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Methods for Research of Effects of Device ABIEM. Applications

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Abstract

This paper presents the results of evaluation of possible biophysical methods and approaches for registering of various non-ionizing radiation (NIR) wave types of the human body in the electromagnetic range after the influence of device ABIEM (Akszjonovics, 2006). Many types of NIR (electromagnetic waves, infrared radiation, thermo radiation, bioluminescence) emitted from the human body were reviewed. In particular the results on of spontaneous biophoton emission and delayed luminescence from the human body are submitted along with infrared thermography (IRT) results. It was shown that 1 cm^2 of skin generally emits ~85 photones for 1s. The intensity of biophoton emission ranges from 10^{-19} to 10^{-16} W/cm^2 (approx. ~1–1000 $photons \cdot cm^{-2} \cdot s^{-1}$). The specific photon emission from part of the human thumb was detected as a spectrum of various colors after influence of ABIEM with the method of Color coronal spectral analysis (Ignatov, 2007) on a device with an electrode made of polyethylene terephthalate (PET hostafan) with applied electric voltage 15 kV, electric impulse duration 10 μs, and electric current frequency 15 kHz. It was established that photons corresponding to a red color emission of visible electromagnetic spectrum have energy at 1.82 eV. The orange color of visible electromagnetic spectrum has energy at 2.05, yellow – 2.14, blue-green (cyan) – 2.43, blue – 2.64, and violet – 3.03 eV. The reliable result measurement norm was at $E \geq 2.53$ eV, while the spectral range of the emission was within 380–495 nm and 570–750 nm±5 nm. Also were estimated some important physical characteristics (energy of hydrogen bonds, wetting angle, surface tension) of water by the methods of non-equilibrium energy (NES) and differential non-equilibrium energy (DNES) spectrum of water (Antonov, 1990; Ignatov, 1998), that helps understand in general how electromagnetic radiation interacts with water and establish the structural characteristics of water.

Keywords: ABIEM, electromagnetic waves, infrared thermal radiation, color coronal spectral analysis, NES, DNES.

1. Introduction

All living organisms have a cellular therefore, a molecular organized structure. The living processes inside of them run on a cellular and a molecular level. Bioelectrical activity is one of the very important physical parameters of living organisms (Ignatov et al., 1998). Bioelectric potentials generated by various cells are widely used in medical diagnostics (Rubik, 2002) and are recorded

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as electrocardiogram, electromyogram, electroencephalogram, etc. It was proved that the human body and tissues emanate weak electromagnetic waves, the electric voltage of which is denoted as resting potential, action potential, omega-potential etc. (Dobrin et al., 1979; Adey, 1981). Between the outer surface of the cell membrane and the inner contents of the cell there is always the electric potential difference which is created because of different concentrations of K^+, Na^+ and Cl^- inside and outside of the cell and their different permeability through the cell membrane (Kiang et al., 2005). Their value in the human body varries ~50–80 mV and is defined by the galvanic contact of a voltmeter input with an object that indicates on the galvanic type of their source (Cleary, 1993). When being excited a living cell changes the membrane electric potential due to changes in membrane permeability and active ion movement through the membrane. In cells of excitable tissues (muscle, nervous), these processes can occur within a very short time intervals (milliseconds) and are called "current action" potential. Its magnitude makes up ~120 mV.

Electromagnetic fields refer to non-ionizing radiation (NIR), i.g. the radiative energy that, instead of producing charged ions when passing through matter, has sufficient energy only for excitation. Nevertheless it is known to cause biological effects (Kwan-Hoong, 2003). The NIR spectrum is divided into two main regions, optical radiations and electromagnetic fields. The optical spectrum can be further sub-divided into ultraviolet, visible, and infra-red. The electromagnetic fields are further divided into radiofrequency (microwave, very high frequency and low frequency radio wave). NIR encompass the long wavelength (> 100 nm) and low photon energy (<12.4 eV) portion of the electromagnetic spectrum, from 1 Hz to $3 \cdot 10^{15}$ Hz.

As a result of research carried out in the 1990-s and subsequent years, it was established the property of animal and plant tissues to generate relatively strong transient NIR electric fields due to mechanical stresses and temperature changes in biological structure (Anderson, 1993). These electric fields are mainly due to the piezoelectric and pyroelectric voltage electric polarization of natural biological structures. Owing to cell metabolism, electric dipoles (polar and ionized molecules) involved in polarization of biostructures are continuously destroyed and restored, i.e. this is a non-equilibrium polarization (Barnes, Greenebaum, 2006). Such type of non-equilibrium electric polarization is known as a main characteristic of electrets (Gubkin, 1978). Electrets include dielectric insulators and semiconductors, which under certain conditions, i.g. under the influence of a strong electrostatic field or ionizing radiation, light and other factors acquire property to generate an external electric field, existing for a long time (days, years) and slowly diminishes because the destruction of their substance by polarization (Sessler, Gerhard-Multhaupt, 1998). Along with the electromagnetic field electrets generate specific elecric currents produced by heating – thermally stimulated current (TSC) (Gross, 1964). Electrets belong to the non-galvanic type of electrical sources, which tend to a strong electric field (up to 10^6 V/m) and the infinitesimal electric current (~10^{-14} A/mm^2). By analogy with the physical fields the electric field emitted from the human body on its physical characteristics resembles the electric field generated by electrets. The electrets play an important role in functioning of many biological structures as they themselves possess electret properties. The bioelectret fild registered on the surface of the human body basically are generated by the basal cells of the epidermis (Marino, 1988). Dermis cells adjacent to the bottom layer of basal cells are surrounded by a conductive interstitial fluid, which electric voltage while grounding of the human body is close to zero (so called ground potential). This interstitial fluid screens off electromagnetic fields of underlying tissues. With the average thickness of the epidermis (~0.1 mm) and the maximum value of electric voltage (~30.0 V), the electric field strength can reach significant values at ~300000 V/m (Seto et al., 1992). The strength of the electric field is quiet sufficient for its influence on the biological processes in cells and surrounding tissues, including the synthesis of proteins and nucleic acids (Liboff at al., 1984; Frey, 1993; Shimizu et al., 1995). This electric field along with the field of transmembrane assymetry of ions concentrated at inside and outside of the membrane (~10^5 V/cm^2) can participate in the cooperative effects in cell membrane structures (Holzel, Lamprecht, 1994; Miller, 1986). Thus, owing to the bioelectret condition of certain subcellular structures in the cell and its surroundings is generated slowly oscillating electric field that is strong enough to influence the biological processes. This field and the electric field due to the piezoelectric voltage and intramembrane electric field formes the total electromagnetic field of the cell and its supracellular structures.

It is known that the human skin emanates electromagnetic waves in close ultraviolet range, optic range and also in close infrared range. Infrared thermal bioradiation is found in the middle

infrared range at wavelengths from 8 to 14 μm. At wavelength of 9.7 μm infrared bioradiation has its maximum value at t = 36.6 °C. At this temperature the skin emission is closest to the emission of absolute black body (ABB) being at the same temperature. Infrared emission penetrates the skin surface at a depth of ~0.1 mm, and is reflected in accordance with the physical laws of reflection of the visible part of the electromagnetic spectrum. Evidently, radiation energy influences tissues while being absorbed by them. Yu.V. Gulyaev and E.E. Godik (Gulyaev, Godik, 1984) determined that the threshold of skin sensitivity for infrared radiation compiled ~10^{-14} W/cm². When thermal influence is applied to the point of threshold skin sensitivity, there is developed a physiological reaction toward the thermal current. The intensity of the radiated thermal current generated by skin makes up ~$2.6 \cdot 10^{-2}$ W/cm².

The second component of electromagnetic waves is bioluminescence (Young, Roper, 1976; Chang et al., 1998). It is supposed that biophotons, or ultraweak photon emissions of biological objects, are weak electromagnetic waves in the optical range of the spectrum (Cohen & Popp, 1997). The typical observed emission of biological tissues in the visible and ultraviolet frequencies ranges from 10^{-19} to 10^{-16} W/cm² (~1–1000 photons·cm^{-2}·sec^{-1}) (Edwards et al., 1989; Choi et al., 2002). This light intensity is much weaker than that one to be seen in the perceptually visible and well-studied spectrum of normal bioluminescence detectable above the background of thermal radiation emitted by tissues at their normal temperature (Niggli, 1993).

Bioelectric emission from parts of the human body as thumbs can be easily detected with the method of Color coronal spectral analysis under applying gas electrical discharge of high voltage and friquency developed by I. Ignatov (Ignatov, 2005). This method has big scientific and practical prospects in biophysics and medical diagnostics (Chiang et al., 2005). Its advantages include safety, sterility, clarity and interpretability of the data obtained, ease of storage and subsequent computer data processing, the ability to monitor the development of processes in time, comparing the structural, functional and temporal processes etc.

The purpose of this research was studying of influence of device ABIEM on human biophysical fields (Figure 1). The research was performed with biophysical methods and approaches for registering various NIR wave's types emitted from the human body (electromagnetic waves, infrared radiation, thermo radiation) and methods of their visualization by different technique including magnetography, infrared thermography and color coronal gas discharge spectral analysis.

Fig. 1. Device ABIEM with author Igor Akszjonovics, 1999

2. Materials and methods

2.1. Infrared Thermography (IRT)

The research was made with using infrared thermographymethod according to V. Marinov. The range of the infrared thermal-imaging camera was in the middle infrared range from 9 μm to 14 μm. The temperature range was from 24.0 °C to 38.0 °C. The first camera was of

Inframetrics/FLIR ThermaCam PM 290 wave type. FLIR ThermaCam PM 290, FLIR 390, Inframetrics PM 250 and Inframetrics PM 350 thermal infrared cameras were of FLIR short wave type, handheld, Focal Plane Array cameras that are capable of temperature measurement. These cameras stored images on a PCMCIA Card, and the images were further analyzed using one of several available FLIR software packages (Thermogram 95, FLIR Reporter 2000 Software, Researcher 2000). The second camera (D.I.T.I.) was a totally non-invasive clinical imaging camera for detecting and monitoring a number of diseases and physical injuries, by revealing the thermal abnormalities present in the human body's patterns. It was used as a tool for diagnosis and prognosis, as well as monitoring therapy progress; the type of this device was TB 04 K.

2.2. Color coronal spectral analysis (Ignatov, 2007)

Experiments were caried out by using selective high-frequency electric discharge (SHFED) on a device with the electrode made of polyethylene terephthalate (PET, hostafan) with an electric voltage on the electrode 15 kV, electric impulse duration 10 μs, and electric current frequency 15 kHz. The electrode of the device was made of hostafan, and was filled up with electro-conductive fluid. The spectral range of the emission was in the range 380–495 nm and 570–750±5 nm. The measurements were measured in electronvolts (eV). Detection of gas disharge glowing was conducted in a dark room equipped with a red filter. On the electrode put a photosensitive paper or color film. The object under study (human thumb) was placed on top of a sheet of photo paper or color film. Between the object and the electrode were generated impulses of the electric voltage 15 kV and electric current frequency – 15–24 kHz; on the reverse side of the electrode was applied the transparent electrically conductive thin copper coating. Under these conditions in the thin contact gas space between the studied object and electrode was generated gas electric discharge in the form of characteristic glow around the object – a corona gas electric discharge in the range of 280–760 nm, illuminates a color photo or a photographic film on which was judged about the bioelectric properties of the studied object. Along with the visible range, for this method were obtained color spectra in UV and IR range. Evaluation of the characteristic parameters of snapshots was based on the analysis of images treated by standard software package. Statistical processing of the experimental data was performed using the statistical package STATISTISA 6 using Student's *t*-criterion (at $p < 0.05$).

2.3. NES and DNES experiments on interaction of electromagnetic field with water (Antonov, 1990; Ignatov, 1998)

The research was made with the method of non-equilibrium spectrum (NES) and differential non-equilibrium spectrum (DNES). The device measures the angle of evaporation of water drops from 72 ° to 0 °. As the main estimation criterion was used the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in water's samples. The spectrum of water was measured in the range of energy of hydrogen bonds 0.08–0.1387 eV or 8.9–13.8 μm with using a specially designed computer program.

3. Results and discussion

3.1. Electric fields

The electric field surrounding the human body with frequency $\nu = 1 \cdot 10^3$ Hz is created by electrochemical processes in the organism and is modulated by the rhythm of internal organs (Ignatov et al., 1998). The spatial distribution of the electric field around the body reflects the teamwork of the different organs and systems in the organism. There are also electric fields, which are generated by accumulation of triboelectric (caused by friction) charge on the epidermis, which depends on epidermal electric resistance and varies from 10^9 to 10^{11} Ω/cm^2. Radiothermal emission is being detected in the centimetre and decimetre range of the spectrum. This type of emission is connected with the temperature and the biorhythms of the internal organs, and is being absorbed by surface layer of skin at depth from 5 cm to 10 cm (Gulyaev, Godik, 1984). Long persistent electric field nearby the human body can be detected with using an electrometer voltmeter after neutralizing electric charges on the skin caused by triboelectric charges. The electric strength of this field is undergoing slow oscillations, and most patients exert its value within the range of 100–1000 V/m at a distance of 5–10 cm from the body. People in a state of clinical death usually have the electric field strength's value reduced to 10–20 V/m after 2–3 hours of cardiac arrest. Intensity vector of the detected electric field is found to be normal to the surface of the skin, and the electric voltage is inversely proportional to the distance. On the skin surface the electric voltage of the field

(the difference of its electric potential with respect to ground potential) reaches essential values of ~10000 mV or more, i.e., is about 1000 times greater than the source electric voltage of the electric unit above the bioelectric potentials. This allows us to characterize the electric field detected nearby the human body as relatively strong electric field emitted from living tissues. Its electric voltage was measured by electrometric methods, indicating on non-galvanic type of its source. If the physical basis of the generation of a relatively strong electric field in the human tissue is non-equilibrium electric polarization of the substance due to metabolic processes, the electric field strength should depend on these processes. As noted above, this dependence is actually observed: inhibition of tissue metabolism due to hypoxia during cardiac arrest was accompanied by drop in the electric field strength. This relationship is confirmed in experiments on animals (Geraldy et al., 2008). For example, in rats inhibition of metabolism of the tissue due to cardiac arrest (death of the animal) or by general anesthesia is accompanied by a significant drop in the electric field strength (Bars, Andre, 1976).

