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BIOACTIVE WATER NAFTUSSYA AND OZOKERITE HAVE THE SAME NEURO-ENDOCRINE-IMMUNE EFFECTS IN MALE RATS CAUSED BY ARYL HYDROCARBONS

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Abstract

Background. It is known that biological activity of curative water Naftussya (N) spa Truskavets' (Ukraine) caused by its organic substances, genetically connected with Oil and Ozokerite (O) from Boryslav's layer, located near from Truskavets'. Because probable aryl hydrocarbon receptor (AhR) agonists are present in both N and O, and AhR-expressing macrophages are localized in lymphoid tissue associated with both the gut (GALT) and the skin (SALT), we hypothesized that both oral use of N, and application to the skin O can have similar immunotropic effects. The study is devoted to testing this hypothesis.

Material and Methods. Experiment was performed on 50 healthy Wistar male rats. Animals of the first group remained intact, using tap water. Instead, the other rats received the same tap water and N through the tube for 6 days. Another group received together with N three applications of O, and the last - only O. The day after the completion of the drinking/application course we recorded the parameters of the neuro-endocrine-immune complex.

Results. Discriminant analysis revealed 23 parameters (5 of the thymus, 3 of the spleen, 6 of blood, 7 endocrine and 2 of HRV), the set of which intact, control and experimental rats differ significantly from each other. Calculation of centroids separately for groups treated with N, O, or both balneofactors showed no differences at all.

Conclusion. The modulating effect on 23 parameters of the neuro-endocrine-immune complex N and O, as well as their combination is almost equivalent which is probably due to the binding of aryl hydrocarbons to AhR of macrophages of GALT and SALT.

Keywords: *aryl hydrocarbons, neuro-endocrine-immune complex, Naftussya, Ozokerite, Truskavets spa.*

Introduction

It is known that biological activity of curative water Naftussya spa Truskavets' (Ukraine) caused by its organic substances, genetically connected with Oil (Naphta in Greek) and Ozokerite from Boryslav's layer, located near (5-7 km) from Truskavets'. Some effects of Naftussya water reproduced by obtained from it organic substances. In biotechnological experiment have been shown that organic substances produced during cultivation sowed from Naftussya water hydrocarbon oxidizing microbes in medium contained water-bearing dirt as well as Oil or Ozokerite [1-4].

In clinical observations we have shown that course drinking of stable water solution of the Ozokerite imitates favorable effects of bioactive Naftussya water on parameters of immune and autonomous nervous systems at volunteers with their dysfunction [5].

Although the aryl hydrocarbon receptor (AhR) was initially recognized as the receptor mediating the pathologic effects of dioxins and other pollutants [6], the activation of AhR by endogenous (bilirubin and biliverdin [7]) and environmental (polycyclic aromatic hydrocarbons, polyphenols, indoles, flavonoids) factors has important physiologic effects, including the regulation of the immune response [8-10]. AhR translocates into the nucleus upon binding of various small molecules into the pocket of its single-ligand binding domain. AhR binding to both xenobiotic and endogenous ligands results in highly cell-specific transcriptome changes and in changes in cellular functions [7]. Both adaptive and innate immune cells require AhR signaling at critical checkpoints. AhR signaling is considered a promising drug and preventive target, particularly for cancer, inflammatory, and autoimmune diseases [11].

Because probable AhR agonists are present in both Naftussya water and Ozokerite, and AhR-expressing macrophages are localized in lymphoid tissue associated with both the gut (GALT) and the skin (SALT), we hypothesized that both oral use of Naftussya water, and application to the skin Ozokerite can have similar immunotropic effects. The study is devoted to testing this hypothesis. Due to the close relationship between the immune, nervous and endocrine systems [12-19], the parameters of the latter have also been studied.

Methods

Experiment was performed on 50 healthy Wistar male rats (body mass M±SD: 260±30 g) divided into 5 equal groups. Animals of the first group remained intact, using tap water from drinking ad libitum. Instead, the other rats received the same tap water and bioactive Naftussya water (Table 1) through the tube at a dose of 1,5 mL/100 g of the body mass for 6 days. Another group of rats received together with Naftussya water three applications on the tail of Ozokerite (t^0 40-42°C, duration 30 minutes, every other day), and the last - only Ozoketite applications.

During the application session, the animals were in individual tight plexiglass chambers, so to neutralize the influence of the immobilization factor, rats of other groups after the introduction of water by the tube were also placed in the chamber for 30 min. To neutralize the influence of other possible factors, one animal from each group was taken daily for procedures (intact rats moved freely in a standard cage).

