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IMMEDIATE NEUROTROPIC EFFECTS OF UKRAINIAN PHYTOCOMPOSITION

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Abstract

Background. Previous experimental and clinical-physiological studies have shown that the Ukrainian phytocomposition "Balm Kryms'kyi" has adaptogenic properties, which are manifested in its vegetative, endocrine, immunotropic, coagulotropic, actotropic and metabolic effects. However, the neurotropic properties of the phytocomposition remain unclear, which is the purpose of this study. **Materials and methods.** The object of observation were 12 women (44±13 years) and 62 men (44±12 years) with dysfunction of neuro-endocrine-immune complex. HRV and EEG parameters were recorded in the morning in basal conditions. Then the members of the main group used 5 ml of phytocomposition dissolved in 45 ml of tap water, instead in the control group used 50 ml of the latter. After 1,5 hours, the test was repeated. **Results**. Discriminant analysis revealed 30 EEG parameters and 4 HRV parameters characteristic of the initial state and after consumption of phytocomposition or tap water. **Conclusion**. Ukrainian phytocomposition "Balm Truskavets" causes immediate neurotropic effects at patients with dysfunction of neuro-endocrine-immune complex.
Keywords: phytocomposition "Balm Truskavets", EEG, HRV, immediate effects.

INTRODUCTION

Previous experimental and clinical-physiological studies have shown that the Ukrainian phytocomposition "Balm Kryms'kyi" [23] has adaptogenic properties, which are manifested in its vegetative, endocrine, immunotropic, coagulotropic, actotropic and metabolic effects [1,5,6,8,9,16,20,22]. However, the neurotropic properties of the phytocomposition remain unclear, which is the purpose of this study.

MATERIAL AND METHODS

The object of observation were 12 women (44±13 years) and 62 men (44±12 years) with dysfunction of neuro-endocrine-immune complex, employees of the clinical sanatoria

“Kryshtalevyĭ Palats” and "Moldova" (Truskavets’, Ukraine). Every of morning before work, carried out initial tests of 6 persons, then the two of them (basic group) used 5 ml of phytocomposition “Balm Truskavets” (ТУ У 15.8-24055046-005:2009, produced by private research and production enterprise "Ukrainian Balms", Mykolayiv, Ukraine), pre-diluted in 45 ml of boiled tap water. This phytocomposition is analogous to the previous “Balm Kryms’kyi”. The other 4 individuals (control group) used 50 ml of the same water at room temperature.

To assess the parameters of heart rate variability (HRV) recorded during 7 min electrocardiogram in II lead (software and hardware complex "CardioLab+HRV", KhAI-MEDICA, Kharkiv). For further analysis the following parameters HRV were selected. Temporal parameters (Time Domain Methods): the standard deviation of all NN intervals (SDNN), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), the percent of interval differences of successive NN intervals greater than 50 msec (pNN_{50}); Triangular Index (TNN). Spectral parameters (Frequency Domain Methods): power spectral density (PSD) bands of HRV - high-frequency (HF, range 0,40÷0,15 Hz), low-frequency (LF, range 0,15÷0,04 Hz), very low-frequency (VLF, range 0,040÷0,015 Hz) and ultralow-frequency (ULF, range 0,015÷0,003 Hz). Calculated classical indexes: LF/HF; (VLF+LF)/HF; LFnu=100%•LF/(LF+HF) [2,4,7].

After 8-13 minutes, the EEG recorded a hardware-software complex “NeuroCom Standard” (KhAI MEDICA, Kharkiv) monopolar in 16 loci (Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, P3, P4, T5, T6, O1, O2) by 10-20 international system, with the reference electrodes A and Ref on the lobes of the ears. The duration of the epoch was 25 sec. Among the options considered the average EEG amplitude (μV), average frequency (Hz), frequency deviation (Hz), index (%) as well as absolute (μV^2/Hz) and relative (%) PSD of basic rhythms: β (35÷13 Hz), α (13÷8 Hz), θ (8÷4 Hz) and δ (4÷0,5 Hz) in all loci, according to the instructions of the device. In addition, calculated coefficient of Asymmetry (As) and Laterality Index (LI) for PSD each Rhythm using formulas:

As, % = 100•(Max – Min)/Min;

LI, % = Σ [200•(Right – Left)/(Right + Left)]/8 [21].

We calculated also for each locus EEG and HRV Shannon’s [31] Entropy (h) of normalized PSD using Popovych’s formulas:

$hEEG = - [PSD\alpha \bullet \log_2 PSD\alpha + PSD\beta \bullet \log_2 PSD\beta + PSD\theta \bullet \log_2 PSD\theta + PSD\delta \bullet \log_2 PSD\delta]/\log_2 4$

$hHRV = - [PSHF \bullet \log_2 PSHF + PSLF \bullet \log_2 PSLF + PSVLF \bullet \log_2 PSVLF + PSULF \bullet \log_2 PSULF]/\log_2 4$

HRV reference values were taken from the instructions for the "CardioLab+HRV" device, and EEG reference from the database of Truskavetsian Scientific School (n=120).

