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VITA intense –Antioxidant and Inhibition Growth of Tumor Cells Effects. Anti Aging Influence. Negative Oxidation-reduction Potential (ORP) Has Important Role in These Effects

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Abstract
I studied the mathematical model of interaction with water VITA intense of LavaVitae company (Austria). In this report are submitted data about the interaction of VITA intense with water, obtained by non-equilibrium (NES) and differential-equilibrium energy spectrum (DNES) of water. The average energy ($\Delta E_{H...O}$) of hydrogen H…O-bonds among individual molecules H_2O after treatment of VITA intense with water measured by NES- and DNES-methods is ΔE=-0.0136±0.0011 eV for VITA intense. This result suggests the restructuring of $\Delta E_{H...O}$ values among H_2O molecules with a statistically reliable increase of local extremums in DNES-spectra. The research is performed for VITA intense, with study of pH and oxidative reduction potential (ORP). There is review of the effects of the chemical composition of VITA intense – anti-inflammatory, antioxidant etc. With methods NES we show the following effects – relaxing effect of nervous system, anti-inflammatory and inhibition of tumor cells. As results of these effects VITA intense has anti aging influence. The base of this influence is anti-inflammatory effect. This article deals with the review of the basic biophysical-biochemical and biological processes underlying the VITA intense. The author is studying their physical-chemical properties and biophysical and biological effects on human organism. Additionally, by using IR, NES, and DNES methods are investigated various samples of water from Bulgarian water springs: the melt water from Glacier Rosenlaui, Swiss Alps, as well as the human blood serum of people with excellent health and cancer patients between 50 and 70 years old. Other experiments were performed on a 1% (v/v) solution of VITA intense in deionized water. As an estimation factor in NES and DNES was measured the values of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules in water samples, as well as a local extremums in the NES and DNES-spectra of various samples of water and the human blood serum at E = -0.1387 eV and λ = 8.95 μm. For a group of people in critical condition of life and patients with malignant tumors the greatest values of local extremums in IR-, DNES-spectra were shifted to lower energies relative to the control healthy group. Further we applied this method for calculation of percent distribution of H_2O molecules in all studied water samples according to energies of hydrogen bonds ranged from (-0.08 to -0.1387 eV). It was shown that mountain water is among the most important factors for human longevity and human health. The variety of ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, SO_4^{2-}, Cl^-, HCO_3^-, CO_3^{2-}), the chemical-physical parameters (pH, electroconductivity) and the decreased content of deuterium in studied water samples renders beneficial effects of these types of water on human health. We are applying the conclusions for the effects of mountain water on human health and longevity as base for the conclusion on the effects of VITA intense. The base is also the results with methods NES and DNES on human serum and expected effects from VITA intense.
VITA intense has negative oxidative reduction potential (ORP) and this is special property of this product for antioxidant effects again free radicals.

Keywords: VITA intense, anti-inflammatory, antioxidant, anti-aging, mathematical model, NES, DNES, negative oxidative reduction potential (ORP).

1. Introduction

Water is the main substance of life. The human body of an adult person is composed from 50 to 55% of water. With aging, the percentage of water in the human body decreases. Hence, the factor of water quality and its amount in organism is an essential factor for the research (Pocock *et al*., 1981; Howard & Hopps, 1986). Water is present in the composition of the physiological fluids in the body and plays an important role as an inner environment in which the vital biochemical processes involving enzymes and nutrients take place. Water also is

the main factor for metabolic processes and aging (Ignatov, 2012). Earlier studies conducted by us have demonstrated the role of water, its structure, the isotopic composition and physical-chemical properties (pH, temperature) on the growth and proliferation of prokaryotes and eukaryotes in water with different isotopic content (Ignatov&Mosin, 2012; Ignatov & Mosin, 2013). These factors, the structure and composition of water are of great importance in many biophysical studies. The peculiarities of the chemical structure of the H_2O molecule and weak bonds caused by electrostatic forces and donor-acceptor interaction between hydrogen and oxygen atoms in H_2O molecules create favorable conditions for formation of directed intermolecular hydrogen bonds (O–H…O) with neighboring H_2O molecules, binding them into complex intermolecular associates which composition represented by general formula $(H_2O)_n$, where n can vary from 3 to 50 (Keutsch & Saykally, 2011). The hydrogen bond is a form of association between the electronegative O-atom and a H-atom, covalently bound to another electronegative O-atom, is of vital importance in the chemistry of intermolecular interactions, based on weak electrostatic forces and donor-acceptor interactions with charge-transfer (Pauling, 1960). It results from interaction between electron-deficient H-atom of one H_2O molecule (hydrogen donor) and unshared electron pair of an electronegative O-atom (hydrogen acceptor) on the neighboring H_2O molecule.

The product of LavaVitae VITA intense is combining of herbs from Alps, Aloe Vera, Green tea, Edelweiss, Aronia, Vitamins and Magnesium, Selenium. The research is with methods NES and DNES. There is research of ORP and pH and there are executing the conclusions from electrochemically activated waters – anolyte and catholyte for anti-inflammatory effects (Ignatov et al., 2014).

The aim of this research is to show the usefully of VITA intense on the base of the following results and conclusions. The research are from various samples of water from Bulgarian water springs: the melt water from Glacier Rosenlaui, Swiss Alps, as well as human blood serum of people with excellent health and cancer patients between 50 and 70 years old. In frames of this research on the water quality were investigated 415 people living in the municipalities of Teteven, Yablanitza. Ugarchin, Lukovit, Lovech district; Dolni Dabnik, Pleven district, Kuklen, Plovdiv district (Bulgaria), where is lived the most of long lived people and their siblings, were studied.

The authors also performed the research of 1% (v/v) solution of VITA intense on the distribution of H_2O molecules according to the energies of hydrogen bonds, as well as studies of the NES and DNES spectrum and the biophysical effect of this type of water on human body.

2. Materials and Methods

2.1. NES and DNES Spectral Analysis

The device for DNES spectral analysis was made by A. Antonov on an optical principle. For this was used a hermetic camera for evaporation of water drops under stable temperature (+22–24 ^{0}C) conditions. The water drops were placed on a water-proof transparent pad, which consists of thin maylar folio and a glass plate. The light was monochromatic with filter for yellow color with wavelength at $\lambda = 580 \pm 7$ nm. The device measures the angle of evaporation of water drops from 72.3^0 to 0^0. The DNES-spectrum was measured in the range of -0.08– -0.1387 eV or $\lambda = 8.9–13.8$ μm using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds among H_2O molecules in water samples and human blood serum.