Electric fields depend on the magnitude of the electric voltage and the distance from the source (Kwan-Hoong, 2003). Generally, the electric voltages are stable and remain the same; however electric fields are easily perturbed and distorted by many surounding objects.

Relatively strong electric field investigated in humans and animals is being formed evidently by skin's biostructures, since the electric fields of the underlying tissues are largely shielded by conductive interstitial fluid (Goodman et al., 1995; Gulyaev, Godik, 1990). The greatest contribution to the detected electric field makes the basal cells of the epidermis – the top layer of the skin. Electric polarization vector of these cells is normal to the surface of the skin, i.e., coincides with the electric voltage's vector field, and yet it is inherent in the metabolism intensity, conditioning the generation of the electric field.

3.2. Magnetic fields

Magnetic field of a living organism can be caused by three following reasons. First of all, it is ion chanels arising from electrical activity of cell membranes (primarily muscle and nervous cells). Another source of magnetic fields are tiny ferromagnetic particles, trapped or specially introduced into the human body. These two sources create their own magnetic fields. In addition, at imposition of external magnetic field there appears inhomogeneity of the magnetic susceptibility of different organs and tissues distorting the external magnetic field (Wikswo, Barach, 1980). The magnetic field in the last two cases is not accompanied by the appearance of the electric field, so the study of the behavior of magnetic particles in the human body and the magnetic properties of various organs are applicable only with using of magnetometric methods. Biocurrents on the contrary except for the magnetic fields create the distribution of electric potentials on a body's surface. Registration of these electric potentials has long been used in research and clinical diagnostics – in electrocardiography, electroencephalography etc (Cohen, 1968). It would seem that their magnetic counterparts, i.e. magnetocardiography and magnetoencephalography recording the signals from the same electrical processes in the body, will give almost the same information about the studied organs. However, as follows from the theory of electromagnetism, the structure of the electric current source in the electric conductive medium (the body) and the heterogeneity of the medium have significantly different impact on the distribution of magnetic and electric fields: some types of bioelectric activity manifest themselves primarily in the electric field, giving a weak magnetic signal, while the others – on the contrary create rather strong magnetic signal (Zhadin, 2001; Anosov, Trukhan, 2003). Therefore, there are many biophysical processes which observation is preferable by using of magnetographic methods.

Magnetography does not require the direct contact with the investigated object, i.e., it allows to carry out measurements over a bandage or other obstructions. It is not only practically useful for diagnostics, but is fundamental advantage over electrical methods towards data recording, as the attachment of the electrodes on the skin can be a source of slowly varying contact electric potentials. There are no such spurious noises while using magnetographic methods, therefore, magnetography allows, in particular, reliably explore slowly occurring processes (with the characteristic time of tens of minutes).

Magnetic fields rapidly diminish with distance from the source of the activity, as they are caused by relatively strong currents running in the body, while the surface potentials are determined mainly by the weaker and "smeared" electric currents in the skin. Therefore, magnetography is more convenient for accurate determination (localization) of bioelectric activity

parts on the human body. And finally, the magnetic field vector is characterized as not only by the absolute value but also by the direction, which also may provide additional useful information. However, it should not be assumed that the electricity and magnetographic methods compete with each other. On the contrary, it is their combination that gives the most complete information about the processes being investigated. But for each of the individual methods, there are practical areas wherein the use of any one of them is preferable.

Water is the main substance of all living organisms and the magnetic field exerts a certain influence on water. This influence is a complex multivariate influence, which the magnetic field exerts on dissolved in water metal cations (Fe^{2+}, Fe^{3+}) and the structure of the hydrates and water associates (Mosin, 2011). Experimentally was proved that the magnetic field acts much weaker on still unmoved water, because water has a conductivity; as water moves in the electromagnetic field it is generated a small electric current (Mosin, 2012).

The research performed with superconductive detectors based on Joseffson junctions shows that magnetic fields around the human body are in the range from 1 to 100 Hz. The magnetic activity of the brain for example makes up ~$30 \cdot 10^{15}$ $T/Hz^{1/2}$. The magnetometric system has a sensitivity of $10 \cdot 10^{15}$ $T/Hz^{1/2}$ in the range of 1 to 100 Hz (Gulyaev, Godik, 1990).

3.3. Chemiluminescence

Chemiluminescence denotes luminescence accompanying chemical reactions, detected in the near infrared, the optical and the near UV-range of the electromagnetic spectrum. When chemiluminescence takes place in living organisms, the phenomenon is called bioluminescence. The term is generally used for higher luminance ATP-dependent luciferin/luciferase systems observed in *Lampyridae* lightning bugs (Rauhut, 1985). Although there bacterial, latia and dinoflagellate luciferin, and coelenterazine found in some bacteria, freshwater snails, dinoflagellates, radiolarians, shrimp, squid and deep-sea fish species (Hastings, 1983).

Chemiluminescence is observed in reactions accompanied by allocation of large amounts of energy, such as the reaction of combining of two radicals, or in reactions involving peroxides, e.g. peroxide oxidation of lipids (Halliwell, Gutteridge, 1989). Peculiar ("ultra-weak") glow of cells and tissues of animals and mammals is caused by free radical reactions: lipid radicals and oxygen, and nitrogen oxides – compounds that play an essential role in cell metabolism, and under certain conditions in the development of a number of pathological conditions.

It is suggested that the major radicals responsible for the light emission are excited triplet carbonyl and excited singlet oxygen, and that these radicals arise through the decomposition of hydroperoxides formed in the process of lipid oxidation (Zlatkevich, Kamal-Eldin, 2005; Porter, Wujek, 1988). The process of lipid peroxidation (LPO) is an important cause of the accumulation of cellular defects caused by radicals. LPO main substrate is polyunsaturated fatty acids in composition of cell membranes and lipoproteins. Their attack by oxygen radicals leads to the formation of hydrophobic radicals, interacting with each other (Vladimirov, 1996). Many different mechanisms have been suggested for the oxidative lipid fragmentation that produce biologically active aldehydes as 4-hydroxynon-2-enal (HNE), oxononanoyl phosphatidylcholine (ON-PC) from linoleic acid (LA) esters, or HNE and oxovaleroyl phosphatidylcholine (OV-PC) (Esterbauer et al., 1990).

Processes of life are almost always accompanied by a very weak radiation, which is sometimes called ultra-low illumination or radiation of cells and tissues (Boveris et al., 1980). Some organisms possess the ability to emit bright light at photon fluxes below about 10^4 $photons \cdot cm^{-2} \cdot s^{-1}$, visible to the naked eye, this phenomenon is denoted "bioluminescence". In biochemical systems, i.e. in tissue's homogenates, cell suspensions or cell organelles, mixtures of enzymes and substrates, chemiluminescence in most cases has an extremely low intensity, and requires particularly sensitive equipment for its detection and measurement (Popp et al., 2002). Some substances – enhancers, have the ability to essentially enhance the chemiluminescence, sometimes many thousands of times (activated, or enhanced chemiluminescence). In addition, weak luminescence is accompanied by the formation of free radicals under the action of a number of physical factors on the object: at ionizing radiation is observed radiochimoluminescence, at ultraviolet or visible light illumination – photochimoluminescence, at passing an electric current – electroluminescence, with ultrasound – sonoluminescence, under the influence of friction forces – triboluminescence.

Chemiluminescence differs from fluorescence in that the electronic excited state is derived from the product of a chemical reaction rather than the more typical way of creating electronic excited states, namely adsorbtion. It photomemical reactions, in which light is used to drive an endothermic chemical reaction, light is generated from a chemically exothermic reaction.

At present time it is known quite a lot of chemical reactions involving the formation of luminescence glow. In most cases they are generally quite a complex processes having many intermediate stages, but the basic processes leading to luminescence glowing in general are similar. They include the separation and transfer of charged particles (electrons and free radicals), the electron transfer (redox reactions) at one of the higher energy levels with the formation of the reaction product in an electron-excited state and further releasing of a photon in the transition of the molecule to the low excited electronic ground state with a lower energy level (luminescence). Theoretically, in this process on each molecule of the reactant should be allocated one photon.

Chemiluminescence accompanies many chemical reactions (ozonation and fluorination reactions, the oxidation of phosphorus and complex organic substances, lipids) and has an impulse mode; the signals of this process usually are very week. Thus, the human skin dissociates few photons per 1 sec. with emission power level ~10 mW/cm^2 (Gulyaev, Godik, 1991).

Luminescence of cells and tissues are accompanied by three types of reactions:

- Reactions with active oxygen;
- Chain reactions of lipid peroxidation;
- Reactions involving nitric oxide ($NO^{\cdot}$).

The more lipid radicals contains the system, e.g. the more energetically occurs the chain reaction of lipid oxidation, the higher the intensity of chemiluminescence accompanying the reaction of radicals. Substances reacting with free radicals and thereby inhibiting the chain lipid oxidation (so-called antioxidants) simultaneously inhibit chemiluminescence. That inhibition of chemiluminescence by cells and tissues by such antioxidants as tocopherol (vitamin E), indicating that chemiluminescence is stipulated by lipid oxidation chain reactions. On the other hand, studying the impact of various natural and synthetic compounds on the time (kinetics) of chemiluminescence, it can be judged on the ability of these substances to protect our body from the harmful effects of free radicals and thereby select candidates to certain medications.

Chemiluminescent methods are used for recording of ultra-weak light waves accopmanying from the chemical and biobiochemical reactions involving the formation of free radicals. They do not require special laboratory conditions and special material preparation for analysis and characterised by high sensitivity, reliability, meet the requirements for rapid methods of express research. Chemiluminescent methods are widely used in biomedical diagnostics for studying of the molecular basis of physiological processes in biological systems and general mechanisms of development of pathological conditions.

3.4. Color coronal spectral analysis

Coronal gas discharge effect is indicated by the glow corona electrical discharge (flooding, crown, streamer) on the surface of objects being placed in the alternating electric field of high frequency (10–150 kHz) and electric voltage (5–30 kV) (Kilrian, 1949). In this process in the ionization zone develops the gas corona discharge sliding on dielectric surface, occurring in a nonuniform electric field near the electrode with a small radius of curvature. In the thin air layer with thickness of ~10–100 μm between the studied object and the electrode are developed the following processes:

1) Excitation, polarization and ionization by electric field of high frequency the main components of air – the molecules of nitrogen (78 % N_2), oxygen (21 % O_2) and carbon dioxide (0.046 % CO_2). In the result of this is formed an ionized gas, i.e. gas with separated electrons having negative charges, creating a conductive medium as plasma;

2) Formation of a weak electric current in the form of free electrons separated from molecules of N_2, O_2 and CO_2, which generate gas discharge between the studied object and the electrode. The form of gas discharge glowing, its density and surface brightness distribution is determined mainly by electromagnetic properties of the object;

3) The transition of electrons from lower to higher energy levels and back again, during which there appears a discrete quantum of light radiation in the form of photon radiation. The transition energy of electrons depends on the external electric field and the electronic state of the studied object. Therefore, in different areas surrounding the electric field, the electrons receive

different energy impulses, i.e. “skipping” at different energy levels that results in emission of photons with different wavelengths (frequencies) and the energy, coloring the contour of the glow in various spectral colors.

Processes outlined above form the total gas electric effect (Ignatov, Mosin, 2012), allows to study the electrical properties of the object at its interaction with an external electromagnetic field (Ignatov & Mosin, 2013a; Ignatov & Mosin, 2013b). It was shown that the electrical conductivity of the object has almost no effect on the formation of the electric images, which mostly depends on the dielectric constant (Pehek et al., 1976).

There is a relationship (1) of the electric discharge per unit area of the recording medium on the following parameters:

$$\sigma = [\alpha - U_p(d_2+\delta)/d_2]\varepsilon_0(d_2+\delta)/\delta d_2, \quad (1)$$

where: $\delta = d_1/\varepsilon_1 + d_3/\varepsilon_3$

α – slope rate of electrical pulse;

T – duration of the electrical pulse;

U_p – breakdown voltage of the air layer between the sobject and the recording medium;

d_1 – the width of the object;

d_2 – width of the zone of influence of the electromagnetic field;

d_3 – width of the recording medium;

ε_0 – dielectric permittivity of the air (ε_0 = 1.00057 F/m);

ε_1 – dielectric permittivity of the studied object;

ε_3 – dielectric permittivity of the medium.

To calculate the breakdown voltage of the air layer is used this formula:

$$U_p = 312 + 6{,}2d_2 \quad (2)$$

As a result of mathematical transformations is obtained a quadratic equation describing the width of the air layer:

$$6{,}2d_2^2 - (\alpha T - 6{,}2\delta - 312)d_2 + 312\delta = 0 \quad (3)$$

This equation has two solutions:

$$d_2 = [\alpha T - 6{,}2\delta - 312] \pm [(\alpha T - 6{,}2\delta - 312)^2 - 7738\delta)^{1/2} \quad (4)$$

The above equations allow to calculate maximum and minimum width of the air layer for the occurrence of elecric discharge under which is being formed the electrical image of the studied object.

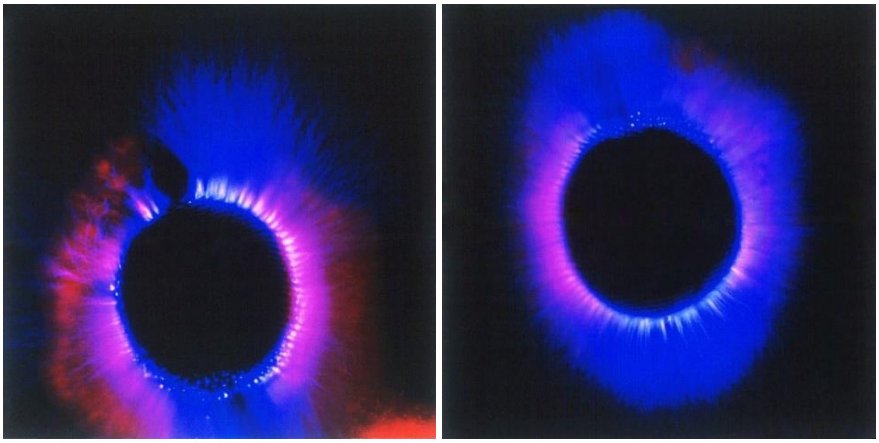
Gas discharge characteristics for various biological objects vary in character and light intensity, size of contour glow and color spectrum and depend both on its own electromagnetic radiation and the dielectric constant of the object. The intensity depends on the electric voltage applied on the electrode.

