Modulating effects of Ukrainian phytocomposition "Balm Truskavets'" on post stress changes in neuroendocrine-immune complex, metabolome, electrocardiogram, and gastric mucosa at rats

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Abstract
Introduction and aim. Earlier our group showed that the Ukrainian phytocomposition "Balm Truskavets'" (UPhCBT) exerts classical adaptogenic effects on parameters of neuro-endocrine-immune complex as well as biophotonics and acupuncture in humans with

maladaptation. The list of important properties of adaptogens includes their stress-limiting effect, which is usually tested in an experiment. Therefore, the purpose of this study was to identify modulating effects of UPhCBT on post-stress changes in neuro-endocrine-immune complex, metabolome, electrocardiogram (ECG), and gastric mucosa at rats.

Material and methods. The experiment is at 18 male and 20 female Wistar rats. 10 animals remained intact with free access to tap daily water. Rats of the control group for 7 days loaded through a tube with same tap daily water (2 mL once), while the animals of main groups received according to a similar scheme 0,1 mL of PhCBT dissolved in 2 mL of daily water or bottled table water "Truskavetska"™. Over the next 10 days, one animal remained intact and 3 other rats were exposed to acute (4 hours) water-immersion and restraint stress. The next day after stressing, EEG, endocrine, immune and metabolic parameters as well as gastric mucosa injuries was recorded.

Results. Acute stress causes in control animals an increase in sympathetic tone, serum levels of catecholamines and corticosterone, combined with a decrease in vagal tone and serum testosterone levels. Such a neuro-endocrine reaction is accompanied by damage to the myocardium and gastric mucosa, an increase in the percentage of macrophages and fibroblasts in the thymus, entropy in the spleen, natural killer cells in the blood. Instead, the mass of the spleen and the percentage of lymphoblasts in it, theophylline-sensitive T-lymphocytes in the blood, the content of α-LP cholesterol in the serum, as well as the catalase and Na,K-ATPase activity of erythrocytes, decrease. Preventive use of phytocomposition, first of all, minimizes or even completely prevents damage to the myocardium and adverse post-stress deviations from the norm of listed parameters. Secondly, it initiates an increase in the level of PTH and the activity of serum acid phosphatase, the percentage of reticulocytes in the spleen and the intensity of phagocytosis of blood neutrophils, but at the same time a decrease in their bactericidal activity, as well as the percentage of monocytes and B-lymphocytes in the blood. Thirdly, it potentiates the post-stress increase in sympathetic tone and damage to the gastric mucosa, as well as natural killers, on the one hand, and the decrease in vagal tone, the level of testosterone in the serum, as well as the mass of the spleen - on the other hand. Fourthly, it reverses the activity of catalase of erythrocytes and the entropy of the splenocytogram.

Conclusion. Ukrainian phytocomposition "Balm Truskavets" has a generally favorable adaptogenic effect on the post-stress state of the neuro-endocrine-immune complex and metabolome. However, there are certain adverse effects as the so-called adaptation fee.

Keywords: Ukrainian phytocomposition "Balm Truskavets", acute water-immersion and restraint stress, neuro-endocrine-immune complex, metabolome, relationships, rats.

Introduction

Earlier our group showed that the Ukrainian phytocomposition "Balm Truskavets'" (UPhCBT; ТУ У 15.8-24055046-005:2009, produced by private research-production enterprise "Ukrainian Balms", Mykolaïv, Ukraine; is analogous to the previous "Balm Kryms'kyi" [1,2,3,4,5]) exerts immediate (in 1,5 hours after use) modulating effects on parameters of EEG and HRV as well as biophotonics (gas discharge visualization, GDV) [6,7]. This gives grounds for finding out the long-term (course) effects of the UPhCBT on these parameters. In a pilot study on 10 volunteers, we found that the use of the UPhCBT for 11 dais causes changes in EEGs parameters accompanied by a sympatho(adreno)mimetic effect. The modulating effects of the UPhCBT on the parameters of the central and autonomous nervous systems are combined with the changes in GDVs parameters [8]. Next study was conducted on a four times larger cohort and with a wider range of methods [9]. A noticeable effect of the UPhCBT on 38 parameters was revealed, grouped into 6 clusters, of which 4 are enhancing and 2 are reducing. In particular, the reduced levels of the adaptation index and phagocytosis

parameters increase significantly, instead, the increased levels of the strain index, testosterone, triiodothyronine, LF band HRV as well as two biophotonics parameters decrease, that is, there is a normalizing/beneficial effect. At the same time, normal levels of HRV-markers of vagal tone decrease, while cortisol and circulating catecholamines as well as the activity of β- and α-rhythm generating neurons increase, but within the normal range. Finally, there is a further increase in the upper limit levels of activity of δ-rhythm generating neurons. Additional use of UPhCBT limits the adverse effects of standard balneotherapy at the Truskavets' Spa in patients with post-radiation encephalopathy by modulating EEG and HRV parameters [10]. Thus, the UPhCBT exerts classical adaptogenic effects on parameters of neuro-endocrine-immune complex as well as biophotonics and acupuncture in humans with maladaptation.

Aim. The list of important properties of adaptogens includes their stress-limiting effect, which is usually tested in an experiment. Therefore, the purpose of this study was to identify modulating effects of UPhCBT on post-stress changes in neuro-endocrine-immune complex, metabolome, electrocardiogram, and gastric mucosa at rats. The study aimed to comprehensively evaluate the adaptogenic properties of this phytocomposition by analyzing its impact on multiple physiological and biochemical parameters in rats subjected to acute stress.

In this article, the following main **research problems** were investigated.

What is the effect of the Ukrainian phytocomposition "Balsam Truskavets" on post-stress changes in the neuroendocrine-immune complex, metabolome, ECG, and gastric mucosa in rats subjected to acute stress?

Does the phytocomposition demonstrate classic adaptogenic effects by mitigating the negative consequences of acute stress in rats?

What are the specific mechanisms of action of the phytocomposition at the physiological and biochemical levels?

Are the effects of the phytocomposition generally beneficial, or are there any adverse side effects?

How do the effects of the phytocomposition compare to the effects of known plant adaptogens, such as ginseng?

What role do aryl hydrocarbon receptors (AhR) play in the mechanism of action of the phytocomposition?

Does the sex of the animals influence the effects of the phytocomposition?

Based on the research problems identified, the following **research hypotheses** appear to be tested in this study.

1. The Ukrainian phytocomposition "Balsam Truskavets" will have a significant modulating effect on post-stress changes in the neuroendocrine-immune complex, metabolome, ECG, and gastric mucosa in rats.

2. The phytocomposition will demonstrate adaptogenic properties by reducing the negative effects of acute stress on various physiological and biochemical parameters in rats.

3. The phytocomposition will act through specific physiological and biochemical mechanisms, potentially involving modulation of stress hormones, immune function, and metabolic processes.

4. The overall effects of the phytocomposition will be predominantly beneficial, but there may be some adverse effects as part of the adaptation process.

5. The effects of "Balsam Truskavets" will be comparable to those of established plant adaptogens like ginseng, particularly in terms of modulating stress responses.

6. Aryl hydrocarbon receptors (AhR) will play a significant role in mediating the adaptogenic effects of the phytocomposition.

7. The adaptogenic effects of the phytocomposition will be consistent across both male and female rats, with potentially minor sex-specific differences.

8. The phytocomposition will demonstrate a protective effect on the myocardium, as evidenced by ECG parameters, while potentially having a different impact on the gastric mucosa.

9. The phytocomposition will modulate specific immune parameters, potentially enhancing some aspects of immune function while suppressing others.

10. The adaptogenic effects of the phytocomposition will be associated with changes in specific metabolic markers and enzyme activities.

These hypotheses are tested through a comprehensive analysis of various physiological, biochemical, and morphological parameters in rats subjected to acute stress, with and without pretreatment with the phytocomposition.

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).

Study design and procedure

In a previous study, it was shown that the severity of post-stress changes in target organs are significantly determined by innate resistance to hypoxia and aerobic muscular performance [11,12,13,14,15]. Therefore, at the preparatory stage, all animals were first tested for resistance to hypoxic hypoxia by the classical method of Berezovskyi VYa [16]. To do this, each rat was placed in a pressure chamber with a transparent lid, in which the pump created a vacuum of air equivalent to a rise to a height of 12 km (20 kPa) and recorded the time of the second agonal breath or seizure. One week later, aerobic muscular performance was determined by the duration of swimming (t^0 water 26^0 C) with a load (5% of body weight) to exhaustion (falling to the bottom of the bath) [17]. After a week of recovery, under light ether anesthesia for 15-20 sec recorded electrocardiogram (ECG) in standard lead II (introducing needle electrodes subcutaneously) followed by calculation of HRV parameters: Mode, Amplitude of Mode (AMo) and Variation scope (MxDMn) as markers of the so-called humoral channel (catecholamines, glucagon, etc) of regulation, sympathetic and vagal tone respectively [18].

On the basis of the received data four qualitatively equivalent groups (equally females and males, practically identical average sizes and variances of swimming and hypoxic tests as well as HRV) were formed. 10 animals remained intact with free access to tap daily water. Rats of the control group (n=10) for 7 days loaded through a tube with same tap daily water (2 mL once), while the animals of main groups received according to a similar scheme 0,1 mL of PhCBT dissolved in 2 mL of daily water (n=8) or bottled table water "Truskavetska"™ (n=10).

Over the next 10 days, one animal remained intact and 3 other rats were exposed to

water-immersion and restraint stress according to the method of Nakamura J et al. [19] in the modification of Popovych IL [20], 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 experiments, 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 (Eo), basophils (Bas), 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 [20,21] on the basis of the classical Shannon's [22] equation:

$$hLCG = -(L \cdot \log_2 L + M \cdot \log_2 M + Eo \cdot \log_2 Eo + Bas \cdot \log_2 Bas + RN \cdot \log_2 RN + PMNN \cdot \log_2 PMNN)/\log_2 6.$$

Then the ECG under light ether anesthesia was re-recorded, 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 [23]) after precipitation nonα-lipoproteins using dextransulfate/Mg^{2+}) as described in the manual [24]. State of lipid peroxidation assessed the content in the serum its products: diene conjugates (spectrophotometry of heptane phase of lipids extract [25]) and malondyaldehide (test with thiobarbituric acid [26]), 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 [28]). 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 in the serum and erythrocytes (flame photometry method) as described in the manual [24]. 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 [29]).

Alanine and asparagine aminotranspherase, alkaline and acid phosphatase as well as creatine phosphokinase determined by uniform methods as described in the manual [24].

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

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 [20]. This scale is based on the qualitative-quantitative scale of Harrington EC [31].

The parameters of immunity were determined, as described in the manual [32]. 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 [30] equation:

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

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), the microbial count (number of microbes absorbed by one phagocyte) and the killing index (percentage of dead microbes) for *Staphylococcus aureus* (ATCC N25423 F49) [33].

The Spleen and Thymus were weighed and made smears-imprints for counting Thymocytogram and Splenocytogram [34,35]. 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 [20,30]:

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

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

Statistical analysis

Statistical processing was performed using a software package "Microsoft Excell" and "Statistica 6.4 StatSoft Inc" (Tulsa, OK, USA).). The level of statistical significance was set at $p < 0.05$ for most analyses.

In this study, the researchers employed a comprehensive set of statistical methods for data analysis and inference.

1. Data Normalization. Variables obtained after acute stress were normalized and expressed as Z-scores. This approach allows for comparison of variables expressed in different units.