We adduce data by Dats'ko OR et al [20] about organic compounds (in mg/L) Naftussya water obtained by Solid Phase Extraction method and Mass-spectroscopy by using as Sorbents Tenacle GC 60/80 and Polysorb-2. Paraffins 4,10 and 4,20; monoolefins 1,67 and 1,75; dienes and monocycloolefins 0,84 and 0,85; alkylbenzene 1,55 and 1,54; alkenylbenzene 0,47 and 0,46; esters of aromatic acids 1,32 and 1,33; alkyl phenols 1,14 and 1,14; polyaromatic hydrocarbons 0,077 and 0,059; oxygene-containing connections (acids) 1,12 and 1,14; sulfur-containing connections 0,30 and 0,31; alkylnaphthalenes 0,53 and 0,53; unidentified polyaromatic hydrocarbons 0,19 and 0,19; connections required subsquent identification 0,48 and 0,50 correspondingly.

The day after the completion of the drinking/application course in all rats, at first, a sample of peripheral blood (by incision of the tip of the tail) was taken for analysis of leukocytogram, i.e., the relative content of lymphocytes, monocytes, eosinophils, basophils, rod-shaped and polymorphonucleary neutrophils.

Then they assessed the state of autonomous regulation. For this purpose, under an easy ether anesthesia, for 15-20 sec ECG was recorded in the lead II, inserting needle electrodes under the skin of the legs, followed by the calculation of the parameters of the HRV: mode (Mo), amplitude of the mode (AMo) and variational swing (MxDMn) as markers of the humoral channel of regulation (circulating catechol amines, steroids, glucagon etc), sympathetic and vagal tones respectively [21].

Animals were then placed in individual chambers with perforated bottom for collecting daily urine. The experiment was completed by decapitation of rats in order to collect as much blood as possible.

The plasma levels of the hormones of adaptation were determined: corticosterone, triiodothyronine and testosterone (by the ELISA) as well as plasma and daily urine sodium and potassium (flamming photometry). The latter also determined the concentration of 17-ketosteroids (by color reaction with m-dinitrobenzene). The analyses were carried out according to the instructions.

The analyzer "Tecan" (Oesterreich) were used with appropriate sets and a flamming spectrophotometer "СФ-47".

According to the parameters of potassium and sodium exchange, mineralocorticoid activity was evaluated by coefficients $(Nap/Kp)^{0,5}$ and $(Ku/Nau)^{0,5}$, based on their classical effects.

In the blood, the parameters of immunity were determined as described in the manual [22]: the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep, their theophylline-resistant and theophylline-susceptible subpopulations (by the test of sensitivity of rosette formation to theophylline); the population of B-lymphocytes (by the test of complementary rosette formation with erythrocytes of sheep). Natural killers were identified as large granules contain lymphocytes. The content of 0-lymphocytes was calculated by the balance method. The blast transformation reaction of T-lymphocytes to Phytohemagglutinin was performed separately.

About the condition of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytosis index, the microbial count and the killing index for Staphylococcus aureus (ATCC N25423 F49) [23].

After decapitation, the spleen, thymus and adrenal glands were removed from the animals. In the adrenal glands after weighing, the thickness of glomerular, fascicular, reticular and medullar zones was measured under a microscope.

Immune organs weighed and made smears-imprints for counting thymocytogram and splenocytogram. The components of the thymocytogram are lymphocytes, lymphoblasts, reticulocytes, macrophages, endotheliocytes, epitheliocytes and Hassal's corpuscles. The splenocytogram includes lymphocytes, lymphoblastes, plasma cells, reticulocytes, macrophages, fibroblasts, microphages and eosinophils.

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The conduct of experiments was approved by the Ethics Committee of the Ukrainian Scientific Research Institute for Medicine of Transport. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

Digital material is statistically processed on a computer using the software package "Statistica 64".

Results

Based on the hypothesis of the same effects, the groups of animals that received Naftussya water and Ozokerite as well as both factors were combined into the group "Balneofactors".

To identify those parameters of the neuro-endocrine-immune complex, the set of which intact, control and experimental rats differ significantly from each other, the available information space was subjected to discriminant analysis by forward stepwise method [24]. The program included 23 parameters (variables) in the discriminant model, in particular, 5 variables of the thymus, 3 variables of the spleen, 6 variables of blood, 7 endocrine variables and two parameters of HRV (Table 2).

Other registered parameters were outside the model, but 8 are still worth noting (Table 3).