RESULTS AND DISCUSSION

In order to identify among the registered parameters those whose constellation is three states of persons: initial and after the use of a solution of phytocomposition or control water - differ significantly from each other, discriminant analysis was used [13]. The forward stepwise program included 34 variables in the discriminant model (Tables 1 and 2). Among them, 3 relate to **delta-rhythm**, 3 - **theta-rhythm**, 15 - **alpha-rhythm**, 6 - **beta-rhythm**, 3 - **entropy** of EEG loci, and the other 4 - HRV.

Table 1. Discriminant Function Analysis Summary for EEGs and HRVs Variables as well as their Reference levels and Coefficients of Variability

Step 34, N of vars in model: 34; Grouping: 3 grps; Wilks' Λ: 0,252; approx. $F_{(68)}$=3,3; $p<10^{-6}$

Variables currently in the model (n=148)	Groups (n)			Parameters of Wilks' Statistics					
	After Balm (20)	Base-line (74)	After CW (54)	Wilks' Λ	Par-tial Λ	F-re-move (2,1)	p-level	Tole-rancy	Refe-rence Cv
ULF/TP, %	2,3 0,7	6,8 0,8	10,4 1,2	0,284	0,886	7,18	0,001	0,405	4,6 0,674
F3-α PSD, %	24,2 2,2	35,7 2,2	41,4 2,3	0,269	0,937	3,79	0,026	0,036	34,5 0,547
P4-δ PSD, %	14,3 1,7	20,2 1,3	19,4 1,6	0,292	0,861	9,07	10^{-3}	0,172	27,1 0,671
C4-β PSD, μV²/Hz	122 12	89 7	71 5	0,295	0,852	9,69	10^{-4}	0,162	84 0,671
T4-β PSD, μV²/Hz	99 10	88 10	64 4	0,298	0,843	10,40	10^{-4}	0,224	72 0,745
Fp1-α PSD, %	27,1 2,5	37,7 2,3	39,7 2,7	0,259	0,971	1,67	0,193	0,088	37,2 0,501
Asymmetry-β, %	21,7 4,3	23,9 2,1	17,6 2,8	0,259	0,970	1,76	0,178	0,659	20,1 0,699
P4-δ PSD, μV²/Hz	47 8	79 9	95 11	0,282	0,891	6,84	0,002	0,130	133 1,440
LF/HF	3,45 0,85	4,04 0,33	5,21 0,41	0,260	0,967	1,93	0,150	0,364	2,80 0,714
O2-θ PSD, %	7,1 1,0	5,2 0,3	5,0 0,4	0,266	0,945	3,29	0,041	0,289	6,0 0,603
Frequency-δ, Hz	1,08 0,04	1,09 0,02	1,03 0,02	0,264	0,952	2,85	0,062	0,675	1,07 0,165
T6-α PSD, %	23,5 2,7	33,6 2,4	37,3 2,9	0,271	0,928	4,35	0,015	0,094	32,2 0,623
C4-β PSD, %	38,9 3,2	30,9 1,8	25,8 2,1	0,281	0,894	6,64	0,002	0,150	26,3 0,493
Frequency-β, Hz	17,2 0,8	19,6 0,5	20,0 0,5	0,264	0,951	2,86	0,062	0,708	19,0 0,200
P3-β PSD, %	41,6 3,7	30,2 1,8	26,8 2,1	0,273	0,923	4,69	0,011	0,135	25,0 0,549
Entropy O2	0,760 0,029	0,688 0,018	0,666 0,022	0,256	0,984	0,89	0,415	0,144	0,727 0,242
O1-θ PSD, μV²/Hz	24 4	36 5	38 5	0,265	0,948	3,09	0,050	0,163	36 1,213
C3-α PSD, %	26,4 2,2	35,4 2,1	42,4 2,3	0,259	0,972	1,60	0,207	0,055	35,3 0,510
O1-θ PSD, %	9,0 1,3	6,6 0,5	6,0 0,5	0,266	0,946	3,17	0,046	0,184	6,7 0,636
Amplitude-α, μV	13,9 1,9	17,8 1,5	20,9 2,1	0,261	0,962	2,21	0,114	0,026	17,7 0,703
Index α, %	34,1 6,2	42,9 4,0	47,7 4,4	0,261	0,965	2,06	0,133	0,203	48,4 0,558
Entropy O1	0,798 0,036	0,718 0,021	0,709 0,023	0,259	0,972	1,60	0,207	0,130	0,738 0,245
F4-α PSD, %	25,8 2,2	34,7 2,2	41,7 2,4	0,264	0,953	2,75	0,068	0,077	32,7 0,564