2. Screening and Pattern Analysis. The researchers used screening to select a constellation of parameters significantly different in intact rats and those subjected to stress with or without phytocomposition consumption. Patterns were grouped into clusters, and the essential (per se) effect of the phytocomposition was calculated as the algebraic difference between post-stress variables in the main and control groups.

3. Discriminant Analysis. Forward stepwise discriminant analysis was used to identify the most characteristic post-stress parameters for each group. The analysis selected 16 variables (including markers of gastric mucosa injuries, ECGs, metabolic, neuro-endocrine, and immune parameters). Discriminant Function Analysis was performed, providing summary statistics including Wilks' Lambda, Partial Lambda, F-remove, p-value, and Tolerance for each variable.

4. Canonical Analysis. The 16-dimensional space of discriminant variables was transformed into a 2-dimensional space of canonical roots. Canonical correlation coefficients were calculated to characterize the discriminating ability of each root. Raw and standardized coefficients for discriminant variables were provided, along with constants for canonical variables.

5. Mahalanobis Distance Calculation. Squared Mahalanobis Distances between clusters were calculated to document the clear demarcation of clusters. F-values and p-levels were provided for these distances.

6. Classification Functions. Coefficients and constants for classification functions were calculated for retrospective identification of animals belonging to each cluster.

7. Cluster Centroid Analysis. Calculation of cluster centroids was performed to demonstrate the presence or absence of sexual differences between groups.

8. Correlation Analysis. Full structural coefficients (correlation coefficients between discriminant roots and variables) were calculated.

9. Canonical Correlation Analysis. This method was used to establish relationships between the severity of post-stress damage to the gastric mucosa and myocardium, and various neuro-endocrine factors.

10. Factor Analysis. Factor analysis was employed to examine the structure of neuro-endocrine canonical roots.

11. Entropy Calculation. Shannon's entropy was calculated for various cell populations (leukocytogram, immunocytogram, thymocytogram, splenocytogram).

This multi-faceted statistical approach allowed the researchers to comprehensively analyze the complex data set, identify significant patterns and relationships, and draw meaningful conclusions about the adaptogenic effects of the phytocomposition.

Results

Due to the purposeful formation of groups, the potential predictors of post-stress reactions of the neuro-endocrine-immune complex and the metabolome were almost identical both in mean values and, to a lesser extent, in variance (SD). In particular, the hypoxic test (sec) was: 136 ± 59; 130 ± 78; and 134 ± 83; swimming test (min): 19 ± 11; 18 ± 14; and 19 ± 19; HRV Stress index (units) as $(AMo/2\bullet Mo\bullet MxDMn)^{1/3}$: $0{,}14\pm0{,}08$, $0{,}14\pm0{,}03$, and $0{,}14\pm0{,}06$ in intact animals and those exposed to acute stress against the background of daily water or UPhCBT consumption, respectively. This gave reason to believe that the possible differences in the post-stress state of the registered parameters are not related to the initial state of innate predictors of reactivity, but are caused only by the factor used in this study - the phytoadaptogen.

Variables obtained after acute stress were normalized and expressed as Z-scores. This approach allows us to compare the variables expressed in different units [36]. By means of screening, a constellation of precisely such parameters, which are significantly different in intact rats and subjected to stress against the background of daily water and phytocomposition consumption, was selected (Fig. 1).
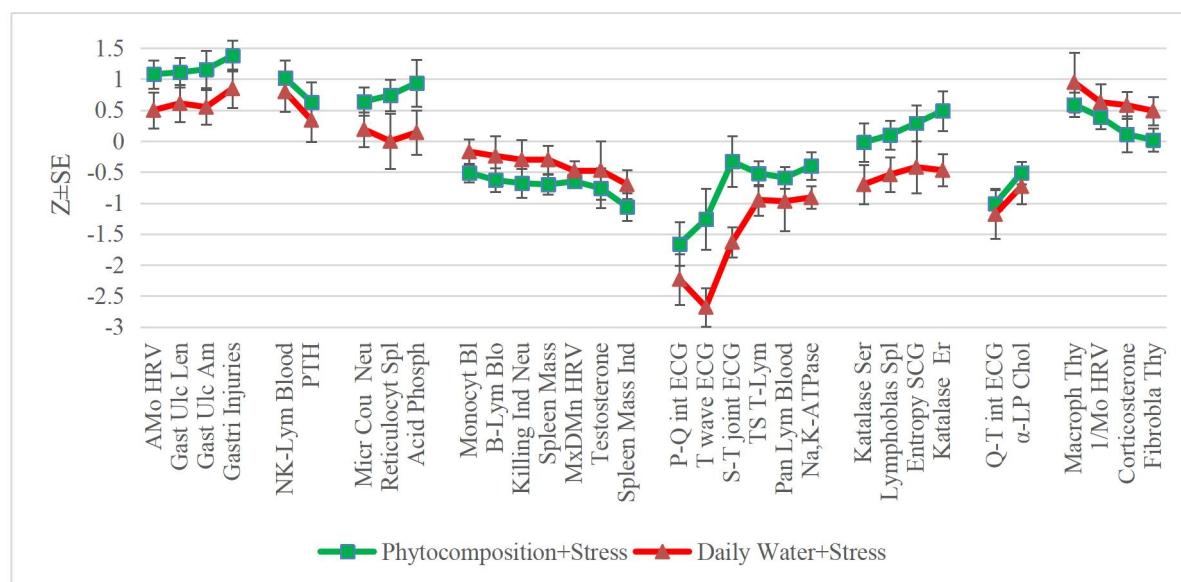

Fig. 1. Patterns of post-stress profiles of rats against the background of daily water and phytocomposition consumption
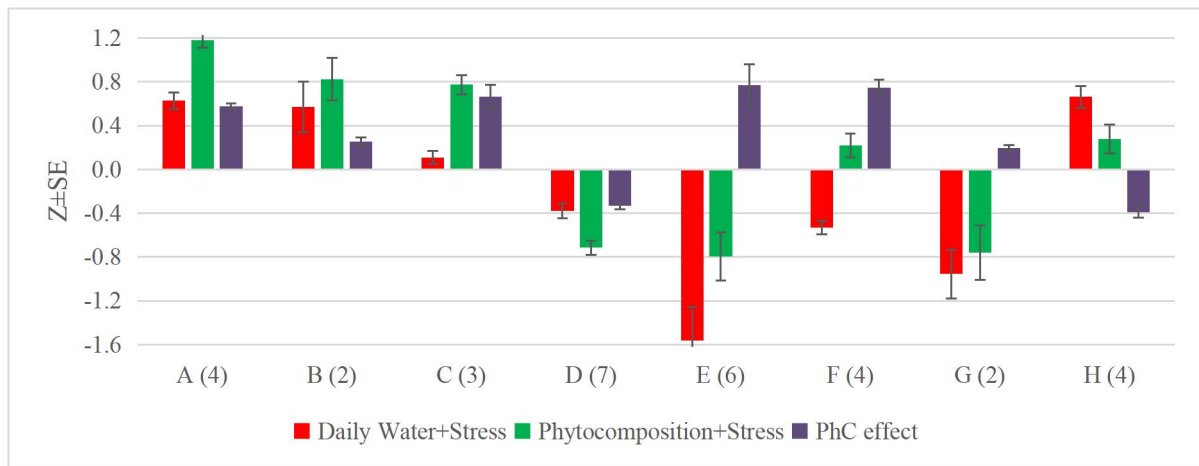
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Fig. 2. Clusters of post-stress variables (n) of rats against the background of **daily water** and **phytocomposition** consumption. Essential (*per se*) effects of phytocomposition calculated as algebraic difference

Further, the patterns were grouped into 8 clusters and for each cluster the essential (per se) effect of the phytocomposition was calculated as the algebraic difference between the post-stress variables in the main and control groups (Fig. 2). It was found that the phytocomposition exerts enhancing effects on the post-stress variables condensed in 6 clusters, while on the other variables - weakening ones.

In order to identify exactly those post-stress parameters (variables) whose constellation is characteristic for each group, the available informational field was subjected to discriminant analysis by the method of forward stepwise [37]. To include in the model (Tables 1 and 2), the program has selected 16 variables (3 **markers of gastric mucosa injuries**, 2 **ECGs**, 3 **metabolic**, 3 **neuro-endocrine**, as well as 5 **immune**).

Table 1. Discriminant Function Analysis Summary

Variables currently in the model	Groups (n)			Parameters of Wilks' Statistics				
	Intact Rats (10)	CW+ Stress (10)	PhC+ Stress (18)	Wilks' Λ	Parti-al Λ	F-re-move (5.6)	p-value	Tole-rancy
Gastric Ulcers Amount	0	1.0 0.5	2.1 0.5	0,050	0,663	5,090	0,016	0,072
Gastric Ulcers Length, mm	0	2.1 1.0	3.8 0.8	0,036	0,918	0,899	0,422	0,063
Gastric Mucosa Injury, points	0	0.21 0.08	0.34 0.06	0,066	0,503	9,884	0,001	0,099
T wave ECG, µV	131 3	61 8	98 13	0,052	0,641	5,605	0,012	0,138
S-T joint ECG, µV	54 5	13 6	45 10	0,042	0,786	2,729	0,090	0,155
Na,K-ATPase of Erythrocytes, M/L•h	0,77 0,06	0,60 0,03	0,69 0,04	0,068	0,485	10,64	0,001	0,180
Katalase of Erythrocytes, µM/L•h	227 17	202 14	253 17	0,050	0,659	5,171	0,015	0,507
Acid Phosphatase, IU/L	31.4 1.9	32.2 2.1	37.0 2.2	0,036	0,901	1,094	0,354	0,449
AMo HRV as Sympathetic tone, %	48 6	73 6	67 4	0,041	0,799	2,520	0,106	0,263
MxDMn HRV as Vagal tone, msec	51 14	16 3	32 5	0,043	0,770	2,995	0,073	0,212
Corticosterone, Z	0 0.32	+0.58 0.22	+0.11 0.29	0,041	0,803	2,458	0,111	0,162
Macrophages	5.39	6.89	6.31	0,057	0,575	7,401	0,004	0,325

Variables				Wilks' Λ	Partial Λ	F to enter	p-value	Tolerancy
of Thymus, %	0.50	0.75	0.31					
Microbial Count of Neutrophils, Bac/Phag	5.5 0.3	5.7 0.3	6.2 0.2	0,050	0,662	5,099	0,016	0,172
Killing Index of Neutrophils, %	47.5 2.9	44.7 3.0	41.1 2.1	0,039	0,851	1,751	0,199	0,506
Theophylline-susceptible T-Lymphocytes, %	15,3 1,1	11.9 0.9	13.4 0.7	0,039	0,853	1,723	0,204	0,636
B-Lymphocytes of Blood, %	13.4 0.8	12.8 0.8	11.8 0.5	0,050	0,654	5,285	0,014	0,327

Step 16, N of vars in model: 16; Grouping: 3 grps; Wilks' Λ: 0.033; appr. $F_{(32.4)}=5.6$; $p<10^{-6}$. In each column, the top row is the average, the bottom is the standard error.