The distinctive information contained in the 23 discriminant variables is condensed into two roots. The first root contains 80% of the discriminatory potential (r^*=0,959; Wilks' Λ=0,021; $\chi^2_{(46)}$=139; $p<10^{-6}$) and the second root contains 20% (r^*=0,861; Wilks' Λ=0,258; $\chi^2_{(22)}$=49; $p<10^{-3}$).

Calculating the values of the discriminant root for each animal as the sum of the product of the raw coefficients on the individual values of the discriminant variables together with a constant (Table 4) makes it possible to visualize each rat in the information space of the roots (Fig. 1).

As we can see, intact, control and experimental animals are very clearly distinguished in the two-dimensional information space of discriminant canonical roots.

Since the rats of the control group were loaded through the tube with the same water that the intact consumed ad libitum, and in addition kept for 30 minutes in a tight chamber, the detected changes are obviously manifestations of chronic aversive stress. The shift along the axis of the first root of the centroid of the cluster of control animals relative to the centroid of intact animals reflects the stress-induced increase in the levels of parameters that correlate with the root **directly** and, accordingly, the decrease in the levels of parameters associated with the root **inverse** (Table 5).

Further shift of the centroid of rats exposed to balneofactors, and more pronounced than in the control (5,00 un. vs 2,79 un.), reflects the strengthening of both **upregulating** and **downregulating** effects of stress. Since the aversion (introduction into the esophagus of the tube, immobilization, etc.) was similar, the detected changes in the parameters can probably be attributed to organic matter in general and ARh agonists in particular.

The following picture emerges. Stress-induced increases in sympathetic tone (AMo) and circulating catechol amines and/or glucagon (Mode as inverse marker) and decreases in vagus tone (MxDMn) and in the size of the glomerular zone of the adrenal cortex are accompanied by an increase in the content of **macrophages/monocytes** in the thymus, spleen and blood and the intensity of phagocytosis by them Staph. aureus with a slight decrease in the activity of phagocytosis. At the same time, the content in the thymus of lymphocytes, in the spleen - lymphoblastes and microphages/neutrophils, in the blood - theophylline-resistant T-lymphocytes decreases in combination with an increase in the content of 0-lymphocytes. The latter can be interpreted as a decrease in lymphocyte expression of CD3 and CD4 receptors.

In addition to the aversarial factors, the body's presentation of organic matter by both the intestinal mucosa and the skin exacerbates these neuro-endocrine and immune manifestations of chronic stress.

Instead, the effect of chronic stress on another constellation of parameters of the neuro-endocrine-immune complex, information about which is condensed in the second root, balneofactors are minimized, nullified or even reversed. Quantitatively, this is expressed by reducing the shift of the centroid of the second root from 5,00 un. to 3,40 un.

The downregulation is performed in relation to stress-induced thickening of the reticular and fascicular areas of the adrenal cortex and glandular mass, plasma levels of corticosterone and testosterone - on the one hand, and thymus mass and it Hassal's corpuscles and epitheliocytes, as well as the phagocytic activity of blood neutrophils - on the other hand.

The upregulation is carried out in relation to the mineralocorticoid activity and medullar zone of adrenals - on the one hand, and to the content of reticulocytes in the spleen and the thymus, endotheliocytes in the latter, rod-shaped neutrophils and NK-lymphocytes in blood as well as blast transformation of T-lymphocytes - on the other hand.

By calculating the algebraic differences between the Z-scores of variable animals loaded with balneofactors and tap water, the effects of aryl hydrocarbons per se can be estimated (Fig. 2).

Calculation of centroids separately for groups of rats treated with Naftussya water, Ozokerite applications, or both balneofactors showed no differences at all (Fig. 3). Therefore, the modulating effect on these parameters of the neuro-endocrine-immune complex Naftussya water and ozokerite, as well as their combination is almost equivalent.

Finally, the canonical correlation between the mentioned neuro-endocrine and immune parameters was analyzed. The placement of sets on the abscissa (argument) or ordinate (function) is quite conditional, because the nervous, endocrine and immune systems **interact** with each other through neurotransmitters, hormones and cytokines, the sources of which are both neurons, endocrinocytes and immunocytes. We have once again confirmed the well-known interaction (Table 6 and Fig. 3).

