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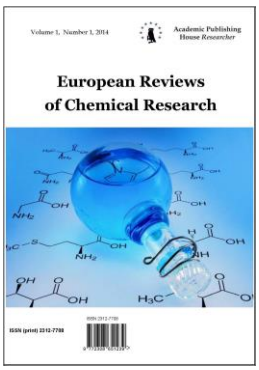

Results with IR Spectroscopy of CortiNon+ on the Development of Experimental *Graffi* Tumor on Hamsters

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Abstract

Studies were conducted with product CortiNon+. CortiNon+ is mix of Liquid Progesterone/DHEA. Progesterone is progestin, a female hormone, produced mostly in the ovaries.

DHEA is an endogenous hormone secreted by the adrenal gland of both males and females. The purpose of the research was to analyze the effects over blood serum of hamsters with *Graffi* tumor (Toshkova, 1995). The analyses have been conducted with Nonequilibrium Energy Spectrum (NES) and Differential Nonequilibrium Energy Spectrum (DNES) methods (Antonov, 1992; Ignatov, 1998). Experiments were carried out about the influence on tumor cells of mice in water. Reduction of DNES spectrum according to the control sample of cells in healthy animals was observed. (Antonov, 1992). Reduction has also been observed in DNES spectrum in blood serum of people having oncological diseases, compared to the one of healthy people (Ignatov, 2012). Such a reduction is most prevalent in (-0.1387 eV; 8.95 μm; 1117 cm^{-1}). The investigation of blood serum from hamsters injected with *Graffi* tumor cells and treated with CortiNon+ demonstrated a reduction in DNES spectrum in the range (-0.08 – -0.14 eV) (8.9 –15.5μm) (645–1129 cm^{-1}) (Toshkova et al., 2019).

Keywords: CortiNon+, *Graffi* tumor, NES, DNES.

1. Introduction

The research demonstrates the effects of CortiNon+ on the *Graffi* tumor of hamsters. The hamsters are separated into 5 groups noted as Gr. 1, Gr. 2, Gr. 3, Gr. 4 and Gr. 5. The first two groups Gr.1 and Gr.2 are for studying the effects of CortiNon+on the tumor development. Gr. 1 includes hamsters that started taking CortiNon+ once daily for 7 days before the injection of *Graffi* tumor cells and then continued taking daily it until the end of the experiment. Gr. 2 is from hamsters that were s.c. injected with the same amount of tumor cells and started taking CortiNon+on the same day as the tumor cell injection.The other groups are used as control. Gr. 3 is of non-treated tumor-bearing hamsters. Gr. 4 is of CortiNon+ treated healthy hamsters, and Gr. 5 is of healthynon-treated animals.

The research is conducted using spectral methods NES and DNES (Antonov, 1990; Antonov, Ignatov, 1998).

The spectrum analyses with methods NES and DNES are conducted on the 10th day after transplantation of *Graffi* tumor cells, which coincides with the appearance (formation) of

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subcutaneous tumor in the trial animals.

2. Materials and Methods

Experimental design. All the animals were divided into 5 groups as follows.

Gr. 1 – The hamsters from this group started taking CortiNon+ once daily for 7 days before the injection with 5x10⁴ *Graffi* tumor cells per hamster in the back area, and then continued taking it once daily until the end of the experiment.

Gr. 2 – These hamsters were s.c. injected with the same amount of tumor cells as Gr. 1 and started taking CortiNon+on the same day of the tumor injection.

Gr. 3 – Hamsters with *Graffi* tumor as a control untreated group.

Gr. 4 – Healthy hamsters takingCortiNon+as a control group.

Gr. 5 – Healthy hamstersas a control untreated group.

1. Experimental animals

Hamsters, breed "Golden Syrian", aged 2-4 months with weight around 90-100 g were used in the trials. The animals were grown in standard conditions in individual plastic cages with free access to food and water.

