**ZUKOW, Walery, FIHURA, Oksana, KORDA, Mykhaylo, KLISHCH, Ivan, RUZHYLO, Sofiya, MELNYK, Oksana, YANCHIJ, Roman and POPOVYCH, Igor. Modulating effects of Ukrainian phytocomposition "Balm Truskavets'" on post stress changes in neuroendocrine-immune complex, metabolome, electrocardiogram, and gastric mucosa at rats. Journal of Education, Health and Sport. 2024;64:55171. eISSN 2391-8306.**
**https://dx.doi.org/10.12775/JEHS.2024.64.55171**
**https://apcz.umk.pl/JEHS/article/view/55171**

**The journal has had 40 points in Minister of Science and Higher Education of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of 05.01.2024 No. 32318. Has a Journal's Unique Identifier: 201159. Scientific disciplines assigned: Physical culture sciences (Field of medical and health sciences); Health Sciences (Field of medical and health sciences).**
**Punkty Ministerialne 40 punktów. Załącznik do komunikatu Ministra Nauki i Szkolnictwa Wyższego z dnia 05.01.2024 Lp. 32318. Posiada Unikatowy Identyfikator Czasopisma: 201159. Przypisane dyscypliny naukowe: Nauki o kulturze fizycznej (Dziedzina nauk medycznych i nauk o zdrowiu); Nauki o zdrowiu (Dziedzina nauk medycznych i nauk o zdrowiu).© The Authors 2024;**
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**The authors declare that there is no conflict of interests regarding the publication of this paper.**
**Received: 10.06.2024. Revised: 10.07.2024. Accepted: 11.07.2024. Published: 13.07.2024.**

# 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:

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

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:

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

About the condition of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytosis index (percentage of cells, in which found microbes), 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]:

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

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

***Statistical analysis***

Statistical processing was performed using a software package "Microsoft Excell" and "Statistica 6.4 StatSoft Inc" (Tulsa, OK, USA). ). 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 $(AMo/2•Mo•MxDMn)^{1/3}$: 0,14±0,08, 0,14±0,03, and 0,14±0,06 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).

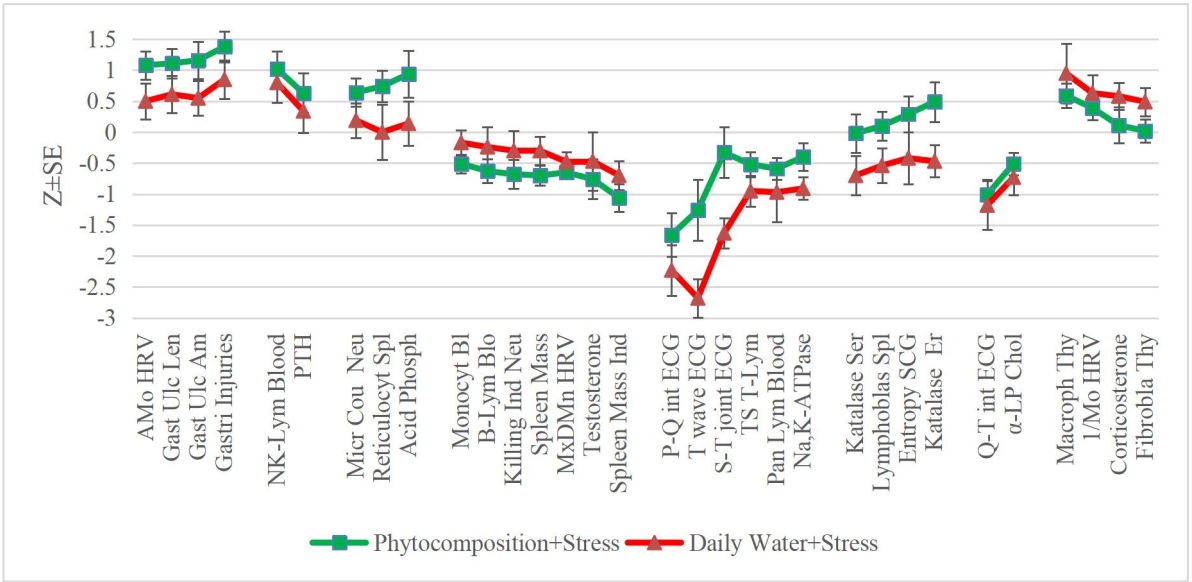


**Fig. 1.** Patterns of post-stress profiles of rats against the background of daily water and phytocomposition consumption