The obtained results, taken together with the existing ideas about neuro-endocrine-immune interactions [12-19], give us grounds for further speculation. When balneofactors are used alone or together, the AhR agonists present in them are likely to activate macrophages of GALT and/or SALT expressing these receptors. Macrophages, in turn, release cytokines that excite chemoreceptors of both the afferent fibers of the vagus nerve of the mucosa and the somatic nerves of the skin, through which the impulses reach the CNS structures responsible for regulating immunity and adaptive responses. In addition, cytokines can reach the CNS through the blood. The ability of macrophages to release hormones, in particular CRH and ACTH, as well as the ability of neurons to release cytokines, is also known.

In conclusion, we consider it necessary to emphasize that in this study, attention was focused on the parameters of the neuro-endocrine-immune complex, the reactions of which are the same for the use of both Naftussya water and Ozokerite. However, in clinical observation it was shown [25,26] that there are also significant differences in the reactions of other parameters due to the presence in the water of autochtone microflora [2,4], which probably interacts with GALT immunocytes through their TL-receptors. This will be the topic of the next experimental study.

Acknowledgments

The authors declsre that there are no conflicts of interest.

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Table 1. Chemical composition of tap water and bioactive Naftussya water

	Tap (Control) Water	Bioactive Naftussya Water
mM/L		
Ca^{2+}	3,4	2,9
Mg^{2+}	0,5	2,3
K^{+}	0,4	0,3
Na^{+}	0,5	0,6
HCO_3^{-}	2,9	8,3
Cl^{-}	3,4	1,0
SO_4^{2-}	1,2	1,0
mg/L		
H_2SiO_3	5	9,5
H_3BO_3	0,25	0,20
F	0,95	0,160
Br	8,3	0,034
J	0,025	0,004
C org	5,0	13,8
N org	0,02	0,33

Table 2. Summary of discriminant analysis of neuro-endocrine-immune parameters at male rats. Variables currently in the model (Step 23, N of vars in model: 23; Grouping: 3 grps; Wilks' Λ: 0,021; approx. $F_{(46)}$=6,4; $p<10^{-6}$)

Variables	**Intact group (10)**	**Tap Water (10)**	**Balneo-factors (30)**	Wilks' Λ	Partial Λ	F-remove (2,25)	p-level	Tolerancy
1	2	3	4	5	6	7	8	9
Macrophages of Thymus, %	4,70 0,144	5,10 +0,59	6,48 +2,63	0,076	0,274	33,2	10^{-6}	0,055
Phagocytic Index of Monocytes of Blood, %	7,8 0,436	6,7 -0,32	5,3 -0,75	0,052	0,402	18,6	10^{-5}	0,308
Lymphocytes of Thymus, %	53,4 0,067	52,2 -0,35	51,9 -0,44	0,064	0,329	25,5	10^{-6}	0,050
Fascicular Zone of Adrenal Cortex, µM	218 0,166	257 +1,08	241 +0,65	0,062	0,337	24,6	10^{-6}	0,095
Reticulocytes of Thymus, %	5,20 0,382	4,90 -0,15	5,72 +0,26	0,046	0,458	14,8	10^{-4}	0,159
Reticulocytes of Spleen, %	14,3 0,124	12,7 -0,90	13,9 -0,24	0,031	0,670	6,16	0,007	0,360
Hassal's corpuscles of Thymus, %	1,70 0,504	2,10 +0,47	1,85 +0,17	0,040	0,517	11,7	10^{-3}	0,150
Endotheliocytes of Thymus, %	7,40 0,182	6,00 -1,04	6,21 -0,89	0,030	0,694	5,51	0,010	0,362
$(Nap/Kp)^{0,5}$ as Mineralo-corticoid Activity	6,10 0,190	5,55 -0,47	5,83 -0,24	0,025	0,820	2,74	0,084	0,373