Table 2. 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
Gastric Mucosa Injury, points	9,444	0,001	0,649	9,44	10^{-3}
S-T joint ECG, μV	6,649	0,004	0,467	7,88	10^{-4}
Na,K-ATPase of Erythrocytes, M/L•h	5,908	0,006	0,344	7,76	10^{-6}
Macrophages of Thymus, %	5,896	0,007	0,251	7,96	10^{-6}
Theophylline-susceptible T-Lymphocytes, %	4,370	0,021	0,196	7,81	10^{-6}
Katalase of Erythrocytes, μM/L•h	3,892	0,031	0,156	7,68	10^{-6}
Gastric Ulcers Amount	4,013	0,029	0,122	7,72	10^{-6}
T wave ECG, μV	2,475	0,102	0,104	7,38	10^{-6}
B-Lymphocytes of Blood, %	2,193	0,131	0,089	7,05	10^{-6}
AMo HRV as Sympathetic tone, %	4,787	0,017	0,065	7,59	10^{-6}
Microbial Count of Neutrophils, Bac/Phag	1,634	0,215	0,058	7,20	10^{-6}
MxDMn HRV as Vagal tone, msec	1,324	0,285	0,052	6,78	10^{-6}
Gastric Ulcers Length, mm	1,566	0,230	0,046	6,51	10^{-6}
Corticosterone, nM/L	1,150	0,335	0,041	6,16	10^{-6}
Killing Index of Neutrophils, %	1,349	0,281	0,037	5,92	10^{-6}
Acid Phosphatase, IU/L	1,094	0,354	0,033	5,63	10^{-6}

The rest of the registered variables were left out of the model (Table 3), although some of them carry discriminant (recognizable) information.

Table 3. Variables currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics				
	Intact Rats (10)	CW+ Stress (10)	PhC+ Stress (18)	Wilks' Λ	Partial Λ	F to enter	p-value	Tolerancy
P-Q interval ECG, msec	55.6 0.8	45.4 1.9	48.0 1.6	0.032	0.992	0.790	0.496	0.405
Q-T interval ECG, msec	104.9 1.2	92.0 4.3	93.8 2.7	0.030	0.955	0.450	0.545	0.430
α-LP Cholesterol, mM/L	0.84 0.05	0.73 0.04	0.76 0.03	0.033	0.970	0.380	0.404	0.342
Katalase of Serum, μM/L•h	143 12	116 12	142 12	0,033	0,998	0,018	0,983	0,501
Mode HRV as inverse Catecholamines, msec	175 10	155 9	163 6	0.034	0.925	0.530	0.618	0.482
Parathyroid hormone normalized by sex, Z	0 0.32	0.30 0.30	0.56 0.30	0,032	0,961	0,386	0,685	0,345
Testosterone normalized by sex, Z	0 0.32	-0.74 0.58	-0.99 0.34	0.035	0.941	0.400	0.596	0.410
Spleen Mass,	773	718	644	0,033	0,996	0,036	0,965	0,350

	58	42	30					
mg								
Spleen Mass Index, mg/100 g Body Mass	375 25	320 18	292 17	0,033	0,997	0,027	0,974	0,450
Reticulocytes of Spleen, %	2.67 0.22	2.67 0.32	3.19 0.18	0,033	0,998	0,016	0,985	0,662
Lymphoblastes of Spleen, %	8.6 10	6.8 0.9	8.9 0.8	0,031	0,932	0,690	0,514	0,601
Entropy of Splenocytogram	0,534 0,019	0,552 0,018	0,509 0,025	0,032	0,969	0,300	0,744	0,589
Fibroblastes of Thymus, %	5.33 0.65	6.33 0.47	5.38 0.38	0,031	0,927	0,749	0,486	0,465
Pan Lymphocytes of Blood, %	51.8 1.5	47.2 2.3	49.0 0.9	0,030	0,920	0,824	0,454	0,485
Monocytes of Blood, %	6.20 0.73	5.81 0.46	5.02 0.35	0,033	0,985	0,147	0,864	0,572
NK-Lymphocytes of Blood, %	5.29 0.35	6.18 0.36	6.43 0.32	0.032	0.965	0.450	0.401	0.457

Note. Testosterone and parathyroid hormone levels are normalized by sex (41.8 ± 1.7 vs 3.53 ± 0.24 nM/L and 154 ± 12 vs 185 ± 3 ng/L in intact male vs female respectively).

Than the 16-dimensional space of discriminant variables transforms into 2-dimensional space of a canonical roots, which are a linear combination of discriminant variables. The discriminating ability of the root characterizes the canonical correlation coefficient (r*) as a measure of the degree of dependence between groups and a roots. It is for Root 1 0.906 (Wilks' $\Lambda=0.033$; $\chi^2_{(32)}=94$; $p<10^{-6}$), for Root 2 0.903 (Wilks' $\Lambda=0.185$; $\chi^2_{(15)}=46$; $p<10^{-4}$). The first root contains 51% of discriminative opportunities, the second 49%.

Table 4 presents raw (actual) and standardized (normalized) coefficients for discriminant variables. The raw coefficient gives information on the absolute contribution of this variable to the value of the discriminative function, whereas standardized coefficients represent the relative contribution of a variable independent of the unit of measurement. They make it possible to identify those variables that make the largest contribution to the discriminatory function value.

Table 4. Standardized and Raw Coefficients and Constants for Canonical Variables

Coefficients	Standardized		Raw	
Variables	**Root 1**	**Root 2**	**Root 1**	**Root 2**
Gastric Mucosa Injury, points	0,894	-2,316	0,785	-2,035
S-T joint ECG, µV	0,439	-1,226	0,014	-0,038
Na,K-ATPase of Erythrocytes, M/L•h	-0,768	1,710	-4,745	10,57
Macrophages of Thymus, %	0,749	-1,019	0,460	-0,625
Theophylline-susceptible T-Lymphocytes, %	0,116	0,520	0,037	0,167
Katalase of Erythrocytes, µM/L•h	0,609	0,672	0,010	0,011
Gastric Ulcers Amount	0,333	2,367	0,185	1,313
T wave ECG, µV	1,231	1,286	0,031	0,032
B-Lymphocytes of Blood, %	-0,867	-0,734	-0,380	-0,322
AMo HRV as Sympathetic tone, %	0,508	0,823	0,028	0,045
Microbial Count of Neutrophils, Bac/Phag	1,533	0,196	1,498	0,191
MxDMn HRV as Vagal tone, msec	1,099	-0,340	0,043	-0,013
Gastric Ulcers Length, mm	0,832	-0,960	0,279	-0,322
Corticosterone, nM/L	1,080	0,564	0,011	0,005
Killing Index of Neutrophils, %	-0,456	-0,389	-0,049	-0,042
Acid Phosphatase, IU/L	0,368	0,365	0,047	0,046
	Constants		-18,30	-9,442
	Eigenvalues		4,60	4,40
Cumulative Proportions			0.511	1

The third discriminant parameter is the full structural coefficients (Table 5), that is, the coefficients of correlation between the discriminant root and variables. The structural coefficient shows how closely variable and discriminant functions are related, that is, what is the portion of information about the discriminant function (root) contained in this variable. The calculation of the discriminant root values for each animal as the sum of the products of raw coefficients to the individual values of discriminant variables together with the constant enables the visualization of each rat in the information space of the roots.

Table 5. Correlations Variables-Canonical Roots, Means of Roots and Z-scores of Variables

Variables	Correlations Variables-Canonical Roots		Intact Rats (10)	CW+ Stress (10)	PhC+ Stress (18)	Effect of PhC	Clus-ter on Fig. 1
Root 1 (51%)	**Root 1**	**Root 2**	-2.41	-1.44	**2.14**		
AMo HRV as Sympathetic tone	**0.202**	-0.034	0	0.50 / 0.29	**1.08** / **0.23**	0.58 / 0.23	A
Gastric Ulcers Length	**0.242**	-0.085	0	0.61 / 0.30	**1.11** / **0.24**	0.50 / 0.24	A
Gastric Ulcers Amount	**0.230**	-0.060	0	0.55 / 0.28	**1.16** / **0.30**	0.61 / 0.30	A
Gastric Mucosal Damage	**0.298**	-0.171	0	0.85 / 0.31	**1.38** / **0.25**	0.53 / 0.25	A
NK-Lymphocytes of Blood			0	0.80 / 0.32	**1.02** / **0.29**	0.22 / 0.29	B
Parathyroid hormone			0	0.30 / 0.30	**0.56** / **0.30**	0.26 / 0.30	B
Microbial Count of Neutrophils	**0.145**	-0.010	0	0.19 / 0.28	**0.64** / **0.23**	0.46 / 0.23	C
Reticulocytes of Spleen			0	0.00 / 0.45	**0.74** / **0.25**	0.74 / 0.25	C
Acid Phosphatase	**0.149**	0.014	0	0.14 / 0.36	**0.94** / **0.38**	0.80 / 0.38	C
Monocytes of Blood			0	-0.17 / 0.20	**-0.51** / **0.15**	-0.34 / 0.15	D
B-Lymphocytes of Blood	**-0.140**	0.023	0	-0.24 / 0.32	**-0.63** / **0.19**	-0.39 / 0.19	D
Killing Index of Neutrophils	**-0.137**	0.031	0	-0.30 / 0.32	**-0.68** / **0.23**	-0.38 / 0.23	D
Spleen Mass			0	-0.30 / 0.23	**-0.70** / **0.16**	-0.40 / 0.16	D
MxDMn HRV as Vagal tone	**-0.173**	0.094	0	-0.48 / 0.16	**-0.65** / **0.08**	-0.17 / 0.08	D
Testosterone			0	-0.74 / 0,58	**-0.99** / **0.34**	-0.25 / 0.34	D
Spleen Mass Index			0	-0.70 / 0.23	**-1.06** / **0.22**	-0.36 / 0.22	D
Root 2 (49%)	**Root 1**	**Root 2**	2.41	**-3.06**	0.37		
P-Q interval ECG			0	**-2.23** / **0.41**	-1.66 / 0.35	0.57 / 0.36	E
T wave ECG	-0.041	**0.312**	0	**-2.68** / **0.31**	-1.26 / 0.49	1.43 / 0.49	E
S-T joint ECG	0.048	**0.239**	0	**-1.63** / **0.24**	-0.33 / 0.41	1.30 / 0.41	E
Theophylline-susceptible T-Lymphocytes	-0.045	**0.192**	0	**-0.95** / **0.25**	-0.52 / 0.20	0.43 / 0.20	E
Pan Lymphocytes of Blood			0	**-0.97** / **0.48**	-0.59 / 0.18	0.38 / 0.18	E
Na,K-ATPase of Erythrocytes	0.009	**0,216**	0	**-0,91** / **0,18**	-0,40 / 0,22	0,51 / 0,22	E

11

Catalase of Serum			0	**-0.70** **0.32**	-0.02 0.31	0.68 0.31	F
Lymphoblastes of Spleen			0	**-0.54** **0.28**	0.10 0.23	0.63 0.23	F
Entropy of Splenocytogram			0	**-0.42** **0.42**	0.29 0.29	0,71 0.42	F
Catalase of Erythrocytes	0.134	**0.100**	0	**-0.47** **0.26**	0.49 0.32	0.96 0.32	F
Q-T interval ECG			0	**-1.18** **0.39**	-1.01 0.24	0.17 0.24	G
α-LP Cholesterol			0	**-0.73** **0.29**	-0.51 0.18	0.22 0.18	G
Macrophages of Thymus	0.055	**-0.150**	0	**0.95** **0.48**	0.59 0.20	-0.37 0.20	H
1/Mode HRV as Catecholamines			0	**0.63** **0.29**	0.39 0.19	-0.25 0.19	H
Corticosterone	0.001	**-0.084**	0	**0.58** **0.22**	0.11 0.29	-0.46 0.29	H
Fibroblastes of Thymus			0	**0.49** **0.23**	0.02 0.19	-0.47 0.19	H