Entropy HRV	0,728 0,025	0,766 0,012	0,785 0,013	0,257	0,979	1,20	0,306	0,325	0,825 0,114
O2-α PSD, μV²/Hz	229 84	463 79	697 128	0,267	0,941	3,50	0,034	0,056	410 1,627
F3-α PSD, μV²/Hz	85 11	156 20	183 24	0,268	0,940	3,60	0,031	0,021	146 1,071
Fp1-α PSD, μV²/Hz	63 9	120 15	140 18	0,259	0,972	1,60	0,207	0,021	109 1,063
T6-α PSD, μV²/Hz	49 6	102 15	124 18	0,263	0,956	2,55	0,083	0,110	100 1,397
T3-α PSD, μV²/Hz	62 8	104 13	119 15	0,258	0,974	1,47	0,234	0,099	97 0,988
T3-α PSD, %	24,0 2,0	31,6 1,9	36,2 2,0	0,261	0,965	2,02	0,137	0,073	30,7 0,546
Fp2-α PSD, %	27,5 2,4	35,3 2,2	37,3 2,5	0,258	0,974	1,49	0,230	0,152	33,2 0,535
O1-α PSD, %	32,7 3,1	40,7 3,0	46,3 3,4	0,257	0,979	1,23	0,298	0,081	39,9 0,591
RMSSD, msec	26,4 4,2	23,5 1,5	19,2 1,0	0,259	0,972	1,64	0,199	0,103	37,0 0,459
pNN_{50}, %	8,3 3,7	5,4 1,2	2,6 0,4	0,258	0,974	1,52	0,222	0,100	9,0 0,858

Note. For groups the average values and standard errors are specified; for the norm - the average values and coefficients of variation.

Table 2. Summary of Stepwise Analysis for EEGs and HRVs Variables. The variables are ranked by criterion Lambda

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
ULF/TP, %	10,8	10^{-4}	0,871	10,8	10^{-4}
F3-α PSD, %	8,05	10^{-3}	0,783	9,36	10^{-6}
P4-δ PSD, %	9,22	10^{-3}	0,694	9,56	10^{-6}
C4-β PSD, μV²/Hz	5,79	0,004	0,641	8,83	10^{-6}
T4-β PSD, μV²/Hz	3,95	0,021	0,607	7,98	10^{-6}
Fp1-α PSD, %	3,60	0,030	0,578	7,37	10^{-6}
Asymmetry-β, %	3,03	0,052	0,554	6,83	10^{-6}
P4-δ PSD, μV²/Hz	2,72	0,069	0,533	6,39	10^{-6}
LF/HF	2,97	0,055	0,510	6,08	10^{-6}
O2-θ PSD, %	2,88	0,059	0,490	5,83	10^{-6}
Frequency-δ, Hz	2,37	0,097	0,473	5,57	10^{-6}
T6-α PSD, %	2,36	0,099	0,457	5,35	10^{-6}
C4-β PSD, %	3,06	0,050	0,437	5,25	10^{-6}
Frequency-β, Hz	2,29	0,105	0,422	5,08	10^{-6}
P3-β PSD, %	3,026	0,053	0,404	5,01	10^{-6}
Entropy O2	2,360	0,098	0,389	4,89	10^{-6}
O1-θ PSD, μV²/Hz	1,811	0,168	0,379	4,74	10^{-6}
C3-α PSD, %	1,906	0,153	0,368	4,61	10^{-6}
O1-θ PSD, %	1,704	0,186	0,358	4,48	10^{-6}
Amplitude-α, μV	2,109	0,126	0,347	4,40	10^{-6}
Index α, %	1,625	0,201	0,338	4,29	10^{-6}
Entropy O1	1,534	0,220	0,330	4,18	10^{-6}
F4-α PSD, %	1,496	0,228	0,322	4,08	10^{-6}

Entropy HRV	1,252	0,290	0,315	3,97	10^{-6}
O2-α PSD, μV²/Hz	1,161	0,317	0,309	3,86	10^{-6}
F3-α PSD, μV²/Hz	1,651	0,196	0,301	3,79	10^{-6}
Fp1-α PSD, μV²/Hz	1,174	0,313	0,295	3,70	10^{-6}
T6-α PSD, μV²/Hz	1,541	0,219	0,288	3,64	10^{-6}
T3-α PSD, μV²/Hz	1,539	0,219	0,280	3,58	10^{-6}
T3-α PSD, %	1,251	0,290	0,275	3,51	10^{-6}
Fp2-α PSD, %	1,296	0,278	0,268	3,45	10^{-6}
O1-α PSD, %	1,093	0,338	0,263	3,38	10^{-6}
pNN_{50}, %	1,524	0,222	0,252	3,27	10^{-6}
RMSSD, msec	1,105	0,335	0,258	3,31	10^{-6}

In addition, a number of other EEG and HRV parameters that were not included in the model are noteworthy (Table 3).

