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Phytoadaptogen reverses the adverse effects of Naftussya bioactive water on dynamic muscle performance in healthy rats

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Summary

Background.. Muscular performance is considered one of the attributes of health and non-specific resistance. Phytoadaptogens occupy an important place in the arsenal of means of increasing non-specific resistance and stress resistance. Many years of research of the Truskavetsian Scientific School of Balneology have demonstrated the adaptogenic properties of the main therapeutic factor of the resort, Naftussya bioactive water, as well as ozokerite and mineral baths. However, in contrast to the beneficial effect of the latter on stress resistance and the neuro-endocrine-immune complex, the effect on the physical performance is ambiguous. The **purpose** of this study is to test the ability of phytocomposition to prevent the adverse actotropic effect of Naftussya bioactive water at rats. **Material and methods.** The experimentt have been carried out at 42 female rats. Rats of the control group for 7 days loaded through a tube with tap daily water (2 mL once), while the animals of the other groups received according to a similar scheme daily water with the addition of 0,1 mL of Balm; bioactive Naftussya water per se or with the addition of 0,1 mL of Balm. The day after completion the course of water loads in the animal determined the urinary excretion of 17-ketosteroids, assessed the mineralocorticoid activity (MCA) by the urine K/Na ratio as well as the state of neutrophil phagocytosis by the number of absorbed latex particles. **Results**. It was found that the weekly use of Naftyssya bioactive water reduces the duration of swimming of rats to exhaustion by 30% compared to the daily water control. Addition of phytoadaptogen to Naftyssya softens its negative actotropic effect by up to -9%, and adding Balsam to daily water prolongs the maximum duration of swimming compared to the control by 11%. A positive correlation of the swimming test with 17-KS excretion and water diuresis was revealed, but a negative correlation with MCA, spontaneous diuresis and neutrophils phagocytosis. **Conclusion**. Phytoadaptogen reverses the adverse effects of Naftussya bioactive water on dynamic muscle performance in healthy rats by mitigating the decrease in the excretion of 17-ketosteroids and increased mineralocorticoid activity.

Keywords: Naftussya bioactive water, phytoadaptogen, swimming test, 17-ketosteroids, mineralocorticoids, phagocytosis, relationships, rats.

INTRODUCTION

Muscular performance is considered one of the attributes of health and non-specific resistance [9,13,55]. Phytoadaptogens (ginseng, eleuterococcus, schizandra, aloe, etc.) occupy an important place in the arsenal of means of increasing non-specific resistance and stress resistance [1,10,11,24,32]. It is significant that the first informative test for the comparative evaluation of the effectiveness of phytoadaptogens was the swimming test [3].

Many years of experimental and clinical research of the Truskavetsian Scientific School of Balneology have demonstrated the adaptogenic properties of the main therapeutic factor of the resort, Naftussya bioactive water, as well as ozokerite and mineral baths, which together make up a standard balneotherapy complex [10,15,24,34,35,37,38].

However, in contrast to the beneficial effect of the latter on stress resistance and the neuro-endocrine-immune complex, the effect on the physical performance of both rats and resort patients is ambiguous [46-48,56,57,60], which prompted the additional use of aerobic training [42,46-48,50-53] and phytoadaptogens, both well-known (ginseng, Bittner's balsam), and the Ukrainian phytocomposition "Balm Kryms'kyi" [1,10,11,16,17,24], the adaptogenic properties of which first discovered by representatives of the Truskavetsian Scientific School of Balneology [1,28,31,33].

We tested immediate neurotropic effects for the first time of phytocomposition "Balm Truskavets" (ТУ У 15.8-24055046-005:2009, produced by private research and production enterprise "Ukrainian Balms", Mykolayïv, Ukraine) [7,43]. This phytocomposition is analogous to the previous "Balm Kryms'kyi".

It has recently been confirmed that weekly use of Naftussya bioactive water caused ambiguous changes in the fitness and the secretion of steroids associated with amines and phenols present in the composition of water [59].

The purpose of this study is to test the ability of this phytocomposition to prevent the adverse actotropic effect of Naftussya bioactive water at rats.