Localization of the cluster of animals exposed to stress against the background of daily water loads in the lowest zone of the axis of the second root (Fig. 3) reflects the maximum for the sample depression of the T wave and the S-T joint and the shortening of the P-Q and Q-T EEG intervals, a decrease the percentage of lymphocytes in general and theophylline-sensitive T-lymphocytes in particular in the blood, lymphoblasts in the Spleen and entropy of splenocytogram, cholesterol levels in α-lipoproteins, as well as erythrocyte Na,K-ATPase and catalase activity. On the other hand, the lowest localization of the cluster reflects the maximum rise of the percentage of macrophages and fibroblastes in the Thymus as well as catecholamines and corticosterone levels in the serum. The intermediate position of rats of the main group relative to control and intact animals, and even partial mixing with the latter, reflects the minimization or even prevention of the effects of stress on the listed variables.
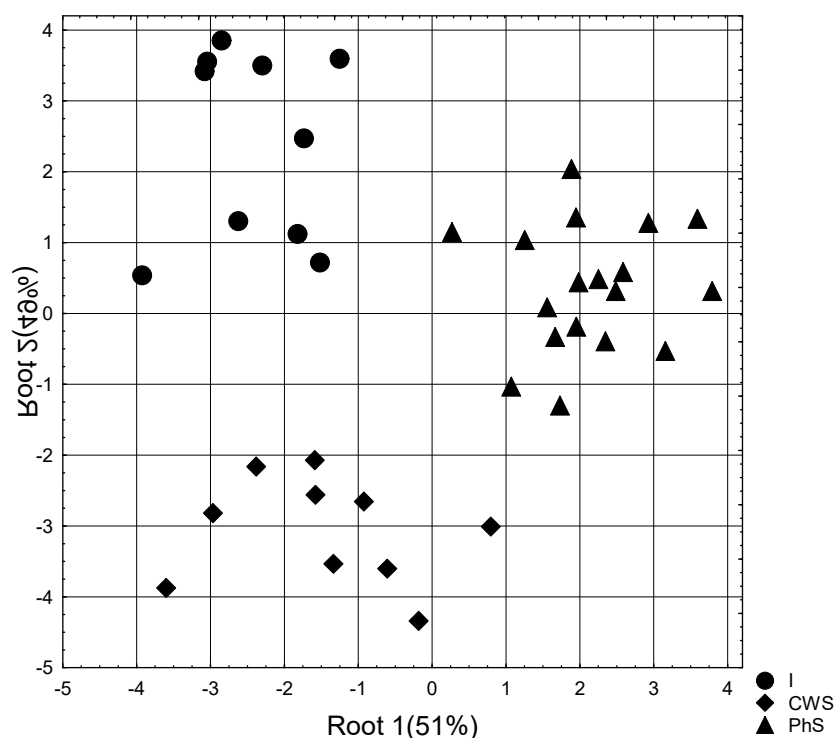
Fig. 3. Individual values of discriminative roots of **intact** rats and one day after acute stress, which was preceded by weekly administration of **daily (control) water** (CWS) and **phytocomposition** (PhS). The roots contain condensed information about 30 parameters

The shift along the first root axis of the cluster of rats of the main group to the right relative to the cluster of both intact and control animals reflects, firstly, the potentiation by phytocomposition the post-stress increase in sympathetic tone and the content of NK-lymphocytes in the blood, as well as damage to the gastric mucosa, on the one hand, and a decrease in vagal tone, the level of testosterone in the serum, and the mass index of the spleen - on the other hand. Secondly, such extreme localization reflects the initiation by the phytocomposition of an increase in the level of PTH and the activity of serum acid phosphatase, the intensity of phagocytosis of blood neutrophils and the percentage of reticulocytes in the Spleen, on the one hand, and a decrease in the bactericidal activity of blood neutrophils, the percentage of monocytes and B-lymphocytes in the blood as well as absolute mass of Spleen - on the other hand.

The apparent clear demarcation of clusters is documented by calculating Mahalanobis distances (Table 6).

Table 6. Squared Mahalanobis Distances between clusters (above the diagonal), F-values (df=16.2) and p-levels (under the diagonal)

Clusters	Intact Rats	CW + Stress	UPhCBT + Stress
Intact Rats (10)	0	30.9	24.9
CW + Stress (10)	5.5 0.0003	0	24.5
UPhCBT + Stress (18)	5.7 0.0002	5.6 0.0002	0

The calculation of cluster centroids demonstrates, firstly, the absence of sexual differences both between intact rats and stressed female rats pretreated by the phytocomposition soluted in daily water; secondly, the moderate difference between the stressed male rats pretreated by

the phytocomposition soluted in the "Truskavetska"™ bottled table water; thirdly, the moderate sexual difference between stressed rats pretreated by the daily water (Fig. 4).
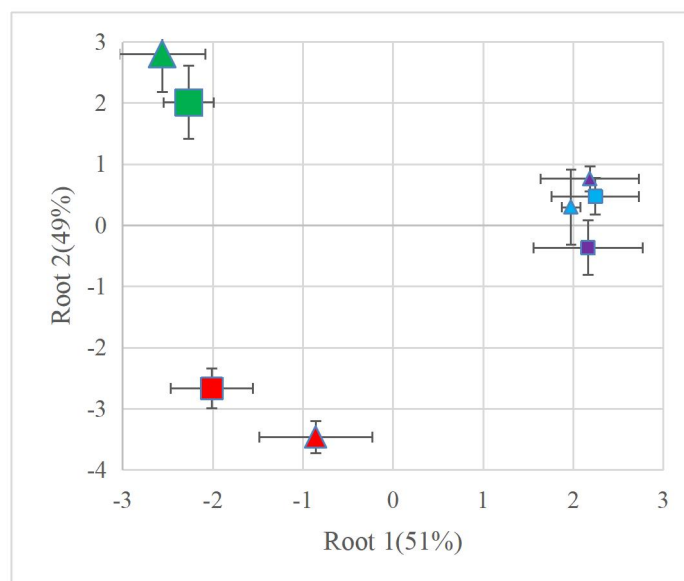

Fig. 4. Mean (M±SE) values of discriminant roots of female (triangles) and male (squares) rats **intact** and one day after acute stress, which was preceded by weekly administration of **daily water** and phytocomposition dissolved in **daily water** or "**Truskavetska**"™ **water**

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 7).

The accuracy of classification (retrospective recognition) is **100%**.

Table 7. Coefficients and Constants for Classification Functions

Clusters	Intact Rats (10)	CW + Stress (10)	UPhCBT + Stress (18)
Variables	p=0.263	p=0.263	p=0.474
Gastric Mucosa Injury, points	-15,668	-3,771	-7,945
S-T joint ECG, µV	-0,302	-0,080	-0,162
Na,K-ATPase of Erythrocytes, M/L•h	52,861	-9,596	9,705
Macrophages of Thymus, %	3,071	6,940	6,438
Theophylline-susceptible T-Lymphocytes, %	6,986	6,109	6,814
Katalase of Erythrocytes, µM/L•h	0,259	0,208	0,283
Gastric Ulcers Amount	16,118	9,117	14,282
T wave ECG, µV	0,860	0,716	0,934
B-Lymphocytes of Blood, %	-8,657	-7,270	-9,730
AMo HRV as Sympathetic tone, %	0,738	0,517	0,773
Microbial Count of Neutrophils, Bac/Phag	56,966	57,387	63,395
MxDMn HRV as Vagal tone, msec	1,045	1,160	1,269
Gastric Ulcers Length, mm	1,627	3,659	3,551
Corticosterone, nM/L	0,625	0,606	0,662
Killing Index of Neutrophils, %	-0,856	-0,674	-0,995
Acid Phosphatase, IU/L	2,851	2,643	2,969
Constants	-439,437	-405,625	-499,458

The article does not present a traditional hypothesis testing process with clearly formulated null and alternative hypotheses and formal statistical tests for each hypothesis. Instead, the researchers applied a more exploratory and descriptive approach to data analysis.

Nevertheless, we can analyze the results in the context of the main research problems and inferred hypotheses.

1. Effect of phytocomposition on post-stress parameters. Hypothesis confirmed: The phytocomposition had a significant impact on many post-stress parameters, as demonstrated by discriminant and canonical analysis. Differences between groups were statistically significant ($p < 10^{-6}$ for the first canonical root).

2. Adaptogenic effects. Hypothesis partially confirmed: The phytocomposition showed classic adaptogenic effects, minimizing or preventing myocardial damage and adverse deviations from the norm of many parameters. However, some adverse effects were also observed.

3. Mechanisms of action. Hypothesis confirmed: Specific mechanisms of action were identified, including modulation of corticosterone levels, testosterone, and sympathetic system activity.

4. Overall benefit of effects. Hypothesis partially confirmed: Most effects were beneficial, but some adverse effects were also observed, e.g., exacerbation of gastric mucosal damage.

5. Comparison with ginseng. Hypothesis confirmed: The effects of the phytocomposition were comparable to those of ginseng, particularly in terms of modulating stress responses.

6. Role of AhR receptors. Hypothesis confirmed: The role of AhR receptors in the mechanism of action of the phytocomposition was discussed, although no direct tests were conducted.

7. Effect of sex. Hypothesis partially confirmed: Some sex differences in response to the phytocomposition were observed, but they were not significant for most parameters.

The verification process was based mainly on discriminant, canonical, and correlation analysis. The statistical significance of differences between groups was assessed using p-values, with $p < 0.05$ considered significant. No formal tests of null hypotheses against alternatives were presented for each hypothesis.

Conclusions were drawn based on observed differences between groups, strength of correlations, and biological significance of observed effects. When effects were consistent with expectations and statistically significant, hypotheses were considered confirmed. When results were mixed or unclear, hypotheses were considered partially confirmed.

Discussion

First of all, by applying the water-immersion and restraint stress (WIRS) model, we reproduced Selye's primary attributes of stress: an increase in the mass of the adrenal glands, an increase in the level of corticosterone, involution of lymphoid tissue (a decrease in the mass of the spleen and the content of lymphocytes as well as eosinophils in the blood) and erosive-ulcerative damage to the gastric mucosa, on the one hand, and Cannon's: an increase in the level of circulating catecholamines and sympathetic tone and a reciprocal decrease in vagal tone, which causes myocardial damage - on the other hand [34,38,39,40,41,42,43]. The moderate severity of the main signs of stress is explained by our use of gentle stressor parameters (water temperature and duration of water immersion). However, the real picture has more colors, and the immune manifestations of stress are ambiguous. Now in more detail.

We found that erosive-ulcerative lesions of the gastric mucosa are combined with changes in EEG parameters, in particular, depression of the T wave and the S-T junction, which indicate myocardial dystrophy [41,43]. Using the method of canonical correlation analysis of the same sample, we previously established that there is a close relationship between the severity of post-stress damage to the gastric mucosa and myocardium (R=0.706; R^2=0.499; $\chi^2_{(12)}$=30.2; p=0.003). This connection is due to the damaging effect on both targets of the post-stress increase in the levels of parathyroid hormone, addosterone and catecholamines as well as sympathetic tone with a simultaneous decrease in testosterone and vagal tone. Such

neuro-endocrine reactions determine the severity of damage to the gastric mucosa and myocardium by 56% (R=0.750 [44].

The absence in the factor structure of the neuro-endocrine canonical root of corticosterone, which indicates the insignificance of its causal influence on post-stress damage to the gastric mucosa and myocardium, turned out to be a surprise. However, this is in excellent agreement with the ambiguous role of corticosterone: some authors consider corticosterone to be a gastroaltering factor [45], while others consider it to be gastroprotective [46,47]. The role of the vagal nerve is likely to be dual, as it can mediate both mucosal damaging and protective effects [45]. Nevertheless, in our experiment, vagal tone was established as a gastroprotective factor, albeit a weak one [44].