Table 3. Discriminant Function Analysis Summary for EEGs and HRVs Variables currently not in the model

Variables	Groups (n)			Parameters of Wilks' Statistics					
	After Balm (20)	Base-line (74)	After CW (54)	Wilks' Λ	Par-tial Λ	F to enter	p-level	Tole-rancy	Refe-rence Cv
F7-α PSD, μV²/Hz	97 3	56 8	63 9	0,250	0,994	0,36	0,698	0,135	59 1,410
Deviation-θ, Hz	1,25 0,14	1,11 0,08	0,94 0,08	0,251	0,998	0,13	0,880	0,695	1,00 0,616
Asymmetry-θ, %	28,6 3,7	23,0 2,1	18,1 1,8	0,249	0,991	0,48	0,619	0,511	23,0 0,699
F3-β PSD, μV²/Hz	108 10	83 7	69 6	0,250	0,994	0,351	0,705	0,144	78 0,667
F4-β PSD, %	32,4 3,5	29,9 1,9	25,2 1,9	0,250	0,992	0,45	0,640	0,195	24,5 0,544
T4-β PSD, %	42,5 4,3	36,7 2,1	30,6 2,3	0,250	0,995	0,28	0,759	0,136	27,9 0,591
O1-β PSD, %	42,3 4,7	36,1 2,5	31,1 2,9	0,250	0,994	0,32	0,726	0,119	26,7 0,656
O2-α PSD, %	38,6 4,4	45,5 3,0	50,1 3,5	0,251	0,999	0,04	0,964	0,057	44,6 0,532
HF, msec²	468 174	291 61	145 15	0,251	0,999	0,03	0,975	0,064	452 0,768
HF/TP, %	20,9 3,9	14,0 1,3	9,3 0,5	0,251	0,998	0,09	0,913	0,191	17,1 1,230
LFnu, %	64,7 4,8	72,9 1,9	79,5 1,5	0,251	0,996	0,203	0,817	0,206	61,8 0,247
T6-β PSD, %	49 5	40 2	26 3	0,249	0,990	0,56	0,572	0,216	30 0,646

Next, the 34-dimensional space of **discriminant variables** transforms into 2-dimensional space of a **canonical discriminant functions** (canonical roots), which are a linear combination of discriminant variables. The discriminating (differentiating) ability of the root characterizes the canonical correlation coefficient (r*) as a measure of connection, the degree of dependence between groups (clusters) and a discriminant function. It is for Root 1 0,765 (Wilks' Λ=0,252; $\chi^2_{(68)}$=177; $p<10^{-6}$), for Root 2 0,627 (Wilks' Λ=0,606; $\chi^2_{(33)}$=64; $p<10^{-3}$). The major root contains 68,5% of discriminative opportunities, the minor - 31,5%.

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. The calculation of the discriminant root values for each person 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 patient in the information space of the roots.

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

Coefficients	Standardized		Raw	
Variables	Root 1	Root 2	Root 1	Root 2
ULF/TP, %	0,685	0,120	0,099	0,017
F3-α PSD, %	1,090	-1,645	0,063	-0,095
P4-δ PSD, %	0,776	-1,079	0,069	-0,096
C4-β PSD, $\mu V^2/Hz$	-1,126	0,656	-0,023	0,013
T4-β PSD, $\mu V^2/Hz$	0,696	-1,028	0,011	-0,016
Fp1-α PSD, %	-0,742	-0,134	-0,040	-0,007
Asymmetry-β, %	-0,119	-0,310	-0,007	-0,018
P4-δ PSD, $\mu V^2/Hz$	0,048	1,455	0,0006	0,019
LF/HF	-0,024	0,481	-0,008	0,158
O2-θ PSD, %	-0,290	0,602	-0,102	0,211
Frequency-δ, Hz	0,058	-0,421	0,339	-2,479
T6-α PSD, %	1,110	-0,352	0,055	-0,018
C4-β PSD, %	0,933	-0,711	0,062	-0,047
Frequency-β, Hz	0,295	-0,211	0,077	-0,055
P3-β PSD, %	-0,720	0,823	-0,045	0,052
Entropy O2	0,069	-0,517	0,445	-3,354
O1-θ PSD, $\mu V^2/Hz$	0,455	-0,710	0,012	-0,019
C3-α PSD, %	0,686	0,756	0,040	0,045
O1-θ PSD, %	-0,706	-0,017	-0,168	-0,004
Amplitude-α, μV	-1,395	0,940	-0,103	0,070
Index α, %	-0,053	-0,663	-0,002	-0,020
Entropy O1	0,518	0,380	2,993	2,195
F4-α PSD, %	-0,001	1,240	-0,0001	0,071
Entropy HRV	-0,313	0,133	-3,052	1,300
O2-α PSD, $\mu V^2/Hz$	1,067	0,983	0,0014	0,0013
F3-α PSD, $\mu V^2/Hz$	-2,090	-0,922	-0,013	-0,006
Fp1-α PSD, $\mu V^2/Hz$	1,497	-0,075	0,012	-0,0006
T6-α PSD, $\mu V^2/Hz$	-0,546	-0,749	-0,0045	-0,0062
T3-α PSD, $\mu V^2/Hz$	0,664	0,050	0,0064	0,0005
T3-α PSD, %	-0,164	1,082	-0,011	0,072
Fp2-α PSD, %	-0,164	-0,628	-0,009	-0,035
O1-α PSD, %	0,002	-0,820	0,0001	-0,034
pNN_{50}, %	0,655	0,252	0,067	0,026
RMSSD, msec	-0,542	-0,485	-0,045	-0,040
	Constants		-3,227	4,454
	Eigenvalues		1,410	0,650
Cumulative Proportion			0,685	1

Table 5 presents the **full structural coefficients**, 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 proportion of information about the discriminant function (root) contained in this variable. There are also average values (centroides) of Roots and Z-scores of Variables. We consider it expedient to include in the table also out-of-model variables in view of their recognizability.