Reticular Zone of Adrenal Cortex, µM	20,0 0,289	26,3 +1,08	22,6 +0,45	0,028	0,734	4,52	0,021062	0,300
1	2	3	4	5	6	7	8	9
Microphages of Spleen, %	11,5 0,137	11,3 -0,13	10,4 -0,70	0,029	0,732	4,58	0,020	0,280
Theophylline-resistant T-Lymphocytes, %	32,3 0,077	32,0 -0,12	30,5 -0,72	0,027	0,781	3,50	0,046	0,404
Lymphoblastes of Spleen, %	5,10 0,235	4,80 -0,25	4,43 -0,56	0,027	0,782	3,48	0,046	0,480
Adrenals Mass Index, mg/100g Body Mass	19,5 0,092	19,8 +0,18	19,1 -0,20	0,026	0,799	3,14	0,061	0,440
Monocytes of Blood, %	4,20 0,548	4,60 +0,17	5,37 +0,51	0,023	0,891	1,54	0,235	0,717
Medullar Zone of Adrenals, µM	87 0,271	75 -0,51	90 +0,16	0,027	0,766	3,81	0,036	0,519
Rod-shaped Neutrophils of Blood, %	3,50 0,151	2,90 -1,14	3,40 -0,19	0,025	0,831	2,55	0,098	0,436
Glomerular Zone of Adrenal Cortex, µM	129 0,279	116 -0,38	112 -0,48	0,025	0,840	2,38	0,113	0,431
AMo HRV as Sympathetic tone, %	53 0,446	58 +0,21	68 +0,63	0,034	0,614	7,84	0,002	0,096
Mode HRV as Humoral Channel, msec	189 0,191	185 -0,10	163 -0,73	0,033	0,627	7,44	0,003	0,117
Corticosterone, nM/L	333 0,404	429 +0,72	405 +0,53	0,025	0,852	2,18	0,135	0,437
NK-Lymphocytes of Blood, %	10,4 0,180	9,2 -0,61	9,4 -0,52	0,025	0,849	2,23	0,128	0,569
Phagocytic Index of Neutrophils of Blood, %	82,3 0,027	83,4 +0,50	81,4 -0,39	0,023	0,905	1,31	0,288	0,525

Note. For animals of all groups, the upper row reflects the average value, the lower row for intact (I) animals reflects the coefficient of variability (Cv), and for loaded (L) animals Z-score: Z = (L-I)/I •Cv.

Table 3. Summary of discriminant analysis of neuro-endocrine-immune parameters at male rats. Variables currently not in the model

Variables	**Intact group (10)**	**Tap Water (10)**	**Balneo-factors (30)**	Wilks' Λ	Partial Λ	F-remove (2,24)	p-level	Tolerancy
Microbial Count of Monocytes of Blood, Bactera/Phagocyte	2,8 0,118	3,1 +0,78	3,5 +2,16	0,020	0,977	0,29	0,753	0,258
Macrophages of Spleen, %	5,50 0,376	6,20 +0,34	7,73 +1,08	0,021	0,999	0,02	0,984	0,096
o-Lymphocytes of Blood, %	29,9 0,160	31,3 +0,31	33,5 +0,79	0,021	0,996	0,05	0,953	0,317
MxDMn HRV as Vagal Tone, msec	45 0,557	41 -0,16	29 -0,65	0,020	0,974	0,32	0,730	0,003
T-lymphocytes Blast Transfor-mation Reaction by PhHA, %	65,8 0,177	59,8 -0,51	62,7 -0,26	0,020	0,970	0,10	0,850	0,333
Testosterone, nM/L	35,1 0,416	50,3 +1,05	36,5 +0,10	0,020	0,974	0,33	0,725	0,630
Thymus Mass Index, mg/100g Body Mass	29 0,231	36 +0,94	33 +0,59	0,021	0,998	0,02	0,979	0,423
Epitheliocytes of Tymus, %	19,9 0,115	21,8 +0,83	20,2 +0,15	0,020	0,969	0,39	0,683	0,047

Table 4. Standardized and Raw coefficients and Constants for neuro-endocrine-immune variables ranked by criterion Λ