2.2. Product of LavaVitae – VITA intense

The product LavaVitae VITA intense of 1 liter is including – Natural and high quality plant extracts from the Swiss Alps, Aronia, Edelweiss, Aloe Vera, Vitamin C (180.0 mg), Vitamin E (15.0 mg), Vitamin A (17.0 mg), Vitamin A (17.0 mg), Vitamin B_1 (1.5 mg), Vitamin B_2 (1.7 mg), Vitamin B_3 (17.0 mg), Vitamins B_5 (10.0 mg), Vitamin B_6 (4.2 mg), Vitamins B_3 (17.0 mg), Water-soluble B Vitamin (17.0 mg) Vitamin B_9, Vitamin M, Vitamin B_{11}, (0.4 mg), Vitamin B_9, (7.4 μg), Vitamin D_3, (5.0 μg), Magnesium (62.5 mg), Selenium (25 μg).

2.3. Studying the Bulgarian Long Living People and Centenarians

Interviews have been conducted with 415 Bulgarian centenarians and long living people and their siblings. Their heredity, body weight, health status, tobacco consumption, physical activity, attitude towards life has been analyzed. With using DNES method was performed a spectral analysis of 15 mountain water springs located in municipalities Teteven and Kuklen (Bulgaria). The composition of water samples was studied in the laboratory

of "Eurotest Control" (Bulgaria). Statistics methods were attributed to the National Statistical Institute of Bulgaria.

2.4. Studying the Human Blood Serum

1% (v/v) solution of human blood serum was studied with the methods of IR-spectroscopy, non-equilibrium (NES) and differential non-equilibrium (DNES) spectral analysis. The specimens were provided by Kalinka Naneva (Municipal Hospital, Bulgaria). Two groups of people between the ages of 50 to 70 were tested. The first group (control group) consisted of people in good clinical health. The second group included people in critical health or suffering from malignant diseases.

2.5. IR-spectroscopy

IR-spectra were registered on Brucker Vertex ("Brucker", Germany) IR spectrometer (a spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; the permission – 0,5 cm^{-1}; the accuracy of wave number – 0,1 cm^{-1} on 2000 cm^{-1}) and on Thermo Nicolet Avatar 360 Fourier-transform IR.

2.6. Statistical Processing of Experimental Data

Statistical processing of experimental data was performed using the statistical package STATISTISA 6.0 using the Student's *t*- criterion (at $p < 0.05$).

3. Results and Discussions

3.1. Applications of VITA intense for Human Health. The information is from the company LavaVitae

Product features: LavaVitae Company –VITA intense product

– Two bottles (2x500 ml) covers up the need for a monthly (~ 33 ml per a day);
– A synergy of essential vitamins and minerals with natural high-quality plant extracts and aromas from the Swiss Alps;
– Highest bioavailability in liquid form;
– Without GMO. Free of animal ingredients and allergens according to regulation (EU) No. 1169/2011;
– Supports a healthy diet and an active lifestyle.

The Table 1 shows the chemical composition of VITA intense

Nutritional Information	Quantity in g per Serving (33 ml)	Quantity in g per 100 ml
Calorific value	18,53 kcal / 77,66 kJ	56,14 kcal / 235,33 kJ
Fat	< 0,1	< 0,1
saturated fatty acids	<0,05	<0,1
Carbohydrates	4,5	13,67
of which sugar	4,43	13,43
Protein	< 0,05	< 0,1
Balla substances	0,3	0,9
Salt	< 0,05	< 0,1

Nutrient	Quantity in mg per Serving (33 ml)
(Vitamins and Minerals)	
Vitamin A	0,750
Vitamin B1	1,5
Vitamin B2	1,7
Niacin	17,0
Pantothenic acid	10,0
Vitamin B_6	4,2
Biotin	0,150
Folic acid	0,400
Vitamin B_{12}	0,0075
Vitamin C	180,0
Vitamin D_3	0,005
Vitamin E	15,0
Magnesium	62,5
Selenium	0.0025

Another Nutrients	Quantity in mg per Serving (33 ml)	Quantity in mg per 100 ml
Organic Aronia juice	1,980	6.000
Organic Aloe Vera extract	17,5	53,0
Edelweiss extract	17,5	53,0
Green tea extract	6,9	21,0

Vitamins and Minerals:

Vitamin A

Improve eyesight and the immune system. Contributes to the maintenance of normal skin.

Vitamin B Complex

It strengthens nerves and ensures continuous supply of power. In the bustle of everyday life and times of increased willingness to perform the essential water-soluble vitamins support the metabolism and increase the stress tolerance. Thus counteract against shiftlessness and fatigue.

Vitamin B_1

Contributes to the normal function of the nervous system, psyche and heart.

Vitamin B_2
Contributes to the normal production of red blood cells and metabolism of iron.
Protect cells against oxidative stress.
Vitamin B_3 or Niacin
Helps maintaining normal skin.
Vitamin B_5 or Pantothenic acid
Contributes to the normal synthesis and metabolism of steroid hormones, Vitamin D and some neurotransmitters.
Vitamin B_6
Contributes to the normal metabolism of proteins and glycogen, to the normal production of red blood cells and regulating hormone balance.
Water-soluble B Vitamin other names: Vitamin H, Coenzyme R, Biotin
Helps maintaining normal skin, hair.
Vitamin B_9, Vitamin M, Vitamin B_{11}
Contributes to the normal synthesis of amino acid, formation of blood and plays an important role in cell division. Contributes to the normal growth of maternal tissues during pregnancy.
Vitamin B_{12}
Contributes to the normal formation of red blood cells and plays an important role in cell division.
Vitamin C
Anti oxidant. Supports formation of collagen for skin, blood vessels and bone and aids in wound healing. Protects cells from oxidative stress.Supports the immune system. Increases the excretion of heavy metals via the kidneys and improves iron absorption.
Vitamin B_3
Contributes to the normal function of the muscles and maintaining normal bone and teeth. Contributes to the normal utilization of calcium and phosphorus and contributes to the normal calcium level in blood.
Vitamin E
Protect cells against oxidative stress and contributes to a young, tight and healthy skin. Increases the amount of collagen in the skin. Supports wound healing through the support of cell division.
Magnesium
Contributes to the normal muscle function and maintaining to normal bone and teeth. Supporting protein synthesis, regulates electrolyte balance and plays an important role in cell division.
Selenium
Contributes to the normal function of the immune system and the thyroid. Protect cells against oxidative stress.
Helps maintaining normal hair and nails as well as for normal formation of sperm. Natural and high quality plant extracts from the Swiss Alps. Extracts from plants provide an effective and long lasting protection against free radicals. For this reason Vita Intense contains a number of effective and health promoting extracts which are carefully matched to achieve an optimum synergistic effect with maximum bioavailability.

Aronia (*Aronia melanocarpa*)
The Aronia (Fig. 1) or "Black chokeberry" originates from North America is now increasingly cultivated in Middle Europe, including Switzerland.

Figure 1: Aronia

The shell of the Aronia is naturally rich in vitamins (such as Vitamin A, C, E, K and the entire group of the B Vitamins), minerals and trace elements (such as calcium, magnesium, potassium, zinc and iron). The Aronia shows record antioxidants levels for protecting cells against oxidative stress (Zheng, Wang, 2003). But the most

interesting active ingredient is hidden in the group of polyphenols, whose main representatives are the flavonoids and anthocyanins. The Aronia contains an unsurpassed amount of flavonoids and the highest proportion of anthocyanins compared to other berries (Table 2) (Zheng, Wang 2003).