Table 5. Factor Structure Matrix and Means of Roots and Variables

Variables, Z	Correlations Variables-Roots		After Balm (20)	Base-line (74)	After CW (54)
Root 1 (68,5%)	R1	R2	-2,74	+0,01	+1,00
ULF/TP, %	0,311	0,137	-0,73	**+0,70**	+1,88
LFnu, %			+0,19	**+0,73**	+1,16
LF/HF	0,153	0,144	+0,33	**+0,62**	+1,20
F4-α PSD, %	0,243	0,097	-0,38	+0,11	+0,49
O2-α PSD, μV²/Hz	0,164	0,090	-0,27	+0,08	+0,43
F3-α PSD, %	0,265	0,039	-0,55	+0,06	+0,36
C3-α PSD, %	0,251	0,101	-0,50	0,00	+0,39
T3-α PSD, %	0,217	0,057	-0,40	+0,05	+0,33
O1-α PSD, %	0,151	0,051	-0,31	+0,03	+0,27
Amplitude-α, μV	0,139	0,056	-0,31	+0,01	+0,26
F3-α PSD, μV²/Hz	0,161	0,003	-0,39	+0,06	+0,24
O2-α PSD, %			-0,25	+0,04	+0,23
T6-α PSD, %	0,183	0,000	-0,43	+0,07	+0,25
T6-α PSD, μV²/Hz	0,164	0,011	-0,36	+0,02	+0,17
Fp1-α PSD, μV²/Hz	0,166	-0,002	-0,39	+0,10	+0,27
Fp2-α PSD, %	0,147	-0,022	-0,32	+0,12	+0,23
Frequency-β, Hz	0,195	-0,047	-0,46	+0,15	+0,27
T3-α PSD, μV²/Hz	0,145	-0,006	-0,36	+0,08	+0,22
Fp1-α PSD, %	0,180	-0,045	-0,54	+0,08	+0,14
F7-α PSD, μV²/Hz			-0,38	-0,04	+0,05
O1-θ PSD, μV²/Hz	0,094	-0,034	-0,26	+0,02	+0,05
Index α, %	0,110	0,023	-0,53	-0,20	-0,03
P4-δ PSD, μV²/Hz	0,171	0,026	-0,45	-0,28	-0,20
P4-δ PSD, %	0,121	-0,122	-0,71	-0,38	-0,43
Entropy HRV	0,148	0,020	-1,03	**-0,62**	-0,43
Entropy O2	-0,163	0,011	+0,19	-0,22	-0,34
O2-θ PSD, %	-0,197	0,077	+0,30	-0,23	-0,28
Entropy O1	-0,137	0,052	+0,33	-0,11	-0,16
O1-θ PSD, %	-0,188	0,029	+0,52	-0,03	-0,17
F3-β PSD, μV²/Hz			+0,56	+0,08	-0,19
C4-β PSD, μV²/Hz	-0,280	-0,055	+0,69	+0,09	-0,23
Deviation-θ, Hz			+0,39	+0,16	-0,10
Asymmetry-θ, %			+0,35	0,00	-0,30
HF/TP, %			+0,18	-0,15	-0,37
T4-β PSD, μV²/Hz	-0,145	-0,143	+0,51	**+0,30**	-0,16
F4-β PSD, %			+0,73	**+0,41**	+0,05
C4-β PSD, %	-0,232	-0,067	+0,97	**+0,36**	-0,04
P3-β PSD, %	-0,250	0,020	+1,21	**+0,38**	+0,13
O1-β PSD, %			+0,94	**+0,53**	+0,25
T6-β PSD, %			+0,98	**+0,51**	+0,29

T4-β PSD, %			**+0,89**	**+0,54**	+0,16
HF, msec²			**+0,04**	**-0,46**	-0,88
pNN$_{50}$, %	**-0,159**	-0,127	**-0,06**	**-0,47**	-0,83
RMSSD, msec	**-0,161**	-0,075	**-0,63**	**-0,79**	-1,05
Root 2 (31,5%)	**R1**	**R2**	+0,79	**-0,80**	+0,80
Frequency-δ, Hz	-0,074	**-0,200**	+0,02	**+0,13**	-0,25
Asymmetry-β, %	-0,064	**-0,194**	+0,11	**+0,27**	-0,18