MATERIAL AND METHODS

It is known data by Dats'ko OR et al [5] about organic compounds (in mg/L) water Naftussya 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; alkylphenols 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.

Usually, due to the high cost of such analyses, the Truskavetsian Hydrogeological Operating Station conducts a simplified analysis. In the Naftussya water used in this study, the content of gross organic carbon (Corg) determined by the method of dry combustion of the sample [12] was 15,5 mg/L, organic nitrogen (Norg) determined by the Kjeldahl method [27] – 0,52 mg/L, bitumen (chromatographic separation in a thin layer of aluminum oxide and their subsequent luminescence measurement [21]) – 1,38 mg/L, carboxylic (fatty) acids (chloroform extraction method) - 50 μeqv/L, phenols (extraction-photometric method APHA [18,27]) - 0,15 mg/L.

Participants. The experimentt have been carried out at 42 female rats Wistar line weighing 180-220 g 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 UkrSR Institute of 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).

Procedure / Test protocol / Skill test trial / Measure / Instruments.

Rats of the control group for 7 days loaded through a tube with tap daily water (2 mL once), while the animals of the other groups received according to a similar scheme daily water with the addition of 0,1 mL of Balm; bioactive Naftussya water per se or with the addition of 0,1 mL of Balm.

The day after completion the course of water loads the animal were placed in individual chambers with perforated bottom for collecting for 10 hours urine, in which determined the concentration of 17-ketosteroids (by color reaction with m-dinitrobenzene). Then the animals were loaded with distilled water (6 mL) through a tube and placed in individual Plexiglas machines to collect two-hour urine, in which the concentration of potassium and sodium was determined (by flaming photometry) in order to assess mineralocorticoid activity (MCA) by the K/Na ratio. In a drop of blood from the tail vein, the state of neutrophil phagocytosis was determined by the number of absorbed latex particles, according to the instructions for the set. The next day, dynamic muscle fitness tested (by the time of swimming to exhaustion in the water t^0 26^0C).

Data collection and analysis / Statistical analysis.

Statistical processing was performed using a software package "Microsoft Excell" and "Statistica 6.4 StatSoft Inc".

RESULTS AND DISCUSSION

It was found that the weekly use of Naftussya bioactive water reduces the duration of swimming of rats to exhaustion by 30% compared to the daily water control. Addition of phytoadaptogen to Naftussya softens its negative actotropic effect by up to -9%, and adding Balsam to daily water prolongs the maximum duration of swimming compared to the control by 11% (Table 1).

In order to identify the parameters characteristic of actotropic effects, a discriminant analysis was performed [22]. All registered parameters are included in the discriminant model, with the exception of one, obviously due to duplication/redundancy of information (Tables 1-2).

Table 1. Discriminant Function Analysis Summary for Variables

Step 5, N of vars in model: 5; Grouping: 4 grps; Wilks' Λ: 0,0250; approx. $F_{(16)}$=17,6; $p<10^{-6}$

Variables currently in the model	Clusters-groups (n)				Parameters of Wilk's Statistics				
	N (5)	**NB (8)**	**DW (20)**	**B (9)**	Wilks' Λ	Parti-al Λ	F-remove (3,34)	p-level	Tole-rancy
Swimming test, min	130 4	169 2	186 1	207 2	0,140	0,179	52,1	10^{-6}	0,600
Phagocytosis, bits/phagocyte	58,2 1,0	38,1 2,0	38,1 1,1	36,4 1,1	0,037	0,684	5,25	0,004	0,803
Ku/Nau as Mineralo-corticoid activity	2,95 0,08	2,23 0,09	1,99 0,05	1,99 0,05	0,033	0,749	3,80	0,019	0,761
Diuresis stimulated, mL/2h	4,52 0,12	5,07 0,06	5,23 0,03	5,42 0,15	0,028	0,887	1,45	0,246	0,679
Diuresis spontaneous, mL/10h	5,61 0,58	5,62 0,19	5,37 0,12	5,21 0,19	0,028	0,899	1,27	0,300	0,774
Variable currently not in model	**N (5)**	**NB (8)**	**DW (20)**	**B (9)**	Wilks' Λ	Parti-al Λ	F to enter	p-level	Tole-rancy
17-Ketosteroids, nM/10h	63,0 2,6	77,7 2,0	83,0 1,1	84,1 1,4	0,024	0,952	0,56	0,647	0,039