Studies have shown that the contours of gas discharge glow at 12 kHz and 15 kHz are homogeneous in their structure. The contour at kHz is 55 % of the contour at 15 kHz and at 24 kHz – only 15 % of the contour at 15 kHz that is important for further analysis and identification of images. The incidence of bioelectrical activity of the body reducing the intensity of gas discharge glow. Pathology in the organism and surrounding tissues also alter the bioelectric activity and the shape and color of gas discharge glow, which is determined mainly by energy of photon emission at the transition of electrons from higher energy levels to the lower ones when being excited by the external electric field. Thus, for red colour of the electromagnetic spectrum this energy compiles 1.82 eV, for orange color – 2.05 eV, yellow – 2.14 eV, blue-green (cyan) – 2.43 eV, blue – 2.64 eV, and violet – 3.03 eV. The reliable result norm is at E ≥ 2.53 eV. The spectral range of the photon emission for different colors is within 380-495 nm and 570–750 nm±5 nm. The photons, corresponding to the emission with green color in the visible electromagnetic spectrum, are not being detected under those experimental conditions. Thus, the more predominant in the color spectrum yellow, orange, blue, blue-green and purple colors, the more pronounced is gas discharge glow and bioelectric properties of the object. According to the data obtained, the incidence of bioelectrical activity of the body reducing the intensity of gas disharge glow.

Studies carried out by A. Antonov and I. Ignatov on 1120 patients shown that the overall drop in the bioelectric activity of the body, as well as pathology in organism alter the bioelectric activity and reduce the apparent size of the gas disharge glow. This dependence is observed for many disorders, although there are not statistical reliable results that this method can be applied in medical diagnostics. Figure 2 shows the results on bioelectrical discharge images of thumbs of

person before and after influence of ABIEM. The research area was from part of the thumb contacted with transparent electrode. The norm of energy of photon emission compiles 2.53 eV. If the value is over than 2.53 eV this is an indicator of normal bioelectrical status. Some people with high energy status possess the values of photon emission over 2.90 eV. The high values of this parameter are possible with practicing of yoga, sport etc. The emission less than 2.53 eV is characteristic for people with low bioelectrical status. These results are interesting from scientific point of view, because they may provide brilliant prospects for further using of this method for biophysical studies.

Through the Method for the color coronal spectral analysis (Ignatov, 2007) was proved statistically reliable results of influence of Akszjonovics device ABIEM on the patient (Figure 2). There was an increasing of the bioelectrical glow of the man. The first color coronal image has 30 % red, 15 % blue and 55 % violet color. A middle biophoton emission is 2,60 eV. After influence from the device there is 10 % red, 5 % blue-green, 20 % blue and 65 % violet color. A middle biophoton emission is 2,80 eV. The difference before and after influence of ABIEM was 2.80-2.60=0.20 eV. These results were shown that is possible achieving the medical effects on human organism on the base biophysical effects achieving through ABIEM.



a). Bioelectrical discharge image before treatment of ABIEM (2.60 eV) b). Bioelectrical discharge image after treatment of ABIEM (2.80 eV)

Fig. 2. Bioelectrical discharge images of thumbs of before (a) and after (b) treatment of ABIEM (I. Ignatov)

3.5. NES and DNES analysis of water

Water seems to be a good model system for studying the interaction with electromagnetic field and structural research. The recent data indicated that water is a complex associated non-equilibrium liquid consisting of associative groups (clusters) containing from 3 to 50 individual H_2O molecules (Keutsch, Saykally, 2011). These associates can be described as unstable groups (dimers, trimers, tetramers, pentamers, hexamers etc.) in which individual H_2O molecules are linked by van der Waals forces, dipole-dipole and other charge-transfer interactions, including hydrogen bonding (Ignatov, Mosin, 2013). At room temperature, the degree of association of H_2O molecules may vary from 2 to 21.

The measurements were performed with using NES and DNES methods. It was established experimentally that the process of evaporation of water drops, the wetting angle θ decreases discreetly to 0, and the diameter of water drop basis is only slightly altered, that is a new physical effect (Antonov, Yuskesselieva, 1983). Based on this effect, by means of 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. A theoretical research established the dependence between the surface tension of water and the energy of hydrogen bonds among individual H_2O-molecules (Antonov, 1995). The hydrogen bonding 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 structure of hydrogen bonding may be

defined as O···H$^{\square+}$–O$^{\square\square}$.

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:

$$f(E) = b \times f(\theta)/1 - (1 + b \times E)^{2})^{1/2}, \quad (4)$$

where $b = 14.33\ eV^{-1}$ (5)

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

$$\theta = \arccos(-1 - 14.33E) \quad (6)$$

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 energy of hydrogen bonds measured by NES is determined as $\bar{E}$ = -0,1067 ± 0,0011 eV.

The difference *Δf(E) = f (samples of water) – f (control sample of water)*

– is called the "differential non-equilibrium energy spectrum of water" (DNES).

Thus, DNES spectrum is an indicator of structural changes of water as a result of various external factors. The cumulative effect of these factors is not the same for the control sample of water and the water sample being under the influence of this factor.

Figure 3 shows NES-spectrum of deionized water that was used as a model system for studying the interaction of electromagnetic field with water. On the X-axis are given three scales. The energies of hydrogen bonds among H_2O molecules are calculated in eV. On the Y-axis is shown the energy distribution function f(E) of H_2O molecules measured in eV^{-1}. It was shown that the window of transparency of the earth atmosphere for the electromagnetic radiation in the middle IR-range almost covers NES-spectrum of water. Arrows A and B designate the energy of hydrogen bonds among H_2O molecules. Arrow C designates the energy at which the human body behaves itself as absolute black body (ABB) at optimum temperature 36.6 °C and adsorbs the thermal radiation. A horizontal arrow designates the window of transparency of the earth atmosphere for the electromagnetic radiation in the middle IR-range.

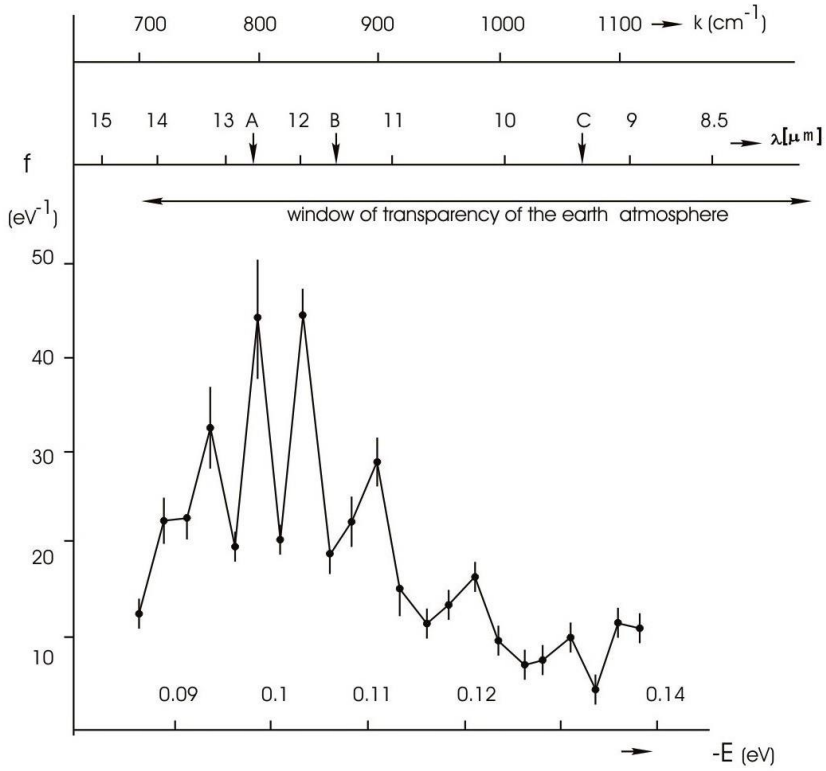


Fig. 3. Non-equilibrium energy spectrum (NES) of water as a result of measurement for 1 year: λ – wavelength, *k* – wave number

Another important physical parameter was calculated with using NES and DNES methods – the average energy ($\Delta E_{H...O}$) of H...O-bonds between H_2O compiled -0.1067±0.0011 eV. The most remarkable peculiarity of H...O-bond consists in its relatively low strength; it is 5–10 times weaker than chemical covalent bond. In respect of energy hydrogen bond has an intermediate position between covalent bonds and intermolecular van der Waals forces, based on dipole-dipole interactions, holding the neutral molecules together in gasses or liquefied or solidified gasses. Hydrogen bonding produces interatomic distances shorter than the sum of van der Waals radii, and usually involves a limited number of interaction partners. These characteristics become more substantial when acceptors bind H atoms from more electronegative donors. Hydrogen bonds hold H_2O molecules on 15 % closer than if water was a simple liquid with van der Waals interactions. The hydrogen bond energy compiles 5–10 kcal/mole, while the energy of covalent O–H-bonds in H_2O molecule – 109 kcal/mole. With fluctuations of water temperature the average energy of hydrogen H...O-bonds in H_2O molecule associates changes. That is why hydrogen bonds in liquid state are relatively weak and unstable: it is thought that they can easily form and disappear as the result of temperature fluctuations. The next conclusion that can be drawn from our research is that there is the distribution of energies among individual H2O molecules.

Further we performed two types of temperature-dependent experiments on heat exchange from the surface of the human body by DNES-method. In first experiment we studied heat exchange when the temperature of the human body was higher than the temperature of the surrounding environment (curve 1a and 1b on Figure 5). In second experiment there was heat exchange when the temperature of the human body was lower than that of the surrounding environment (curve 2a and 2b on Figure 5). In both experiments it was detected a local maximum at 9.7 μm on curve 1 and curve 2 (Figure 5). This local maximum corresponds to the maximal level of heat emission from the surface of the human body and lays within the "transparency window" of Earth atmosphere to electromagnetic radiation in the mid IR-range of the electromagnetic spectrum. In this range, the electromagnetic radiation emitted by the earth in the surrounding space is being absorbed by the Earth atmosphere. There is a statistical difference between the results of heat emission from the surface of the human body to the surrounding environment and back to the human body according to the *t*-criterion of Student at $p < 0.01$. The local maximum on curve 1a is detected at 7.3 eV^{-1}, while the local maximum on curve 2a – at 2.4 eV^{-1} (Figure 4).

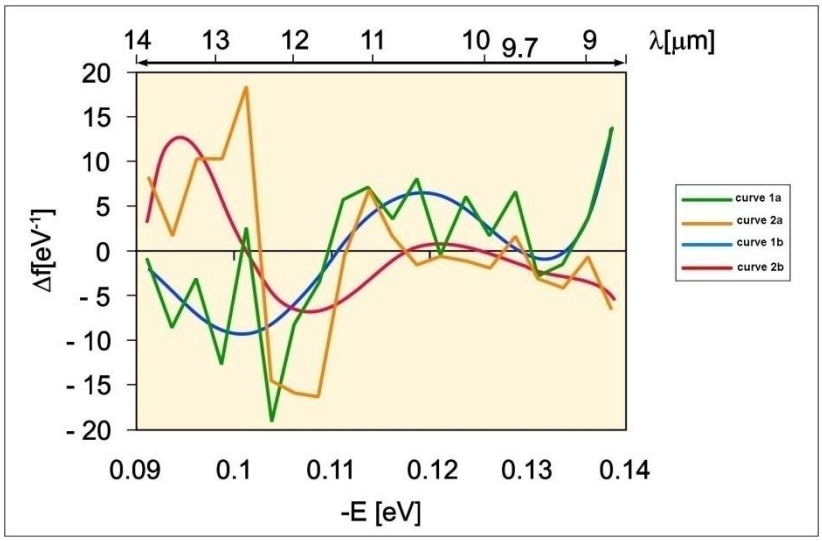


Fig. 4. Differential non-equilibrium energy spectrum (DNES) reflecting the heat exchange of the human body with surrounding environment

There was measured the effect of ABIEM on middle energy of hydrogen bonds among water molecules. The result was +3.7±1.1 eV meV. The effect is relaxation. The stimulating of nervous conductivity was connected with the biggest local extremum at (-0.11) eV. The results show screening effect of the fields from the environment on the man.

3.6. Infrared thermography

The human body as a biological body having a temperature in the range from 36.1 °C to 37.8 °C, therefore being preferably a source of infrared radiation that percieves by organism as thermoradiation. The main part of this radiation predominantly falls on human skin with a long wavelength range from 4 to 50 μm. Maximum of spectral density covers the range approx. ~10 μm i.e. the long wavelength IR range. Specifically the infrared radiation of the human skin can be distributed as follows: radiation with a wavelength of up to 5 μm covers 1 %, the radiation with a wavelength of 5 to 9 μm – 20 %, the radiation with a wavelength of 9 to 16 μm – 30 % and at a long wavelength – 41 %.

The physical essence of the thermal radiation consists in the presence of charged particles (electrons and ions), which are in random motion and have the properties of electrical or magnetic polarity. Infrared radiation is emitted or absorbed by excited atoms or ions when they change their rotational-vibrational movements. Electromagnetic waves propagate throughout the body and reach the surface, passing through the skin and partly emitted into the environment. The intensity of these processes is proportional to the body temperature. That is why any heated body having a temperature above absolute zero (273 °K) emits electromagnetic waves in a broad frequency spectrum. Because particle motion is random, they generate different wavelengths. The wavelength of infrared radiation emitted by the body depends on the heating temperature: the higher the temperature, the shorter is the wavelength and therefore the higher the emission intensity

Studies have shown that in the long wavelength infrared region (8–14 μm) the human skin radiates as a black body, regardless of age, degree of pigmentation and other features. Therefore, the emissivity of the human skin can be considered equal to 1 absolute unit. In practice, it is proved that the difference between the emission characteristics of the human skin and blackbody still exist, however, it is small and depends essentially on the influence of the surrounding background.