A previously conducted analysis of the metabolic and immune support of the gastric mucosa and myocardium in intact and stressed rats revealed the following [48]. Serum levels of Phosphates, Catalase and α-LP cholesterol as well as erythrocyte level of Potassium (as marker of kalihistia) and Na,K-ATPase activity of the shadows of erythrocytes (as marker of membranes of myocardiocytes) are positively correlated with ECG markers of myocardial damage, and negatively correlated with visual markers of damage to the gastric mucosa, i.e. reflect intactness (normality) of both targets of stressors. Erythrocyte level of Sodium (as marker of natrihistia) and serum levels of Potassium and Alkaline Phosphatase reflect the intactness of the gastric mucosa only. While serum level of Calcium reflects damage to the gastric mucosa. Taken together, the listed metabolic factors determine the morpho-functional state of the gastric mucosa and myocardium by 72% (R=0.851).

The metabolome, in turn, is determined by the constellation of neuro-endocrine effectors of stress [48]. In particular, enzymes as markers of cytolysis, α-LP Cholesterol, Potassium of serum and Sodium of erythrocytes are upregulated by Testosterone and Calcitonin while are downregulated by Corticosterone, Aldosterone, PTH and, probably, judging by the negative correlation with the Sex index, female sex hormones such as Progesterone and Estradiol. Superoxide dismutase, Diene conjugates, Calcium and Sodium of serum as well as Potassium of erythrocytes and Na,K-ATPase of the shadows of erythrocytes are downregulated by Testosterone, Calcitonin, Catecholamines and Sympathetic tone while are upregulated by Corticosterone, Aldosterone, PTH, Vagal tone and, probably, female sex hormones. Catalase and Malondialdehyde are downregulated by Corticosterone and PTH while upregulated by Calcitonin and Vagal tone. Phosphates are downregulated by PTH and Calcitonin. The canonical correlation between the listed neuro-endocrine and metabolic parameters is very strong: R=0.974.

Damage to the gastric mucosa and myocardium is more severe, the lower the bactericidal activity of blood neutrophils, and the greater the mass of the Thymus. The spleen mass and the content of fibroblasts in the thymus are negatively correlated only with the severity of damage to the gastric mucosa, while the percentages of reticulocytes and lymphoblasts in the Spleen are positively correlated with it. Finally, the higher the percentage of macrophages in the Thymus, the deeper the damage to the myocardium. The canonical correlation between the listed immune parameters and markers of the two targets of stressors is very strong (R=0.809) [48].

Dhabhar FS [49,50] noted that stress may suppress immune function under some conditions while enhancing it under others: acute stress enhances while chronic stress suppresses immune function. We discovered that the constellation of neuro-endocrine effectors of stress determine the immunity at naïve and stressed rats on 95% (R=0.976) [48]. This is perfectly consistent with the concept of a triune neuro-endocrine-immune complex [51] and the results of experimental and clinical studies of the Truskavetsian Scientific School, conducted in line with this concept [20,21,30,33,36,52,53,54,55,56,57,58,59,60,61].

The main goal of this study is to find out the essential effects of the phytocomposition. One of the approaches tested in our previous studies is the calculation of algebraic differences between the Z-scores of variable animals that received an aqueous solution of the phytocomposition and only water-solvent. The revealed effects of the phytocomposition were collected in 7 morpho-functional-metabolic clusters (Fig. 5).
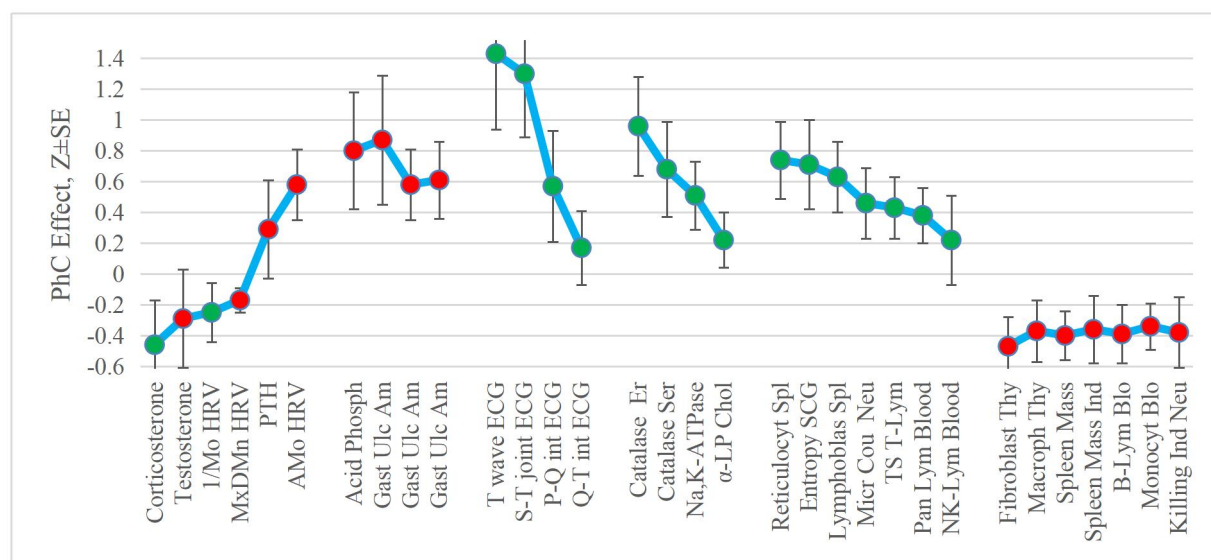

Fig. 5. Clusters of simulated **favorable** and **unfavorable** effects of phytocomposition per se (essentially) on post-stress parameters of rats. See please Table 4

Among the registered neuro-endocrine effectors of acute stress, the phytocomposition most significantly affected the serum corticosterone level, which is an attribute of adaptogens [62]. The inhibitory effects on testosterone, catecholamines and vagal tone levels were less noticeable. Instead, the sympathetic tone and the serum PTH level increased slightly. Such modulation by the phytocomposition of the post-stress constellation of neuro-endocrine factors has a noticeable cardioprotective effect (judging by the T wave and ST joint ECG), which is accompanied by a significant increase in the activity of catalase in both erythrocyte shadows (a marker of the membranes of myocardiocytes and other cells) and serum, but at the same time slightly burdens stressor damage to the gastric mucosa, which is accompanied by a significant increase in the activity of acid phosphatase (a marker of cytolysis), which is contrary to expectations [63,64]. However, it should be kept in mind that under this model of acute stress, damage to the gastric mucosa was insignificant, while damage to the myocardium was pronounced (see please Fig. 1). Therefore, we consider the insignificant burden of phytoadaptogen damage to the gastric mucosa as a kind of payment (sacrifice) of the body for appreciable minimization of myocardial damage.

The same interpretation applies to the combination of an increase in the percentage of reticulocytes and lymphoblasts in the spleen, T- and natural killer cells in the blood, as well as the intensity of phagocytosis of blood neutrophils with a decrease in the mass of the spleen, the percentage of fibroblasts and macrophages in the thymus, B-lymphocytes and monocytes in the blood, as well as completion of phagocytosis of blood neutrophils.

To what substances does this phytocomposition owe its adaptogenicity?

The most investigated medicinal herbs for their adaptogenic activity are *Panax ginseng, Eleutherococcus senticosus, Withania somnifera, Schisandra chinensis, Rhaponticum carthamoides, Lepidium meyenii, and Rhodiola spp.* The main phytochemical classes isolated from different plant parts were phytosteroids, phytosterols, flavoloids, flavolignans, alkaloids,

glucosinolates, saponins, phenolic acids, salidroside, ginsenosides, andrographolide, methyl jasmonate, cucurbitacin R, dichotosin, dichotosininare and others that have shown a considerable adaptogenic activity. Flavonoids are substances with a phenolic structure, and over 8000 flavonoids are known. Flavonoids are divided into the subclasses flavonols, flavones, flavanones, catechins, and their glycosides. An important property of phenolic compounds is the ability to oxidize; they are especially easily oxidized in an alkaline environment. Antioxidant activity is associated with the presence of a large number of hydroxyl groups in flavonoids. Flavonoids differ in the degree of oxidation: the most reduced of them are catechins, the most oxidized are flavonols. Others chemicals are Phenolic acids: Protocatechuic, Benzoic, Hydroxyphenylacetic, Hydroxybenzoic, Salicylic, Gentisic, Elagic, Chlorogenic, Vanillic, Coumaric, Synapic, Caffeic, Ferulic, Gallic, Syringic [reviews: 65,66,67,68,69].

The principal active constituents of adaptogenic plants can be divided into three main chemical groups: compounds with a tetracyclic skeleton like cortisol and testosterone - terpenoids, ginsenosides, sitoindosides, cucurbitacines, and withanolides; structural analogues of catecholamines or tyrosine - lignans (schizandrin B, eleutheroside E), phenylpropane derivatives (rosavin and syringin), phenylethane derivatives (tyrosol and salidroside); structural analogues of resolvins - oxylipins (polyhydroxylated polyunsaturated fatty acids [review: 70].

The ginsenosides act primarily on the hypothalamus and pituitary, stimulating ACTH secretion, followed by increased corticosterone biosynthesis in the adrenal cortex. On the contrary, ginseng has an inhibitory effect on the hyperactivity of the HPA axis induced by stresses and increased corticosterone levels associated with metabolic and psychiatric disorders, e.g., Ginsenoside Rd, inhibits corticosterone secretion in the cells, and inhibits ACTH-induced corticosterone biosynthesis through downregulation of proteins in the cAMP/PKA/CREB signaling pathway in adrenocortical cells. In other words, ginseng acts as a mild stressor ("stress vaccine"), increasing the range of adaptive homeostasis that adjusts the stress response in mental disorders and metabolic diseases. That is a typical adaptogenic activity to activate the body's defense system and metabolic rate resulting in increased resilience and survival in response to stressful factors, including infections. Key mechanisms of action of ginseng and other adaptogens are related to their effects on adaptive intracellular signaling pathways involved in the regulation of cell growth, differentiation, apoptosis, and survival under the stressful stimulus, factors including hormones, neurotransmitters, xenobiotics, pathogens, and physical factors (UV, osmotic, etc.) [review: 71,72,73].

Here are the components of the phytocomposition "Balm Truskavets'": *Nepeta cataria, Mentha×piperita, Salvia officinalis, Echinacea purpurea, Cichorium inthybus, Achillea millefolium, Artemisia balchanorum, Acorus calamus, Althaea officinalis, Silybum marianum, Rubus idaeus, Rosa majalis.*

Currently, we do not have data on its chemical composition. In the composition of its predecessor and analogue "Balm Kryms'kyi", polyphenols were detected in the amount of 4 mg/L compared to 7 mg/L in ginseng tincture (produced by "Lubnykhimfarm", Ukraine) [4]. It is interesting that polyphenols in amounts of 5.28 mg/L (alkylbenzene 1.55; alkenylbenzene 0.47; esters of aromatic acids 1.32; alkyl phenols 1.14; polyaromatic hydrocarbons 0.08; alkylnaphthalenes 0.53; unidentified polycyclic aromatic hydrocarbons 0,19) also found in the composition of Naftussya bioactive water [74,75,76], the adaptogenic properties of which have long been known [4,5,77].

In a comparative study of effects of the phytocomposition "Balm Truskavets'" and the bioactive Naftussya water on patients with maladaptation, 39 parameters (18 EEGs, 8 HRVs, 5 biophysical, 4 phagocytosis, as well as the Popovych's leukocytary adaptation index,

triiodothyronine, testosterone and cortisol) were identified, the physiologically favorable changes of which are common to both adaptogenic means [83].