Table 1: Composition of different berries (Zheng, Wang 2003)

ORAC, Anthocyan- und Phenolgehalt der Beeren im Vergleich (nach Zheng und Wang 2003)

Beerensorte	ORAC (!mol Trolox Equivalent/g FG	Anthocyane (mg Cyanidin-3-Glukoside/g FG)	Phenole (mg Gallussäureequivalent/gFG)
Blaubeere	28,9	1,20	4,12
Cranberries	18,5	0,32	3,15
Preiselbeere	38,1	0,45	6,52
Apfelbeere	160,2	4,28	25,56

Figure 2 shows the results of anti oxidative parameter of Aronia (mmol/l) for TEAC

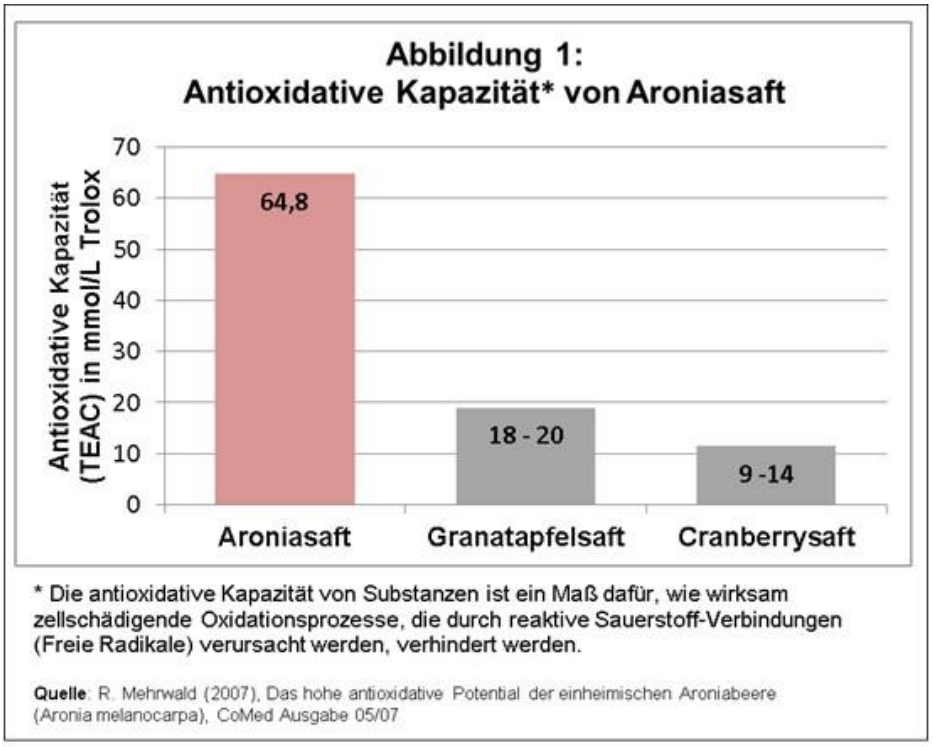

Figure 2: Oxidative parameter of Aronia (mmol/l) for TEAC

The Aronia has antioxidant (Olas et al., 2008), anti-inflammatory (Ohgami et al. 2005, Borisova et al., 1994), anti oxidative stress (Valcheva-Kuzmanova et al., 2005) antibacterial and antiviral effect (Kokotkiewicz et al., 2010). Further these antioxidants show positive effects on blood sugar (Ryszawa et al. 2005) and of blood lipid levels (Valcheva-Kuzmanova et al. 2006). They protect the heritage (~antimutagenic effect: Gasiorowski et al., 1997; Dimova et al., 1997), the cardiovascular system (Ryszawa et al. 2006), the gastrointestinal tract (Valcheva-Kuzmanova et al., 2005; Matsumoto et al., 2004) and the liver (Borisova et al., 2004). While flavonoids improve blood circulation and the cardiovascular system (via relaxation of the vessel walls), anthocyanins reduce platelet formation (Ryszawa et al., 2006). Both flavonoids show unsurpassed antiviral, antimicrobial, antiallergic and anticarcinogenic effects (Bermudez-Soto et al. 2006; Lala, Marlik, 2006; Zhao et al., 2004; Marlik 2003, Zhao et al. 2003).

Green tea *(Camelia sinensis)*

Green tea (Fig. 3) has been shown to preventative against many diseases. Green tea has been proven for everyday primary care, prevention of mild illnesses or weakened constitution, improving fitness and metabolism or for setting an ideal weight. (Lambert, Yang. 2003).

Figure 3: Green tea

The extract of Green tea (*Camelia sinensisis)* naturally rich in many vitamins, minerals and trace elements (calcium, iron, fluoride, potassium, manganese and magnesium), tea polyphenols and catechins (especially EGCG = Epigallocatechingallate). The combination of these powerful ingredients provides an antioxidative (Chan et al. 1997), anti-inflammatory, cardiotonic (Hollman, Tjiburg, Yang, 1997) and cancer preventive effects (Lambert et al., 2003). Green tea contributes to the dental health, reduces deposits in the blood vessels and protects against atherosclerosis (Chacko et al., 2010). While the antioxidant properties are generally ascribed to the high content of tea polyphenols (Balentine et al., 1997), the positive influence on the body weight is attributable to the caffeine and catechins. The caffeine/catechins combination stimulates metabolism, increases energy expenditure, fat digestion and resorption in the stomach and the intestine (Bartista et al., 2009). Furthermore, the German Medical Journal reported in April 2010 that the catechins contained in green tea (EGCG) harmless "toxic" plaques in Alzheimer's.

Aloe Vera

Sumerian and Egyptian records occupied that the "plant of immortality" or the "blood of the gods" has been used medicinally 5,000 years ago. Scientists were able to detect more than 200 active ingredients till today. The Aloe Vera (Fig. 4) is naturally rich in polysaccharides, glycoproteins, vitamins, amino acids, enzymes and phytochemicals (such as essential oils, saponins, tannins, salicylic acid, sterols and aloin) in an unique pharmacological combination (Choi et al., 2001).

Figure 4: Aloe Vera

Esua and Rauwald reported 2005 of a novel bioactive glucan, which attributed to the significant anti-inflammatory effect. The Aloe further harmonized the metabolism in all organs by regulation of the basic substances and the acid-base balance. In folk medicine the “wonderful desert lily” shows an excellent effect in inflammation (especially in the gastrointestinal tract), skin diseases (especially in Eczema, Dermatitis and Psorias), sunburns and wound raptures (Vogler, Ernst 1999).

Edelweiss (*Leontopodii alpinum*) – Pure ingrediens, real effect! The most famous and most symbolic flower of the Alps is naturally rich in antioxidants, terpenes, caffee acid derivatives (Leontopodic acid) and Leoligin. So it is very popular in the cosmetic- (especially in UV-protective- and anti-aging products), food- and dietary

supplement industries. In the Alpine region it is particularly known Edelweiss (Fig. 5) as "Bellyache flower" (such as abdominal pain, indigestion, diarrhea or intestinal colic).

