Truskavets'and Pomyarky layers.

A day after the end of course animals test groups subjected to water immersion restraint stress by the method of J Nakamura et al. [28] as modified IL Popovych [33], which is to reduce the duration of stay of rats in cold water (t^o 20-21^o C) from 8 to 4 hours.

The day after acute stress took samples of peripheral blood (through a cut tail) to analyze leukocytogram. An hour under light ether anesthesia for 15-20 sec recorded ECG in standard lead II (introducing needle electrodes subcutaneously) to determine parameters of heart rate variability (HRV) [3,23,36]. Then the animals were decapitated, for the purpose of collecting blood in which was determined some endocrine, immune and metabolic parameters.

Among endocrine parameters determined plasma concentration of corticosterone, testosterone and triiodothyronine (by ELISA, reagents from JSC "Alkor Bio", RF [17]).

Immune parameters were determined by tests I and II levels of WHO as described in the handbook [24] and the previously developed algorithm [34-36]. On the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) judged by phagocytic index, microbial (phagocytic) number and index of killing regarding museum culture Staphylococcus aureus (ATCC N 25423 F49) [6,9], with the calculation of derivative indices: microbial capacity (number of microbes that are able to absorb phagocytes contained in 1 L of blood) and bactericidal capacity (number of microbes that are able to neutralize neutrophils or monocytes contained in 1 L of blood) [6,35,36].

Among the parameters immunogram determined the relative amount of blood population of T-cells by spontaneous rosette test with sheep erythrocytes by M Jondal et al. [18], their theophylline resistant (T-helpers) and theophylline sensitive (T-cytotoxic) subpopulations (by test sensitivity rosette to theophylline by S Limatibul et al. [26]), the population of B-lymphocytes by test complementary rosette of sheep erythrocytes by C Bianco [5]. Natural killers identified as big containing granules lymphocytes.

On lipid metabolism judged by the level of plasma triacylglycerides (metaperiodate-acetylacetone colorimetric method), total cholesterol (direct method by reaction Zlatkis-Zach) and its distribution as part of α-lipoprotein (applied enzymatic method after precipitation nonα-lipoproteins using dextransulfate/Mg^{2+}) as well as nonα-lipoprotein (turbidometric method Burstein-Samay) as described in the handbook [14].

State of lipid peroxidation assessed the content in the serum its products: diene conjugates (spectrophotometry of heptane phase of lipids extract) [13] and malonic dyaldehid (test with tiobarbiture acid) [1], as well as the activity of antioxidant enzymes: catalase serum and red blood cells (by the speed of decomposition hydrogen peroxide) [22], superoxide dismutase erythrocytes (by the degree of inhibition of nitroblue tetrazolium recovery in the presence of N-methylphenazone metasulfate and NADH) [10,27].

On electrolyte metabolism judged by the level in the plasma of calcium (by the reaction with arsenazo III), phosphate (phosphate molibdate method), chloride (mercury rodanide method), sodium and potassium (also in the erythrocytes) (flame photometry method) as described in the handbook [14].

Based on obtained data expected number of hormonal activities: mineralocorticoid $MCA=(Na/K)^{0,5}$, parathyrine $PTA=(Ca/P)^{0,5}$ and calcitonine $CTA=(1/Ca•P)^{0,5}$, based on the classical principles and guidelines IL Popovych [33,36], as well as Ca/K ratio, which is considered a marker of sympatho-vagal balance [11].

Alanine and asparagine aminotranspherase, alkaline and acid phosphatase as well as creatinephosphokinase determined by uniform methods as described in the handbook [14].

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

After a blood sample was removed spleen, thymus, adrenal glands and stomach. Immune organs weighed and made them smears for counting of splenocytogram and thymocytogram [4,6] as well as its entropy [2,34,50]. The stomach was cut along the greater curvature, mounted it on gastroluminoscope and under a magnifying glass detected erosions and counted the number of ulcers and their length was measured, evaluated erosive and ulcerative damage on scale by VN Shatalov et al [42].

Digital material it is processed using the package of softwares “Statistica 5.5”.

RESULTS AND DISCUSSION

Preliminary analysis using previously described [45] approach showed that the parameters of animals that received water Naftussya from Truskavets’ (NT) and Pomyarky (NP) layers did not significantly differ. On the other hand, did not reveal significant differences between the parameters of rats that received distilled water (DW) and table water "Truskavets’ka" (TW). This gave a reason for the next stage of the analysis create combined groups NTPS and DTWS. When comparing poststressory (S) changes in the parameters of the animals NTPS, DTWS and control (CWS) groups relatively intact animals were created nine clusters (Tables 1-9).

Table 1. Variables poststressory increase which decreases under the influence of water Naftussya and increases caused by distilled water

Variables of Cluster I	Intact (10)	NTPS (20)	CWS (10)	DTWS (18)
Th-Lymphocytes of Blood, %	29,7±0,3	+0,72±0,65	+2,04±0,95	+3,10±0,80
Sympatho-Vagal Balance as Cap/Kp	0,81±0,09	+0,22±0,27	+0,44±0,44	+0,64±0,33
Parathyrine Activity as $(Cap/Pp)^{0,5}$	1,53±0,07	+0,10±0,24	+0,34±0,45	+1,10±0,41
Shatalov's Injuries Gastric Mucosa Ind	0	1,10±0,26	1,40±0,40	2,50±0,23
Mean of Changes as Z-score	0	+0,54±0,23	+1,06±0,41	+1,84±0,58

Notes. For the intact group given the actual parameters (Mean±SE). For the poststressory groups parameters expressed in Z-score±SE, Shatalov's Injuries Gastric Mucosa Index expressed in points±SE.