Notes. In each column, the first line is the average, the second – SE for variables

Table 2. Summary of Stepwise Analysis for physiological. The variables are ranked by criterion Lambda

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level
Swimming test, min	181	10^{-6}	0,065	181	10^{-6}
Phagocytosis, bits/phagocyte	7,34	0,001	0,041	48,6	10^{-6}
Ku/Nau as Mineralocorticoid activity	3,88	0,017	0,031	30,9	10^{-6}
Diuresis stimulated, mL/2h	1,35	0,275	0,028	22,3	10^{-6}
Diuresis spontaneous, mL/10h	1,27	0,300	0,025	17,6	10^{-6}

The identifying information contained in the 5 discriminant variables is condensed into three roots. The major root contains 95,8% of discriminatory opportunities (r*=0,975; Wilks' Λ=0,025; $\chi^2_{(15)}$=135; p<10^{-6}), while minor root – 3,4% only (r*=0,637; Wilks' Λ=0,508; $\chi^2_{(8)}$=25; p=0,002), and the third is not worth paying attention to (0,8%; p=0,126).

Calculating the values of discriminant roots for each rat by the raw coefficients and the constant (Table 3) allows visualization of each animal in the information space of roots.

Table 3. Standardized, Structural and Raw Coefficients and Constants for Canonical Variables

Coefficients	**Standardized**		**Structural**		**Raw**	
Variables currently in the model	Root 1	Root 2	Root 1	Root 2	Root 1	Root 2
Swimming test, min	1,150	-0,521	**0,859**	-0,143	0,187	-0,085
Diuresis stimulated, mL/2h	-0,340	0,295	**0,235**	0,120	-1,298	1,127
Ku/Nau as Mineralocorticoid activity	-0,393	-0,252	**-0,331**	-0,556	-1,882	-1,208
Phagocytosis, bits/phagocyte	0,070	-0,914	**-0,318**	-0,833	0,016	-0,204
Diuresis spontaneous, mL/10h	0,328	-0,080	**-0,047**	0,139	0,500	-0,122
				Constants	-26,45	20,96
				Eigenvalues	19,33	0,685
				Cumulative Proportions	0,958	0,992

Judging by the structural coefficient, the major discriminant root reflects, first of all, the swimming test. The extreme left localization (centroid: -9,7) of the members of the Naftussya cluster (Fig. 1) reflects the duration of swimming, which is the minimum for the sample. This is accompanied by the minimal levels of water-load-stimulated diuresis and 17-ketosteroids excretion and maximally elevated levels of spontaneous diuresis and mineralocorticoid activity as well as intensity of phagocytosis. Rats of the Balm cluster are located at the opposite pole of the root axis (centroid: +4,7). This reflects their maximal/minimal levels mentioned parameters.

Figure 1 illustrates that the addition of a phytoadaptogen to Naftussya water brings the state of these rats as close as possible to such a control (centroids: -2,1 and +1,2 respectively).