The limit of effective temperature measurement is equal to the thickness of the emitting layer (skin layer) and is defined as the distance at which electromagnetic waves propagate from the object's surface before the layer in which the intensity decreases in ~2.5 times. Under equal conditions the greater is the wavelength, the greater is the depth, which can detect the temperature perturbations. The maximum intensity of thermal radiation at normal ambient temperature is located in infrared rage of the spectrum (wavelength ~10 μm at t = 36.6 °C). The threshold of skin sensitivity according to Yu.V. Gulyaev and E.E. Godik compiles ~10^{-14} W/cm² (Gulyaev, Godik, 1990). This led to the feasibility of establishing IR thermal imaging (thermography) for the study of the temperature anomalies. However, the measurement of the thermal radiation of the human body in the IR range gives the true temperature for only the top layer of skin with thickness of ~1 mm; after that the thermal radiation is reflected back into the environment. The temperature of the underlying tissues and organs can be judged indirectly when the temperature changes are "projected" on the skin.

Infrared thermography is a scientific method for registering the thermogram – infrared image showing the distribution pattern of infrared waves emitted from the objects (Ring, Hughes, 1986). Thermographic cameras detect radiation in the infrared range of the electromagnetic spectrum (approx. ~0.9–14 μm), and on its basis are obtained thermographic images (thermograms) allowing to determine the locations of patterns having different temperatures. Thermograms therefore are actually visual displays of the amount of infrared energy emitted, transmitted, and reflected from the surface of the object. Since infrared radiation is emitted by all objects with the temperature according to Planck's formula for black body radiation, thermography allows to "see" the environment with or without visible illumination. The intensity of the thermal radiation of the body increases with the temperature, therefore thermography allows to see the temperature distribution on the surface of the body. As a result warm objects are seen better on the cooler environment background; mammals and warm-blooded animals are better visible on the environment. That is why the thermography may find many diagnostic applications and is often being used for breast diagnostics, tumour detection etc.

Most thermographic cameras use CCD and CMOS image sensors having most of their spectral sensitivity in the visible light wavelength range. The most commonly used is a matrix of indium antimonide(InSb), gallium arsenide (GaAs), mercury telluride (HgTe), indium (In) and cadmium (Cd). The latest technology allows to use the inexpensive uncooled microbolometer

sensors. Their resolution is varried from 160×120 or 320×240 up to 768×1024 pixels in the most advanced camera's models. Often the thermogram reveals temperature variations so clearly that a photograph is not necessary for further analysis. Usually a block of the focal planes of thermo imager can detect radiation in the medium (3 to 5 μm) and long (8 to 15 μm) infrared wave band, designated as MWIR and LWIR corresponding to two infrared windows with high coeficient of transmittance. Improperly selected temperature range on the surface of the objects, indicates a potential problem.

It should be noted that the intensity of the thermal radiation of the human body in the microwave (MW) range is much more smaller on magnitude than in the infrared part of the spectrum (Sisodia, 2007). In particular at a wavelength of 17 cm the intensity is less in ~10 times, so the heat reception signals in this range of the spectrum require equipment with higher sensitivity. However, the advantage of this method is that the measurement range and the depth of radiation penetration is much greater, therefore it is possible to obtain data on the temperature parameters of the internal organs and structures of the human body, but the resolution is significantly reduced, therefore it is impossible to obtain reliable thermal image of the study area. Infrared thermography registered a thermal infrared radiation emitted by the capillary network of the skin, which is used in medicine for thermovisual diagnostics. The closer an ailing organ is to the skin, the more accurate the diagnosis based on a thermal signal.

Today it seems to be an established fact that some biotherapists possess the ability to increase the temperature of the treated area of the human body. In this connection there should be noted two important empirical thermography results obtained by V. Marinov, which allow carry out the medical diagnostics of various human organs and monitor their condition and malfunction by this method. In the diagnostic of the patient was clinically healthy and the first image was shown the results before treatment with ABIEM and second after treatment of ABIEM (Figure 5). The effected DITI thermovisual diagnostics confirms this.

Fig. 5. Thermovisual results before treatment with ABIEM on person and second after treatment of ABIEM

Experiment was performed for the influence of ABIEM device Igor Akszjonovics, A predominately facial hyperthermic reaction was ascertained with the following values:

-buccal (oral cavity zone) – from minus 1.55 to minus 1.05

-nasal (nasal cavity zone)- from minus 3.95 to minus 2.05

-cervical – from plus 1.35 to plus 2.50

The thermovisual diagnostics device has a reference interval outside the scope from -0.5 to 0.5. The result was medically reliable outside the scope from 2.0 to -2.0. The hypothermic reaction to spine level Th3-5 where an initial scoliosis marking is observed is interesting. A hypothermic reaction was read from plus 0.85 to plus 0.50. It was recommended to make this test before and after ABIEM application to patients with bone-joint pathology who have demonstrated a good clinical influence.

4. Conclusion

In frames of this research many types of NIR radiation (electromagnetic waves, infrared radiation, thermo radiation, bioluminescence) emitted from the human body were studied and carefully scrutinized. The approaches and methods for detecting various types of radiation employed in this research for biophysical proofs for influence of ABIEM device with author Igor Akszjonovics. There were results with influence on spectrum of water, color coronal glow and infrared thermal emission. The effects of ABIEM were relaxing, bio harmonization, improvement of nervous conductivity, energizing effect on whole human body.

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The Roots and Mechanisms of Oncology

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Abstract

The theory of somatic mutations is incorrect, and the concept of "mutation" is incorrectly used. Etiological classification of tumors: 1) Tumors of malignant proliferation. Tumor cells are not specific. Tumor growth of malignant proliferation is provided by the reproduction of normal cells. The trigger mechanism of division is the interrelated actions of all the constituent parts of the tissue. The tumor mass increases due to the division of normal cells during its stimulation and dysfunction of its inhibitors and due to the predominance of cell proliferation over apoptosis. Calcium contributes to the formation of an independent structure. A tumor is a hierarchical system of its tissue and distant parts (metastases) that carry out the mutual influence. 2) Transgenic (infectious) tumors – a hybrid of a somatic cell and a microbe. They may be contagious. 3) Gestational tumors. 4) Tumors of genetic aberrations.

Keywords: classification of tumors, malignant proliferation, transgenic tumors, gestational tumors.

1. Introduction

A large number of theories have been put forward to explain the etiology of tumors (Ermolenko, 2012). Now two ideas are being discussed in science. At the beginning of the 20th century, (Boveri, 1914) put forward the idea of DNA mutations, trying to explain carcinogenesis. The idea turned out to be a viable organism. The real criticism pointing to its flaws was not paid attention, at the same time it explained many properties of carcinogenesis and won the trust of many researchers. At present, the idea is called the theory of somatic mutations (SMT) and occupies a leading position in world science. In the past 20 years, its position has become precarious due to the fact that this theory is confronted with a low level of therapeutic results (Godlee, 2016) and a discrepancy between scientific data and theory (Brinster, 1974; Mintz, Illmensee, 1975). In parallel with SMT, less intensively, the tissue theory of carcinogenesis was developed, in which it was suggested that carcinogenesis occurs not at the cellular but at the tissue level of biological organization. This approach is clearly consistent with extracellular theory, according to which a cell is completely dependent on the extracellular matrix (Ermolenko, Perepada, 2007).

The tissue theory of carcinogenesis was first proposed by C.H.Waddington (Waddington, 1935). N. N. Petrov (Petrov, 1958) found SMT errors, other researchers (Hodges, 1977) supported the idea of Waddington. In the future, this idea continued to develop (Cherezov, 1997; Sonnenschein, Soto, 1999). Recently, this theory has gained wide support from researchers (Ermolenko, 2012; Sarode et al., 2016). However, the supporters of SMT use it as a tool to explain many of the phenomena of cancer and do not plan to give up their beliefs. They propose to consider these two theories, explaining the mechanisms of carcinogenesis, as independent and compatible.

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Such an approach is consistent with integrative views, and it is considered that they have a higher value than the two theories taken separately. In my opinion, in order to defeat oncology, the Rubicon must be crossed — it is necessary to abandon SMT once and for all.

I intend to identify misses of the main provisions of modern oncology and give a new interpretation of the nature of tumors, their classification, stages and mechanisms.

2. Discussion

Classification of tumors

Oncologists use the generally accepted informative international classification indicating the exact nomenclature. However, today there is no etiological scientifically based classification of oncological diseases, so I created an etiological classification of tumors:

Root №1. Tumors of malignant proliferation.

Tumor cells do not exist in reality. The cells in the tumor are materially identical to other normal cells (Petrov, 1958). The tumor mass increases due to the division of normal cells during its stimulation and dysfunction of its inhibitors and due to the predominance of cell proliferation over apoptosis (Repina, 2004). A special place in this type of oncology is occupied by the tumors of hormone-producing organs, whose growth occurs due to the violation of the interaction between stimulating organs and target tissues.

Root №2. Transgenic (infectious) tumors are a hybrid of a somatic cell and a microbe.

There is an impressive amount of scientific publications on horizontal gene transfer (Syvanen, 1994). These data suggest that it is possible to combine the genetic material of micro- and macroorganisms. The virogenetic hypothesis suggests that viruses transform a cell into a tumor cell. Some researchers suggest that the hybrid is identical to other tumors. I am convinced that the newly formed separate organism (hybrid) has new properties. Its life is determined by a number of factors: the influence of the surrounding microorganisms; the functioning of the immune system of the macroorganism; absence of division inhibitors; the state of the extracellular matrix of tissue; carcinogens disorganize the tissue, thus may contribute to the formation of a hybrid.

Root №3 Gestational tumors include tumors of dysembryogenesis, as well as germ cell tumors, trophoblastic and coelomic epithelium tumors.. Enchondromatosis is a hereditary disorder of bone enchondral development. With such a disorder, chondrosarcomas often develop. The remains of the fetal chord are the source material of the chordoma. The parasitic twin (ischiopagus) shows itself either as separate organs or as highly differentiated tissues (for example, hair, muscle, bone tissue, a detached eye with the unusual localization for them; a germ in the intestine or in the lung). Oncologists call this phenomenon teratoma. These formations have similarities – well-differentiated tissues, but the origin of parasitic twins can be different: disturbances in the development of the embryoblast or the formation is due to the disembryogenesis of primary germ cells. There is the assumption unproved by the science that identical twins develop from the first two blastomeres. It is more logical to assume that they develop from the inner cell mass. My judgment is confirmed by the fact that the armadillo (*Dasypodidae*) naturally produces four identical twins due to the division of the embryoblast (Carlson, 1983). With its proper division, normal twins develop, and with incomplete separation – Siamese twins are formed, facultative tissues and organs – the ishiopag (ischiopagus). With minor failures, hamartomas are formed. These neoplasms are considered to be tumors. Some variants of the syndrome of MEN are believed to be the result of neural crest disambiogenesis, the cells of which are capable of migration and intensive diffusion (Pinsky, Beloborodov, 1999).

Germ cell tumors are caused by abnormal development, as well as by the associated with it disruption of primordial germ cells migration during ontogenesis. At the same time localization affects their morphology. Initially in multicellular organisms the germ cells were located in an organ which during phylogenesis is transformed into the primary intestine. Later becomes natural the migration of the primary germ cells from the outgrowth of the intestine, called the yolk sac (saccus vitellinus) to the place of permanent residence – in the gonads (Carlson, 1983). The symmetrical organ for the gonads is the lungs (Ermolenko, Perepada, 2007). In the process of embryo development, the primary germ cells sometimes migrate to phylogenetically related places — the intestines and lungs. Due to the impaired induction, the development of the primary germ cells is distorted, they slow down in the development. They can migrate to the mediastinum, brain and other parts of the body (Willis, 1962).

It is known that these tumors, regardless of their nature, are characterized by the presence of the 12p[i(12p)] isochromosome without a long arm, and with a short arm duplication (Schmoll, 2002). The NANOG and OCT 3/4 genes reflect polypotency and serve as accurate indicators of the malignancy of germinative cells (Medvedev, 2008). Consequently, there are genes that determine the development of germ cell tumors.

Distorted cells of the trophoblast and coelomic epithelium can be further expressed as tumors (Karseladze, 2000).

"Branchiogenic tumors", interdigital membranes, additional fingers of the hands are the result of a deviation in embryogenesis. Such deformities are difficult to attribute to oncology.

Root №4. Tumors of genetic abnormalities.

In scientific publications about 1000 types of gene abnormalities are described which are the cause of many hereditary biochemical disorders (Vasiliev, 2006). There is documented evidence that defects in DNA recovery genes can lead to hereditary syndromes of premature aging, oncology of many organs (Lombard. et al., 2005).

The chromosomal abnormality found in myeloblastic leukemia is caused by the “Philadelphia chromosome”. It is known that aberrations in the gene CHEK2 (Li-Fraumeni syndrome) increase the likelihood of developing oncology of the mammary, prostate, colon (Cybulski et al., 2011). REQL4 gene aberrations are shown by bone tumors, as well as Rothmund – Thomson syndrome. Due to the dysfunction of the RB1 gene, a congenital form of retinoblastoma develops. Due to the breakdown of EXT genes with tumor suppressor function, chondrosarcomas can develop (Hecht et al., 1997). Hereditary multiple endocrine neoplasia (MEN) is also known. Some variants of the syndrome of MEN are believed to be genetically determined (Caldas, Ponder, 1997). I did not set myself the goal of reviewing scientific reports on the relationship of chromosomal changes and hereditary human cancers. More detailed scientific publications on this topic can be found in broad reviews, in particular from E.N. Imianitov and R.P. Hanson (Imianitov, Hanson, 2004).

The staging of malignant fission tumors.

Tumor development is usually divided into two stages – initiation and promotion. In the initiation stage, there are several substages. In the second stage (promotion) the final substage – progression – is identified. The proposed division of carcinogenesis is derived from clinical observations. This interpretation does not explain the processes occurring in the structure of the tissue, and does not reflect the essence of the phenomenon. I also believe that the formation of a tumor has two stages, but they do not have additional gradations. The processes occurring in the structure of tissue are radically different from each other. Initially, there are hidden conditions that contribute to the occurrence of a tumor. This stage ends with the formation of a tumor germ. The promotion stage is characterized by the expansion of the tumor, the uniformity of events, the progressiveness of the process acceleration, therefore, it is clinically shown in different ways.

Mechanisms of development of malignant fission tumors.

There are several mechanisms for the formation of malignant tumors.