As a preamble, we present existing views on the interpretation of HRV parameters. Well-known markers of Vagal tone are HF, RMSSD and pNN$_{50}$. LFnu is admitted HRV marker of Sympathetic tone, LF/HF ratio reflects the sympathetic-vagal balance. It is speculated that absolute SPD LF band reflects mainly Sympathetic outflow or both Sympathetic and Vagal origin [2,4,7]. The interpretation of the other two bands remains the most controversial. VLF band (0,040÷0,0033 Hz) associated with oscillation blood levels of renin (0,04 Hz) and epinephrine (0,025 Hz), reflects thermoregulatory cycles, cerebral ergotropic and metabolotropic outflows, activation of cerebral sympatho-adrenal system, sympathetic activity; ULF band (<0,0033 Hz) associated with oscillation blood level of norepinephrine (0,0020 Hz) as well as 17-OCS (0,0019 Hz) [2,4,7,11,12,14,15]. Because in our device ULF band (range 0,015÷0,003 Hz) is integrated into the lower zone of VLF band, what has been said about the latter also applies to the former.

The above gives grounds to state in our contingent a sympathotonic shift of the autonomic balance due to both an increase in sympathetic tone and a decrease in vagal tone. This is accompanied by increased activity of beta-rhythm-generating cortical and subcortical structures.

It is traditionally believed that loci C3/C4 projected hippocampus, loci T3/T4 reflect the activity of the amygdala [30], loci F3/F4 - caudal anterior cingulate cortex. These cortical structures affect the activity of the vagus and sympathetic nuclei [reviews: 18,26,27,29].

Figures 1 and 2 show that the use of balm causes a shift in the information field of the discriminant roots of the HRV and EEG parameters to the left and up. The shift to the left reflects, first of all, the normalizing decrease of increased sympathotonic markers and the increase of decreased vagotonic markers.

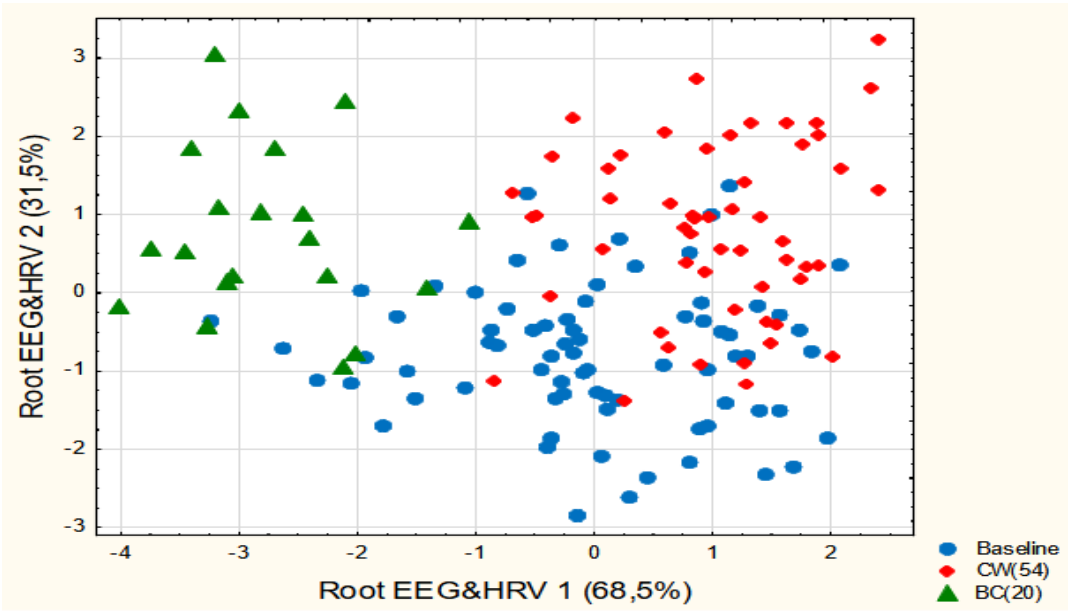

Fig. 1. Individual values of the first and second the EEG&HRV roots of the patients before (Baseline) and 1,5 hours after application of control water or balm

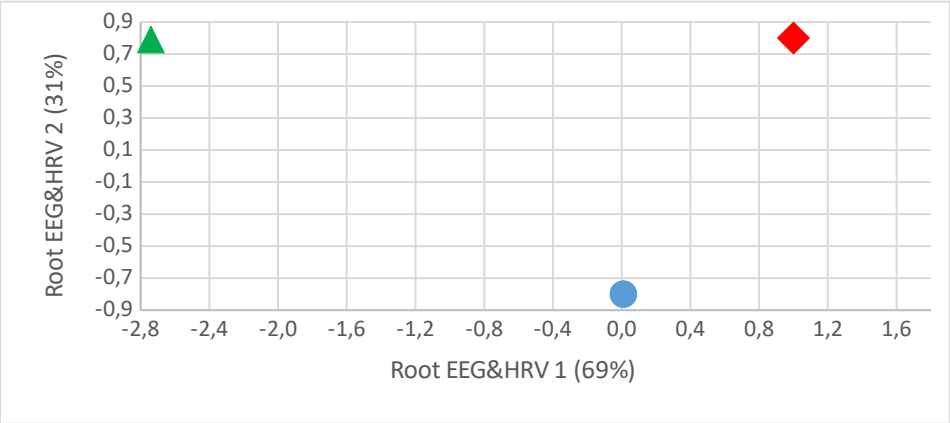

Fig. 2. Average (Mean±SD) of the first and second EEG&HRV roots of the patients before and 1,5 hours after application of control water or balm

This is in line with the concept we put forward back in 1993 about ambivalence-equilibratory character of influence on organism of human of curative water Naftussya [3], which is now considered a generally accepted adaptogen [1,5,6,18,28,29].