2.2. Experimental tumor model

Tumor cells (1-2.10⁶) from the experimental *Graffi* solid tumor are transplanted subcutaneously in the back of hamsters. Between days 7 and 15 after the transplantation tumor appears, grows progressively and the hamsters die around 30-35 days. In this tumor model 100 % tumor transplantation and 100 % mortality are observed. No spontaneous tumor's regression takes place (Toshkova, 1995).

2.3. Ethical aspects

All experiments were conducted in accordance with the European convention for protection of vertebrate animals, used for experimental and other scientific purposes (OJ L 222) and approved from the National Veterinary Medical Office.

2.4. NES and DNES Spectral Analyses

The device for DNES spectral analysis based on an optical principle was designed by A. Antonov. For this, a hermetic camera for evaporation of water drops under stable temperature (+22–24 °C) conditions was used. The water drops were placed on a water-proof transparent pad, which consisted of thin maylar folio and a glass plate. The light was monochromatic with filter for yellow color with wavelength at λ = 580±7 nm. The device measures the angle of evaporation of water drops from 72.3° to 0°. The DNES-spectrum was measured in the range of -0.08– -0.1387 eV or λ = 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 between H_2O molecules in water samples and hamster serum blood.

3. Results and discussion

3.1. Parameters of NES and DNES spectrums of CortiNon+

The spectrum analysis is conducted on the 10th day after transplantation of *Graffi* tumor cells, which coincides with the appearance (formation) of subcutaneous tumor in the trial animals. 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 *Δf(E) = f (samples of water) – f (control sample of water)* – is called the "differential non-equilibrium energy spectrum of water" (DNES). The Figure 1 shows that on the X-axis are depicted three scales. The energies of hydrogen bonds among H_2O molecules are calculated in electronvolts (eV). On the Y-axis is depicted the function of distribution of H_2O molecules according to energies f(E), measured in reciprocal electronvolts unit eV^{-1}. The local extremums of water samples are detected at E = -0.1112 eV, E = -0.1212 eV and E = -0.1387 eV. The value measured at E = -0.1212 eV is characteristic for anti-inflammatory effect (Ignatov et al., 2014) The value measured at E = -0.1112 eV is characteristic for the presence of Ca^{2+} ions in water

(Antonov, 1995). The value measured at E = -0.1387 eV is characteristic for inhibiting the growth of tumor cells (Ignatov, Mosin, 2012). Experiments conducted by Antonov with cancer cells of mice in water demonstrated a reduction of this local extremum to a negative value in DNES spectra.

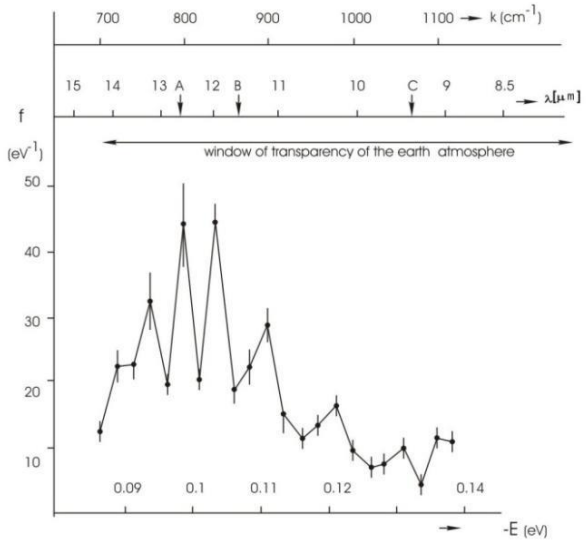

Fig. 1. The NES-spectrum of deionized water (chemical purity – 99.99 %; pH – 6,0–7,5; electric conductivity – 10 μS/cm): the horizontal axis shows the energy of the H…O hydrogen bonds in the associates (– E (eV)); the vertical axis – the energy distribution function – f (eV^{-1}); *k* – the vibration frequency of the H–O–H atoms (cm^{-1}); λ – wavelength (μm)

The following results of the effects of CortiNon+ with NES and DNES methods are obtained:

3.1.1. The difference of DNES spectra between Gr. 1 and Gr. 3 is

ΔE = (-0.1194 eV)- (-0.1156 eV) = -3.8±1,1 meV. The difference is essential and shows effect of CortiNon+ on *Graffi* tumor of molecular and cell level

3.1.2. The difference of DNES spectra between Gr. 1 and Gr. 5 is

ΔE = (-0.1194 eV)-(-0.1179 eV) = -1.5±1,1 meV. This result is achieved for the products with anti tumor effect.