Table 2. Variables poststressory decrease which decreases under the influence of water Naftussya and increases caused by distilled water

Variables of Cluster III	Intact (10)	NTPS (20)	CWS (10)	DTWS (18)
Moda HRV, msec	170±9	+0,31±0,27	-0,51±0,31	-0,61±0,14
Potassium, mM/L	4,10±0,20	-0,35±0,28	-0,48±0,38	-0,63±0,32
Vagal tone as MxDMn HRV, msec	42±14	-0,07±0,17	-0,30±0,16	-0,55±0,06
Phosphate, mM/L	1,32±0,02	-0,52±0,41	-0,92±0,65	-2,95±0,89
Eosinophiles of Blood, %	4,90±0,72	-0,33±0,25	-0,80±0,25	-0,77±0,16
Cholesterol of α-Lipoproteines, mM/L	0,84±0,05	-0,20±0,23	-0,73±0,29	-0,77±0,20
Mean of Changes as Z-score	0	-0,19±0,12	-0,62±0,09	-1,05±0,38

Table 3. Variables poststressory increase which unchanged under the influence of water Naftussya and decreases caused by distilled water

Variables of Cluster II	Intact (10)	NTPS (20)	CWS (10)	DTWS (18)
Hassal's corpuscules of Thymus, %	1,00±0,00	+1,40±0,40	+1,53±0,51	+1,02±0,28
Macrophages of Thymus, %	5,39±0,50	+0,88±0,18	+0,95±0,47	+0,60±0,27
Alanine Aminotranspherase, μKat/L	0,53±0,05	+0,92±0,42	+1,23±0,72	+0,38±0,26
Fibroblastes of Thymus, %	5,3±0,6	+0,11±0,14	+0,49±0,23	0,00±0,20
Mean of Changes as Z-score	0	+0,83±0,27	+1,05±0,22	+0,50±0,21

Table 4. Variables poststressory decrease which unchanged under the influence of water Naftussya and decreases caused by distilled water

Variables of Cluster V	Intact (10)	NTPS (20)	CWS (10)	DTWS (18)
Cholesterol nonα-Lipoproteines, mM/L	1,04±0,07	-1,02±0,29	-1,08±0,40	-0,66±0,28
Klimov's Atherogenity Coefficient	1,27±0,10	-0,74±0,34	-0,40±0,52	-0,07±0,32
Malonic dyaldehid, μM/L	63,5±5,6	-0,36±0,17	-0,43±0,22	-0,42±0,14
Mean of Changes as Z-score	0	-0,71±0,19	-0,64±0,22	-0,38±0,17

Table 5. Variables poststressory decrease which reduced under the influence of all waters

Variables of Cluster IV	Intact (10)	NTPS (20)	CWS (10)	DTWS (18)
Entropy of Splenocytogram, $\cdot 10^{-3}$	534±19	+0,37±0,24	-0,42±0,42	+0,58±0,25
Tc-Lymphocytes of Blood, %	15,3±1,1	-0,40±0,19	-0,95±0,25	-0,58±0,18
Entropy of Immunocytogram, $\cdot 10^{-3}$	807±8	+0,04±0,28	-0,50±0,52	+0,09±0,32
Lymphoblastes of Thymus, %	7,5±1,0	+0,05±0,20	-0,54±0,27	+0,17±0,21
Katalase, μM/L•h	143±12	+0,30±0,29	-0,70±0,31	-0,18±0,31
Mean of Changes as Z-score	0	+0,07±0,14	-0,62±0,09	+0,02±0,19

Table 6. Variables that are not subordinate to a change in control, but the increase caused by water Naftussya

Variables of Cluster VI	Intact (10)	NTPS (20)	CWS (10)	DTWS (18)
Acid Phosphatase, IU/L	31,4±1,9	+1,07±0,41	+0,14±0,36	+1,03±0,48
Reticulocytes of Spleen, %	2,67±0,22	+0,71±0,33	0,00±0,45	+0,86±0,34
Microbial Capacity of Neutrophils, 10^9Bac/L	15,5±2,5	+1,29±0,24	+0,63±0,31	+0,64±0,33
NK-Lymphocytes of Blood, %	5,3±0,35	+1,17±0,23	+0,80±0,32	+0,88±0,38
Microbes Count of Neutrophils, Bac/Ph	5,5±0,3	+1,11±0,21	+0,19±0,27	+0,33±0,18
Phagocytose Index of Neutrophiles, %	55,2±1,8	+0,76±0,37	-0,09±0,34	-0,02±0,31
Bactericidal Capacity of Neutroph., 10^9Bac/L	7,5±1,4	+0,68±0,23	+0,40±0,32	+0,13±0,27
Basophiles of Thymus, %	2,78±0,39	+0,63±0,33	-0,18±0,39	-0,23±0,34
Mean of Changes as Z-score	0	+0,93±0,09	+0,24±0,12	+0,45±0,17

Table 7. Variables that are not subordinate to a change in control, but the decrease caused by water Naftussya

Variables of Cluster VII	Intact (10)	NTPS (20)	CWS (10)	DTWS (18)
Corticosteron normalized by sex, Z-score	0±0,42	-0,54±0,21	+0,58±0,22	+0,51±0,22
Testosterone normalized by sex, Z-score	0±0,42	-1,21±0,29	-0,47±0,47	-0,44±0,25
Nonα-Lipoproteines, units	4,5±0,3	-0,91±0,24	-0,54±0,48	-0,67±0,43
Monocytes of Blood, %	6,2±0,7	-0,43±0,17	-0,17±0,20	-0,44±0,18
B-Lymphocytes of Blood, %	13,4±0,8	-0,42±0,21	-0,24±0,31	-0,38±0,20
Lymphocytes of Spleen, %	68,4±1,6	-0,22±0,26	+0,48±0,42	-0,40±0,26
Killing Index of Neutrophiles, %	47,5±2,9	-0,67±0,26	-0,30±0,32	-0,84±0,25
Mean of Changes as Z-score	0	-0,63±0,13	-0,09±0,17	-0,38±0,16