Coefficients	Standardized		Raw		Parameters of Wilks' Statistics				
Variables urrently in the model	Root 1	Root 2	Root 1	Root 2	F to enter	p-value	**Λ**	F-value	p-value
Macrophages of Thymus	3,787	0,133	2,468	0,087	6,38	0,004	0,786	6,38	0,004
Phagocytic Index of Monocytes	-1,428	0,295	-0,607	0,125	5,13	0,010	0,643	5,68	10^{-3}
Lymphocytes of Thymus	3,740	-0,917	0,779	-0,191	6,76	0,003	0,494	6,33	10^{-4}
Fascicular Zone Adrenal Cortex	2,592	1,048	0,055	0,022	5,42	0,008	0,397	6,46	10^{-6}
Reticulocytes of Thymus	1,895	-0,381	1,032	-0,208	4,78	0,013	0,325	6,50	10^{-6}
Reticulocytes of Spleen	0,906	-0,468	0,501	-0,259	9,32	10^{-3}	0,225	7,77	10^{-6}
Hassal's corpuscles of Thymus	1,287	1,519	1,456	1,719	4,30	0,020	0,186	7,73	10^{-6}
Endotheliocytes of Thymus	-0,682	-0,750	-0,439	-0,484	5,36	0,009	0,147	8,06	10^{-6}
$(Nap/Kp)^{0,5}$ as Mineralocort Act	0,718	0,105	0,907	0,133	4,71	0,015	0,118	8,28	10^{-6}
Reticular Zone Adrenal Cortex	-0,697	0,768	-0,104	0,115	2,75	0,077	0,103	8,03	10^{-6}
Microphages of Spleen	-0,808	0,693	-0,359	0,308	2,45	0,100	0,091	7,78	10^{-6}
Theophylline-resistant T-Lym	-0,716	0,306	-0,272	0,116	2,36	0,109	0,081	7,57	10^{-6}
Lymphoblastes of Spleen	-0,701	0,068	-0,643	0,062	1,94	0,158	0,072	7,31	10^{-6}
Adrenals Mass Index	0,604	0,404	0,016	0,011	1,96	0,156	0,065	7,10	10^{-6}
Monocytes of Blood	0,407	-0,005	0,210	-0,003	1,89	0,166	0,058	6,91	10^{-6}
Medullar Zone of Adrenals	-0,626	-0,349	-0,023	-0,013	2,16	0,131	0,051	6,83	10^{-6}
Rod-shaped Neutrophils Blood	0,181	-0,695	0,181	-0,698	1,41	0,260	0,047	6,58	10^{-6}
Glomerular Zone Adren Cortex	0,605	-0,219	0,024	-0,009	2,55	0,095	0,040	6,64	10^{-6}
AMo HRV as Sympathetic tone	1,492	1,629	0,064	0,070	1,49	0,243	0,036	6,46	10^{-6}
Mode HRV as Humoral Chann	1,164	1,614	0,032	0,045	3,54	0,043	0,029	6,80	10^{-6}
Corticosterone	0,244	0,619	0,001	0,003	1,62	0,216	0,026	6,69	10^{-6}
NK-Lymphocytes of Blood	-0,363	-0,443	-0,237	-0,290	1,63	0,215	0,023	6,60	10^{-6}
Phagocytic Index of Neutrophils	0,366	0,278	0,096	0,073	1,31	0,288	0,021	6,43	10^{-6}
	Constants		-86,06	-16,97					
	Eigenvalues		11,38	2,87					

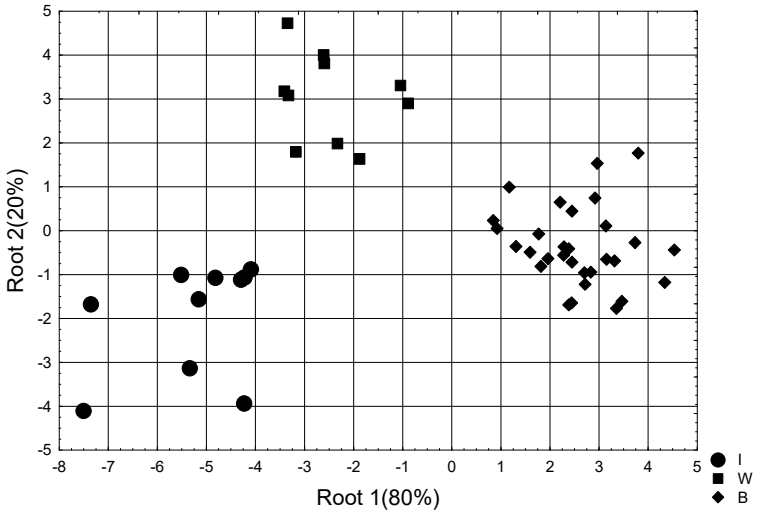


Fig. 1. Individual values of neuro-endocrine-immune roots of intact male rats, loaded with tap water and receiving divers balneofactors