Figure. 5. Edelweiss

The secondary plant substances of the “Edelweiss” support in the prevention and treatment of gastrointestinal complaints (Dobner et al., 2003) and in respiratory-, neurologic-, muscular- and cardiovascular diseases.
In addition to the outstanding antiinflammatory, antibacterial (Dobner et al. 2004) - even with infections (Stuppner, 2000) - and analgesic effects (Speroni et al., 2006) the “Edelweiss” shows a superior property as a scavenger and antioxidant (Schwalger et al., 2005) and preserves so cells from damage (also by molds; Costa et al., 2009). While Leoligin (Fig. 6) proved highly effective against thickening of the inner wall of blood vessels (Reisinger et al., 2009), an unknown terpene shows a significant cancer hostile activity against human leukemia cells (Wang et al. 2007). Moreover (Hornick et al., 2008) can showed after taking an extract from the whole root an improvement in the capacity of memory and kick off a discussion for taking this extract in the prevention and treatment of dementia.

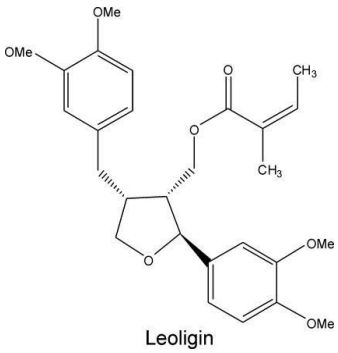

Figure. 6. The structure of Leoligin in Edelweiss

3.2. Comparative analysis between longevity of long living people, centenarians and their siblings and the quality of water

In frames of the research on the water quality 121 long living people from Bulgaria over 90 years of age have been studied together with their 294 siblings. The average lifespan of long lived people and centenarians in mountain areas is 94.1 years. For the average lifespan of long lived people in plain areas the result is 90.6 years. The most adult person from mountain areas is 104 years old and for plain areas is 97 years old. For the brothers and sisters of long live people from mountain areas the average lifespan is 88.5 years. For the brothers and sisters of long live people from plain areas the average lifespan is 86.4 years. The difference in life expectancy of the two groups of people is reliable and it corresponds to the Student’s *t*-criteria at $p < 0.05$ with a confidence level of $t = 2.36$. There are distances of no more than 50–70 km between these places and the only difference is the mountain water and air.

There have been 21519 residents in Teteven and 142 of them were born before 1924. Figure 7 demonstrates the interrelation between the year of birth (1912–1924) of long living people (age) and their number (Teteven municipality, Bulgaria).

Figure 7. Interrelation between the year of birth of long living people (age) and their number in Teteven municipality, Bulgaria.

From the standpoint of genetics, the process of aging is associated with disruption of the genetic program of the organism and gradual accumulation of errors during the process of DNA replication. Aging may be associated with the accumulation of somatic mutations in the genome and be influenced by free radicals (mainly oxygen and primary products of oxidative metabolism) and ionizing radiation on DNA molecules as well (Woodhead, 1984; Adelman *et al*., 1988). Such mutations can reduce the ability of cells to the normal growth and division and be a cause of a large number of various cell responses: inhibition of replication and transcription, impaired cell cycle division, transcriptional mutagenesis, cell aging that finally result in cell death. Cells taken from the elderly people show a reduction in transcription when transferring information from DNA to RNA.

From the standpoint of dynamics, aging is a non-linear biological process, which increases over time. Accordingly, the rate of aging increases with time. The accumulation of errors in the human genome increases exponentially with time and reaches a certain stationary maximum at the end of life. L. Orgel shows that, for this reason, the probability of cancer occurrence increases with age (Orgel, 1963). Figure 8 shows L. Orgel's results on the interrelation between age and the number of cancer cases. The accumulation of errors in synthesis of abnormal proteins increases exponentially over time with age. Cells taken from elderly people show the reduced levels of transcription or transmission of information from DNA to RNA. Therefore, the probability of cancer increases with age. The interrelation between the number of Bulgarian centenarians in the mountainous municipality of Teteven and their age is close to exponential.

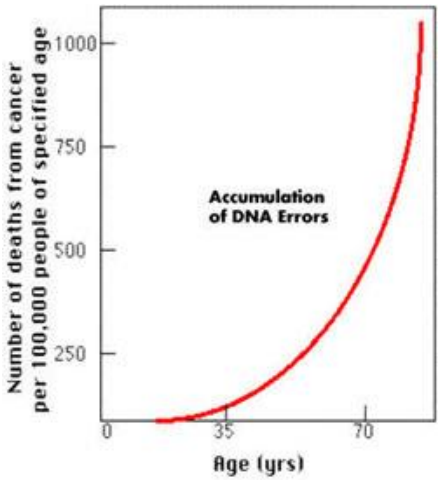

Figure 8: Interrelation between age and the number of cancer patients (Orgel, 1963).

Here are submitted the data for Bulgaria:
1) Varna district – 44 centenarians per 1 million of inhabitants, plain and sea regions;
2) Pleven district – 78 centenarians per 1 million of inhabitants, plain regions;
3) Teteven district – 279 centenarians per 1 million of inhabitants, hills and mountainous regions;
4) Bulgaria – 47 centenarians per 1 million of inhabitants.

3.3. Clinical studies with human blood serum testing

A convenient method for studying of liquids is non-equilibrium differential spectrum. It was established experimentally that the process of evaporation of water drops, the wetting angle θ decreases discreetly to zero, and the diameter of the water drop basis is only slightly altered, that is a new physical effect (Antonov, 1995; Antonov & Yuskesselieva, 1983). Based on this effect, by means of the measurement of the wetting angle within equal intervals of time is determined the function of distribution of H_2O molecules according to the value of f(θ). The distribution function is denoted as the energy spectrum of the water state. The theoretical research established the dependence between the surface tension of water and the energy of hydrogen bonds among individual H_2O-molecules (Antonov, 1995).

For calculation of the function f(E) represented the energy spectrum of water, the experimental dependence between the wetting angle (θ) and the energy of hydrogen bonds (E) is established:

$$f(E) = \frac{14{,}33 f(\theta)}{[1-(1+bE)^2]^2} \qquad (1)$$

where $b = 14.33\ eV^{-1}$

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H_2O molecules is calculated by the formula:

$$\theta = arcos\ (-1 - 14.33E) \qquad (2)$$

The energy spectrum of water is characterized by a non-equilibrium process of water droplets evaporation, therefore, the term non-equilibrium spectrum (NES) of water is used.

The difference $\Delta f(E) = f$ (*Esamples of water*) – f (*Econtrol sample of water*) – is called the "differential non-equilibrium energy spectrum of water" (DNES).