Table 8. Variables that are not subordinate to a change in control, but the decrease caused by distilled water

Variables of Cluster VIII	Intact (10)	NTPS (20)	CWS (10)	DTWS (18)
Spleen Mass, mg	773±58	-0,38±0,18	-0,30±0,23	-0,61±0,17
Sodium of Erythrocytes, mM/L	27,4±3,0	-0,11±0,24	-0,18±0,27	-0,47±0,27
Potassium of Erythrocytes, mM/L	88±5	-0,18±0,17	-0,15±0,20	-0,47±0,16
Entropy of Leukocytogram, $\cdot 10^{-3}$	682±17	-0,22±0,15	-0,33±0,17	-0,47±0,21
Phagocytose Index of Monocytes, %	5,9±0,5	+0,10±0,27	+0,38±0,41	-0,39±0,19
Mean of Changes as Z-score	0	-0,16±0,08	-0,12±0,13	-0,48±0,04

Table 9. Variables that are not subordinate to a change in control, but the increase caused by distilled water

Variables of Cluster IX	Intact (10)	NTPS (20)	CWS (10)	DTWS (18)
Mineralocorticoide Activity as $(Nap/Kp)^{0,5}$	5,75±0,17	+0,38±0,30	+0,43±0,41	+0,71±0,39
Sympathetic tone as AMo HRV, %	58±8	+0,01±0,20	-0,01±0,21	+0,58±0,17
Mean of Changes as Z-score	0	+0,20±0,19	+0,21±0,22	+0,65±0,06

Patterns of poststressory changes in all clusters shown in Fig. 1.

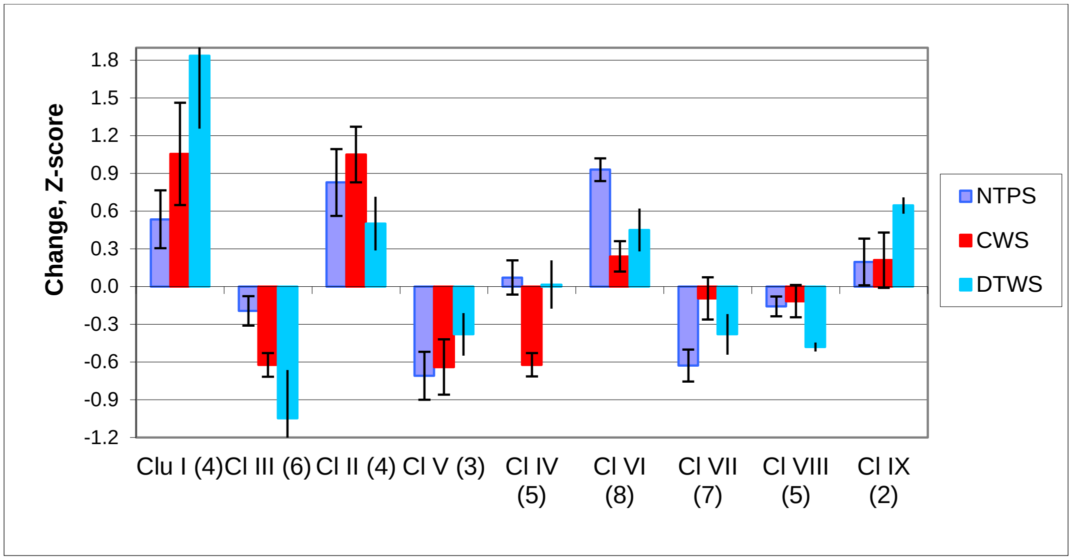


Fig. 1. Patterns of poststressory changes in the parameters (n) of the primary clusters

As you can see, clusters I and III, II and V, VI and VII, VIII and IX are almost mirror, so the next stage of the analysis, they were united in pairs (Fig. 2). It is evident that preventive use of BAWN significantly reduces caused by stress abnormal 10 parameters, united in the cluster A.

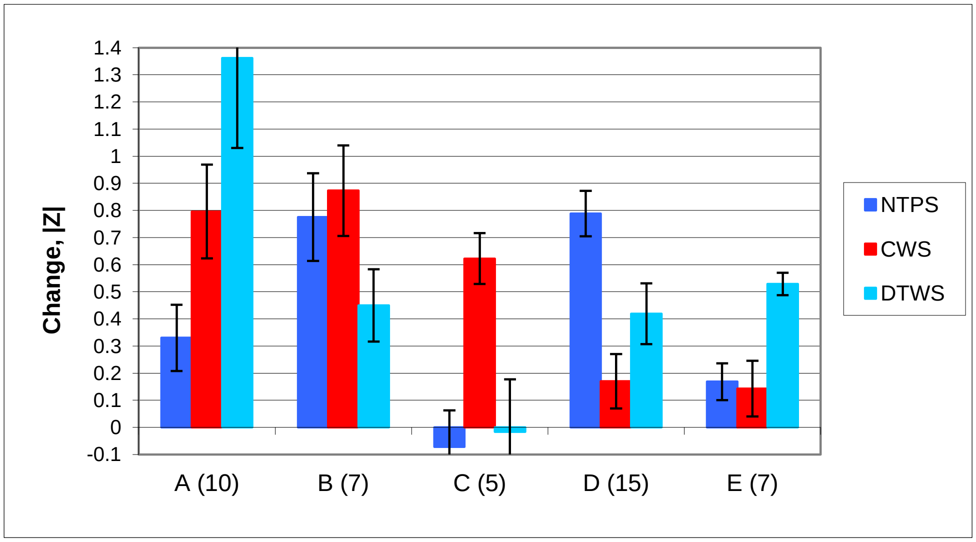


Fig. 2. Patterns of poststressory changes in the parameters (n) of the twin clusters

If these pathogenic manifestations of acute stress as ulceration of the stomach, sympatotonic shift of sympato-vagal balance, lower blood levels of potassium and eosinophils known, while raising Th-Lymphocytes and reduce Cholesterol of α-Lipoproteines unexpected for us. Nevertheless, the presence of significant correlations of these parameters with Injuries Gastric Mucosa Index (r=0,30 and -0,33 respectively) gives reason to treat them as also pathogenic manifestations of stress. Was also unexpected complication pathogenic manifestations of stress prior use distilled water as well as water "Truskavets'ka". But this can be explained by a concomitant increase Parathyrine Activity, which correlates with Injuries Gastric Mucosa Index (r=0,32).