The adrenomimetic effect of both "Balm Kryms'kyi" and ginseng tincture on the isolated heart of a frog [3], due to inhibition of catechol-o-methyltransferase activity [78], is associated with polyphenols. However, we are inclined to the neurogenic mechanism of the adreno-sympathomimetic effect of the phytocomposition revealed in this study. This is consistent with literature data on the direct neurotropic effect of phytoadaptogens in vitro and in vivo [79,80,81,82] as well as our group data on changes in EEG parameters [83].

In conclusion, we will consider the issue of receptors through which the effects of physiologically active chemicals of adaptogens are realized. Based on the structural analogue [70], the corresponding chemicals act through cortisol, testosterone, catecholamines, and polyunsaturated fatty acids receptors. However, the most authoritative group on the study of adaptogens, led by Panossian A, to our surprise, ignored both the very existence of the aryl hydrocarbon receptors (AhR) and their role in the effects of the favorite adaptogen ginseng.

As a preamble, we note that although AhR was initially recognized as a receptor that mediates the pathological effects of dioxins and other environmental pollutants [84,85], AhR activation by endogenous (bilirubin and biliverdin [86]), pseudoendogenous (products of tryptophan biotransformation by intestinal microflora [87]) and the same environmental (polycyclic aromatic hydrocarbons, halogenated biphenyls, polyphenols, indoles, flavonoids [88]) agonists have important physiological effects, including regulation of immune [89,90] and endocrine [91,92,93,94] responses.

It is important that the AhRs are also, or rather primarily, expressed in the neurons of hippocampus and cerebral cortex [95,96,97]. Although AhR expression decreases from the embryonic period into adult life, several physiological functions remain in the adult brain, which include the regulation of synaptic plasticity, neurogenesis, neurotransmitter levels and blood-brain barrier functions [98,99,100,101].

AhR signaling is considered a promising drug and preventive target, especially in cases of cancer, inflammatory and autoimmune diseases. Binding of AhR to both xenobiotic and endogenous ligands leads to highly transcriptome-specific cell changes and changes in cellular functions [102]. It is becoming increasingly clear that the physiological activity of the AhR is nuanced, involving a complex cooperative/competitive "interaction" and changing the AhR from a *toxic mediator to an important sensor of physiological homeostasis* [87,103,104,105].

In our opinion, among the given list of organic compounds of Naftussya bioactive water [74], there is a high probability that at least one AhR agonist is present. In favor of such an assumption, the data show that AhR, due to the peculiarities of its site, can bind and be activated or inhibited by very different structural compounds [106,107,108].

The discovery of Wang Y et al. [109] was a new stage in the research of the mechanisms of adaptogenic action of ginseng. It is known that transcriptional activation of the CYP1A1 gene (coding for cytochrome P450 1A1) is mediated by the AhR. The authors have examined interaction of the ginsenoside Rg1 and Rb1 with the carcinogen activation pathway mediated by the AhR in HepG2 cells. The results showed that in HepG2 cells CYP1A1 mRNA expression was significantly increased in a concentration- and time- dependent manner by ginsenoside Rg1 and Rb1. Ginsenoside Rg1 and Rb1 activated the DNA-binding capacity of the AhR for the xenobiotic responsive element of CYP1A1. Rg1 and Rb1 were able to activate the ability of the AhR to bind to an oligonucleotide containing the xenobiotic-responsive element (XRE) of the Cyp1a1 promoter. These results indicate that Rg1 and Rb1's effects on CYP1A1 induction are mediated by the AhR. Since CYP1A1 and AhR play important roles in carcinogenesis, development, differentiation and many other essential physiological functions, these results suggest that the chemopreventive effect of Panax

ginseng may be due, in part, to ginsenoside Rg1 and Rb1's ability to compete with aryl hydrocarbons for both the AhR and CYP1A1. Rg1 and Rb1 may thus be natural ligands and substrates of the AhR or have relationship with AhR pathway. These properties might be of help for future studies in P. ginseng and chemoprevention in chemical-induced cancer.

Later Hu Q et al. [110] examined the ability of a series of ginsenosides extracted from ginseng to bind to and activate/inhibit the AhR and AhR signal transduction. The authors demonstrated the ability of selected ginsenosides to directly bind to and activate the guinea pig cytosolic AHR, and to stimulate/inhibit AHR-dependent luciferase gene expression in a recombinant guinea pig cell line. Comparative studies revealed significant species differences in the ability of ginsenosides to stimulate AhR-dependent gene expression in guinea pig, rat, mouse and human cell lines. The endogenous gene CYP1A1 could be inducted in all cell line. The authors concluded that the ability of these compounds to stimulate AhR signal transduction demonstrated that these *ginsenosides are a new class of naturally occurring AhR agonists*.

Incidentally, we cannot deny ourselves the pleasure of stating that as early as 1994, the Truskaveysian Scientific School, during a comparative study of the adaptogenic properties of ginseng tincture, the phytocomposition "Balm Kryms'kyi" and Naftussya bioactive water, showed that a four-day treatment of female rats shortened the duration of Nembutal sleep from 159±8 min in control (tap water) to 131±8, 87±8, and 65±5 min respectively [1,3,43]. This indirectly indicates the activation of microsomal hydroxylation, which is mediated by the cytochrome P450 and AhR complex.

In conclusion, let us add that in previous studies, the actoprotective ability of the phytocomposition was revealed [76,111], which is considered one of the attributes of adaptogens [17,62].

Conclusion

Ukrainian phytocomposition "Balm Truskavets" has a generally favorable adaptogenic effect on the post-stress state of the neuro-endocrine-immune complex and metabolome. However, there are certain adverse effects as the so-called adaptation fee.

Based on the results and hypothesis testing presented in this article, the following conclusions can be drawn.

1. Adaptogenic Effects. The Ukrainian phytocomposition "Balsam Truskavets" demonstrates significant adaptogenic properties. It modulates the post-stress state of the neuro-endocrine-immune complex and metabolome in rats, generally mitigating the negative effects of acute stress.

2. Cardioprotective Action. The phytocomposition shows notable cardioprotective effects, as evidenced by the normalization of ECG parameters in stressed rats. This suggests potential applications in preventing or reducing stress-induced cardiac damage.

3. Complex Gastric Effects. While the phytocomposition shows some protective effects on the gastric mucosa, it also slightly exacerbates stress-induced gastric damage in some cases. This dual action requires further investigation and careful consideration in potential therapeutic applications.

4. Immune Modulation. The phytocomposition significantly affects various immune parameters, enhancing some aspects of immune function while suppressing others. This complex immunomodulatory effect could be beneficial in stress-related immune disorders.

5. Neuroendocrine Regulation. The study confirms the phytocomposition's ability to modulate key stress hormones, particularly corticosterone, suggesting its potential in managing stress-related endocrine imbalances.

6. Metabolic Impact. The phytocomposition influences various metabolic parameters, indicating potential applications in metabolic disorders associated with chronic stress.

7. Similarity to Known Adaptogens. The effects of "Balsam Truskavets" are comparable to those of established adaptogens like ginseng, validating its classification as an adaptogenic substance.

8. Sex Differences. While some sex-specific differences in response to the phytocomposition were observed, they were not significant for most parameters, suggesting broad applicability across genders.

9. AhR Receptor Involvement. The discussion on the role of aryl hydrocarbon receptors (AhR) in mediating the effects of the phytocomposition opens new avenues for understanding its mechanism of action.

10. Adaptation Cost. Some adverse effects were observed, which the researchers interpret as an "adaptation fee." This concept highlights the complex nature of adaptogenic responses and the need for balanced approaches in their application.

Applicability of Results.

1. Stress Management. The phytocomposition could be developed into a natural remedy for managing acute stress and its physiological consequences.

2. Cardiovascular Health. Its cardioprotective properties suggest potential use in preventing stress-induced cardiac issues.

3. Immune Support. The immunomodulatory effects could be beneficial in conditions where stress negatively impacts immune function.

4. Endocrine Disorders. The ability to modulate stress hormones indicates potential applications in treating stress-related endocrine imbalances.

5. Metabolic Health. The metabolic effects suggest possible uses in managing stress-induced metabolic disturbances.

6. Personalized Medicine. The observed sex differences, although minor, hint at the potential for tailored adaptogenic therapies.

7. Drug Development. The insights into the mechanism of action, particularly regarding AhR receptors, could guide the development of new adaptogenic compounds.

8. Holistic Health Approaches. The complex, multi-system effects of the phytocomposition support its potential integration into holistic health and wellness strategies.

These conclusions and potential applications highlight the significance of "Balsam Truskavets" as a promising adaptogenic agent. However, further research, including clinical trials, would be necessary to fully validate its efficacy and safety for human use in various stress-related conditions.

Acknowledgments

REFERENCES

1. Panasyuk YM, Levkut LH, Popovych IL, Alekseyev OI, et al. Experimental study of adaptogenic properties of "Crimean" balm [in Ukrainian]. Fiziol Zh. 1994;40(3-4):25-30.

2. Pat. 10271 Ukraine MKI A 61 K 31/00. Adaptogenic agent [in Ukrainian]. Panasyuk YM, Levkut LG, Popovych IL, Shumakov MF, et al. 1996. Bull № 4.

3. Alyeksyeyev OI, Popovych IL, Panasyuk YeM, Barylyak LG, et al. Adaptogens and Radiation [in Ukrainian]. Kyïv: Naukova dumka;1996:126.

4. Kostyuk PG, Popovych IL, Ivassivka SV (Editors). Chornobyl, Adaptive and Protection Systems, Rehabilitation. Adaptive, metabolic, hemostasis and

immunological aspects of diagnostics and balneo- and phytorehabilitation in Truskavets' spa of persons exposed to Chornobyl accident factors [in Ukrainian]. Kyïv: Computerpress;2006:348.

5. Hrinchenko BV. Adaptive balneophytotherapy as a sanogenetic basis for rehabilitation of neuro-endocrine-immune system dysfunction [in Ukrainian]. Medical Hydrology and Rehabilitation. 2008;6(1):85-97.

6. Ruzhylo SV, Fihura OA, Zukow W, Popovych IL. Immediate neurotropic effects of Ukrainian phytocomposition. Journal of Education, Health and Sport. 2015;5(4):415-427.

7. Fihura OA, Ruzhylo SV, Żukow X, Popovych IL. Immediate effects of Ukrainian phytocomposition on biophotonics (GDV), EEG and HRV parameters. Journal of Education, Health and Sport. 2021;11(7):349-365.

8. Fihura OA, Ruzhylo SV, Popovych IL. Ukrainian adaptogenic phytocomposition "Balm Truskavets'" modulate EEG, HRV and biophotonics (GDV) parameters. Journal of marine medicine. 2022;2(95):99-108.

9. Fihura OA, Ruzhylo SV, Korda MM, et al. The influence of the Ukrainian phytocomposition "Balm Truskavets'" on parameters of neuro-endocrine-immune complex and biophotonics in humans with maladaptation. Journal of Education, Health and Sport. 2023;13(1):326-337.

10. Fihura OA. Amelioration by phytoadaptogene of effects of balneofactors of Truskavets' Spa on patients with post-radiation encephalopathy. Journal of Education, Health and Sport. 2023;19(1):36-58.

11. Ordynskyi YuM, Riabokon MO, Denefil OV. Stress effect on male and female rats' organism with various hypoxia resistance [in Ukrainian]. Bukovinian Medical Herald. 2017;21(3):36-43.

12. Ordynskyi YuM, Riabokon MO, Denefil OV, Bolyukh OO. Stress-limiting mechanisms of adaptation to immobilization stress in high-resistant and low-resistant to hypoxic hypoxia female and male rats [in Ukrainian]. Art of Medicine. 2019;1(9):95-99.