Thus, the DNES spectrum is an indicator of structural changes in water, because the energy of hydrogen bonds in water samples differ due to the different number of hydrogen bonds in water samples, which may result from the fact that different waters have different structures and composition and various intermolecular interactions – various associative elements etc (Ignatov et al, 2014; Ignatov et al., 2015). The redistribution of H_2O molecules in water samples according to the energy is a statistical process of dynamics.

Figure 9 shows the average NES-spectrum of deionised water. On the X-axis are depicted three scales. The energies of hydrogen bonds among H_2O molecules are calculated in eV. On the Y-axis is depicted the function of distribution of H_2O molecules according to energies f(E), measured in reciprocal unit eV^{-1}.

Arrow A designates the energy of hydrogen bonds among H_2O molecules, which is accepted as most reliable in spectroscopy.
Arrow B designates the energy of hydrogen bonds among H_2O molecules the value of which is calculated as:

$$\bar{E} = -0.1067 \pm 0.0011 \text{ eV} \qquad (3)$$

Arrow C designates the energy at which the thermal radiation of the human body, considered like an absolute black body (ABB) with a temperature +36.6 ^{0}C, is at its maximum.
A horizontal arrow designates the window of transparency of the Earth atmosphere for the electromagnetic radiation in the middle infrared range of the Sun toward the Earth and from the Earth toward the surrounding space. It can be seen that the atmosphere window of transparency almost covers the NES-spectrum of water.

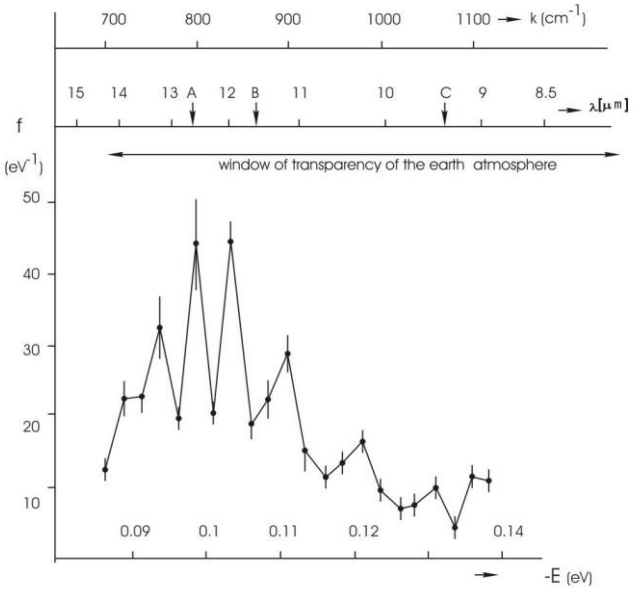

Figure 9: The NES-spectrum of deionized water (chemical purity – 99.99 %; pH – 6,5–7,5; total mineralization – 200 mg/l; electric conductivity – 10 μS/cm): the horizontal axis shows the energy of the H...O hydrogen bonds in the associates – E (eV); the vertical axis – the energy distribution function – f (eV^{-1}); *k* – the vibration frequency of the H–O–H atoms (cm^{-1}); λ – wavelength (μm)

We have conducted studies of 1% (v/v) solution of human blood serum taken from two groups of people between 50 and 70 years of age by IR, NES and DNES spectral analysis. The first group consisted of people in excellent health. The second group consisted of people in a critical state and patients with malignant tumors. The average energy of hydrogen bonds ($\Delta E_{H...O}$) between H_2O molecules in the blood serum was investigated as the main biophysical parameter. The result was registered as a difference between the NES-spectrum of 1% solution of human blood serum and the NES-spectrum of deionized water control sample – DNES-spectrum, measured as the difference Δf(E) = f (samples of water) – f (control sample of water). The DNES-spectrum obtained from the first group has a local extremum energy ($\Delta E_{H...O}$) at E = -9.1±1.1 meV and from the second group at E = -1.6±1.1 meV. The results between the two groups have a statistical difference in Student's criterion at $p < 0.05$. For the control group of healthy people the value of the largest local maximum in the DNES-spectrum was detected at E = -0.1387 eV, or at a wavelength λ = 8.95 μm. For the group of people in a critical health state and the patients with malignant tumors, the analogous values of the largest local maximums of the DNES-spectrum shifted to lower energies compared with the control group of people. For a group of people in critical health condition and patients with malignant tumors the greatest values of local extremum in the IR-spectrum are shifted to lower energies relative to the control group. In IR-spectrum of human blood serum are detected 8 local maxima at λ = 8.55, 8.58, 8.70, 8.77, 8.85, 9.10, 9.35 and 9.76 μm (Krasnov, Gordetsov, 2009). The resulting

peak at λ = 8.95 μm in the IR-spectrum (Ignatov, 2012) approaching the peak at λ = 8.85 μm was monitored by Russian researchers. In the control group of healthy people the average value of the energy distribution function f(E) at λ = 8.95 μm compiles E = 75.3 eV, and in a group of people in critical condition – E = 24.1 eV. The norm has statistically reliable result for human blood serum for the control group of people having cancer at the local extremum of f(E) ~24.1 eV^{-1}. The level of reliability of the results is $p< 0.05$ according to the Student's t-test. In 1995 were performed DNES-experiments with an impact on tumor mice cells in water solutions containing Ca^{2+} (Antonov, 1995). There was a decrease in the DNES-spectrum compared with the control sample of cells from a healthy mouse. The decrease was also observed in the DNES-spectrum of human blood serum of terminally ill people relative to that of healthy people. With increasing of age of long-living blood relatives, the function of distribution of H_2O molecules according to energies at -0.1387 eV decreases. In this group of tested people the result was obtained by the DNES-method at E = -5.5±1.1 meV; the difference in age was of 20–25 years in relation to the control group. It should be noted that many of Bulgarian centenarians inhabit the Rhodopes Mountains areas. Among to the DNES-spectrum of mountain waters the similar to the DNES-spectrum of blood serum of healthy people at λ = 8.95 μm, was the DNES-spectrum of water in the Rhodopes. The mountain water from Teteven, Boyana and other Bulgarian provinces has similar parameters. Tables 1, 2 and 3 show the composition of mountain water springs in Teteven and Kuklen (Bulgaria) and local extremums in NES-spectra of water samples. The local extremums is water samples were detected at E = -0.11 eV and E = -0.1387 eV. The value measured at E = -0.11 eV is characteristic for the presence of Ca^{2+} in water. The value measured at E = -0.1387 eV is characteristic for inhibiting the growth of cancer cells. Experiments conducted by A. Antonov with cancer cells of mice in water with Ca^{2+} demonstrated a reduction of this local extremum to a negative value in spectra. Analysis by the DNES-method of aqueous solutions of natural mineral sorbents – shungite (carbonaceous mineral from Zazhoginskoe deposit in Karelia, Russia) and zeolite (microporous crystalline aluminosilicate mineral from Most village, Bulgaria) showed the presence of a local extremum at E = -0.1387 eV for shungite and E = -0.11 eV for zeolite (Mosin & Ignatov, 2013, Ignatov & Mosin, 2014a). It should be noted that owing to the unique porous structures both the natural minerals shungite and zeolite are ideal natural water adsorbers effectively removing from water organochlorine compounds, phenols, dioxins, heavy metals, radionuclides, and color, and gives the water a good organoleptic qualities, additionally saturating water with micro-and macro-elements until the physiological levels (Mosin & Ignatov, 2013). It is worth to note that in Bulgaria the main mineral deposits of Bulgarian zeolites are located in the Rhodope Mountains, whereat has lived the greatest number of Bulgarian centenarians. It is believed that water in these areas is cleared out in a natural way by mineral zeolite.