Instead, these waters tangible than BAWN reduce poststressory increased Alanine Aminotranspherase activity in plasma and content in the thymus Hassal's corpuscules, Macrophages and Fibroblastes as well as reduce Cholesterol nonα-Lipoproteines (Cluster B).

Only minimize poststressory low levels of blood T cytotoxic Lymphocytes, the activity of Katalase, as well as full preventing the reduction Lymphoblastes level in the thymus and Entropy of Immunocytogram, and reversion reduction Entropy of Splenocytogram expressed approximately equally in both groups (Cluster C).

A particular situation occurs in relation to parameters, which are collected in Cluster VI. The vast majority of them the day after acute stress did not significantly deviates from the norm, and NK-Lymphocytes, Microbial and Bactericidal Capacity of Neutrophils in blood increased moderately. Previous use BAWN initiate significant increase in these parameters and potentiates the further improvement of other parameters. Distilled water "Truskavets'ka" influencing these parameters weaker (Fig. 1). IL Popovych [35,36] interprets these immune and metabolic responses as compensatory and sanogenic aimed at strengthening antimicrobial protection, with which we agree.

On the other hand (Cluster VII), use BAWN initiate significant decrease in level Monocytes and B-Lymphocytes of Blood, potentiates further reduce levels of Testosterone, nonα-Lipoproteines and Killing Index of Neutrophiles and reverses poststressory increase Corticosteronemia and level of Lymphocytes in Spleen. Distilled water and water "Truskavets'ka" influencing these parameters weaker too (Fig. 1). Unique interpretation of described reactions as compensatory and sanogenic causes some reservations, but can be accepted in view of the ambiguous interrelationships between adaptation hormones and immunity [7,8,12,15,16,19,21,23,31,32,36-38,40,41,43,46-49].

In another constellation of parameters collected Clusters VIII and IX, affects not stress too, both in control and with the prior BAWN use, instead of the previous use of distilled water and water "Truskavets'ka" cause a reduction Spleen Mass, Phagocytose Index of Monocytes, Entropy of Leukocytogram as well as levels Na and K in Erythrocytes combined with increased Mineralocorticoide Activity and Sympathetic tone. We interpret this as an initiation pathogenic reactions.

At the final stage held discriminant analysis to identify precisely those parameters, the totality of which poststressory states three groups of rats differ significantly from each other. Methods used forward stepwise [20]. The program is included in the model of 22 variables (Table 10).

Table 10. Discriminant Function Analysis Summary and Summary of Stepwise Analysis

Step 22, N of vars in model: 22; Grouping: 4 grps
Wilks' Lambda: 0,023; approx. $F_{(67)}$=3,85; $p<10^{-6}$

Variables currently in the model	Wilks Λ	Part Λ	F remov	p-level	Tolerancy	F to ent	p-level	Λ	F-val	p-level
Shatalov's Injuries Gastric Muc Ind	,043	,519	10	10^{-4}	,341	13	10^{-5}	,587	13	10^{-5}
Corticosteron normalized	,032	,708	4,5	,009	,101	5,5	,002	,449	8,7	10^{-6}
Microbes Count of Neutrophiles	,024	,945	0,6	,591	,115	4,0	,013	,365	7,2	10^{-6}
Moda HRV	,040	,562	8,6	10^{-3}	,327	3,5	,023	,303	6,4	10^{-6}
Hassal's corpuscules of Thymus	,033	,677	5,2	,005	,649	2,8	,051	,260	5,8	10^{-6}
Malonic dyaldehid	,023	,963	0,4	,742	,496	2,6	,063	,225	5,4	10^{-6}
Entropy of Immunocytogram	,026	,869	1,7	,195	,385	2,5	,071	,194	5,1	10^{-6}
Macrophages of Thymus	,037	,608	7,1	10^{-3}	,385	2,1	,113	,171	4,8	10^{-6}
Th-Lymphocytes of Blood	,034	,661	5,6	,003	,395	2,9	,043	,144	4,7	10^{-6}

Katalase	,041	,545	9,2	10^{-4}	,275	2,0	,135	,127	4,5	10^{-6}
Testosterone normalized	,031	,731	4,0	,015	,204	2,1	,116	,111	4,4	10^{-6}
Entropy of Splenocytogram	,034	,657	5,8	,003	,273	3,0	,039	,092	4,4	10^{-6}
Spleen Mass	,037	,605	7,2	10^{-3}	,222	2,7	,060	,077	4,4	10^{-6}
Acid Phosphatase	,030	,747	3,7	,020	,494	2,1	,113	,067	4,3	10^{-6}
Cholesterol of nonα-Lipoproteines	,029	,783	3,1	,042	,539	2,1	,119	,058	4,3	10^{-6}
Tc-Lymphocytes of Blood	,033	,683	5,1	,005	,335	1,9	,145	,051	4,2	10^{-6}
Fibroblastes of Thymus	,026	,866	1,7	,185	,603	1,9	,144	,044	4,1	10^{-6}
Sympathetic tone as AMo HRV	,028	,816	2,5	,079	,360	1,9	,141	,038	4,1	10^{-6}
Phosphate	,024	,921	0,9	,433	,203	1,6	,213	,034	4,0	10^{-6}
Cholesterol of α-Lipoproteines	,028	,807	2,6	,066	,471	1,5	,238	,030	3,9	10^{-6}
Sodium of Erythrocytes	,026	,882	1,5	,239	,322	2,0	,133	,025	3,9	10^{-6}
Basophiles of Thymus, %	,025	,891	1,3	,276	,517	1,3	,275	,023	3,9	10^{-6}

Information about these variables condensed in three Canonical Roots. First Root has 60,3% discriminant properties (Canonical R=0,92; Wilks' Λ=0,023; $\chi^2_{(66)}$=167; $p<10^{-6}$), second Root 31,5% (Canonical R=0,86; Wilks' Λ=0,147; $\chi^2_{(42)}$=84; $p<10^{-3}$), third Root 8,2% (Canonical R=0,66; Wilks' Λ=0,571; $\chi^2_{(20)}$=25; p=0,214).