I. One of them is due to the disorganization of the tissue, which is explained by the following factors:

a) As a result of slowing down the excretion of products of cellular metabolism, changes in tissue homeostasis occur, and then tissue dysfunction. Slowing the export of cell metabolism products increases the size of cells and the intercellular space, leading to compaction of cells, interfering with the functioning of the tissue. Under stress, autophagy and sisterhood levels decrease and TOR (Target of Rapamycin) production increases. An increase in the total number of aging cells reduces the proportion of stem cells, thereby stimulating division, leading to the onset of carcinogenesis. In conditions when dividing cells interact with healthy cells and extracellular structures, the process develops slowly. When the stimulation of cell division coincides with the decrease of the function of factors inhibiting the proliferation, the expression of carcinogenesis begins.

b) The aging of the organism is due to the genetically programmed process of cell life. As a result, the quantity and quality of proteins is disturbed, the structure of collagen of the connective tissue is distorted. The local influence on the tissue of radiation, toxic chemicals, aggressive microorganisms causes local aging.

c) The accumulation of substances peroxidation in the body deteriorates the properties of membranes, which leads to disruption of the transport of ions, primarily protons and ionized

calcium. Active forms of oxygen damage not only the membranes, but also DNA, nucleotide coenzymes, radically change the cell metabolism and the entire tissue architecture.

d) Toxic substances penetrate the extracellular medium, causing pronounced distortions in the production of protein in cells, differentiation of connective tissue, and thus disrupt the structure of the tissue.

e) The immunopathological process is shown in the form of autoimmune inflammation due to the reaction of antibodies and T-cells with its own tissues, and as the final – damage and destruction of normal tissues. Autoimmune carcinogenesis develops due to necrosis of its own tissues. Immunopathological process that disrupts tissue organization is also expressed as an allergy, in which inflammatory humoral and cellular modulators enter the pathological zone, causing exudation, cell emigration, proliferation, increase of vascular wall permeability, increase of chemotaxis of microorganisms, and potentiate the development of edema in the site, increase emigration there leukocytes and microorganisms.

f) Diseases of the deposits in soft tissues (hyaline, amyloid, pigments, urates, purines, calcium, iron) change the structure of organs, disrupting their trophism and metabolism: the result of progressive amyloidosis is atrophy and necrosis; The abnormal exchange of stable complexes of nucleic acids with proteins leads to disintegration into the original constituents, even up to uric acid, which can accumulate in soft tissues and tendons and cause necrosis around which inflammatory infiltration develops with the presence of "giant multicore cells". If there is an impairment of phosphorus and calcium metabolism, calcium phosphate can be deposited in tissues with the formation of macrophage granulomas. The number of pigments can be increased by deposition in soft tissues in the form of nevi and jaundice. With all the variety of substrates that accumulate in organs, a common characteristic is their ability to change metabolism and trophism of tissues and influence the proliferation.

g) Medical statistics show that patients with diabetes mellitus are much more likely than the general population to suffer from oncology. At the same time, a higher risk of developing oncoprocess is only for some localizations – pancreatic and liver cancer is most often detected (Bowker et al., 2006). Ways of the formation of malignant tumors in diabetes are varied: tissue structure disorders; disruption of the insulin receptors and the associated stimulation of insulin secretion; pathological metabolism; genetic aberrations. In diabetes, dystrophic processes slowly develop, disrupting the organization of the pancreas and then other internal organs. In the pancreas there is an accumulation of amyloid until it completely replaces the islets of Langerhans which can be replaced by non-functional connective tissue. Diabetes mellitus is characterized by increased plasma osmolarity due to hyperglycemia, which causes dehydration of cells throughout the body and, as a result, hypoxia. Hypoxia is aggravated by microangiopathy of the microcirculatory bed, resulting from the addition of sorbitol and fructose and other metabolic products characteristic of diabetes mellitus. In the non-enzymatic reaction of glucose with proteins, foreign substances with antigenic properties are formed, causing autoimmune damage and tissue ischemia. The end result of such reactions is necrosis, which is a precursor of oncology. Glucose is the main energy substrate of life processes. When insulin deficiency or resistance to it, the flow of glucose into the cells decreases and its content in the blood increases. This stimulates the production of insulin and glucose. This is the way to stimulate the proliferation of interested organs.

The beginning of type 2 diabetes mellitus is often accompanied by chronic hyperinsulinemia, which is also associated with long-term insulin therapy which contributes to the development of tumors. With hyperinsulinemia, the tumor process is more severe, postoperative complications occur, survival decreases and cancer mortality increases (Bowker et al., 2006). Its trigger is the activation of the mTOR signaling pathway. As a result, inhibitors of apoptosis and factors of angiogenesis increase, cell growth and proliferation is accelerated, and a tumor is generally formed. Moreover, studies have shown that the risk of developing liver and pancreatic cancer associated with the stimulation of their work is the highest. Some researchers estimate pathological metabolism as an independent factor of increased cancer risk. Hyperestrogenia, characteristic of patients with an increased accumulation of adipose tissue, is a leading mechanism aimed at increasing the incidence of cancer (Cleary, Grossmann, 2009). The authors point to the effect of aberration of genes associated with insulin production and sensitivity to it on the risk of tumors.

h) Chronic trauma.

The significance of acute tissue damage for oncology has not been proven, but there is much evidence of the involvement of chronic trauma in the development of tumors. It is known that permanent thermal trauma causes oncology (kangri burncarcinoma) (Chaklin, 1963). Statistics show that cholelithiasis is accompanied by cancer of the gallbladder (Darawish, 2006), and a prolonged mechanical effect of stones on the gallbladder mucosa is assumed. There is also a similar connection between the occurrence of tumors in the oral mucosa in chronic trauma to it by its defective teeth or their substitutes (Bulyakov, 2005). It can be assumed that prolonged use of excessively hot food can have the same effect. It is believed that not only mechanical and thermal, but also ultraviolet, X-ray and radioactive radiation can cause oncology. This also applies to chronic chemical and infectious agents that cause inflammation (Podilchak, 1965). It should be noted that chemical and infectious effects, like chronic stimuli, are not associated with DNA changes. Trauma changes the structure of the tissue and the interaction of the epithelium and stroma. The result is tissue proliferation in response to the damage. R. Virchow attached great importance to chronic inflammation in carcinogenesis.

II. Cancer, which is based on the mechanisms of hormonal imbalance.

The function of hormone glands is balanced by stimulation and inhibition of hormone producing cells using negative feedback. The number of metabolic products of hormones in target cells regulates their synthesis, directly affecting the glands themselves, as well as through the hypothalamus. The production of tropic hormones is regulated by the hormonal level of the glands themselves. Distorted metabolism contributes to the formation of defective hormones, thereby destroying the feedback and the entire balancing mechanism. As a result, hormone-producing cells are stimulated. The mechanisms of interaction of glands and target tissues using feedback work in the regulation of the functions of the gonads, adrenal glands, thyroid gland.

III. Cancer formation, which is based on the mechanisms of interstitial imbalance.

Chronic infection creates conditions for prolonged stimulation of the immune system and leukopoiesis, and in combination with the non-functionality of the fission inhibitors can be a trigger for carcinogenesis of the lymphatic system. It can be assumed that some antibiotics and carcinogens can react with fission inhibitors, inactivating their function.

Available scientific publications on oncology do not give reasonable ideas about the nature of carcinogenesis. Scientists and oncologists practically do not use basic research in biology, physics, biochemistry — studies of the development of the organism and the life of the cell, induction and field effects, and information intercellular interactions. SMT does not provide a clear understanding of the term "mutation": deviations in the structure of DNA (genetic aberrations) and regular changes in DNA that ensure the functioning of cells are considered as mutations. The medical encyclopedia gives a specific definition: “mutation is a permanent change in the properties of a cell or organism that can be inherited by the descendants of a given cell or organism, mutations do not have a reverse development” (Great medical encyclopedia. Ed. Acad. B.V. Petrovsky, 1981). Due to mutations, the evolutionary process developed and new classes of animals were formed. The experiments of R.L. Brinster (Brinster, 1974) with enucleated mouse egg show the absence of mutations in oncology, the “tumor” characteristics are not transmitted to the daughter cells. These findings have been confirmed by other researchers (Mintz, Illmensee, 1975), (Li et al., 2003). I.N. Shvemberger (Shvemberger, 1987) showed that tumor cells are able to regress. And modern studies also claim that tumor processes are reversible (Telerman, Amson, 2009).

Chemical compounds in DNA are deterministic and complementary, but SMT claims that many chemicals can react with it, causing cell division. The cell cycle of division has been studied thoroughly, and there are no mechanisms for the influence on it of the "carcinogens" (Nurse, 2002). According to SMT (including the theory of somatic mosaicism), various carcinogens produce DNA mutations in a limited cell volume, they trigger spontaneous, irrepressible division of cells and their descendants.

It is known that the "carcinogens" in most cases are inert. However, according to the judgment of SMT supporters, after metabolic transformations, they are able to react with nucleic acids. However, xenobiotics are able to penetrate the cell and react with DNA (Biochemistry ed. by E.S. Severin, 2003), besides, as a result of random replication failures, nucleotide deamination disorders, depurination, DNA structure changes occur. These processes occur regularly, but since the multilevel system of DNA structure repair works, the process is normalized. Otherwise, the cell

may inadequately function or die, but the division cycle does not start in the damaged cell. SMT claims that countless chemical and physical factors trigger carcinogenesis, and on this basis it is concluded that it is poly-etiologic. Since, according to this theory, genetic mutations are the only way to form a tumor, this is the theory of mono-etiologic carcinogenesis. The SMT claims that radiation causes certain changes that cause the cell to divide. The idea of chemical carcinogenesis is logical, but unfounded, and radiation carcinogenesis is complete absurdity. X-rays and radiation have a random damaging effect on matter, and SMT ascribes to them creative properties – creation of a tumor. Practice shows that the incidence of oncology is correlated with the prevalence of the use of certain chemicals and radiation, which led to the conclusion that these factors cause cancer. However, it can be assumed that they are not the cause of cancer, but only participate in cancer development.

The clonal-selective idea of carcinogenesis states that random DNA changes in a single cell or in a limited number of them can transform them into a clone of cells with high genetic variability, and then, by natural selection, more and more autonomous and aggressive subclones survive and accumulate (Bachtin et al., 1987). Such a conclusion is not justified, since natural selection extracts only viable organisms and does not create new species (Vorontsov, 1984). It can be assumed that at the cellular level there are no mechanisms for creating specialized cells with separate characteristics. Infectious agents, being one of the mechanisms of carcinogenesis, are capable of disrupting the structure of the tissue (see paragraph “h” of this article). This type of oncology is usually not considered to be a transmitted disease. By its very nature, SMT does not suggest the possibility of transmitting cancer from one individual to another, however, there is a transmissible oncology: in dogs, the venereal sarcoma (the canine transmissible venereal tumour – CTVT); facial tumors (Devil facial tumor disease – DFTD) of Tasmanian devils (*Sarcophilus harrisii*); Syrian hamster tumors (*Mesocricetus auratus*), as well as disseminated neoplasia in marine bivalve mollusks (*Mya arenaria*). Infection occurs through the transmission of live "cancer cells"A.A. (Bostanci, 2005), according to our classification these are transgenic tumors.

The SMT paradigm is that intranuclear processes trigger carcinogenesis. It is known that the cell and its components do not live by themselves, but closely interact with the extracellular matrix, are susceptible to the induction effects of the stroma of the tissue, are in a system of certain intercellular mechanisms similar to the interaction in microbial populations (cell sensing systems (CSS) (Oleskin, 1963). Moreover, the initiation of carcinogenesis is considered to be a chain of spontaneous reactions. In fact, carcinogenesis is a natural process caused by the functioning of tissues. The chemical components determine the course of the reaction. The presence or absence of any substances leads to certain interrelations, reactions of components. The tumor tissue in its chemical composition is not different from normal tissues, but it is not normal, since its function is not coordinated with the vital activity of the surrounding tissues and the whole organism. The imbalance of substances, although characteristic of normal tissue, determines impaired function. SMT does not allow the probability of hierarchy of the tumor process, when induction and informational interaction of tissues can contribute to the separation of a part of the tissue into an independent structure. This theory does not explain the formation of independent growth centers after the elimination or disintegration of the central part of the tumor. Tumors can occur simultaneously and sequentially in different tissues under appropriate conditions.

Modern oncology theory suggests that the immune system is able to recognize and kill "tumor cells." In fact, natural immunity has no mechanisms for performing such a function, it is not able to distinguish tumor cells from other normal cells, to reveal “mutated” DNA. On the contrary, its negative role in carcinogenesis is proven. The autoimmune process causes fibrinoid necrosis due to the fact that autoantibodies bind to the components of tissues (Potekhin, Malyshev, 2000), and cell death enhances proliferation. In the compromised tissue, the chalons function inadequately, which also enhances the malignant proliferation. The role of immunity in oncology is very large – it is able to eliminate infectious tumors, and induced immunity makes a priority in the therapeutic process of oncology.

3. Conclusion

It is known from embryology experiments that the upper lip of the blastopore and then the chord determine the differentiation and division of the cells of the developing organism. From this fact, it is possible to make a reasonable conclusion that there is a non-chemical effect of tissues on the mitotic process. A.G. Gurwitsch (Gurwitsch, 1922) introduced the concept of a morphogenetic

field and mitogenetic radiation into the science. C.Sonnenschein (Sonnenschein, Soto, 1999) broadened the idea and suggests that disruption of the interaction of tissues can lead to mitotic cell activity and tumor development. However, the existence of tissue fields has only been proven indirectly, there is no information about their parameters, confirmed by devices, therefore, there is no evidence of the formation of a tumor due to morphogenetic fields. This theory does not take into account other (ultrasound, ultraviolet, electromagnetic) interactions. In modern ideas the leading importance is given to the nature of tumors and extracellular structures (De Clerck et al., 2017).

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Drug Interactions of Acetaminophen (Paracetamol) involving CYP and UGT Enzymes

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Abstract

Acetaminophen (Paracetamol) is the most frequently used Over-The-Counter (OTC) antipyretic and analgesic drug, worldwide. The metabolism of Acetaminophen is mediated by phase II reactions (UDP-glucuronosyl transferases (UGT)-mediated glucuronidation and sulfation) and phase I oxidative reactions mediated by Cytochrome P450 (CYP) enzymes including CYP2E1 and CYP1A2. The drugs inducing CYP2E1 and CYP1A2 enzymes and the drugs inhibiting UDP-glucuronosyl transferases (UGTs) may increase the exposure of NAPQI resulting in elevated risk of hepatotoxicity. The risk of acetaminophen-associated hepatotoxicity might be elevated due to concomitant use of certain medications including Isoniazid, Antiepileptic drugs, Tyrosine kinase inhibitors and Alcohol. Acetaminophen may increase the international normalized ratio (INR) by potentiating the anticoagulant effect of warfarin. To prevent possible adverse drug interactions, the prescribers and pharmacists must discuss with their patients about the use of OTC Acetaminophen.