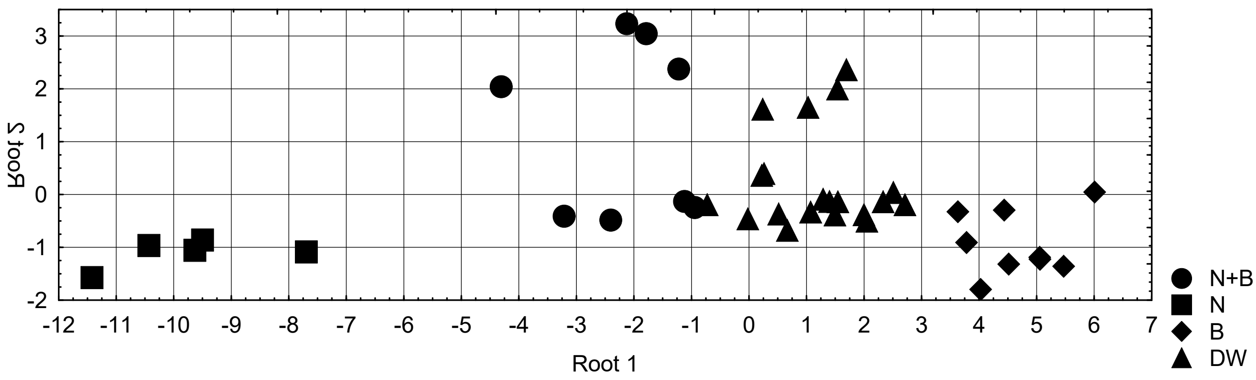

Fig. 1. Diagram of scattering of individual values of discriminant Roots of rats loaded by Daily Water (DW), Naftussya Bioactive Water (N), Balm (B) and Naftussya together with Balm (N+B)

All four clusters are quite clearly demarcated along the axis of even one root which is documented by calculating Mahalanobis distances (Table 4).

Table 4. Squared Mahalanobis Distances between groups (over diagonal), F-values and p-levels (under diagonal)

Groups	NB (8)	N (5)	B (9)	DW (20)
Naftussya + Balm		63,2	50,8	12,8
Naftussya	34,8 10^{-4}		208	121
Balm	38,5 10^{-4}	119 10^{-6}		13,9
Daily Water	13,1 10^{-3}	86,8 10^{-5}	15,5 10^{-3}	

Classification accuracy is 100% (Table 5).

Table 5. Classification matrix

Group	Rows: Observed classifications Columns: Predicted classifications				
	Percent Correct	N+B p=,19048	N p=,11905	B p=,21429	DW p=,47619
N+B	100	8	0	0	0
N	100	0	5	0	0
B	100	0	0	9	0
DW	100	0	0	0	20
Total	100	8	5	9	20

Of particular interest is the inverse relationship between the swimming test and the intensity of phagocytosis (Fig. 2).

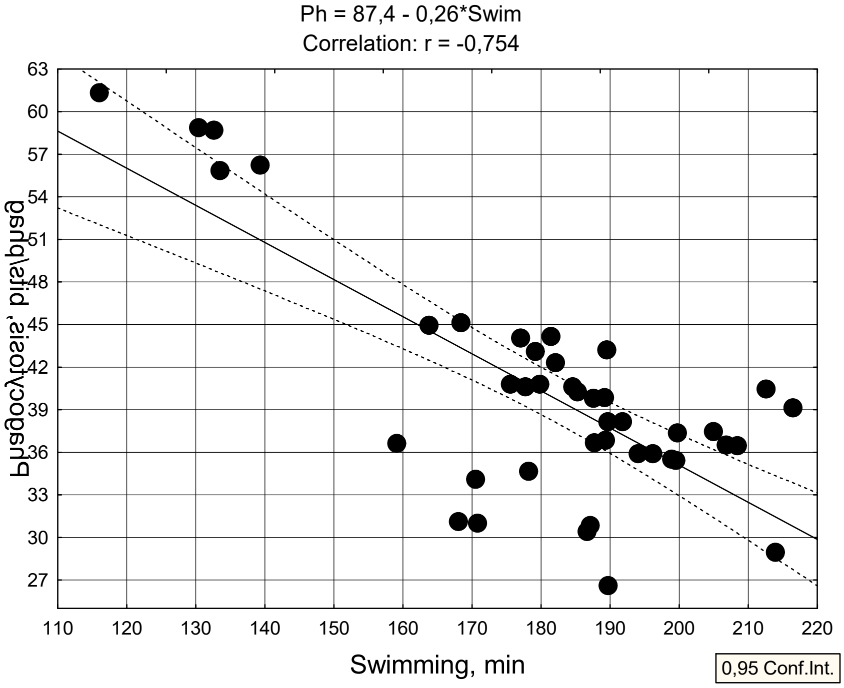

Fig. 2. Scatterplot of correlation between the swimming test (X-line) and intensity of phagocytosis (Y-line) in rats

This is consistent with recently published data on the combination of a decrease in the level of the cycle ergometric test with an increase in the intensity of phagocytosis of Staphylococcus aureus by neutrophils and monocytes of people who received Naftussya water and mineral baths [60].