Table 11 shows the Standardized and Raw Coefficients for Canonical Variables. Standardized Coefficients reflect the **relative** contribution of Variable, independent of measurement units, while the Raw Coefficients give information about the **overall** contribution of this variable in the discriminant function values.

Table 11. Standardized and Raw Coefficients and Constants for Canonical Variables

Variables currently in the model	Standardized			Raw		
	Root 1	Root 2	Root 3	Root 1	Root 2	Root 3
Shatalov's Injuries Gastric Muc Ind	1,113	-,691	-,093	1,078	-,669	-,090
Corticosteron normalized by sex, Z	-,310	-1,898	,530	-,353	-2,160	,603
Microbes Number of Neutrophiles	,242	-,699	,400	,252	-,729	,417
Moda HRV, msec	,052	1,335	-,191	,002	,046	-,007
Hassal's corpuscules of Thymus, %	,560	,396	,520	,949	,670	,882
Malonic dyaldehid, μM/L	,081	-,097	-,378	,006	-,007	-,028
Entropy of Immunocytogram, $\bullet 10^{-3}$	,280	,593	,175	7,984	16,93	4,996
Macrophages of Thymus, %	1,065	,277	-,010	,638	,166	-,006
Th-Lymphocytes of Blood, %	,999	,074	,146	,417	,031	,061
Katalase, μM/L•h	1,069	,946	-,225	,023	,020	-,005
Testosterone normalized by sex, Z	1,097	-,413	-,627	,971	-,366	-,554
Entropy of Splenocytogram, $\bullet 10^{-3}$	,873	,599	-,899	13,66	9,375	-14,07
Spleen Mass, mg	-,584	-1,380	,438	-,004	-,009	,003
Acid Phosphatase, IU/L	,247	,786	-,080	,025	,080	-,008
Cholesterol of nonα-LP, mM/L	-,576	-,224	-,446	-2,004	-,778	-1,552
Tc-Lymphocytes of Blood, %	-,902	-,584	,107	-,296	-,191	,035
Fibroblastes of Thymus, %	-,319	-,235	,472	-,200	-,148	,296
Sympathetic tone as AMo HRV, %	-,103	,671	-,625	-,005	,031	-,029
Phosphate, mM/L	-,517	-,462	,090	-2,999	-2,678	,522
Cholesterol of α-LP, mM/L	-,693	,056	-,070	-4,868	,395	-,492
Sodium Erythrocytes, mM/L	,638	-,134	,148	,065	-,014	,015
Basophiles of Thymus, %	-,407	-,266	,208	-,281	-,183	,143
			Constants	-22,98	-16,83	-,644
			Groups	**Means of Roots**		
			Intact	-4,19	-0,85	-0,88
			Naftussya from Truskavets' and Pomyarky + Stress	-0,22	2,11	0,40
			Control (daily) Water + Stress	-0,05	-2,30	1,41
			Distilled Water and Water "Truskavets'ka" + Stress	2,60	-0,59	-0,74

Canonical roots are poorly structured and correlated with discriminant variables in different ways (Table 12).

Table 12. Factor Structure Matrix (Correlations Variables - Canonical Roots); the differences between the averages roots of rats subjected stress and intact; Z-scores of discriminant variables

Variables and Roots (discriminant properties)	Root 1	Root 2	Root 3	NTPS	CWS	DTWS
Root 1 (60,3 %)				**+4,0**	**+4,1**	**+6,8**
Shatalov's Injuries Gastric Muc Ind	**,348**	-,083	-,133	1,10±0,26	1,40±0,40	**2,50±0,23**
Th-Lymphocytes of Blood	**,164**	-,111	-,057	+0,72±0,65	+2,04±0,95	**+3,10±0,80**
Sympathetic tone as AMo HRV	**,110**	-,043	-,233	+0,01±0,20	-0,01±0,21	**+0,58±0,17**
Moda HRV	**-,102**	,205	,085	+0,31±0,27	-0,51±0,31	**-0,61±0,14**
Phosphate	**-,177**	,076	,212	-0,52±0,41	-0,92±0,65	**-2,95±0,89**
Cholesterol of α-Lipoproteins	**-,121**	,110	-,045	-0,20±0,23	-0,73±0,29	**-0,77±0,20**
Spleen Mass	**-,112**	-,020	,030	-0,38±0,18	-0,30±0,23	**-0,61±0,17**
Sodium Erythrocytes	**-,067**	,027	,069	-0,11±0,24	-0,18±0,27	**-0,47±0,27**
Root 2 (31,5 %)				**+3,0**	**-1,5**	**+0,3**
Microbes Number of Neutrophiles	,040	**,283**	,197	**+1,11±0,21**	+0,19±0,27	+0,33±0,18
Basophiles of Thymus	-,037	**,166**	,115	**+0,63±0.33**	-0,18±0,39	-0,23±0,34
Acid Phosphatase	,079	**,118**	-,051	**+1,07±0,41**	+0,14±0,36	+1,03±0,48
Corticosteron normalized	,096	**-,277**	-,090	**-0,54±0,21**	+0,58±0,22	+0,51±0,22
Testosterone normalized	-,047	**-,168**	-,213	**-1,21±0,29**	-0,47±0,47	-0,44±0,25
Root 3 (8,2 %)				**+1,3**	**+2,3**	**+0,1**
Hassal's corpuscules of Thymus	,102	,060	**,332**	+1,40±0,40	**+1,53±0,51**	+1,02±0,28
Macrophages of Thymus	,075	,052	**,276**	+0,88±0,18	**+0,95±0,47**	+0,60±0,27
Fibroblastes of Thymus	-,003	-,048	**,229**	+0,11±0,14	**+0,49±0,23**	0,00±0,20
Cholesterol of nonα-Lipproteins	**-,072**	-,060	**-,296**	-1,02±0,29	**-1,08±0,40**	-0,66±0,28
Tc-Lymphocytes of Blood	-,098	,083	**-,257**	-0,40±0,19	**-0,95±0,25**	-0,58±0,18
Entropy of Splenocytogram	,076	,100	**-,231**	+0,37±0,24	**-0,42±0,42**	+0,58±0,25
Entropy of Immunocytogram	,011	,060	**-,149**	+0,04±0,28	**-0,50±0,52**	+0,09±0,32
Katalase	-,025	,164	**-,105**	+0,30±0,29	**-0,70±0,31**	-0,18±0,31
Malonic dyaldehid	-,081	-,005	**-,108**	-0,36±0,17	**-0,43±0,22**	-0,42±0,14