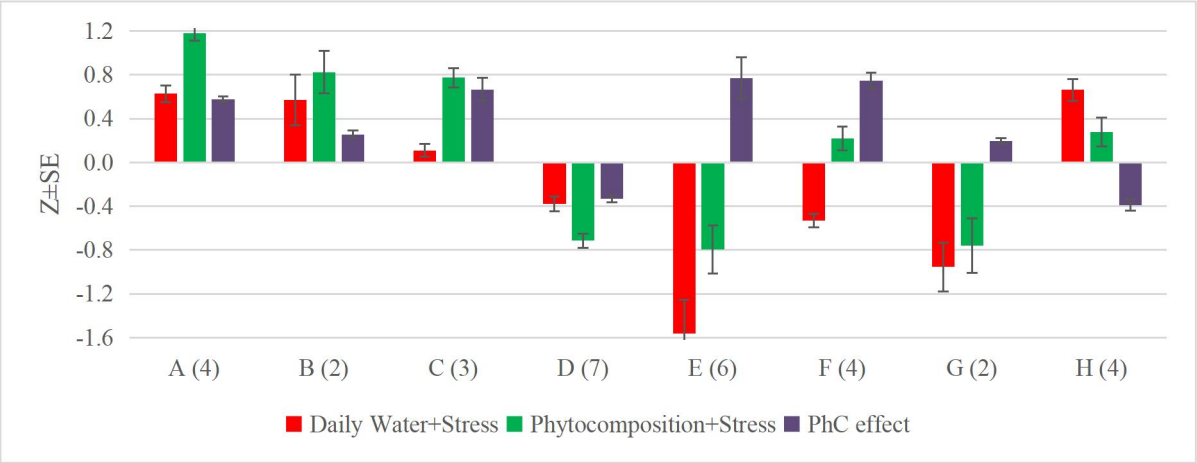


**Fig. 2.** Clusters of post-stress variables (n) of rats against the background of **daily water** and **phytocomposition** consumption. Essential (*per se*) effects of phytocomposition calculated as algebraic difference

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

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

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| of Thymus, % | 0.50 | 0.75 | 0.31 | | | | | |
| Microbial Count of Neutrophils, Bac/Phag | 5.5<br>0.3 | 5.7<br>0.3 | 6.2<br>0.2 | 0,050 | 0,662 | 5,099 | 0,016 | 0,172 |
| Killing Index of Neutrophils, % | 47.5<br>2.9 | 44.7<br>3.0 | 41.1<br>2.1 | 0,039 | 0,851 | 1,751 | 0,199 | 0,506 |
| Theophylline-susceptib-le T-Lymphocytes, % | 15,3<br>1,1 | 11.9<br>0.9 | 13.4<br>0.7 | 0,039 | 0,853 | 1,723 | 0,204 | 0,636 |
| B-Lymphocytes of Blood, % | 13.4<br>0.8 | 12.8<br>0.8 | 11.8<br>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