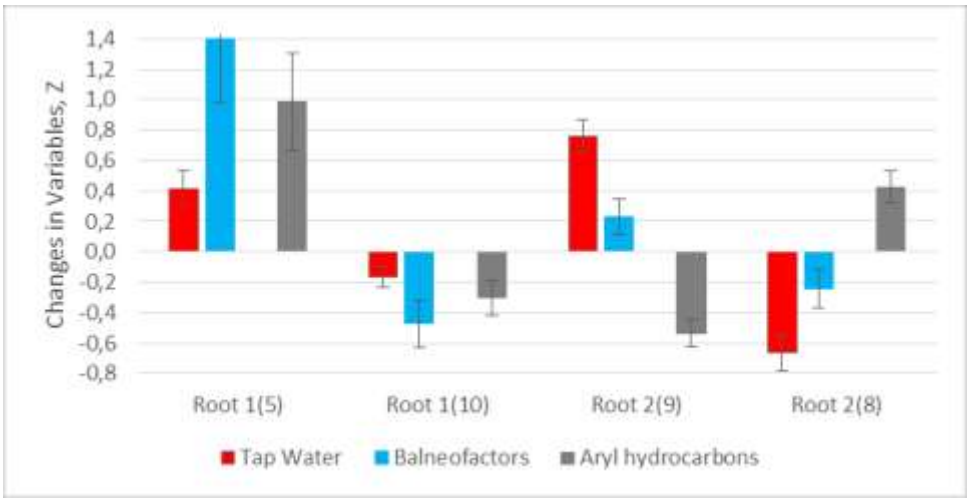


Fig. 2. Simulated effects of aryl hydrocarbons on the parameters of the neuro-endocrine-immune complex of male rats

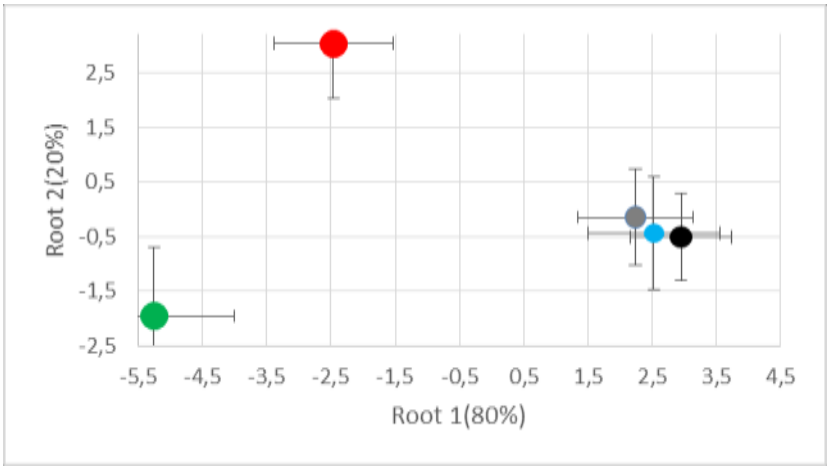


Fig. 3. Mean values (M±SD) of neuro-endocrine-immune roots of intact male rats, loaded with tap water and Naftussya water, as well as receiving applications of ozokerite and ozokerite and Naftussya

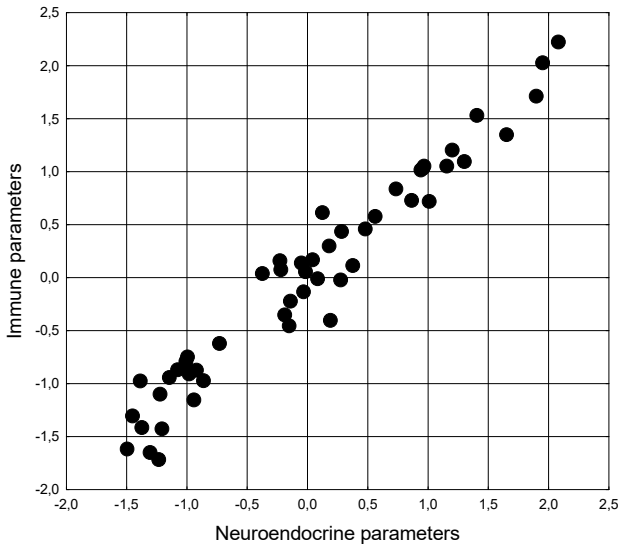
Table 5. Correlations Variables - Canonical Roots, Centroids of Roots and Z-scores of neuroendocrine-immune variables at male rats