3.4. Results of 1% (v/v) solution in deionized water of VITA intense

The research with the NES method of water drops is received with 1% solution VITA intense, and deionized water as control sample. The mathematical models of 1% (v/v) solution VITA intense gives the valuable information for the possible number of hydrogen bonds as percent of H_2O molecules with different values of distribution of energies (Table 3 and Fig. 10). These distributions are basically connected with the restructuring of H_2O molecules having the same energies.

Table 2: The distribution (%, $(-E_{value})/(-E_{total\ value})$ of H_2O molecules in 1% water solution of VITA intense (product of LavaVitae, Austria) and control deionized water

-E(eV) x-axis	1% water solution VITA intense (LavaVitae) y-axis (%$((-E_{value})$ */ $(-E_{total\ value})$**	Control Sample Deionized water y-axis (%$((-E_{value})$*/ $(-E_{total\ value})$**	-E(eV) x-axis	1% water solution VITA intense (LavaVitae) y-axis (%$((-E_{value})$ */ $(-E_{total\ value})$**	Control Sample Deionized water y-axis (%$((-E_{value})$*/ $(-E_{total\ value})$**
0.0937	0	0	0.1187	0	0
0.0962	0	14.3	0.1212	**17.7**[2]	0
0.0987	0	0	0.1237	0	0
0.1012	0	14.3	0.1262	11.7	3.6
0.1037	0	0	0.1287	0	3.6
0.1062	11.7	14.3	0.1312	5.7	7.1
0.1087	0	0	0.1337	11.7	0
0.1112	**5.7**[1]	**14.3**	0.1362	5.7	7.1
0.1137	0	0	0.1387	**30.1**[3]	7.1
0.1162	0	14.3	–	–	–

E= -0.1112 eV is the local extremum for relaxing effect on nervous system

E=-0.1212 eV is the local extremum for anti-inflammatory effect

E= -0.1387 eV is the local extremum for inhibition of development of tumor cells of molecular level

Notes:

* The result $(-E_{value})$ is the result of hydrogen bonds energy for one parameter of (-E)

** The result $(-E_{total\ value})$ is the total result of hydrogen bonds energy

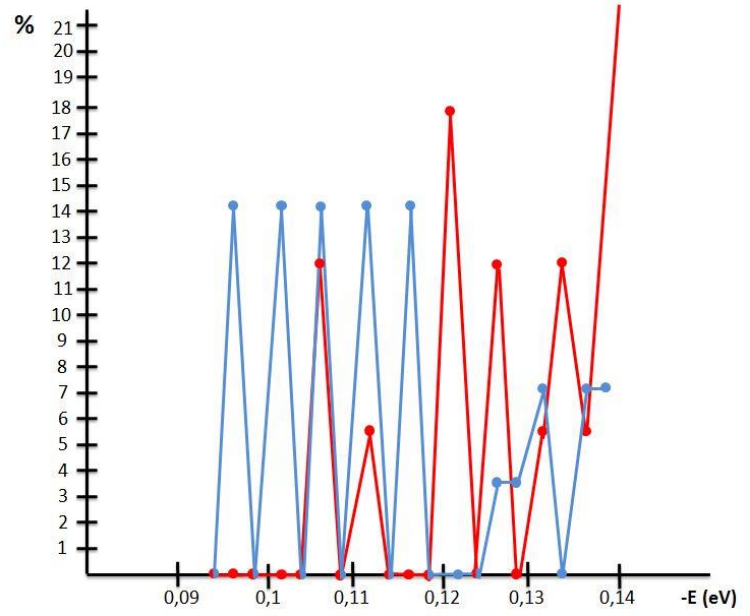

Figure 10: Mathematical model (Ignatov, Mosin, 2013) of 1% water solution of VITA intense (product of LavaVitae, Austria).

Figure 10 shows the distribution (%, $(-E_{value})/(-E_{total\ value})$ of H_2O molecules in and 1% (v/v) of water solution of VITA intense (product of LavaVitae, Austria) (red line) and control sample deionized water (blue line).

Notes:

E= -0.1112 eV is the local extremum for relaxing effect on nervous system

E=-0.1212 eV is the local extremum for anti-inflammatory effect
E= -0.1387 eV is the local extremum for inhibition of development of tumor cells of molecular level

The experimental data obtained testified the following conclusions from the mathematical model of in 1% (v/v) water solution of VITA intense (product of LavaVitae, Austria) and control deionized water. The distribution (%, $(-E_{value})/(-E_{total\ value})$ of water molecules in mathematical model of in 1% (v/v) water solution of VITA intense (product of LavaVitae, Austria) and control deionized water. The distribution (%, $(-E_{value})/(-E_{total\ value})$ of water molecules in VITA intense (product of Lava Vitae, Austria) according control sample is different. However, for the value E = -0.1387 eV or λ = 8.95 μm there is the biggest local extremum (30.1 (%, $(-E_{value})/(-E_{total\ value})$) corresponding to the re-structuring of hydrogen bonds among H_2O molecules for inhabitation of development of tumor cells of molecular level. This difference may indicate on the different number of hydrogen bonds in water samples, as well as their physical parameters **(**pH, ORP**),** resulting in different distribution of H_2O molecules and different values of H_2O molecules with ratios of $(-E_{value})/(-E_{total\ value})$. Particularly it was observed the statistical re-structuring of H_2O molecules in water samples according to the energies. The experimental data may prove that stipulates the restructuring of H_2O molecules on molecular level and may be used for the prophylaxis of inhibition of development of tumor cells. For the value E = -0.1112 eV or λ = 11.15 μm there is the local extremum with sight minus (5.7 (%, $(-E_{value})/(-E_{total\ value})$) according control sample (14.3 (%, $(-E_{value})/(-E_{total\ value})$) corresponding to the re-structuring of hydrogen bonds among H_2O molecules. The experimental data may prove that influence stipulates the restructuring of H_2O molecules on molecular level and has biophysical effect for relaxing effect on nervous system. For the value E = -0.1212 eV or λ = 10.23 μm there is bigger local extremum (17.7 (%, $(-E_{value})/(-E_{total\ value})$) corresponding to the re-structuring of hydrogen bonds among H_2O molecules for anti-inflammatory effect. The experimental data for Lava Intense may prove that stipulates the restructuring of H_2O molecules on molecular level and the biophysical effects are:

E= -0.1112 eV is the local extremum for relaxing effect on nervous system
E=-0.1212 eV is the local extremum for anti inflammatory effect
E= -0.1387 eV is the local extremum for inhibition of development of tumor cells of molecular level.