Note. Shatalov's Injuries Gastric Mucosa Index is expressed in points

The calculation of individual Roots values based on Raw Coefficients for discriminant variables and Constant (Table 11) allows to visualize the status of each rat on information space of Roots (Fig. 3 and 4).

Calculating averages Roots for each group of animals allows to visualize their location (Fig. 5).

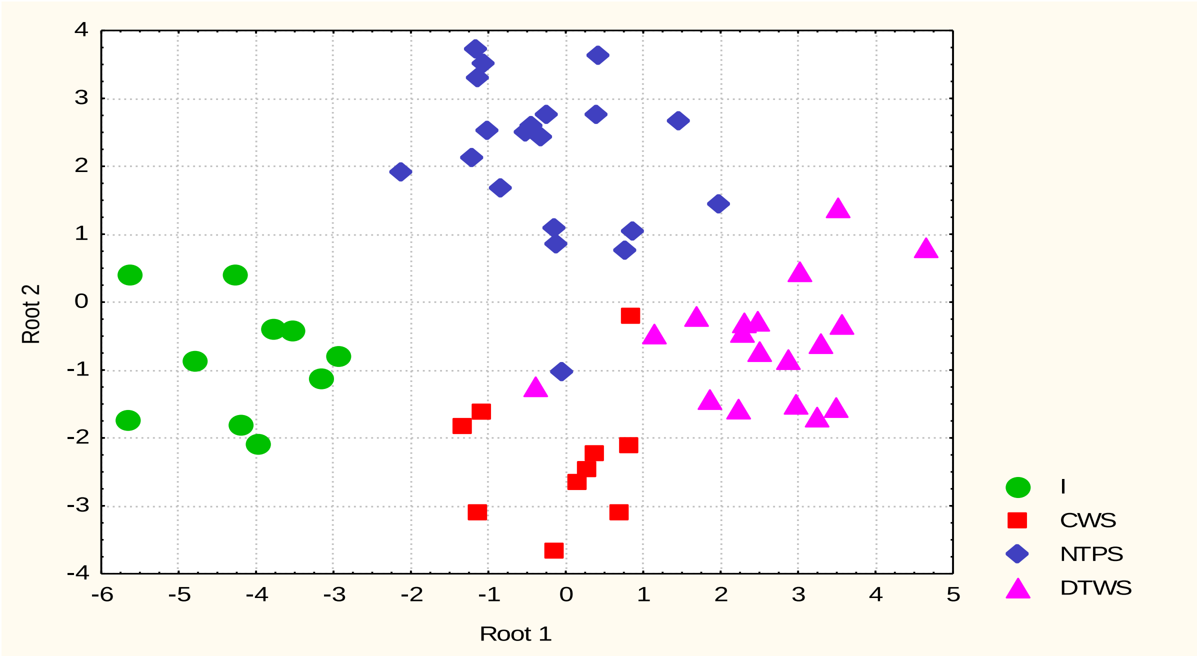


Fig. 3. Individual values first and second canonical roots intact (I) rats and subjected stress (S) with previous use of control water (CW), waters Naftussya (NTP) and distilled water