3.1.3. The difference of DNES spectra between Gr. 1 and Gr. 4 is

ΔE = (-0.1194 eV)-(-0.1177 eV) = -1.7±1,1 meV. This result is achieved for the products with anti tumor effect. For Gr. 1 CortiNon+ with implanted *Graffi* tumor after 7 days the result is higher according to healthy hamsters from Gr. 4 with CortiNon+.

3.1.4. The difference of DNES spectra between DNES spectrum of CortiNon+ and control sample of deionized water is ΔE = (-0.1230 eV)- (-0.1083 eV)= -14.7 ±1,1 meV. This is the maximal effect of the product for structuring of hydrogen bonds among water molecules.

3.2. The mathematical models of CortiNon+

The mathematical models of CortiNon+ of Gr.1, Gr.3 and Gr.5 give valuable information for the possible number of hydrogen bonds as percent of H_2O molecules with different values of distribution of energies (Table 1 and Fig. 2). These distributions are basically connected with the restructuring of H_2O molecules having the same energies.

The average energy ($E_{H...O}$) of hydrogen H…O- bonds among H_2O molecules of the samples of blood serum of hamsters was measured for the following groups.

Gr. 1 – The result of NES for Gr.1 is E = - 0.1194 eV.

Gr. 2 – The result of NES for Gr. 2 is E = - 0.1168 eV.

Gr. 3 –The result of NES for Gr. 3 is E = - 0.1156 eV.

Gr. 4 – The result of NES for Gr. 4 is E = - 0.1177 eV.

Gr. 5 – The result of NES for Gr. 5 is E = - 0.1179 eV.

The Table 1 and Figure 2 show the mathematical Models of CortiNon+ for Gr. 1, Gr. 3 and Gr. 5.

Table 1. Mathematical Models of CortiNon+ for Gr. 1, Gr. 3 and Gr. 5

-E(eV) x-axis	1st group CortiNon+ 7 days before *Graffi* tumor (%((-Evalue) */ (-Etotal value)**	3d group *Graffi* tumor (%((-Evalue) */ (-Etotal value)**	5th group Healthy hamsters (%((-Evalue) */ (-Etotal value)**	-E(eV) x-axis	1st group CortiNon+ 7 days before *Graffi* tumor (%((-Evalue) */ (-Etotal value)**	3d group *Graffi* tumor (%((-Evalue) */ (-Etotal value)**	5th group Healthy hamsters (%((-Evalue) */ (-Etotal value)**
0.0937	0	0	0	0.1187	4.5	9.7	9.5
0.0962	0	0	0	0.1212	**13.6**	**6.1**	**0**
0.0987	0	5.1	4.7	0.1237	0	9.7	0
0.1012	9.1	5.1	4.7	0.1262	0	5.1	9.5
0.1037	9.1	9.7	9.5	0.1287	9.1	0	0
0.1062	9.1	9.7	19.4	0.1312	4.5	5.1	0
0.1087	0	5.1	4.7	0.1337	4.5	5.1	9.5
0.1112	**18.1**	**5.1**	**0**	0.1362	4.5	0	9.5
0.1137	0	9.7	0	0.1387	**13.9**	**0**	**9.5**
0.1162	0	9.7	9.5	–	–		–