13. Fil V, Zukow W, Kovalchuk G, et al. The role of innate muscular endurance and resistance to hypoxia in reactions to acute stress of neuroendocrine, metabolic and ECGs parameters and gastric mucosa in rats. JPES. 2021;21(Sup. 5):3030-3039. doi:10.7752/jpes.2021.s5403.

14. Zukow W, Fil VM, Kovalchuk HY, et al. The role of innate muscular endurance and resistance to hypoxia in reactions to acute stress of immunity in rats. JPES. 2022;22(7):1608-1617. doi: 10.7752/jpes.2022.07202.

15. Melnyk OI, Chendey IV, Zukow W, et al. The features of reactions to acute stress of neuro-endocrine-immune complex, metabolome, ECG and gastric mucosa in rats with various state of innate muscular endurance and resistance to hypoxia. JEHS. 2023;38(1):96-128.

16. Berezovskyi VYa. Personality traits in response to hypoxia [in Ukrainian]. Fiziol Zhurn. 1975;21(3):371-376.

17. Brekhman II. Eleutherococcus [in Russian]. Leningrad:Nauka;1968:186.

18. Baevsky RM, Berseneva AP. Use KARDIVAR system for determination of the stress level and estimation of the body adaptability. Standards of measurements and physiological interpretation. Moscow-Prague;2008:41.

19. Nakamura J, Takada S, Ohtsuka N, et al. An assessment of gastric ulcers in vivo: enhancement of urinary recovery after oral administration of phenolsulfonphthalein in rats. J of Pharmacobiodynamics. 1984;7(7):485–491. doi.org/10.1248/bpb1978.7.485

20. Popovych IL. Factor and canonical analyzes of the parameters of the neuro-endocrine-

immune complex, metabolism and erosive-ulcerative lesions of the gastric mucosa in rats under acute water-immersion stress [in Ukrainian]. Medical Hydrology and Rehabilitation. 2007;5(2):68-80.

21. Gozhenko AI, Korda MM, Popadynets' OO, Popovych IL. Entropy, Harmony, Synchronization and Their Neuro-Endocrine-Immune Correlates [in Ukrainian]. Odesa:Feniks;2021:232.

22. Shannon CE. A mathematical theory of information. Bell Syst Tech J. 1948;27:379-423.

23. Hiller G. Test for the quantitative determination of HDL cholesterol in EDTA plasma with Reflotron®. Klin Chem. 1987;33:895-898.

24. Goryachkovskiy AM. Clinical biochemistry [in Russian]. Odesa:Astroprint,1998:608.

25. Gavrilov VB, Mishkorudnaya MI. Spectrophotometric determination of plasma levels of lipid hydroperoxides [in Russian]. Laboratornoye Delo. 1983;3:33-36.

26. Andreyeva LI, Kozhemyakin LA, Kishkun AA. Modification of the method for determining the lipid peroxide in the test with thiobarbituric acid [in Russian]. Laboratornoye Delo. 1988;11:41-43.

27. Korolyuk MA, Ivanova MI, Mayorova IG, Tokarev VYe. The method for determining the activity of catalase [in Russian]. Laboratornoye Delo. 1988;1:16-19.

28. Dubinina YY, Yefimova LF, Sofronova LN, Geronimus AL. Comparative analysis of the activity of superoxide dismutase and catalase of erythrocytes and whole blood from newborn children with chronic hypoxia [in Russian]. Laboratornoye Delo. 1988;8:16-19.

29. Makarenko YeV. ATPase activity of erythrocytes in patients with chronic liver and stomach disease [in Russian]. Laboratornoye Delo. 1987;2:14-17.

30. Popovych IL, Gozhenko AI, Zukow W, Polovynko IS. Variety of Immune Responses to Chronic Stress and their Neuro-Endocrine Accompaniment. Riga:Scholars' Press;2020:172. doi.org/10.5281/zenodo.3822074.

31. Harrington EC. The Desirability Function. Industrial Quality Control. 1965;21:494-498.

32. Perederiy VG, Zemskov AM, Bychkova NG, Zemskov VM. Immune Status, Principles of its Evaluation and Correction of Immune Disorders [in Russian]. Kyïv:Zdorovya;1995:211.

33. Bilas VR, Popovych IL. Role of microflora and organic substances of water Naftussya in its modulating influence on neuroendocrine-immune complex and metabolism [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009;7(1):68-102.

34. Horizontov PD, Belousova BI, Fedotova MI. Stress and the Blood System [in Russian]. Moskva:Meditsina;1983:240.

35. Bilas VR, Popadynets' OO, Flyunt ISS, et al. Entropies of thymocytogram, splenocytogram, immunocytogram and leukocytogram in rats are regulated by sex and the neuroendocrine parameters while regulates immune parameters. Journal of Education, Health and Sport. 2020;10(7):266-288.

36. Polovynko IS, Zajats LM, Zukow W, et al. Quantitative evaluation of integrated neuroendocrine and immune responses to chronic stress in rat male. Journal of Education, Health and Sport. 2016;6(8):154-166.

37. Klecka WR. Discriminant Analysis (seventh printing, 1986) [trans. from English in Russian]. In: Factor, Discriminant and Cluster Analysis. Moskva:Finansy i Statistika;1989:78-138.

38. Selye H: A syndrome produced by diverse nocuous agents. 1936. *J Neuropsychiatry Clin Neurosci.* 1998;10(2):230–231. doi: 10.1176/jnp.10.2.230a

39. Szabo S, Yoshida M, Filakovszky J, Juhasz G. "Stress" is 80 Years Old: From Hans

Selye Original Paper in 1936 to Recent Advances in GI Ulceration. Curr Pharm Des. 2017;23(27):4029-4041. doi: 10.2174/1381612823666170622110046.

40. Berger EN. Neurohumoral Mechanisms of Disturbed Tissue Trophism [in Russian]. Kyïv:Zdorovya;1980:104.

41. Zavodskaya IS & Moreva YeV. Pharmacological Analysis of Mechanism of Stress and its Effects [in Russian]. Moskva:Meditsina;1981:216.

42. Meerson FZ. Adaptation, Stress and Prophylaxis [in Russian]. Moskva:Nauka;1981:279.

43. Markova OO, Popovych IL, Tserkovnyuk AV, Barylyak LG. Adrenaline Myocardiodystrophy and Reactivity of the Organism [in Ukrainian]. Kyïv:Computerpress;1997:126.

44. Fihura OA, Melnyk OI, Ruzhylo SV, et al. Relationships between neuro-endocrine, electrocardiogram, and gastric mucosal damage parameters in naïve and stressed rats. Journal of Education, Health and Sport. 2023;18(1):162-190.

45. Zhao DQ, Xue H, Sun HJ. Nervous mechanisms of restraint water-immersion stress-induced gastric mucosal lesion. World J Gastroenterology. 2020;26(20):2533–2549. doi.org/10.3748/wjg.v26.i20.2533

46. Filaretova LP, Filaretov AA, Makara GB. Corticosterone increase inhibits stress-induced gastric erosions in rats. Am J Physiology. 1998;274(6):G1024–G1030. doi.org/10.1152/ajpgi.1998.274.6.G1024

47. Filaretova LP, Bagaeva TR, Amagase K, Takeuchi K. Contribution of glucocorticoids to protective influence of preconditioning mild stress against stress-induced gastric erosions. ANYAS. 2008;1148:209–212. doi.org/10.1196/annals.1410.005

48. Fihura OA, Korda MM, Klishch IM, et al. Metabolic and immune accompaniments of electrocardiographic and morphologic gastric mucosa parameters in naïve and stressed rats. Journal of Education, Health and Sport. 2024;61:54947. doi.org/10.12775/JEHS.2024.61.54947

49. Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. NeuroImmunoModulation. 2009;16:300–317.

50. Dhabhar FS. The short-term stress response – mother nature's mechanism for enhancing protection and performance under conditions of threat, challenge, and opportunity. Front Neuroendocrinol. 2018;49:175–192.

51. Besedovsky H & del Rey A. Immune-neuro-endocrine interactions: facts and hypotheses. Endocrine reviews. 1996;17(1):64-102. doi.org/10.1210/edrv-17-1-64

52. Kozyavkina OV. The state of post-stress parameters of autonomic homeostasis and endocrine, metabolic and immune status and the relationship between them in rats with alternative types of pre-stress autonomic homeostasis induced by bioactive water Naftussya [in Ukrainian]. Medical Hydrology and Rehabilitation. 2009;7(2):40-56.

53. Kozyavkina OV, Kozyavkina NV, Gozhenko OA, et al. Bioactive Water Naftussya and Neuro-Endocrine-Immune Complex [in Ukrainian]. Kyïv:UNESCO-SOCIO;2015:349.

54. Kul'chyns'kyi AB, Gozhenko AI, Zukow W, Popovych IL. Neuro-immune relationships at patients with chronic pyelonephrite and cholecystite. Communication 3. Correlations between parameters EEG, HRV and Immunogram. Journal of Education, Health and Sport. 2017;7(3):53-71.

55. Kul'chyns'kyi AB, Kyjenko VM, Zukow W, Popovych IL. Causal neuro-immune relationships at patients with chronic pyelonephritis and cholecystitis. Correlations between parameters EEG, HRV and white blood cell count. Open Medicine. 2017;12(1):201-213.

56. Popovych IL, Kul'chyns'kyi AB, Korolyshyn TA, Zukow W. Interrelations between changes in parameters of HRV, EEG and cellular immunity at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2017;7(10):11-23.

57. Popovych IL, Kul'chyns'kyi AB, Gozhenko AI, et al. Interrelations between changes in parameters of HRV, EEG and phagocytosis at patients with chronic pyelonephritis and cholecystitis. Journal of Education, Health and Sport. 2018;8(2):135-156.

58. Mel'nyk OI, Zukow W, Hrytsak MV, et al. Canonical analysis of neuroendocrine-metabolic and neuroendocrine-immune relationships at female rats. Journal of Education, Health and Sport. 2021;11(5):356-369.

59. Polovynko IS, Zajats LM, Popovych AI, Popovych IL. Integral quantification of neuroendocrine and immune responses to chronic stress in male rats [in Ukrainian]. In: Pathophysiology and Pharmacy: ways of integration: Abstracts VII National Congress pathophysiologists Ukraine with international participation (5-7 October 2016). Kharkiv:NPhU;2016:182-182.

60. Zajats LM, Polovynko IS, Zukow W, et al. Neuroendocrine-immune relatioships in rat females. Journal of Education, Health and Sport. 2017;7(10):59-78.

61. Mel'nyk OI, Struk ZD, Zukow W, Popovych IL. Vegetative, endocrine and metabolic accompaniments of individual immune responses to adaptogenic balneotherapy. Journal of Education, Health and Sport. 2019;9(12):207-229.

62. Dardymov IV. Ginseng, Eleutherococcus (To the mechanism of physiological action) [in Russian]. Moskva:Nauka;1976:189.

63. Sun XB, Matsumoto T, Yamada H. Anti-ulcer activity and mode of action of the polysaccharide fraction from the leaves of Panax ginseng. Planta Med. 1992;58(5):432-435. doi: 10.1055/s-2006-961507.

64. Lu S, Wu D, Sun G, et al. Gastroprotective effects of Kangfuxin against water-immersion and restraint stress-induced gastric ulcer in rats: roles of antioxidation, anti-inflammation, and pro-survival. Pharmaceutical biology. 2019;57(1):770–777. doi.org/10.1080/ 13880209. 2019.1682620

65. Gerontakos SE, Casteleijn D, Shikov AN, Wardle J. A critical review to identify the domains used to measure the effect and outcome of adaptogenic herbal medicines. Yale J Biol Med. 2020;93(2):327-346. PMID: 32607092; PMCID: PMC7309667.