Keywords: drug interactions, Acetaminophen, Paracetamol, CYP2E1, CYP1A2, UGT enzymes.

1. Introduction

Acetaminophen (Paracetamol) is an antipyretic and analgesic medication and it is the most frequently used Over-The-Counter (OTC) drug worldwide (Kontogiorgis et al., 2016). It is found in more than 200 OTC products used to treat pain, common cold and cough. The antipyretic effect of Acetaminophen occurs through the selective inhibition of cyclooxygenase (COXs) pathway in the brain as Acetaminophen crosses the blood brain barrier easily. Moreover, it has been proposed that Acetaminophen modulates endogenous cannabinoid system to produce analgesic effect (Ghanem et al., 2016).

Acetaminophen is metabolised majorly (>90 %) by phase II reactions (UDP-glucuronosyl transferases (UGT)-mediated glucuronidation and sulfation) to form non-toxic metabolites and a small fraction (5-10 %) is metabolised by Cytochrome P450 (CYP) enzymes including CYP2E1 and CYP1A2 to form toxic metabolite called N-acetyl-p-benzoquinoneimine (NAPQI) (Mazaleuskaya et al., 2015). The NAPQI formed by therapeutic doses of Acetaminophen, is detoxified by glutathione. However, intentional higher dose or non-intentional misuse may induce overdose or toxicity of paracetamol resulting in the formation of higher levels of NAPQI leading to hepatocellular injury (Caparrotta et al., 2017). The pediatric dose of 150 mg/kg of Acetaminophen could induce hepatic failure in children while a single dose of >7 gm of paracetamol needed to induce hepatocellular damage in adults (Hodgman et al., 2012).

Modification of effects of one drug (object drug) by the administration of another (precipitant) drug, supplement, tobacco smoke or alcohol is termed drug interaction (Maideen et

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al., 2018; Maideen, 2018) and the adverse drug interaction is defined as the drug interaction resulting in decreased therapeutic efficacy or increased rate of adverse effects (Pakkir Maideen NM, 2018). The risk of drug interactions is enhanced as the number of concurrent medications increases (Maideen et al., 2017). The addition of precipitant drugs alters the pharmacokinetic or pharmacodynamic profile of an object drug (Pakkir Maideen et al., 2018).

The drugs inducing CYP2E1 and CYP1A2 enzymes and the drugs inhibiting UDP-glucuronosyl transferases (UGTs) may increase the exposure of NAPQI resulting in elevated risk of hepatotoxicity.

2. Results

Isoniazid

Isoniazid (INH) is a first-line Antitubercular drug and it is an inducer of CYP2E1 enzyme (Hassan et al., 2018). Administration of Acetaminophen in patients taking INH resulted in enhanced risk of hepatotoxicity (Murphy et al., 1990; Moulding et al., 1991) which might be caused by CYP2E1-mediated metabolism of Acetaminophen leading to increased exposure to toxic acetaminophen metabolites (NAPQI). Caution is advised and administration of higher doses of Acetaminophen should be avoided in patients taking INH (Namdar et al., 2018).

Antiepileptic Drugs

The antiepileptic drugs such as Carbamazepine, phenytoin and phenobarbital induce many CYP enzymes (Zaccara et al., 2014). Acute liver failure occurred in a patient taking carbamazepine and Acetaminophen concomitantly and it is recommended to monitor the signs and symptoms of liver failure when chronic use of both drugs is necessary (Jickling et al., 2009).

Phenytoin and phenobarbital may also increase the risk of acetaminophen-associated hepatotoxicity through the inhibition of glucuronidation of acetaminophen via Uridin-glucuronyl transferases (UGTs) inhibition (Kostrubsky et al., 2015). The patients taking antiepileptic drugs such as carbamazepine, phenytoin or phenobarbital along with acetaminophen are advised to monitor for the signs and symptoms of hepatotoxicity.

Alcohol

Alcohol is a substrate of CYP2E1 enzyme (Cederbaum, 2012) and the chronic ingestion of alcohol increase the synthesis and activity of CYP2E1 enzyme along with decreased glutathione stores. Hence, the risk of acetaminophen-associated hepatotoxicity is enhanced in chronic alcoholics due to increased formation of NAPQI and decreased glutathjione levels (Yoon et al., 2016). The chronic alcoholics should be advised to consider dose reduction of acetaminophen from 4gm/day to 2gm/day (Lourenco, 2017).

Tyrosine kinase inhibitors

Tyrosine kinase inhibitors (TKIs) are anticancer agents useful to treat various malignancies and they include imatinib, gefitinib, erlotinib, sorafenib, sunitinib, and dasatinib (Hartmann et al., 2009). The TKIs such as imatinib, sorafenib and dasatinib may increase the risk of paracetamol hepatotoxicity through the inhibition of UGT (UGT1A9, UGT2B15 and UGT1A1)-mediated glucuronidation of Acetaminophen (Liu et al., 2011). Monitoring of liver function tests is advised in patients taking imatinib for longer period especially with concomitant use of Acetaminophen (Ridruejo et al., 2007).

Warfarin

Warfarin is an oral anticoagulant used effectively to prevent thromboembolic events in patients with atrial fibrillation, venous thromboembolism, and other conditions (Holbrook et al., 2005). Warfarin prevents the synthesis of hepatic synthesis of coagulation factors II, VII, IX, and X through the inhibition of vitamin K epoxide reductase (VKOR) which is responsible for the activation of vitamin K (Wu et al., 2018).

Concomitant use of warfarin and Acetaminophen may result in increased international normalized ratio (INR) (Olsen et al., 2018). It has been hypothesized that Acetaminophen may potentiate anticoagulant effect of warfarin by interfering the enzymes responsible for the synthesis of vitamin K-dependent coagulation factors (Mahé et al., 2006). The vitamin K cycle might be disrupted by the toxic metabolite of Acetaminophen (NAPQI) through the oxidation of vitamin K-hydroquinone, inhibition of vitamin K-dependent carboxylation and blockade of vitamin K-epoxide reductase (VKOR) activity (Lopes et al., 2011).

Monitoring of INR is recommended in patients taking warfarin and higher dose of Acetaminophen concomitantly for more than three consecutive days (Ornetti et al., 2005). Acetaminophen is the drug of choice as an analgesic in patients taking oral anticoagulants such as warfarin, acenocoumarol or phenprocoumon. Clinically relevant interaction may occur in patients taking more than 2gm/day of paracetamol with oral anticoagulants and monitoring of INR is recommended (Gschwind et al., 2013; Caldeira et al., 2015).

Food

Orally administered Acetaminophen is absorbed by passive diffusion into the bloodstream and hence the rate of gastric emptying determines the absorption of acetaminophen (Raffa et al., 2014). The rate of absorption of Acetaminophen might be decreased by the presence of food (DIVOLL et al., 1982; Bushra et al., 2011). It is recommended to administer oral Acetaminophen one hour before or two hours after food, to avoid such interaction (Ismail et al., 2009).

3. Conclusion

The drugs inducing CYP enzymes such as Isoniazid and antiepileptic drugs (Carbamazepine, Phenytoin and Phenobarbital) and the drugs inhibiting UDP-glucuronosyl transferases (UGTs) including Tyrosine kinase inhibitors (TKIs) (Imatinib, Sorafenib and Dasatinib) and antiepileptic drugs (Phenytoin and Phenobarbital) may increase the exposure of NAPQI resulting in elevated risk of hepatotoxicity. Acetaminophen may increase the international normalized ratio (INR) by potentiating the anticoagulant effect of warfarin. The rate of absorption of Acetaminophen might be decreased by the presence of food. To prevent possible adverse drug interactions, the prescribers and pharmacists must discuss with their patients about the use of OTC Acetaminophen.

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A Prospective Observational Study on Assessment of Adverse Drug Reactions in Patients taking First Line Antitubercular Drugs at Tertiary Care Teaching Hospital, Nellore

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Abstract

Tuberculosis (TB) is a mycobacterial infection, which remains a leading infectious killer globally. Patients' non-compliance, therapeutic failure and drug resistance may result due to the occurrence of adverse drug reactions of Antitubercular drugs. The aim of present study was to identify and analyse the adverse drug reactions caused by first-line antitubercular drugs in DOTS centre and tertiary care hospital in Nellore.

This was a prospective observational study carried out in the Department of Pulmonology at AC Subba Reddy Government Medical College, a tertiary care teaching hospital, Nellore, Andhra Pradesh over a period of six months. During the study period, all the patients receiving treatment with first-line Antitubercular drugs who met the study criteria were included and monitored for adverse drug reactions and subjected to causality assessment.

A total of 260 patients were followed during the study period, out of which 155 patients developed adverse drug reactions. Higher incidence of adverse drug reactions was observed in females (66.66 %) than males (57.77 %). Gastrointestinal system was affected most commonly by the ADRs of first-line antitubercular drugs. Most of the reported ADRs belonged to "possible" category as per WHO and Naranjo scales of causality assessment. And it has been identified that majority of ADRs fall in "mild" category according to Hartwig' scale of severity assessment.

The present study suggests that the involvement of clinical pharmacists in the monitoring and assessment of ADRs of Antitubercular drugs may help to minimize morbidity and improve patient compliance and achieve better therapeutic outcome.

Keywords: tuberculosis, first-line antitubercular drugs, adverse drug reactions, causality assessment, severity assessment.

1. Introduction

Tuberculosis (TB) is a mycobacterial infection, which remains a leading infectious killer globally. It has been estimated that 10.4 million new cases of tuberculosis identified across the world and almost two-thirds of them belong to countries like India, Indonesia, China, Philippines, Pakistan, South Africa and Nigeria, in the year of 2016. In addition, around 1.7 million deaths were reported to be caused by tuberculosis in 2016 (Floyd et al., 2018). TB is classified mainly as

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Pulmonary TB (PTB) and Extra-pulmonary TB (EPTB). The PTB accounts almost for 85 % of TB cases worldwide and EPTB affects organs such as pleura, lymph nodes, spine, joints, genitor-urinary, nervous system or abdomen and it represents 10 % to 15 % of all the TB cases in the world (Ranzani et al., 2017).

The first line anti- tubercular agents include Isoniazid, Rifampicin, Pyrazinamide, Ethambutol, and Streptomycin (Pourakbari et al., 2016) and the second line anti-tubercular agents include Ethionamide, Prothionamide, Cycloserine, Terizidone, Para-aminosalicylic acid (PAS), Rifabutin, Thiacetazone, Fluoroquinolones (Ofloxacin, Moxifloxacin, Ciprofloxacin) and injectables (Kanamycin, Amikacin) (Park et al., 2015). Second line anti-tubercular agents are reported to have low efficacy or higher toxicity or both and hence they used as reserve drugs.

An unpleasant or unintended reaction resulting from the use of a drug administered in normal doses is termed as adverse drug reaction (ADR) (Patton et al., 2018). ADRs are classified into six types including type A (Augmented reactions – dose-related), type B (Bizarre reactions – non-dose-related), type C (Chronic reactions – dose-related and time-related), type D (Delayed reactions – time-related), type E (End of use reactions – withdrawal related), and type F (Failure of therapy) (Edwards et al., 2000). In addition, an adverse drug reaction occurring due to interaction with concomitant medications, is known as adverse drug interactions (Pakkir Maideen et al., 2018; Maideen, 2018).

Identification of an ADR can be useful for the prevention, early detection and management of ADRs. Causality assessment of ADRs is an important step in the ADR monitoring programs and Naranjo's algorithm scale are commonly used to carry out the assessment of causality of the ADRs. Being a source of significant morbidity and mortality, ADRs are responsible for 6 % of hospital admissions and they occur in 10-20 % of hospitalised patients (Cabré et al., 2018). The healthcare costs might be increased by ADRs due to frequent hospitalization, prolongation of hospital stay and additional investigations (Schnippel et al., 2018). It has been reported that ADRs are developed in more than 5 % of patients taking Antitubercular drugs and they include gastrointestinal diturbances, hepatotoxicity, allergic reactions, arthralgia, neurological disorders, and many others (Agrawal et al., 2018).

Monitoring and reporting of ADRs of Antitubercular drugs is very much essential as they can affect the compliance of patients resulting in abrupt discontinuation of treatment leading to multidrug resistance. Clinical pharmacists can play a major role in the assessment of ADRs related to the use of Antitubercular drugs, by which the patient compliance could be improved and the occurrence of multidrug resistance minimised. Hence, this study aimed to involve clinical pharmacists in monitoring and evaluation of ADRs in patients taking first line Antitubercular drugs, in a DOTS centre and tertiary care teaching hospital of Nellore, India.

2. Materials and methods

A prospective observational study was conducted in the Department of Pulmonology at AC Subba Reddy Government Medical College, a 1000 bedded tertiary care teaching hospital, Nellore, Andhra Pradesh and DOTS centre.

Duration of study

The study was conducted for six months from August 2017 to January 2018.

Ethical approval

Institutional Ethics committee of Ratnam Institute of Pharmacy, Nellore and Department of Pulmonology, ACSR Medical College & Govt. General Hospital, Nellore, approved the study.

Patients' selection criteria

The study included the patients receiving first-line anti-tubercular agents, willing to participate in the study and above 12 years of age and the study excluded the patients below 12 years of age, pregnant and lactating women, patients in the categories of MDR-TB and XDR-TB, not willing to participate and the patients having renal and hepatic impairment.

Study Procedure

Pharmacists monitor for the ADRs in patients taking first-line Antitubercular drugs daily, during the study period. Upon the occurrence of suspected ADRs, the concerned physician has been notified and the information such as type of reaction to the drug, outcome of therapy of the patient, etc. documented in the adverse drug reactions monitoring and reporting form.

The causality assessment of ADRs was done by using WHO probability scale (Definite, probable, possible, unclassifiable, unlikely, conditional) and Naranjo's scale (Definite, probable, possible, unlikely), at the end of the study.

Statistical analysis

The data obtained was entered into Microsoft Excel sheet. Descriptive statistics was used to analyse the data.

3. Results

A total number of 260 patients who were on Directly Observed Treatment Short course (DOTS) therapy were enrolled in our study, of which 206 patients (79.23 %) were male and 54 patients (20.76 %) were female (Table 1).