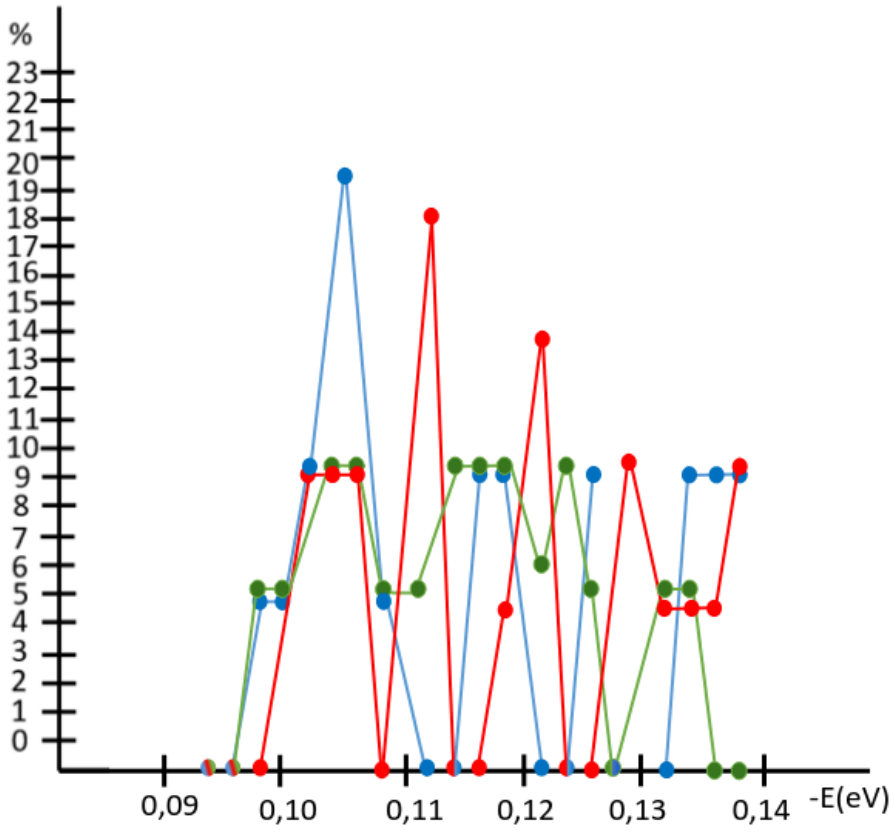

Fig. 2. Mathematical Models of CortiNon+ for Gr. 1, Gr. 3 and Gr. 5

Notes:

E = -0.1112 eV is the local extremum for stimulating effect on nervous system and improvement of nervous conductivity.

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.

* ($-E_{value}$) stands for the value of hydrogen bonds energy for one parameter of (-E).

** ($-E_{total\ value}$) stands for the total value of hydrogen bonds energy.

Figure 2 shows the distribution (%, ($-E_{value}$)/($-E_{total\ value}$) of H_2O molecules of CortiNon+ of Gr. 1, Gr. 3 and Gr. 5 respectively.

E = -0.1112 eV is the local extremum for stimulating effect on nervous system and improvement of nervous conductivity. The effect of CortiNon+ is 18.1 % for Gr.1, 5.1 % for Gr. 3 and 0 % for Gr. 5.

E = -0.1212 eV is the local extremum for anti inflammatory effect. The effect is 13.6 % for Gr. 1, 6.1 % for Gr. 3 and 0 % for Gr. 5.

E = -0.1387 eV is the local extremum for inhibition of development of tumor cells of molecular level. The effect is 13.9 % for Gr. 1, 0 % for Gr. 3 and 9.5 % for Gr. 5.

4. Conclusion

The influence of CortiNon+ on the development of *Graffi* tumor implanted in hamsters is assessed. The results show restructuring of water molecules in clusters with distribution that influences beneficially hamster (and human) health on molecular and cellular level.

The results from the application of NES and DENS spectrums and mathematical models confirm the effects of CortiNon+ on the nervous and endocrine systems, and anti-inflammatory and anti-tumor effects, as well. One interesting observation is that higher effect from the application of CortiNon+ on the tumor-bearing hamsters from Gr. 1 that have been treated 7 days before the injection is obtained relative to even the result of healthy hamsters from Gr. 4.

Product Name: CortiNon+

Inventor: Georgi D. Dinkov.

Trademark: Submitted to USPTO (USA) on December 10, 2017.

Product Distribution: IdeaLabs, LLC, 1200 18th Street NW #700, Washington, DC, USA.

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