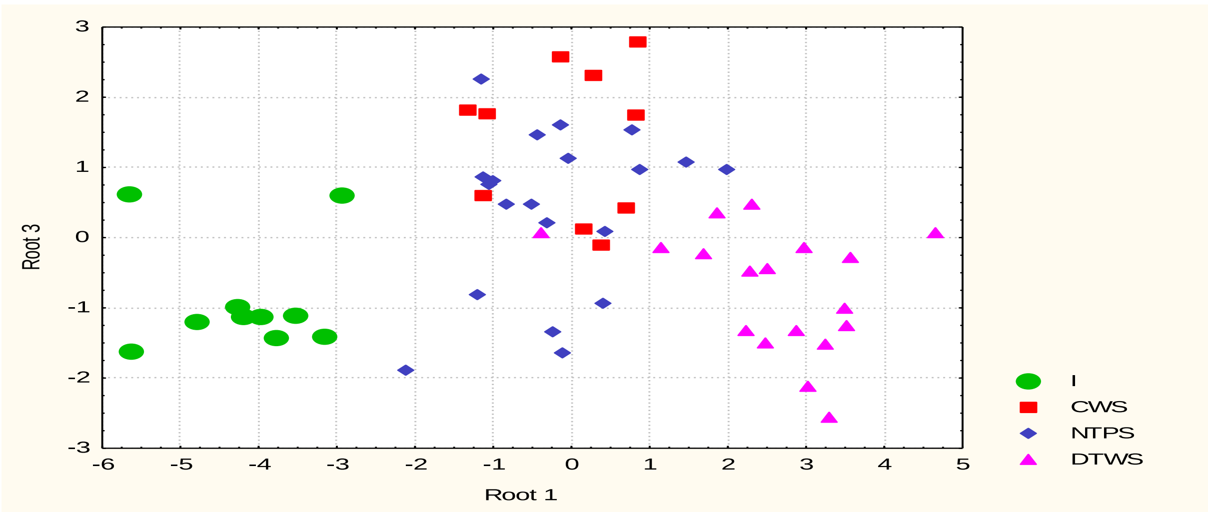
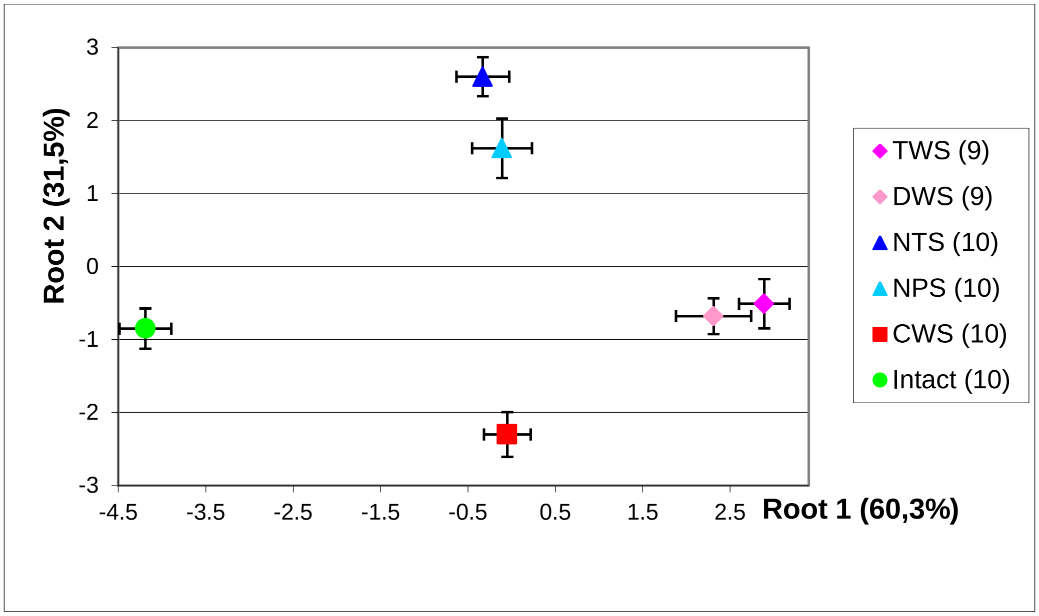


Fig. 4. Individual values first and third canonical roots intact (I) rats and subjected stress (S) with previous use of control water (CW), waters Naftussya (NTP) and distilled water



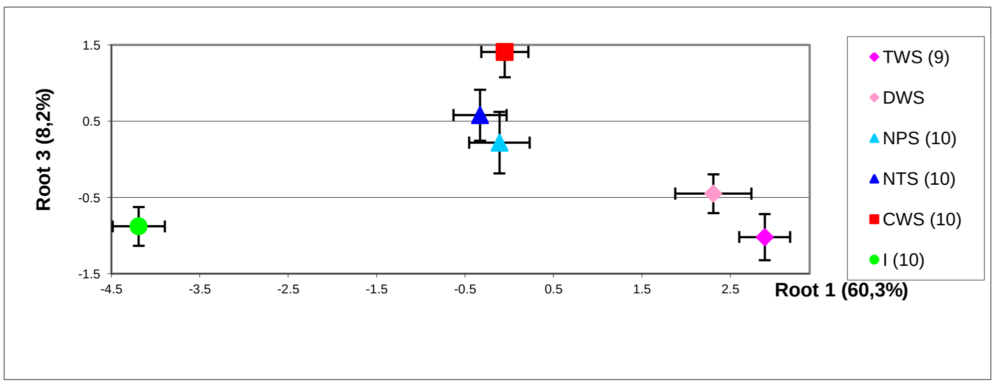


Fig. 5. Mean values canonical roots intact rats and subjected stress (S) with previous use of control water (CW), waters Naftussya Truskavets' (NT), Naftussya Pomyarky (NP), distilled water (DW) and water "Truskavets'ka" (TW)

The noticeable shift along the axis Root 1 control group rats centroid relatively intact group centroid (+4,1) (Fig. 5 top) shows the stress caused significant deviation from this root-related parameters that characterize pathogenic manifestations of stress. Previous use BAWN both fields barely affects the whole of these manifestations (+4,0), while distilled water and water "Truskavets'ka" to increase these (+6,8).

On the other hand, the displacement along the axis Root 2 centroid BAWN relatively centroid intact group up (+3,0), while the control group centroid down (-1,5) reflects sanogenic effects BAWN, while distilled water and water "Truskavets'ka" ineffective (+0,3).

Finally, a less pronounced displacement along the axis Root 3 centroid BAWN relatively centroid intact group bias than the control group centroid (+1,3 vs +2,3) reflects the limitations poststressory deviation of other immune and metabolic parameters. Stresslimiting effect distilled water and water "Truskavets'ka" on these parameters perceptible (+0,1).

Fig. 6 is the same as set out facts and draws attention to the absence of significant differences between the effects BAWN both fields.