Physiologically favorable vegetotropic effects of the balm are accompanied by a further increase in the initially increased activity of beta-rhythm-generating cortical and subcortical structures as well as activation of theta-rhythm generating and inhibition of alpha- and delta-rhythm generating nuclei whose initial activity was within normal limits.

The cluster of individuals in the control group was shifted along the axis of the first root in the opposite direction, which reflects the sympathotonic shift of autonomous balance and opposite changes in EEG parameters. It is unlikely that the reason for such changes in neurodynamics is the use of 50 ml of tap water. The neurotropic effects of individuals' occupational activity within 1,5 hours between tests and/or the ultradian biorhythm of the autonomic nervous system and cortisol are more obvious.

Interestingly, slight displacements along the axis of the second root were almost the same in both groups.

In general, all EEG&HRV clusters on the planes of two roots are quite clearly delineated, which is documented by calculating the Mahalanobis distances (Table 6).

Table 6. Squared Mahalanobis Distances between EEG&HRV Clusters, F-values (df=34) and p-levels

Groups	Base-line (74)	After CW (54)	After Balm (20)
Baseline (74)	0	3,55	10,04
After CW (54)	2,52 10^{-3}	0	14,01
After Balm (20)	3,59 10^{-6}	4,64 10^{-6}	0

The same discriminant parameters can be used to identify (classify) the belonging of one or another person to one or another cluster. This purpose of discriminant analysis is realized with the help of classifying (discriminant) functions (Table 7). These functions are special linear combinations that maximize differences between groups and minimize dispersion within groups. The coefficients of the classifying functions are not standardized, therefore they are not interpreted. An object belongs to a group with the maximum value of a function

calculated by summing the products of the values of the variables by the coefficients of the classifying functions plus the constant.

Table 7. Coefficients and Constants for Classification Functions

Clusters	Base-line (74)	After CW (54)	After Balm (20)
Variables	p=,500	p=,365	p=,135
ULF/TP, %	-1,417	-1,291	-1,660
F3-α PSD, %	0,187	0,098	-0,137
P4-δ PSD, %	2,120	2,036	1,779
C4-β PSD, μV²/Hz	-0,050	-0,052	0,034
T4-β PSD, μV²/Hz	0,056	0,041	0,001
Fp1-α PSD, %	0,692	0,641	0,789
Asymmetry-β, %	-0,238	-0,275	-0,248
P4-δ PSD, μV²/Hz	-0,093	-0,061	-0,064
LF/HF	0,927	1,172	1,199
O2-θ PSD, %	-1,526	-1,290	-0,912
Frequency-δ, Hz	60,59	56,96	55,73
T6-α PSD, %	1,135	1,162	0,955
C4-β PSD, %	1,013	0,999	0,768
Frequency-β, Hz	2,391	2,380	2,092
P3-β PSD, %	0,897	0,935	1,105
Entropy O2	127,3	122,4	120,8
O1-θ PSD, μV²/Hz	-0,059	-0,077	-0,122
C3-α PSD, %	0,476	0,588	0,436
O1-θ PSD, %	-0,289	-0,464	0,166
Amplitude-α, μV	2,202	2,210	2,595
Index α, %	0,192	0,158	0,164
Entropy O1	39,25	45,75	34,51
F4-α PSD, %	0,147	0,261	0,260
Entropy HRV	191,3	190,3	201,7
O2-α PSD, μV²/Hz	-0,009	-0,006	-0,011
F3-α PSD, μV²/Hz	-0,134	-0,156	-0,108
Fp1-α PSD, μV²/Hz	0,097	0,108	0,063
T6-α PSD, μV²/Hz	0,051	0,037	0,054
T3-α PSD, μV²/Hz	-0,001	0,006	-0,017
T3-α PSD, %	-0,304	-0,199	-0,159
Fp2-α PSD, %	-0,325	-0,391	-0,356
O1-α PSD, %	-0,199	-0,253	-0,253
pNN_{50}, %	-0,227	-0,119	-0,369
RMSSD, msec	0,417	0,308	0,477
Constants	-279,5	-276,4	-268,6

In this case, we can retrospectively recognize members in the initial state with 14 errors, after using control water - with 11 errors, and after applying the balm - with 4 errors. Overall classification accuracy is 81,1% (Table 8).