Phagocytosis, in turn, is upregulated by mineralocorticoids (Fig. 3) and downregulated by 17-Ketosteroids (Fig. 4).

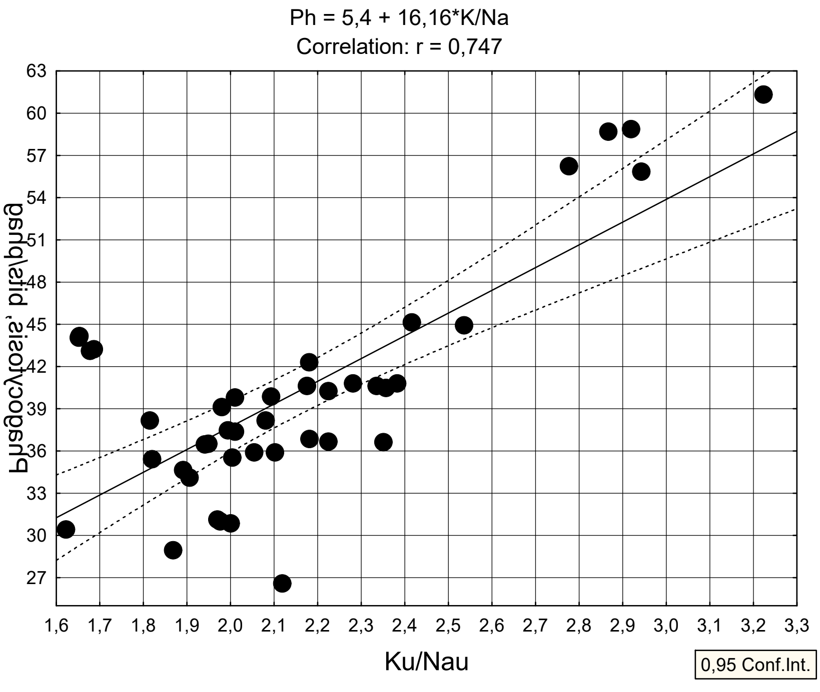

Fig. 3. Scatterplot of correlation between the mineralocorticoid activity (X-line) and intensity of phagocytosis (Y-line) in rats

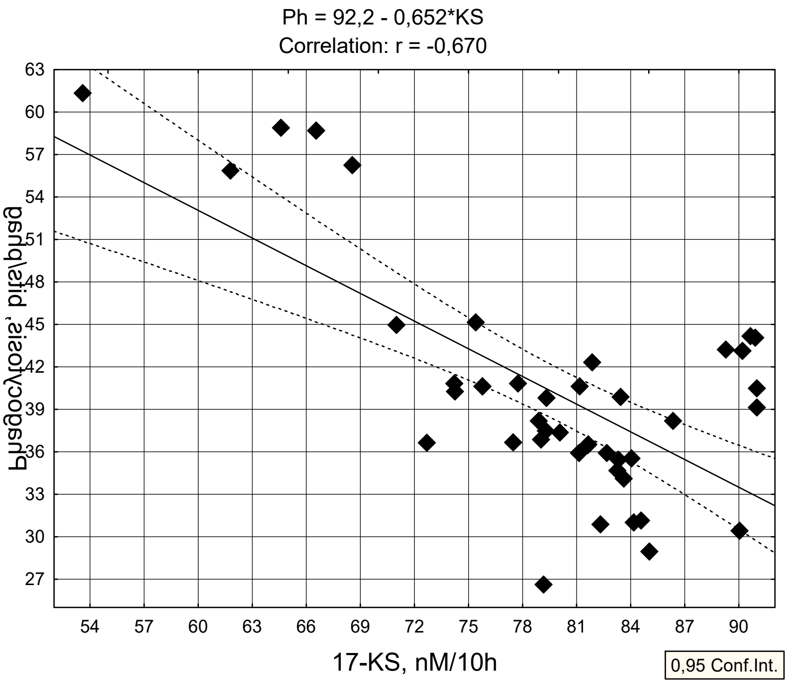

Fig. 4. Scatterplot of correlation between the urine excretion of 17-Ketosteroids (X-line) and intensity of phagocytosis (Y-line) in rats

It is well known that in females 17-ketosteroids are almost entirely metabolites of androgens secreted by the reticular zone of the adrenal cortex and the ovaries. We have recently [44] shown that in women, but not in men, PWC is downregulated (r=-0,24) by aldosterone levels. In addition, a similar negative correlation (r=-0,31) with the cortisol level was found.