Higher number of patients recruited in the age group between 33-42 years (26.1 %) among that 53 were male and 15 were female, followed by the age group 43-52 years (24.6 %) among them 53 were male and 11 were female. In the age group of 23-32 (18.8 %) 36 patients were male and 13 patients were female and 53-62 years (14.2 %) among them 31 patients were male and 6 female patients were moderately affected. The age group between 63-72 (10.7 %) of total 28 patients, out of that 24 were male and 4 were female and age group of 13-22 (5.3 %) comprises of 9 males and 5 females, the least affected age group (Table 1).

Table 1. Age and Gender wise distribution of patients enrolled in the study

Age	Male	Female	Total No of Patients	Percentage (%)
13-22	9	5	14	5.3
23-32	36	13	49	18.8
33-42	53	15	68	26.1
43-52	53	11	64	24.6
53-62	31	6	37	14.2
63-72	24	4	28	10.7
Total	**206**	**54**	**260**	

The patients affected with tuberculosis (TB) in the study were divided according to the type of TB. Out of 206 male patients 181 patients were affected by pulmonary TB (87.8 %) and 25 patients (12.2 %) were affected by extra pulmonary TB. Out of 54 female patients 43 (79.6 %) were affected by pulmonary TB and 11 (20.4 %) were affected by extra pulmonary TB and total of 86.15 % of pulmonary TB and 13.85 % of Extra pulmonary TB (Table 2).

Table 2. Pattern of TB of both genders

Age Group	Pulmonary TB		Extra Pulmonary TB	
	Male	Female	Male	Female
13-22	6 (3.3)	2 (4.6)	3 (12)	3 (27.2)
23-32	30 (16.5)	8 (18.6)	6 (24)	5 (45.4)
33-42	51 (28.1)	12 (27.9)	2 (8)	3 (27.2)
43-52	45 (24.8)	11 (25.5)	8 (44)	0 (0)
53-62	25 (13.8)	6 (13.9)	6 (24)	0 (0)

63-72	24 (13.2)	4 (9.3)	0 (0)	0 (0)
Total	181 (87.8 %)	43 (79.6 %)	25 (12.2 %)	11 (20.4 %)

The patients enrolled in our study received Category-I and Category-II type of treatments. The drugs such as Isoniazid (H), Rifampicin (R), Pyrazinamide (Z) and Ethambutol (E) comprises Category-I treatment while the drugs like Isoniazid (H), Rifampicin (R), Pyrazinamide (Z), Ethambutol (E) and Streptomycin (SM) included in Category- II treatment. Out of 206 male patients, 154 patients (74.75 %) received Category-I type of treatment and 52 patients (25.24 %) were received Category-II type of treatment. Out of 54 female patients, 44 were received Category-I (81.48 %) and 10 received Category- II (18.52 %) type of treatments (Table 3).

Table 3. Type of treatment for both gender

Age	**Category –I** (H, R, Z & E)		**Percentage %**		**Category-II** (H, R, Z, E & SM)		**Percentage %**	
	Male	Female	Male	Female	Male	Female	Male	Female
13-22	8	5	5.19	11.36	1	0	1.92	0
23-32	31	11	20.12	25.00	5	2	9.61	20.00
33-42	41	13	26.62	29.54	12	2	23.07	20.00
43-52	36	9	23.37	20.45	17	2	32.69	20.00
53-62	22	5	14.28	11.36	9	1	17.30	10.00
63-72	16	1	10.38	2.27	8	3	15.38	30.00
Total	**154**	**44**			**52**	**10**		

Tobacco is a known risk factor of tuberculosis and tobacco use in the form of smoking and chewing, among our patients has been recorded. Smoking is known to affect the immune system and can render the smokers more susceptible to infections. The results of our study implies that nearly 62.3 % of patients using tobacco in the form of chewing and smoking. Out of these 147 patients were male (90.74 %) and 15 patients were female (9.25 %). Overall 37.69 % of people are nontobacco patients, which comprises 59 male patients (60.2 %) and 39 female (39.79 %) (Table 4).

Table 4. Smoking / chewing tobacco history of patients

Age	**Smoking / Tobacco chewing**		**Percentage**		**Non Smoking / No Tobacco chewing**		**Percentage**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
13-22	7	0	4.76	0	2	5	3.38	12.82
23-32	20	2	13.60	13.33	16	11	27.11	28.20
33-42	34	3	23.12	20.00	19	12	32.20	30.76
43-52	43	5	29.25	33.33	10	6	16.94	15.38
53-62	21	3	14.28	20.00	10	3	16.94	7.69
63-72	22	2	14.96	13.33	2	2	3.38	5.12
Total	**147**	**15**			**59**	**39**		

The alcohol consumption among our patients has also been recorded and it was found that out of 260 patients, 133 patients (51.15 %) consumed alcohol and 127 were non-alcoholic (48.85 %) (Table 5).

Table 5. Gender wise distribution based upon alcohol consumption

Gender	Alcoholic	Non alcoholic
Male	131	75
Female	2	52
Total	133	127
Percentage (%)	**51.15**	**48.85**

Family history is the causative genetic factor in TB and the family history of the enrolled patients were analysed, in which 78 patients have the family history of TB. In this 62 were male (30.09 %) and 16 (29.62 %) were female and that of no family history male 69.90 % and female 70.37 % respectively. Out of the 78 patients age group 33-42 (29.03 %) in male and 43-52 (31.25 %) in female have more affected (Table 6).

Table 6. Patients' family history status

Gender	Family history	No family history
Male	62	144
Female	16	38
Total	**78**	**182**
Percentage (%)	**30**	**70**

Among 260 TB patients recruited for the study, 211 patients (81.16 %) were without any comorbid conditions, while remaining 49 (18.84 %) had one of the co-morbid conditions HIV and Diabetes mellitus. Out of 49 patients (18.84) with comorbidity, 36 were male (73.46 %) and 13 were female (26.53 %). The patients suffering from comorbid conditions included 27 patients of HIV, of which 20 males and 7 females, 22 patients with diabetes mellitus, of which 16 males and 6 females. Patients of age group 43-52 in males (30.55 %), 53-62 (38.46 %) in females have more percentage of co-morbid conditions (Table 7).

Table 7. Co-morbidities status of patients

Age	Co-morbidities		No co-morbidities	
	Male (%)	Female (%)	Male (%)	Female (%)
13-22	0 (0)	0 (0)	9 (5.29)	5 (12.19)
23-32	10 (27.77)	2 (15.38)	26 (15.29)	11 (26.82)
33-42	7 (19.44)	3 (23.07)	46 (27.05)	12 (29.26)
43-52	11 (30.55)	1 (7.69)	42 (24.70)	10 (24.39)
53-62	7 (19.44)	5 (38.46)	24 (14.11)	1 (2.43)
63-72	1 (2.77)	2 (15.38)	23 (13.52)	2 (4.87)
Total	36	13	170	41

During the study period the total of 260 patients were monitored. In which, 155 patients shown ADRs (59.61 %), out of these 119 patients (57.77 %) were male and 36 patients (66.66 %) were female (Figure 1).

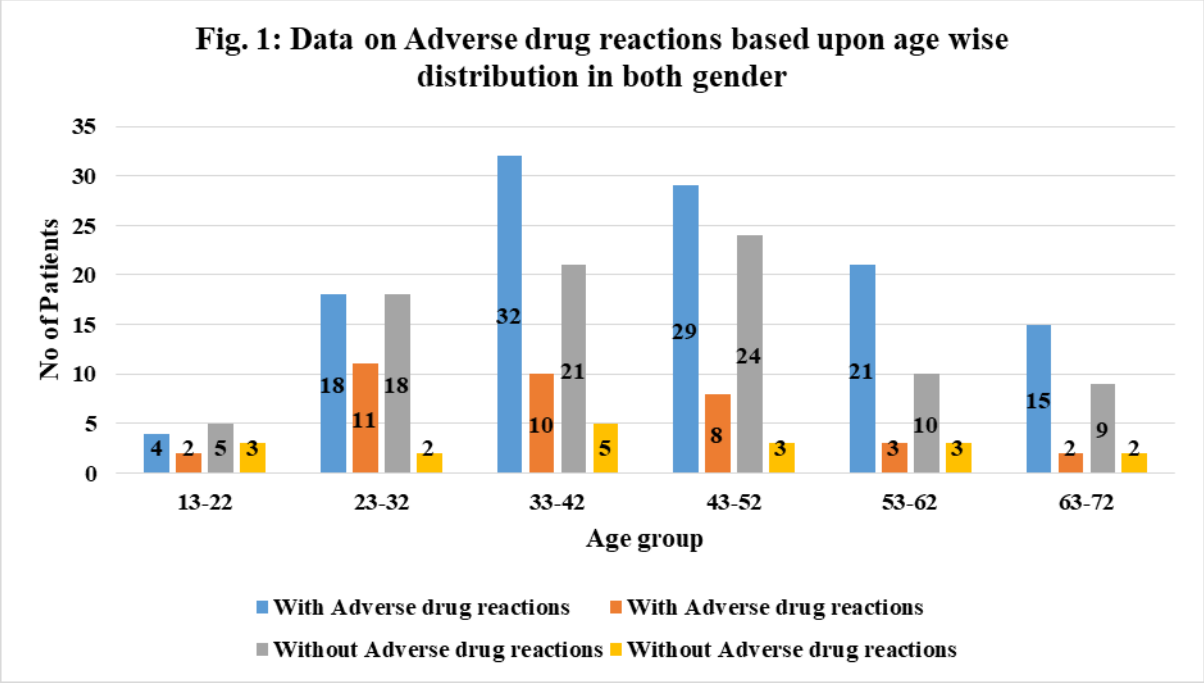

Fig. 1. Data on adverse drug reactions based upon age wise distribution in both genders

In the present study, 76 (49.03 %) patients (65 male and 11 female) developed at least one adverse reaction. A total of 53 patients (41 male and 12 female) had two ADRs and 26 patients (13 male and 13 female) with multiple ADRs. Study reveals that females were more affected by ADRs as compared to male patients. Age group of 33-42 male patients and 23-32 female patients have reported single ADR. Age group of 43-52 male patients and 23-32 female patients were reported two ADRs. Age group of 43-52 of both gender have reported multiple ADRs (Figure 2).

Gastrointestinal system (Gastritis) was the most common system affected followed by Skin (Rashes). Serious ADRs are hepatitis and Odema observe in male patients (0.64 %). Greatest number of ADRs were seen with the use of Isoniazid and Rifampicin. Gastritis (32.9 %), vomiting (21.2 %), giddiness (29 %), headache (2.5 %), anemia (10.9 %), Pruritus (10.9 %), skin rashes (10.9 %), arthralgia (16.1 %), ototoxicity (3.87 %), fever (5.8 %) were the commonest. Serious ADRs like hepatitis and edema (0.64 %) were also observed.

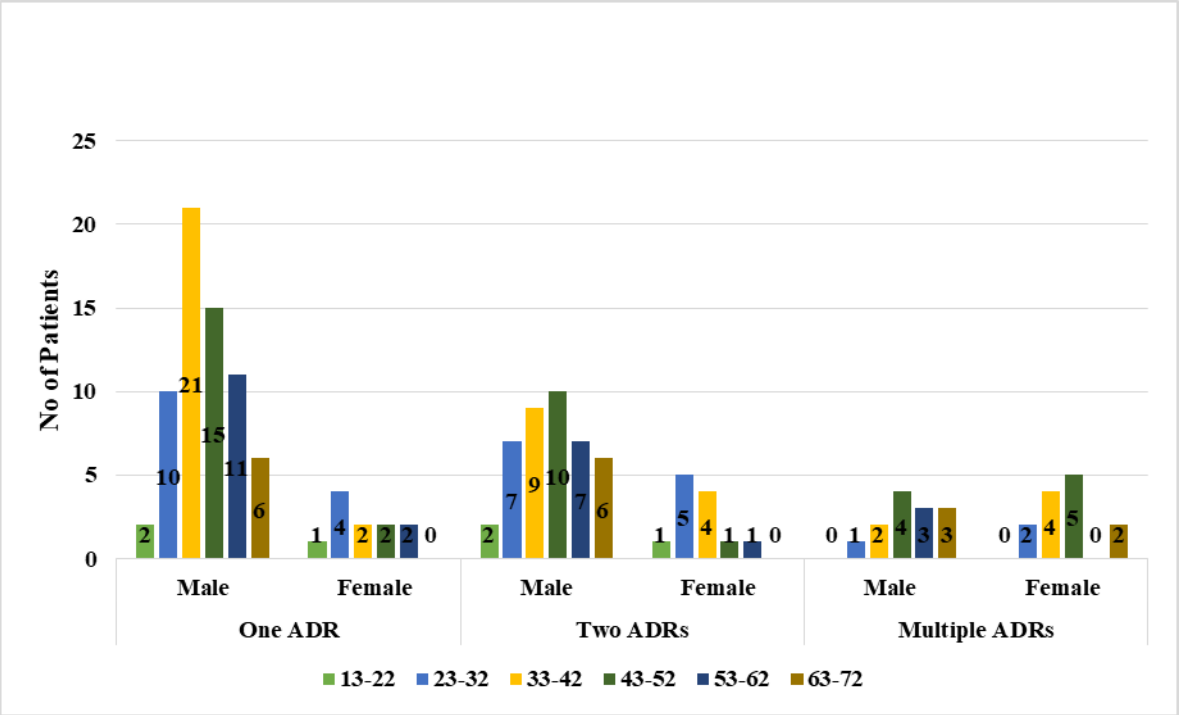

Fig. 2. Number of adverse drug reactions based upon age wise distribution in both genders

Table 8 reveals the ADRs with causative drugs. In which, vomiting is commonly associated with ethambutol and other Anti-tubercular drugs. Rifampicin and Isoniazid produced gastritis. Isoniazid is the causative drug for hepatitis. The incidence of ADRs was increased with increase in the number of drugs in the prescription. The greatest number of ADRs was seen with the use of Isoniazid and Rifampicin. GIT was the commonly involved system.