Table 8. Classification Matrix for EEG&HRV Clusters
Rows: Observed classifications; Columns: Predicted classifications

	Clusters	Base-line	After CW	After Balm
Clusters	% Correct	p=,500	p=,365	p=,135
Baseline (74)	82,4	**61**	10	3
After CW (54)	79,6	11	**43**	0
After Balm (20)	80,0	4	0	**16**
Total	81,1	76	53	19

The digital data of Table 5 are visualized in Figure 3.

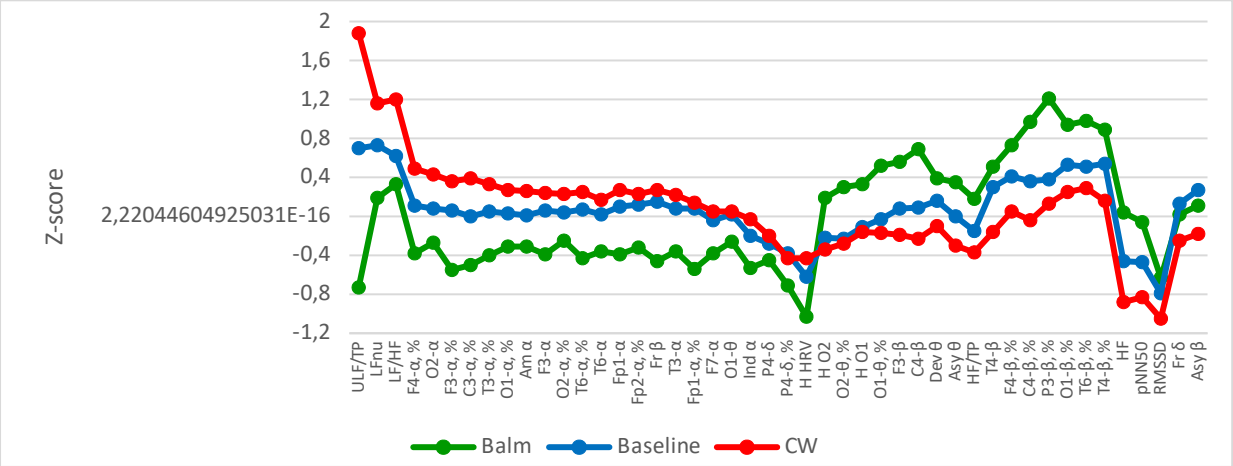

Fig. 3. Profiles of Z-scores of EEGs and HRVs variables in the initial state and 1,5 hours after drinking control water or balm

A clear divergence of profiles is visible, however, it is heterogeneous. Therefore, for more detailed analysis, the profiles were structured in 7 homogeneous patterns. This approach also makes it possible to model the own (per se) neurotropic effects of the balm as algebraic sums of effects in the main and control groups.

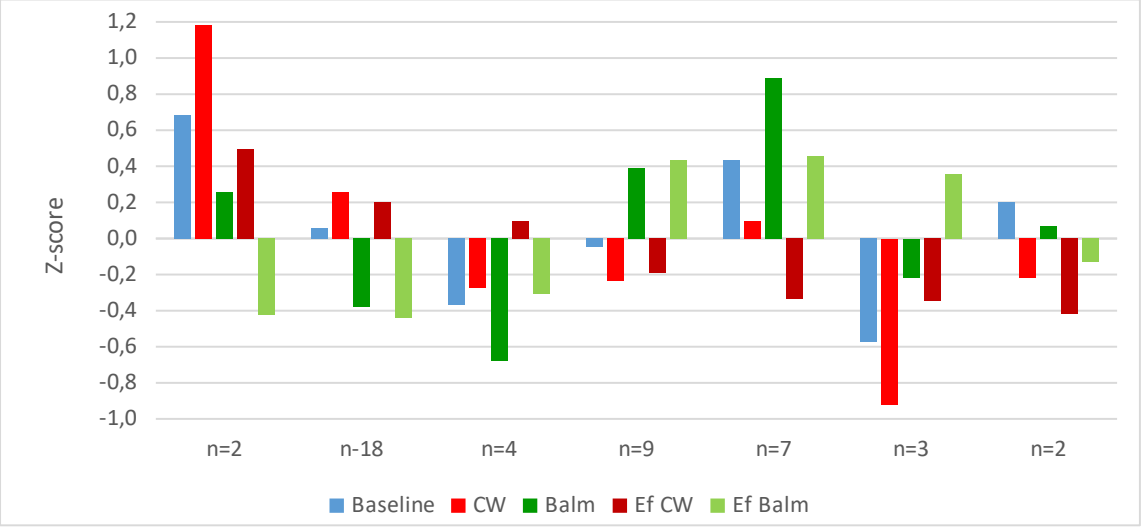

Fig. 4. Patterns of EEG&HRV (Mean±SE) parameters before and 1,5 hours after application of control water or balm and simulated effects per se. The members of the patterns are separated in table 5 by spaces

Regarding the factors of neurotropic effects of the balm, it is possible to assume the presence of polyphenols, which are present in both the balm [1] and bioactive water Naftussya [10], whose neurotropic and endocrine effects are well known [5,6,16,17,25,29,32].

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ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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