The long-known increase in spontaneous diuresis under the influence of Naftussya water is due, among other factors, to a decrease in the level of antidiuretic hormone/arginine vasopressin in the blood. Hence, we assume that dynamic fitness is upregulated by reactivity of source of this hormone. This source are parvocellular neurons of the paraventricular nuclei of the hypothalamus. Some parvocellular neurons contain and secrete both arginine vasopressin (AVP) and corticotropin-releasing hormone (CRH) that in turn stimulates the secretion of ACTH. AVP alone has very little ACTH secretagogue activity but is apotent synergistic factor with CRH. AVP and CRH may act synergistically on other target tissue with AVP and CRH receptors in the CNS and perhaps the periphery [4], including, let's add, in skeletal muscles.

Previously, it was shown that the weekly use of Naftussya water increases the level of corticosterone in female rats to 619 nM/L vs 375 nM/L in daily water control, the thickness of the fascicular zone to 394 nM vs 386 nM, the glomerular zone to 192 nM vs 187 nM, the reticular zones up to 45 nM vs 42 nM, however, testosterone plasma level decrease to 4,11 nM/L vs 5,98 nM/L [38].

So, there are reasons to assume that in this experiment, Naftussya water stimulates the release of both mineralocorticoids and corticosterone into the blood, which, in turn, has a negative actotropic effect, as we have shown in humans [44].

Regarding the mechanism of stimulation by Naftussya water of mineralocorticoid and glucocorticoid, while suppression of androgenic functions of the adrenal cortex, there are two hypotheses. The first hypothesis allows for a direct activating effect of hydrophobic organic substances, in particular bitumen, on 21-hydroxylase of endocrinocyte microsomes with a subsequent increase in the biosynthesis of deoxycorticosterone and corticosterone, and a decrease in androgen secretion, apparently, due to a shift in the direction of use of pregnolol -

a common precursor of all three steroids - towards deoxycorticosterone and corticosterone [19,37,39]. Direct activation of phagocyte by Naftussya fatty acids is also allowed [60].

An alternative hypothesis, much more substantiated, considers the endocrine and immune effects of Naphtussya water in the context of its modulating effect on the neuro-endocrine-immune complex [2,8,14,15,23,25,26,36,37,40,41,58].

At least some of the listed organic substances (alkylbenzene, alkenylbenzene, alkylnaphthalenes, alkyl phenols, esters of aromatic acids, polyaromatic hydrocarbons) are, obviously, agonists of aryl hydrocarbon receptors (AhR), which are expressed by almost all types of cells of living organisms. The activation of AhR by endogenous and environmental factors has important physiologic effects, including the regulation of the endocrine and immune response [6,30].

We will say that one gets the impression that a decrease in fitness under the influence of balneofactors is compensated by their increase in phagocytosis, while the body “pays” for the increase in fitness by weakening it. This is consistent with the long-known principle of the “physiological price” of adaptation [29] as well as with the textbook fact of a decrease in athletes' resistance to a banal infection at the peak of cardiorespiratory fitness. Phytoadaptogen reverses the adverse effects of Naftussya bioactive water on dynamic muscle performance in healthy rats by mitigating the decrease in the excretion of 17-ketosteroids and increased mineralocorticoid activity. This is probably due to its sympathotonic effect [1,10,28]

The similar constellation of organic substances was found in the composition of other medicinal waters of Ukrainian Carpathians and Podolia [49] as well as Siberia [20]. This gives reason to predict their effects, similar to those of Naftussya bioactive water.

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