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

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| mg | 58 | 42 | 30 | | | | | |
| Spleen Mass Index, mg/100 g Body Mass | 375<br>25 | 320<br>18 | 292<br>17 | 0,033 | 0,997 | 0,027 | 0,974 | 0,450 |
| Reticulocytes of Spleen, % | 2.67<br>0.22 | 2.67<br>0.32 | 3.19<br>0.18 | 0,033 | 0,998 | 0,016 | 0,985 | 0,662 |
| Lymphoblastes of Spleen, % | 8.6<br>10 | 6.8<br>0.9 | 8.9<br>0.8 | 0,031 | 0,932 | 0,690 | 0,514 | 0,601 |
| Entropy of Splenocytogram | 0,534<br>0,019 | 0,552<br>0,018 | 0,509<br>0,025 | 0,032 | 0,969 | 0,300 | 0,744 | 0,589 |
| Fibroblastes of Thymus, % | 5.33<br>0.65 | 6.33<br>0.47 | 5.38<br>0.38 | 0,031 | 0,927 | 0,749 | 0,486 | 0,465 |
| Pan Lymphocytes of Blood, % | 51.8<br>1.5 | 47.2<br>2.3 | 49.0<br>0.9 | 0,030 | 0,920 | 0,824 | 0,454 | 0,485 |
| Monocytes of Blood, % | 6.20<br>0.73 | 5.81<br>0.46 | 5.02<br>0.35 | 0,033 | 0,985 | 0,147 | 0,864 | 0,572 |
| NK-Lymphocytes of Blood, % | 5.29<br>0.35 | 6.18<br>0.36 | 6.43<br>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 | Cluster 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<br>0.29 | **1.08**<br>**0.23** | 0.58<br>0.23 | A |
| **Gastric Ulcers Length** | **0.242** | -0.085 | 0 | 0.61<br>0.30 | **1.11**<br>**0.24** | 0.50<br>0.24 | A |
| **Gastric Ulcers Amount** | **0.230** | -0.060 | 0 | 0.55<br>0.28 | **1.16**<br>**0.30** | 0.61<br>0.30 | A |
| **Gastric Mucosal Damage** | **0.298** | -0.171 | 0 | 0.85<br>0.31 | **1.38**<br>**0.25** | 0.53<br>0.25 | A |
| **NK-Lymphocytes of Blood** | | | 0 | 0.80<br>0.32 | **1.02**<br>**0.29** | 0.22<br>0.29 | B |
| **Parathyroid hormone** | | | 0 | 0.30<br>0.30 | **0.56**<br>**0.30** | 0.26<br>0.30 | B |
| **Microbial Count of Neutrophils** | **0.145** | -0.010 | 0 | 0.19<br>0.28 | **0.64**<br>**0.23** | 0.46<br>0.23 | C |
| **Reticulocytes of Spleen** | | | 0 | 0.00<br>0.45 | **0.74**<br>**0.25** | 0.74<br>0.25 | C |
| **Acid Phosphatase** | **0.149** | 0.014 | 0 | 0.14<br>0.36 | **0.94**<br>**0.38** | 0.80<br>0.38 | C |
| **Monocytes of Blood** | | | 0 | -0.17<br>0.20 | **-0.51**<br>**0.15** | -0.34<br>0.15 | D |
| **B-Lymphocytes of Blood** | **-0.140** | 0.023 | 0 | -0.24<br>0.32 | **-0.63**<br>**0.19** | -0.39<br>0.19 | D |
| **Killing Index of Neutrophils** | **-0.137** | 0.031 | 0 | -0.30<br>0.32 | **-0.68**<br>**0.23** | -0.38<br>0.23 | D |
| **Spleen Mass** | | | 0 | -0.30<br>0.23 | **-0.70**<br>**0.16** | -0.40<br>0.16 | D |
| **MxDMn HRV as Vagal tone** | **-0.173** | 0.094 | 0 | -0.48<br>0.16 | **-0.65**<br>**0.08** | -0.17<br>0.08 | D |
| **Testosterone** | | | 0 | -0.74<br>0,58 | **-0.99**<br>**0.34** | -0.25<br>0.34 | D |
| **Spleen Mass Index** | | | 0 | -0.70<br>0.23 | **-1.06**<br>**0.22** | -0.36<br>0.22 | D |
| **Root 2 (49%)** | **Root 1** | **Root 2** | 2.41 | **-3.06** | 0.37 | | |
| **P-Q interval ECG** | | | 0 | **-2.23**<br>**0.41** | -1.66<br>0.35 | 0.57<br>0.36 | E |
| **T wave ECG** | -0.041 | **0.312** | 0 | **-2.68**<br>**0.31** | -1.26<br>0.49 | 1.43<br>0.49 | E |
| **S-T joint ECG** | 0.048 | **0.239** | 0 | **-1.63**<br>**0.24** | -0.33<br>0.41 | 1.30<br>0.41 | E |
| **Theophylline-susceptible T-Lymphocytes** | -0.045 | **0.192** | 0 | **-0.95**<br>**0.25** | -0.52<br>0.20 | 0.43<br>0.20 | E |
| **Pan Lymphocytes of Blood** | | | 0 | **-0.97**<br>**0.48** | -0.59<br>0.18 | 0.38<br>0.18 | E |
| **Na,K-ATPase of Erythrocytes** | 0.009 | **0,216** | 0 | **-0,91**<br>**0,18** | -0,40<br>0,22 | 0,51<br>0,22 | E |