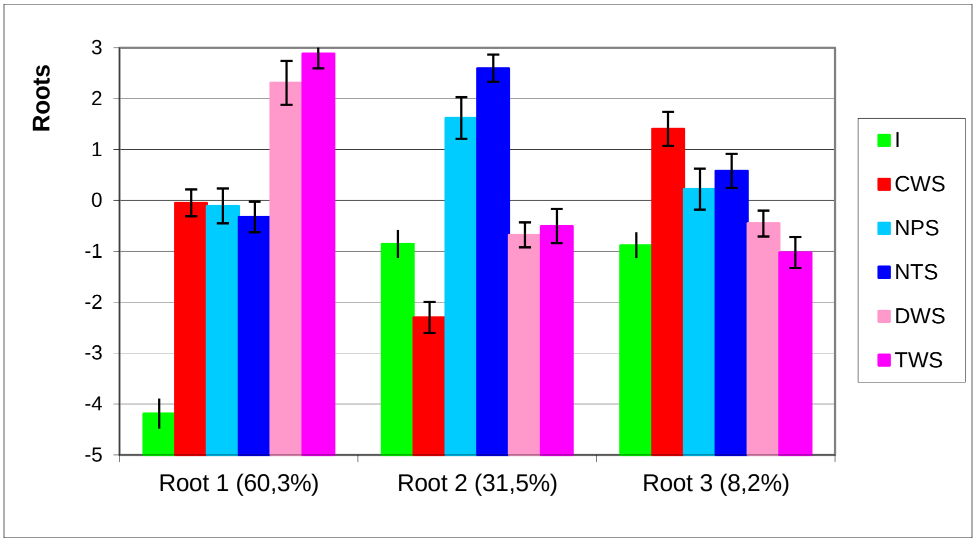


Fig. 6. Mean values first, second and third canonical roots intact rats and subjected stress (S) with previous use of control water (CW), waters Naftussya Pomyarky (NP), Naftussya Truskavets' (NT), "Truskavets'ka" (TW) and distilled water (DW)

In general, all four groups clearly delineated that documented calculations Squared Mahalanobis Distances between groups (Table 13).

Table 13. Squared Mahalanobis Distances between groups (on the diagonal), F-values (df=22) and p-levels (in the diagonal)

Groups (n)	I	CWS	NTPS	DTWS
Intact (10)	0	26	28	50
Control (daily) Water + Stress (10)	3,29 $p<10^{-3}$	0	22	16
Naftussya from Truskavets' and Pomyarky + Stress (20)	4,78 $p<10^{-4}$	3,73 $p<10^{-3}$	0	18
Distilled Water and Water "Truskavets'ka" + Stress (18)	8,11 $p<10^{-6}$	2,55 $p<10^{-2}$	4,42 $p<10^{-4}$	0

Calculation of Classification Functions based Coefficients and Constants (Table 14) allows retrospectively recognize rats from NTPS and DTWS groups with one mistake and intact and control stressed rats without mistake. Total accuracy of classification makes 96,6% (Table 15).

Table 14. Coefficients and Constants for Classification Functions

Variables currently in the model	I p=,172	CWS p=,172	NTPS p=,345	DTWS p=,310
Shatalov's Injuries Gastric Mucosa, points	19,85	25,08	22,04	26,98
Corticosteron normalized, Z	8,03	11,08	1,01	5,16
Microbes Number of Neutrophiles	52,35	55,40	51,73	53,93
Moda HRV, msec	1,14	1,07	1,28	1,17
Hassal's corpuscules of Thymus, %	22,66	27,64	29,55	29,40
Malonic dyaldehid, μM/L	0,28	0,25	0,24	0,31
Entropy of Immunocytogram, $\cdot 10^{-3}$	1,348	1,368	1,436	1,407
Macrophages of Thymus, %	15,11	17,50	18,13	19,48
Th-Lymphocytes of Blood, %	8,92	10,74	10,74	11,76

Katalase, μM/L•h	0,808	0,863	0,953	0,969
Testosterone normalized, Z	43,02	46,30	45,08	49,44
Entropy of Splenocytogram, $\bullet 10^{-3}$	0,440	0,451	0,504	0,533
Spleen Mass, mg	-0,07	-0,07	-0,11	-0,10
Acid Phosphatase, IU/L	0,70	0,67	1,03	0,89
Cholesterol nonα-Lipoproteines, mM/L	-29,35	-40,08	-41,61	-43,38
Tc-Lymphocytes of Blood, %	-7,89	-8,76	-9,59	-9,95
Fibroblastes of Thymus, %	-1,45	-1,39	-2,30	-2,81
Sympathetic tone as AMo HRV, %	0,20	0,07	0,23	0,17
Phosphate, mM/L	59,16	51,81	39,98	38,17
Cholesterol of α-Lipoproteines, mM/L	-167,8	-189,6	-186,6	-200,8
Sodium of Erythrocytes, mM/L	0,61	0,94	0,85	1,05
Basophiles of Thymus, %	-10,18	-10,75	-11,65	-12,11
Constants	-1031	-1097	-1165	-1185

Table 15. Classification Matrix. Rows: Observed classifications. Columns: Predicted classifications

Groups	% Correct	Intact	CWS	NTPS	DTWS
Intact	100	**10**	0	0	0
CWS	100	0	**10**	0	0
NTPS	95,0	0	1	**19**	0
DTWS	94,4	0	1	0	**17**
Total	96,6	10	12	19	17

CONCLUSION

BAWN both Truskavets' and Pomyarky layers minimizes or obviates abnormal 15 parameters caused by stress, but does not affect the poststressory deviation of 7 parameters. On the other hand, BAWN causes deviation of 15 other parameters which little or do not change under stress. Distilled water and water "Truskavets'ka" affect some parameters the opposite way, other less favorable, but some more favorably than BAWN. BAWN both Truskavets' and Pomyarky layers limit pathogenic effects of stress, and initiate or enhance its compensatory and sanogenic effects.

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