66. Todorova V, Ivanov K, Ivanova S. Comparison between the biological active compounds in plants with adaptogenic properties (Rhaponticum carthamoides, Lepidium meyenii, Eleutherococcus senticosus and Panax ginseng). Plants (Basel). 2021;11(1):64. doi: 10.3390/plants11010064. PMID: 35009068; PMCID: PMC8747685.

67. Sergeeva I, Kiseleva T, Pomozova V, Shkrabtak N, Frolova N, Vereshchagin A. Experimental studies of the effect of schisandra chinensis extract on the state of adaptive capabilities of rats under chronic and general exposure to cold. Int J Environ Res Public Health. 2021;18(22):11780. doi: 10.3390/ijerph182211780.

68. Esmaealzadeh N, Iranpanah A, Sarris J, Rahimi R. A literature review of the studies concerning selected plant-derived adaptogens and their general function in body with a focus on animal studies. *Phytomedicine*. 2022;105:154354. doi: 10.1016/j.phymed.2022.154354.

69. Kumar P, Banik SP, Goel A, Chakraborty S, Bagchi M, Bagchi D. Chemical, microbial and safety profiling of a standardized Withania somnifera (Ashwagandha) extract and Withaferin A, a potent novel phytotherapeutic of the millennium. Functional Foods in Health and Disease. 2023;13(2):36-51. doi.org/10.31989/ffhd.v13i2.1071

70. Panossian AG, Efferth T, Shikov AN, Pozharitskaya ON, Kuchta K, Mukherjee PK, Banerjee S, Heinrich M, Wu W, Guo DA, Wagner H. Evolution of the adaptogenic concept from traditional use to medical systems: Pharmacology of stress- and aging-related diseases. Med Res Rev. 2021;41(1):630-703. doi: 10.1002/med.21743.

71. Panossian A, Efferth T. Network Pharmacology of Adaptogens in the Assessment of Their Pleiotropic Therapeutic Activity. Pharmaceuticals (Basel). 2022;15(9):1051. doi: 10.3390/ph15091051.

72. Panossian A. Challenges in phytotherapy research. Front Pharmacol. 2023;14:1199516. doi: 10.3389/fphar.2023.1199516.

73. Jin W, Ma R, Zhai L, et al. Ginsenoside Rd attenuates ACTH-induced corticosterone secretion by blocking the MC2R-cAMP/PKA/CREB pathway in Y1 mouse adrenocortical cells. *Life Sci*. 2020;245:117337. doi: 10.1016/j.lfs.2020.117337.

74. Dats'ko, OR, Bubnyak, AB, Ivassivka SV. The organic part in mineral water Naftussya. Development of knowledges about its composition and origination [in Ukrainian]. Medical Hydrology and Rehabilitation. 2008;6(1):168-174.

75. Ivassivka SV. Biologically Active Substances of Naftussya Water, their Genesis and Mechanisms of Physiological Action [in Ukrainian]. Kyïv:Naukova dumka;1997:110.

76. Zukow W, Muszkieta R, Hagner-Derengowska M, et al. Role of organic substances of Naftussya bioactive water in its effects on dynamic and static fitness in rats. *JPES*. 2022;22(11):2733-2742.

77. Vis'tak HI, Popovych IL. Vegetotropic effects of bioactive water Naftussya and their endocrine-immune support in female rats [in Ukrainian]. Medical Hydrology and Rehabilitation. 2011;9(2):39-57.

78. Lupandin AV. On the role of catecholaminergic synapses in the mechanism of adaptation formation with the participation of polyphenolic adaptogens [in Russian]. Fiziiol Zh SSSR. 1989;75(8):1082-1088.

79. Asea A, Kaur P, Panossian A, Wikman KG. Evaluation of molecular chaperons Hsp72 and neuropeptide Y as characteristic markers of adaptogenic activity of plant extracts. Phytomedicine. 2013;20(14):1323-1329.

80. Panossian A, Wikman G. Effects of adaptogens on the central nervous system and the molecular mechanisms associated with their stress-protective activity. Pharmaceuticals (Basel). 2010;3(1):188-224.

81. Panossian A, Seo EJ, Efferth T. Novel molecular mechanisms for the adaptogenic effects of herbal extracts on isolated brain cells using systems biology. Phytomedicine. 2018;50:257-284.

82. Panossian A, Seo EJ, Efferth T. Effects of anti-inflammatory and adaptogenic herbal extracts on gene expression of eicosanoids signaling pathways in isolated brain cells. Phytomedicine. 2019;60:152881.

83. Popovych IL. Similarity of adaptogenic effects of bioactive Naftussya water and phytocomposition "Balm Truskavets'". Journal of Education, Health and Sport. 2022;12(12):344-356.

84. Nebert DW & Bausserman LL. Genetic differences in the extent of aryl hydrocarbon hydroxylase induction in mouse fetal cell cultures. J Biol Chem. 1970;245(23):6373-82. PMID: 5484816.

85. Poland A, Glover E, Kende AS. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. Evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase. J Biol Chem. 1976;251(16):4936-46. PMID: 956169.

86. Phelan D, Winter GM, Rogers WJ, et al. Activation of the Ah receptor signal transduction pathway by bilirubin and biliverdin. Arch Biochem Biophys. 1988;357(1):155-163. doi: 10.1006/abbi.1998.0814

87. Murray IA & Perdew GH. How Ah Receptor Ligand Specificity Became Important in Understanding Its Physiological Function. Int J Mol Sci. 2020;21(24):9614. doi: 10.3390/ijms21249614.

88. Busbee PB, Rouse M, Nagarkatti M, Nagarkatti PS. Use of natural AhR ligands as potential therapeutic modalities against inflammatory disorders. Nutrition reviews. 2013;71(6):353–369. doi:10.1111/nure.12024.

89. Quintana FJ & Sherr DH. Aryl hydrocarbon receptor control of adaptive immunity. Pharmacol Rev. 2013;65(4):1148-61. doi:10.1124/pr.113.007823.

90. Yang X, Liu H, Ye T, et al. AhR activation attenuates calcium oxalate nephrocalcinosis by diminishing M1 macrophage polarization and promoting M2 macrophage polarization. Theranostics. 2020;10(26):12011-12025. doi:10.7150/thno.51144

91. Andric SA, Kostic TS, Stojilkovic SS, Kovacevic RZ. Inhibition of rat testicular androgenesis by a polychlorinated biphenyl mixture aroclor 1248. Biol Reprod. 2000;62(6):1882-1888. doi: 10.1095/biolreprod62.6.1882.

92. Li Lih-Ann & Wang Pei-Wen. PCB126 Induces Differential Changes in Androgen, Cortisol, and Aldosterone Biosynthesis in Human Adrenocortical H295R Cells. *Toxicological Sciences.* 2005;85(1):530–540. doi.org/10.1093/toxsci/kfi105

93. Ye L, Zhao B, Hu G, et al. Inhibition of human and rat testicular steroidogenic enzyme activities by bisphenol A. Toxicol Lett. 2011;207(2):137-42. doi: 10.1016/j.toxlet.2011.09.001.

94. Trego ML, Hoh E, Kellar NM, et al. Comprehensive Screening Links Halogenated Organic Compounds with Testosterone Levels in Male Delphinus delphis from the Southern California Bight. Environ Sci Technol. 2018;52(5):3101-3109. doi: 10.1021/acs.est.7b04652.

95. Eckers A, Jakob S, Heiss C, et al. The aryl hydrocarbon receptor promotes aging phenotypes across species. Sci Rep. 2016;6:19618. doi:10.1038/srep19618.

96. Kimura E & Tohyama C. Embryonic and Postnatal Expression of Aryl Hydrocarbon Receptor mRNA in Mouse Brain. Front Neuroanat. 2017;11:4. doi:10.3389/fnana.2017.00004.

97. Ojo ES & Tischkau SA. The Role of AhR in the Hallmarks of Brain Aging: Friend and Foe. Cells. 2021;10(10):2729. doi:10.3390/cells10102729

98. Wang X, Hawkins BT, Miller DS. Aryl hydrocarbon receptor-mediated up-regulation of ATP-driven xenobiotic efflux transporters at the blood-brain barrier. FASEB J. 2011;25:644–652. doi:10.1096/fj.10-169227.

99. Chen Y, Xu L, Xie HQH, et al. Identification of differentially expressed genes response to TCDD in rat brain after long-term low-dose exposure. J Environ Sci. 2017;62:92-99. doi:10.1016/j.jes.2017.07.010.

100. Chen WC, Chang LH, Huang SS, et al. Aryl hydrocarbon receptor modulates stroke-induced astrogliosis and neurogenesis in the adult mouse brain. J Neuroinflamm. 2019;16:187. doi:10.1186/s12974-019-1572-7.

101. Keshavarzi M, Khoshnoud MJ, Ghaffarian Bahraman A, Mohammadi-Bardbori A. An Endogenous Ligand of Aryl Hydrocarbon Receptor 6-Formylindolo[3,2-b]Carbazole (FICZ) Is a Signaling Molecule in Neurogenesis of Adult Hippocampal Neurons. J Mol Neurosci. 2020;70:806–817. doi:10.1007/s12031-020-01506-x.

102. Esser C & Rannug A. The aryl hydrocarbon receptor in barrier organ physiology, immunology, and toxicology. Pharmacol Rev. 2015;67(2):259-79. doi:10.1124/pr.114.009001.

103. Avilla MN, Malecki KMC, Hahn ME, et al. The Ah Receptor: Adaptive Metabolism, Ligand Diversity, and the Xenokine Model. Chem Res Toxicol. 2020;33(4):860-879. doi:10.1021/acs.chemrestox.9b00476.

104. Kou Z & Dai W. Aryl hydrocarbon receptor: Its roles in physiology. Biochem Pharmacol. 2021;185:114428. doi: 10.1016/j.bcp.2021.114428.

105. Rejano-Gordillo CM, Marín-Díaz B, Ordiales-Talavero A, et al. From Nucleus to Organs: Insights of Aryl Hydrocarbon Receptor Molecular Mechanisms. Int J Mol Sci. 2022;23(23):14919. doi: 10.3390/ijms232314919.

106. Denison MS, Pandini A, Nagy SR, et al. Ligand binding and activation of the Ah receptor. Chem Biol Interact. 2002;141(1-2):3-24. doi:10.1016/s0009-2797(02)00063-7

107. Denison MS, Soshilov AA, He G, et al. Exactly the same but different: promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor. Toxicol Sci. 2011;124:1–22.

108. Giani Tagliabue S, Faber SC, Motta S, et al. Modeling the binding of diverse ligands within the Ah receptor ligand binding domain. Sci Rep. 2019;9(1):10693. doi:10.1038/s41598-019-47138-z

109. Wang Y, Ye X, Ma Z, et al. Induction of cytochrome P450 1A1 expression by ginsenoside Rg1 and Rb1 in HepG2 cells. Eur J Pharmacol. 2008;601(1-3):73-78. doi: 10.1016/j.ejphar.2008.10.057.

110. Hu Q, He G, Zhao J, et al. Ginsenosides are novel naturally-occurring aryl hydrocarbon receptor ligands. *PLoS One*. 2013;8(6):e66258. doi:10.1371/journal.pone.0066258

111. Fihura OA, Ruzhylo SV, Zakalyak NR. Phytoadaptogen reverses the adverse effects of Naftussya bioactive water on dynamic muscle performance in healthy rats. Quality in Sport. 2022;8(2):45-55.