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

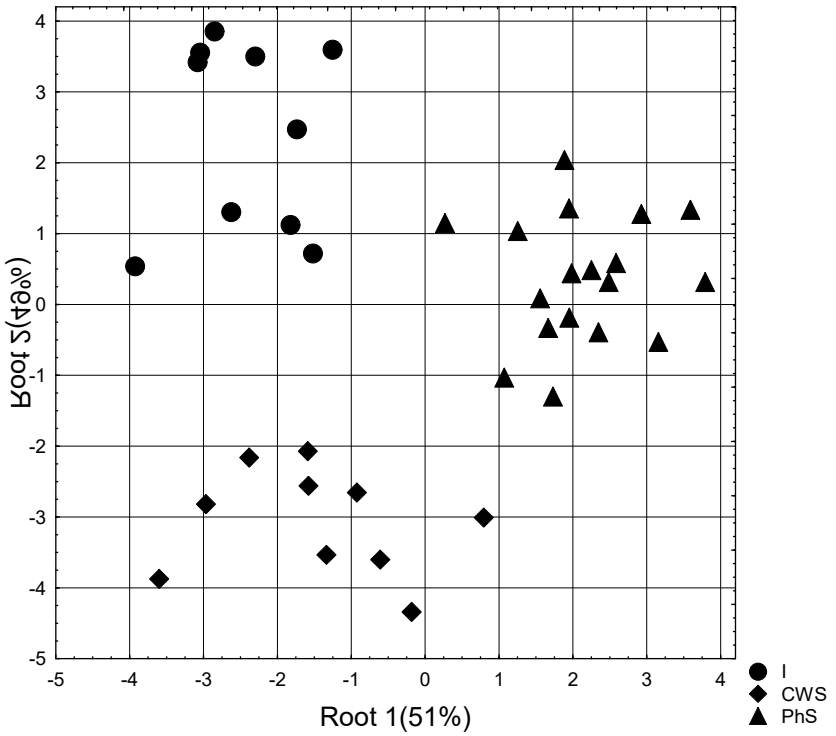


**Fig. 3**. Individual values of discriminative roots of **intact** rats and one day after acute stress, which was preceded by weekly administration of **daily (control) water** (CWS) and **phytocomposition** (PhS). The roots contain condensed information about 30 parameters

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<br>0.0003 | 0 | 24.5 |
| **UPhCBT + Stress (18)** | 5.7<br>0.0002 | 5.6<br>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).