Variables currently as well as currently not in the model	**Correlations Variables - Canonical Roots**		**Z-scores for Variables**		
			Intact group (10)	**Tap Water (10)**	**Balneo-factors (30)**
Root 1 (80%)	**R1**	**R2**	**-5,25**	**-2,46**	**+2,57**
Macrophages of Thymus, %	**0,154**	-0,034	**0**	**+0,59**	**+2,63**
AMo HRV as Sympathetic tone, %	**0,082**	-0,003	**0**	**+0,21**	**+0,63**
Monocytes of Blood, %	**0,077**	-0,002	**0**	**+0,17**	**+0,51**
Microbial Count Monocytes of Blood, Bac/Phag			**0**	**+0,78**	**+2,16**
Macrophages of Spleen, %			**0**	**+0,34**	**+1,08**
o-Lymphocytes of Blood, %			**0**	**+0,31**	**+0,79**
Phagocytic Index of Monocytes of Blood, %	**-0,134**	-0,017	**0**	**-0,32**	**-0,75**
Mode HRV as Humoral Channel, msec	**-0,101**	0,036	**0**	**-0,10**	**-0,73**
Theophylline-resistant T-Lymphocytes, %	**-0,093**	0,029	**0**	**-0,12**	**-0,72**
Lymphoblastes of Spleen, %	**-0,076**	-0,013	**0**	**-0,25**	**-0,56**
Glomerular Zone of Adrenals Cortex, µM	**-0,074**	-0,068	**0**	**-0,38**	**-0,48**
Microphages of Spleen, %	**-0,066**	0,019	**0**	**-0,13**	**-0,70**
Lymphocytes of Thymus, %	**-0,035**	-0,032	**0**	**-0,35**	**-0,44**
MxDMn HRV as Vagal Tone, msec			**0**	**-0,16**	**-0,65**
Root 2 (20%)	**R1**	**R2**	-1,96	**+3,04**	-0,36
Reticular Zone of Adrenal Cortex, µM	0,013	**0,180**	0	**+1,08**	+0,45
Fascicular Zone of Adrenals Cortex, µM	0,034	**0,148**	0	**+1,08**	+0,65
Hassal's corpuscles of Thymus, %	0,003	**0,089**	0	**+0,47**	+0,17
Phagocytic Index Neutrophiles of Blood, %	-0,046	**0,084**	0	**+0,50**	-0,39
Corticosterone, nM/L	0,029	**0,078**	0	**+0,72**	+0,53
Adrenals Mass Index, mg/100g Body Mass	-0,018	**0,027**	0	**+0,18**	-0,20
Testosterone, nM/L			0	**+1,05**	+0,10

Thymus Mass Index, mg/100g Body Mass			0	**+0,94**	+0,59
Epitheliocytes of Thymus, %			0	**+0,83**	+0,15
Reticulocytes of Spleen, %	0,006	**-0,181**	0	**-0,90**	-0,24
Endotheliocytes of Thymus, %	-0,069	**-0,142**	0	**-1,04**	-0,89
Rod-shaped Neutrophils of Blood, %	0,013	**-0,128**	0	**-1,14**	-0,19
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	-0,018	**-0,128**	0	**-0,47**	-0,24
NK-Lymphocytes of Blood, %	-0,057	**-0,116**	0	**-0,61**	-0,52
Medullar Zone of Adrenals, µM	0,039	**-0,109**	0	**-0,51**	+0,16
Reticulocytes of Thymus, %	0,048	**-0,060**	0	**-0,15**	+0,26
T-lymphocyte Blast Transformation Reaction, %			0	**-0,51**	-0,26

Table 6. Factor load on neuro-endocrine (left set) and immune (right set) canonical roots at male rats

Left set	**R**
AMo HRV as Sympathetic tone, %	**0,924**
$(Nap/Kp)^{0,5}$ as Mineralocorticoid Activity	**0,371**
Corticosterone, nM/L	**0,193**
MxDMn HRV as Vagal Tone, msec	**-0,866**
Mode HRV as Humoral Channel, msec	**-0,698**
Adrenals Mass Index, mg/100g Body Mass	**-0,355**
Glomerular Zone of Adrenal Cortex, µM	**-0,301**
Fascicular Zone of Adrenal Cortex, µM	**-0,246**
Reticular Zone of Adrenal Cortex, µM	**-0,184**
Right set	**R**
Macrophages of Spleen, %	**0,864**
Hassal's corpuscles of Thymus, %	**0,273**
Endotheliocytes of Thymus, %	**0,271**
Rod-shaped Neutrophils of Blood, %	**0,257**
Theophylline-resistant T-Lymphocytes, %	**0,248**
Reticulocytes of Spleen, %	**0,196**
Epitheliocytes of Thymus, %	**0,194**
Macrophages of Thymus, %	**0,185**
NK-Lymphocytes of Blood, %	**0,172**
o-Lymphocytes of Blood, %	**-0,446**
Microphages of Spleen, %	**-0,406**
Phagocytic Index of Monocytes of Blood, %	**-0,350**
Reticulocytes of Thymus, %	**-0,217**
Lymphocytes of Thymus, %	**-0,192**
Microbial Count Monocytes of Blood, Bac/Phag	**-0,135**



R=0,973; R^2=0,948; $\chi^2_{(209)}$=285; p=0,0004; Λ Prime=0,0002

Fig. 4. Scatterplot of canonical correlation between Neuroendocrine (X-line) and Immune (Y-line) parameters in male rats