As a result of different energies of hydrogen bonds, the surface tension of 1% (v/v) solutions of water samples with VITA Intense is increasing. The increasing of surface tension is regarding the control samples. This effect is connected with preservation of the energy in human body as result of biochemical process among water molecules and bio molecules.

5. VITA intense (product of the company LavaVitae)

The average energy ($E_{H...O}$) of hydrogen H…O-bonds among individual H_2O molecules in 1% (v/v) solution of VITA Intense is measured at E=-0.1261 eV. The result for the control sample (deionized water) is E=-0.1125 eV. The results obtained with the NES method are recalculated with the DNES method as a difference of the NES (1% (v/v) solution of VITA intense) minus the NES (control sample with deionized water) equaled the DNES spectrum of 1% solution of VITA Intense. Thus, the result for 1% solution of VITA intense recalculated with the DNES method is ΔE=-0.0136±0.0011 eV. The result shows the increasing of the values of the energy of hydrogen bonds in 1% (v/v) solution of VITA intense regarding the deionized water. This is effect of stimulation on human body. The results show restructuring of water molecules in configurations of clusters, which influence usefully on human health on molecular and cellular level. The effects are describing with mathematical model of 1% solution of VITA intense.

3.6. Results with pH and ORP

There are valid the following results of pH as indicator for acid alkaline medium of the products of LavaVitae. There are the results also of ORP or Oxidation-reduction potential.
The results are for 1% (v/v) of solutions of products, which are made from deionized water. This research is performed with Georgi Gluhchev from Bulgarian Academy of Science. The results of pH of deionized water is 6.05 and of ORP is 119.7. Table 4 shows the results of pH and ORP.

Table 4. Results of products of company LavaVitae for pH and ORP

Product	pH	ORP (mV)	Coordinates Fig. 11
VITA intense	4.07 ±0.02	- 104.5	Point 1 (4,07; -104.5)
BOOST	3.60 ±0.02	+113.6	Point 2 (3,90;113.6)
ZEOLITH detox	8.01 ±0.02	+109.5	Point 3 (8,01;103.3)
Deionized water	6.05 ±0.02	+119.7	

Figure 11 shows the dependence between the acidity and basicity (pH) of electrochemically activated solutions and the oxidation-reduction potential (ORP). The pH value within the interval from 3 to 10 units and the ORP within the interval from -400 mV to +900 mV characterize the area of the biosphere of microorganisms. Outside these ranges of pH and ORP the microorganisms will hardly survive.

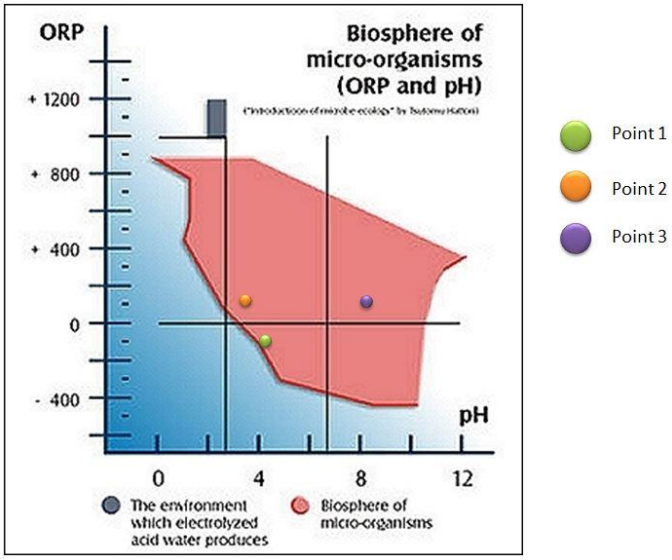

Figure 11: The dependence between acidity and basicity (pH) of solutions and the ORP on the biosphere of micro-organisms (point 1; VITA intense), (point 2; BOOST), point 3; ZEOLITH detox).

The result of 1% (v/v) solution of VITA intense is 4.07 or acidic medium. The result of ORP is (-104.5). The result of ORP with negative charge is connected with charge with negative value, which has antioxidant and permanent antioxidant activity. In the VITA intense there are the following antioxidants – Vitamins C, E, D. Figure 11 shows the dependence between acidity and basicity (pH) of solutions and the ORP on the biosphere of micro-organisms. The result of VITA intense with point 1 with coordinates (4,07; -104.5) is the biosphere of micro-organisms. VITA intense is useful for human health also with liquid form.

3.7. Effects of Magnesium and Selenium, Vitamins C and E in VITA intense again oxidative stress

VITA intense is product with negative Oxidation-reduction potential (ORP) or (-104.5) mV. This results shows that there are particles in VITA intense with negative charge again free radicals. There are also active antioxidants as Vitamin C and Vitamin E. There are special role in the liquid product the ions of Magnesium - Mg^{2+}. These ions neutralize one of the most danger radicals – hydroxyl radical OH (Glamello et al.;1993). Magnesium deficiency increased cytotoxicity of the added oxyradicals. These results suggest that increased oxidative endothelial cell injury may contribute to vascular injury during Mg deficiency (Dickens et al.; 1992). The magnesium deficiency and oxidative stress have both been identified as pathogenic factors in aging and in several age-related diseases. The link between these two factors is unclear in humans although, in experimental

animals, severe Mg^{2+} deficiency has been shown to lead to the increased oxidative stress (Begona et al.; 2000). A negative correlation between magnesium balance and oxidative stress was observed suggesting that the same etiological factor (chronic stress) initiate decreases in both free and total magnesium concentrations and simultaneously increase oxidative stress intensity. These findings support the need for magnesium supplementation with antioxidant vitamins for people living in conditions of chronic stress (Cernak et al.; 2000).
Selenium has role for anti oxidative stress in the enzymes, which are connected with anti oxidant effects. (Stewart, M. et al.;1999)
Vitamin E, as a powerful antioxidant residing mainly in biomembranes, may provide effective protection against oxidative membrane damage and resultant age-related deterioration, especially in the elderly. We hypothesized that appropriate levels of vitamin E supplementation would protect erythrocyte membranes from oxidative stress and thus improve membrane fluidity in healthy middle-aged and elderly people (Sun, Y. et al.; 2012).
The combination of antioxidative treatment with vitamins E and C decreases fetal malformation rate and diminishes oxygen radical-related tissue damage (Cederberg, Siman, Eriksson ; 2001)

3.8. Effects of Ca^{2+}, Mg^{2+}, Zn^{2+} and Mn^{2+} in water on biophysical and biochemical processes in the human body