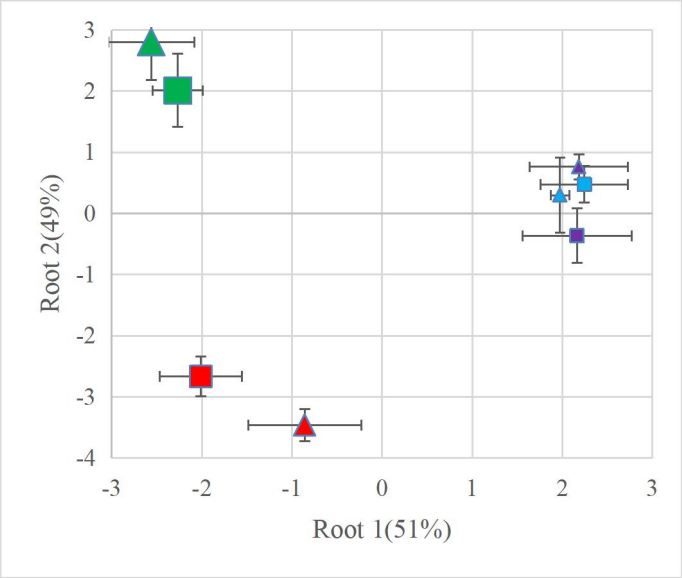


**Fig. 4.** Mean (M±SE) values of discriminant roots of female (triangles) and male (squares) rats **intact** and one day after acute stress, which was preceded by weekly administration of **daily water** and phytocomposition dissolved in **daily water** or "**Truskavetska"™ water**

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).

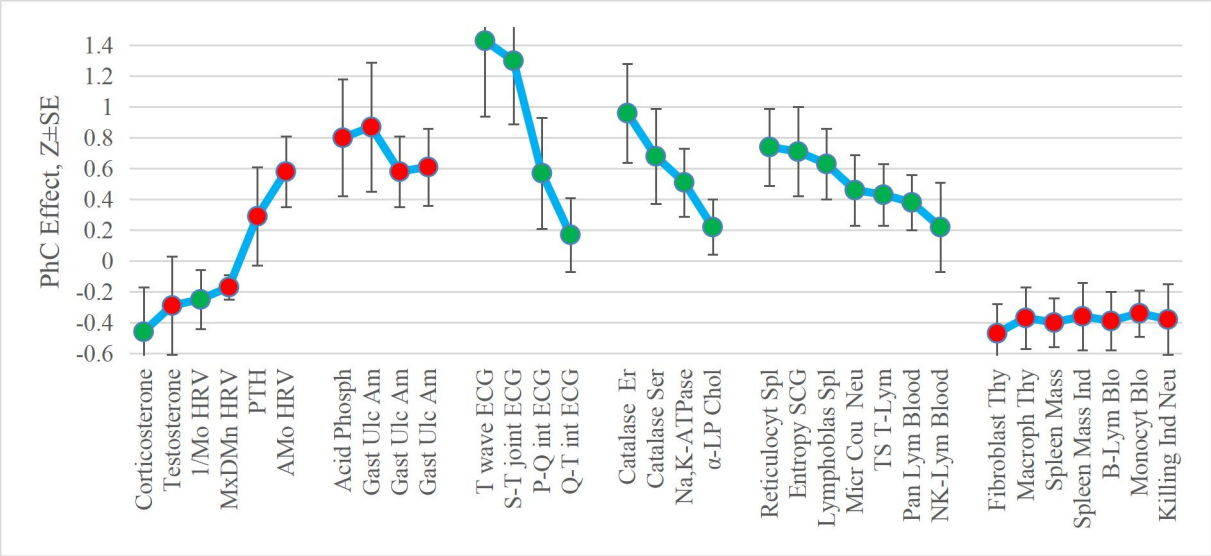


**Fig. 5.** Clusters of simulated **favorable** and **unfavorable** effects of phytocomposition per se (essentially) on post-stress parameters of rats. See please Table 4

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**

We express sincere gratitude to PhD Volodymyra R. Bilas and Galyna Yo. Matiyishyn for help in carry out of immune and biochemical analyses.

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