The research of distribution of local extremums (eV^{-1}) in spectra of various water samples as a function of distribution of H_2O molecules according to energy f(E) at λ = 8.95 μm shows the analogue extremum at analogous values of f(E), E and λ, which was detected in water with Zn^{2+} and Mn^{2+} ions earlier demonstrated inhibiting the growth of cancer cells. Magnesium (Mg^{2+}), zinc (Zn^{2+}) and manganese (Mn^{2+}) ions dissolved in water have influence on enzymes, which are antioxidants (Ignatov & Mosin, 2015). The research of China team was categorized three groups of elements from the rice and drinking water according to their effect on longevity: Sr, Ca, Al, Mo, and Se, which were positively correlated with longevity: Fe, Mn, Zn, Cr, P, Mg, and K, which had a weak effect on local longevity, and Cu and Ba, which had a negative effect on longevity (Lv et al., 2011). There was a positive correlation between the eSOD activity and the age and a negative correlation between the eSOD activity and concentration of Zn^{2+} in plasma. An inverse correlation was also found between the content of Zn^{2+} ions in plasma relative to the age. The prevalence of Zn^{2+} deficiency is increased with age; with normal Zn^{2+} levels it is observed in about 80% of adult people and only in 37 % of the non-agenarians. Aging is an inevitable biological process that is associated with gradual and spontaneous biochemical and physiological changes and the increased susceptibility to diseases. Because the nutritional factors are involved in improving the immune functions, metabolic balance, and antioxidant defense, some nutritional factors, such as Zn, may modify susceptibility to disease and promote healthy aging. *In vitro* (human lymphocytes exposed to endotoxins) and *in vivo* (old or young mice fed with low zinc dietary intake) studies revealed that zinc is important for immune efficiency (innate and adaptive), antioxidant activity (superoxide dismutase), and cell differentiation *via* clusterin/apolipoprotein J. The intracellular Zn homeostasis is regulated by metallothioneins (MT) *via* an ion release through the reduction of thiol groups in the MT molecule (Mocchegiani, 2007). Zinc in composition of water improves the antioxidative enzymes in red blood cells (Malhotra & Dhawan, 2008).
The antioxidants against free radical damage include tocopherol (vitamin E), ascorbic acid (vitamin C), β-carotene, glutathione, uric acid, bilirubin, and several metalloenzymes including glutathione peroxidase (Se), catalase (Fe), and superoxide dismutase (Cu, Zn, Mn) and proteins such as ceruloplasmin (Co). The extent of the tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defense system (Machlin & Bendich, 1988). There was reported the antioxidant effects of water on rats (Abdullah, 2012). The norm in water for Zn^{2+} and Mn^{2+} according to the World Health Organization (WHO) should be less than 20 μg. For the Na^{+} content the norm according to the WHO is less than 20 mg.
The interesting results on the concentration of Ca^{2+} in water were obtained in USA and Canada. According to the statistical information the most number of centenarians in Canada per 1 million of population is observed in Nova Scotia (210 of centenarians per 1 million). In the water from Nova Scotia the Ca^{2+} content makes up 6.8 mg/l. N. Druzhyak, Russia showed that in the places wherein live the most number of centenarians the Ca^{2+} content in water was 8–20 mg/l. The only risk factor regarding the increased Ca^{2+} content in water is cardiovascular diseases.
The following reactions occur in water if there are high concentrations of Ca^{2+} and Mg^{2+} ions: the reaction of limestone ($CaCO_3$) and gypsum ($CaSO_4 \cdot 2H_2O$) with water to separate the calcium (Ca^{2+}), carbonates (CO_3^{2-}) and

sulfate (SO_4^{2-}) ions. By increasing the mineralization of water the content of Ca^{2+} ions decreases. During the concentration of the solutions Ca^{2+} ions are precipitated. With the increase of carbon dioxide (CO_2) in water and decreasing of the pH value the content of Ca^{2+} increases. The reaction of interaction of dolomite ($CaCO_3 \cdot MgCO_3$) with water makes the formation of Mg^{2+} ions. Hydrocarbonates (HCO_3^-) and carbonates (CO_3^{2-}) ions are formed by reaction of interaction of karst rocks, CO_2 and water. For example, in Zamzam water there is Ca^{2+} – 299.7 mg/l; Mg^{2+} – 18.9 mg/l; Zn^{2+} – 0.001 mg/l.

4. Conclusion

From the NES and DNES spectrum and mathematical model of 1% (v/v) solution of VITA Intense and deionized water as control sample are valid the following conclusions for biophysical effects for VITA Intense (LavaVitae company)

- relaxing effect on nervous system;
- anti inflammatory effect;
- inhibtion of development of tumor cells of molecular level;

In 1% (v/v) solution of VITA intense there is restructuring of water molecules in configurations of clusters, which influence usefully on human health on molecular and cellular level.

The biophysical effects of VITA intense are connected also with antioxidant effects. The VITA intense is recommended as anti aging solution for prophylaxis (Ignatov, Gluhchev, Karadzhov et al. 2015). The scientific studies show that the inflammations are one of the basic reasons for aging. The recommendation is connected with additionally using or including in VITA intense of the additional mineral. The structuring of water clusters with highest energy of hydrogen bonds at 8.95 μm makes the water in human body more “active” as medium of biochemical and biophysical processes. This is similar like the human organism to be younger (Ignatov, Mosin, 2012). The quality of the water with which will be using is very important. There are types of water which will increase the effects. For these effects is recommended additional scientific project with pH is in progress.

As a result of different energies of hydrogen bonds, the surface tension of 1% (v/v) solutions of water samples with VITA intense is increasing. The increasing of surface tension is regarding the control sample. This effect is connected with preservation of the energy in human body as result of biochemical process among water molecules and bio molecules;

It worth to note that IR-spectrum of VITA Intense is most similar to the IR-spectrum of blood serum of healthy group of people with a local extremum at λ=8.95 μm. The similar spectral characteristics possess mountain water from Teteven and other Bulgarian sources electrochemically activated water catholyte (Ignatov et al., 2014). The basic research is with Kangen device (Ignatov, Mosin, Kirov, 2016) Studying the human blood serum by NES and DNES-methods show that by measuring the average energy of hydrogen bonds among H_2O molecules and the distribution function of H_2O molecules on energies it is possible to show a vital status of a person and associated life expectancy. Our data indicates that water in the human body has the IR-spectrum resembling the IR-spectrum of human blood serum. On the characteristics of the IR-spectrum of water also exerts an influence the presence of deuterium in water samples. In the research there is the optimal composition of mountain and melt water from areas where are lived the long live people and centenarians. The decreased content of deuterium in studied water samples with residual deuterium content of 60-100 ppm, the variety of ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, SO_4^{2-}, Cl^-, HCO_3^-, CO_3^{2-}), and chemical-physical parameters (pH, electroconductivity) of studied water samples renders beneficial effects of this type of water on human health. We have also obtained new proofs for biophysical and biochemical effects of Ca^{2+}, Mg^{2+}, Zn^{2+} and Mn^{2+} in composition of water on human organism and DNES-spectra of water. There are obtained new results of chemical composition of water from Glacier Rosenlaui, Swiss Alps.

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