Table 8. Adverse drug reactions (ADRs) associated with first-line Antitubercular drugs

ADRs	Causative Drugs
Gastritis	Rifampicin, Isoniazid
Anorexia	Rifampicin, Isoniazid, Ethambutol, Pyrazinamide, Streptomycin
Vomiting	Rifampicin, Isoniazid, Ethambutol, Pyrazinamide, Streptomycin
Skin Rashes	Rifampicin, Isoniazid, Ethambutol, Pyrazinamide, Streptomycin
Head ache	Isoniazid, Pyrazinamide
Dizziness	Rifampicin, Isoniazid, Ethambutol, Streptomycin
Optic neuritis	Ethambutol
Peripheral neuritis	Isoniazid
Fever	Pyrazinamide
Redness and Watering of Eye	Rifampicin
Hepatitis	Rifampicin, Isoniazid, Ethambutol, Pyrazinamide

Severity of ADRs encountered during the study period was determined by using the Hartwig Assessment Scale. The results of the assessment revealed that most of the ADRs were mild. 85 patients (63 male, 22 female) showed only mild ADR. 63 patients (49 male and 14 female) showed moderate and 7 male patients showed severe ADR. Age group of 33-42 in male patients showed both mild and moderate ADR whereas 53-62 age group shown severe ADR. In case of female patients, 23-32 age group showed mild and 33-42 age group showed moderate. No severe ADRs reported in female patients (Figure 3).

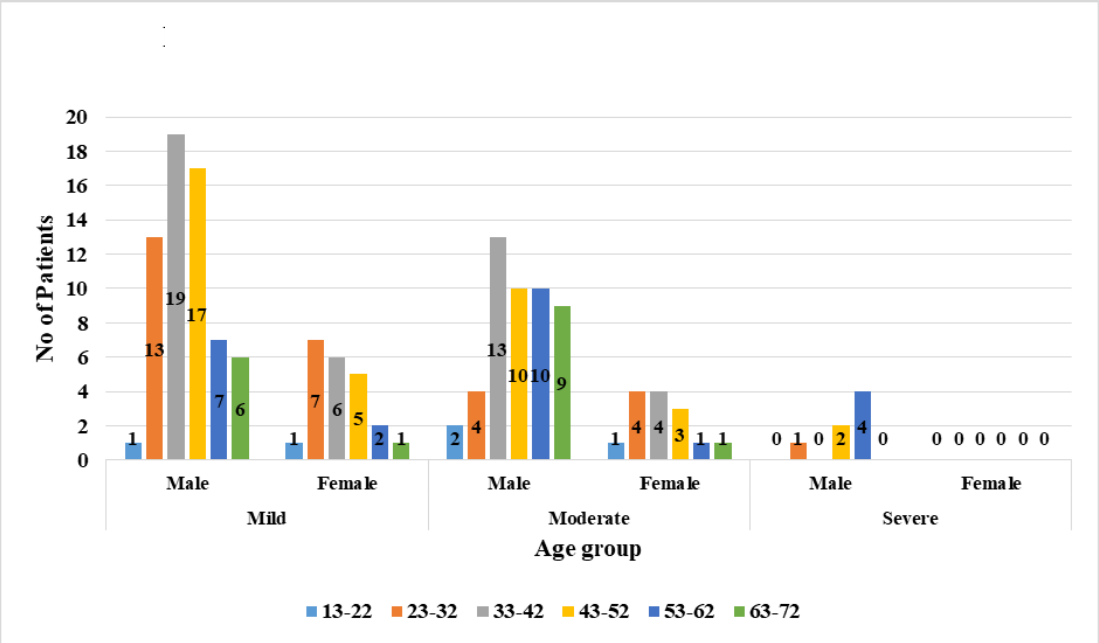

Fig. 3. Severity of adverse drug reactions based upon age wise distribution in both genders

Figure 4 shows the Causality assessment of ADR based on Naranjo's Causality assessment scale. The result showed that most of the encountered ADR were possible (54.83). Age group of 33-42 male patients showed more possible, whereas for female patients both 23-32 and 33-42 age groups were more possible for ADRs.

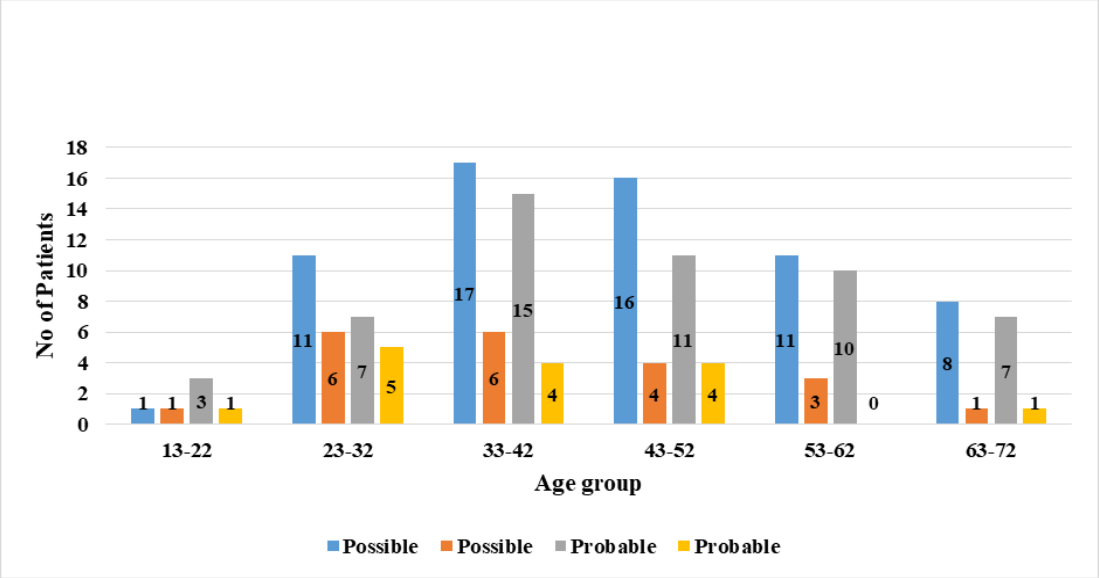

Fig. 4. Assessment of adverse drug reactions based upon age wise distribution in both gender

4. Discussion

It is necessary to evaluate pattern of adverse reactions, as there can be no hope of eliminating all the adverse effects of drugs. There is a special need for systemic collection of information on ADRs in India due to wide variation in genetic (family history), life style (smoking, drinking alcohol) and disease patterns. ADR monitoring and reporting in hospitals is an important program to identify and quantify the risks associated with use of drugs. This information may be useful in identifying and preventing suspected ADRs while generally enhancing the knowledge of prescribers to deal with ADRs more effectively. Early detection of ADRs helps to modify the doses or the drug

regimen to minimize toxic effects. Similar studies can be used as reference for iatrogenic ADRs and assessment of prevention of expected adverse drug reactions. ADR reporting and monitoring in a tertiary care hospital should be continuous and ongoing process to keep a record of newly marketed drugs and medicinal products. This helps in providing baseline data regarding the safety of those drugs. Serious ADRs account for prolonged hospitalization, increased morbidity and also economic burden. Hence, ADR monitoring is considered as an important activity, as it justifies the benefit versus risk ratio of drugs.

This study was performed with the ultimate aim, to generate information about ADRs to anti tubercular drugs prescribed at DOTS centre in Nellore, Andhra Pradesh, to add knowledge about the safety of medicines and prevention of ADRs. During the study period from August 2017 – January 2018, 62 patients with ADRs to anti-tubercular drugs were detected by spontaneous reporting from the health care professionals of the DOTS therapy centre, Nellore. This was accomplished using the notification slip, telephonic conversation or communicating personally. The patients suffering from ADRs were examined by physician and information about the adverse event was recorded in the ADR form. Information about ADR in patients satisfying the inclusion criteria were recorded in the case report.

Our Study reveals that females were more affected by ADRs as compared to male patients. Previous studies have revealed similar findings (Nemagouda, 2014; Maqusood et al., 2016; Shareef et al., 2018). Female patients have reduced body size to body weight ratio, compared to males, which might be the reason for high prevalence of ADRs among them. Our present study also found that gastrointestinal system was involved commonly in the ADRs of first-line Antitubercular drugs. Various studies have also noted the same (Gor et al., 2008; Sinha et al., 2013; Singh et al., 2015).

In our present study, WHO Causality assessment scale (Zaki, 2011) and Naranjo causality algorithm scale (Naranjo et al., 1981) have been employed to evaluate causality assessment of the suspected adverse drug reactions. Majority of the reported ADRs belonged to the possible category and the rest of the ADRs were found as probable, as per WHO Causality assessment scale and Naranjo's scale.

Hartwig Assessment Scale was used to carry out the severity assessment of ADRs and the scale is categorized into mild, moderate and severe levels (Hartwig et al., 1992). Hartwig's scale helps to decide whether hospitalization is required or not for an adverse drug reactions developed. Though the incidence of ADRs due to first line antitubercular drugs used in the DOTS centre, Nellore was high, majority of the reported ADRs were classified as mild and did not need modification of treatment or administration of specific antidotes. In addition, majority of the patients were seen to have mistaken the symptoms of ADR with the disease being treated.

5. Conclusion

Our current study indicates that gastrointestinal system was affected more commonly by the ADRs of first-line Antitubercular drugs. As per the Hartwig's scale, majority of the reported ADRs were classified as mild. In addition, most of the ADRs had a "possible" relationship with the suspected drugs, as per the causality assessment done by using WHO Causality assessment scale and Naranjo causality algorithm scale. The current study stresses on more intensive implementation of ADR monitoring and reporting to provide optimum patient care and to obtain therapeutic outcome. The involvement of clinical pharmacists in detecting and monitoring of adverse drug reactions might help to improve the patient adherence, minimize drug resistance and achieving better therapeutic outcome.

6. Acknowledgement

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Effects of Catholyte Water on the Development of Experimental *Graffi* Tumor on Hamsters

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Abstract

The paper describes the results of a pilot study aimed at the investigation of the influence of catholyte (electrolyzed alkaline water) on the development of tumors. In the experiments solid *Graffi* tumor was transplanted subcutaneously in the back of the experimental group of *Golden Syrian* hamsters. Tumor appearance and growth were registered every day. Blood parameters were measured on the 10th and 25th day after transplantation and blood smears were prepared. Hamsters treated with catholyte developed tumor with some delay compared to untreated (drinking tap water) ones. Also, the tumor growth was slow and the survival rate was increased. The analysis of blood parameters and cell morphology has shown significant differences in the value of some hematometric parameters and morphological changes of some blood cells. The obtained results suggest about the beneficial influence of catholyte and the possible use of it as a supporting non-invasive therapy of cancer diseases.

Keywords: Graffi tumor, catholyte, tumor growth, survival rate, mortality, blood parameters.

1. Introduction

Water is a natural and necessary medium for many biological molecules. Alterations in its composition and structure can produce stimulating or inhibitory effects on the processes in the living things. Influenced by different factors water can change its acidity pH, ORP (Oxidation Reduction Potential), and its physical structure. When electrochemical activation or electrolysis is applied the obtained catholyte receives pH more than 8.0 and negative ORP in milivolts which leads to increased antioxidant effect (Prilutsky, Bakhir, 1997). Due to this reason it could be expected that the catholyte would have protective and positive effect for oxidative stress-related diseases like diabetes and cancer.

Clinical examinations carried out by different scientists have demonstrated positive effect in case of diabetes type 2, telomere shortening in cancer cells and inhibition of their growth, suppression of side effects caused by the use of anticancer medications, favorable influence on the blood system (Hayashi, Kawamura, 2002; Shirahata, 2012; Aschbah, 2019; Gluhchev, Ivanov, 2014; Sanetaka et al., 2012; Ignatov, Gluhchev., 2019). Along with this it was proved that the activated water was not toxic for cells and tissues, and did not have mutagenic, cancerogenic, embryotoxic or immunotoxic effects (Sanetaka et al., 2012).

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For the evaluation of the influence of some medicine or therapy on tumor malignancies different parameters are used based on measurements of tumor development, survival rate, mortality, blood cells changes and others. In the last decade, the main hematometric indices (biomarkers) have been evaluated as diagnostic tools and prognostic parameters in patients with malignancies – cancer and leukemia (Zvetkova, Fuchs, 2017; Hirahara et al., 2016; Azab et al., 2013).

Recent data from the scientific oncological literature evaluated that the NLR (neutrophil to lymphocyte ratio) is superior for predicting the long term survival of cancer patients (Jia et al., 2015; Dezayee, Al-Nimer, 2016; Qin et al., 2018). E.g., lower NLR (≤ 2.0) is associated with good prognosis for breast cancer patients; a higher peripheral blood NLR (≧5) was considered to indicate – significantly and independently, a poor prognosis for breast cancer patients, gastric cancer patients, etc. Simultaneously, the total WBC count, neutrophil and lymphocyte counts alone could be also statistically significant predictors of 5-year cancer patients' mortality (Azab et al., 2013).

At present, the data about the effects of electrolyzed alkaline water (catholyte) on tumor growth and haematological parameters in experimental tumor-bearing animals are absent.

Recently the authors carried out a research about the influence of electromagnetic fields and infrared thermal fields on *Graffi* tumor (Toshkova et al., 2019).

The aim of this study is to examine the biological effects of catholyte on the tumor growth parameters, hematometric biomarkers (including main WBC count; granulocyte and lymphocyte count; lymphocyte percent, GLR (granulocyte to lymphocyte ratio), and blood cell morphology in hamsters with experimental Graffi myeloid tumor.

2. Materials and methods

Experimental animals. In the trials hamsters, breed "Golden Syrian", aged 2-4 months, male and female, with weight around 100 g, grown in individual plastic cages with free access to food and water were used.

Experimental tumor. The experimental *Graffi* solid tumor is maintained on a monthly basis *in vivo* in hamsters from the research team at IEMPAM-BAS (Toshkova, 1995) via subcutaneous **(s.c.)** transplantation of live tumor cells ((1-2.10^6) in the area of the back. Between days 7 and 15 in the spot of injection appear tumors, which grow progressively, and the hamsters die aproximately 30-35 days after the injection. In such a tumor model it is observed 100 % appearance (transplantability) of umor and 100 % mortality rate. Spontaneous regression, i.e. spontaneous shrinking and disappearance of the tumor is not observed.

Catholyte water. During the experiment catholyte water was produced every day using the Actvator-2 device, developed in the Institute of Information and Communication Technologies at the Bulgarian Academy of Sciences. In this way acidity pH of the water was kept between 9.0 and 9.5 and ORP < 0.

2.1. Experimental design. All the animals were divided into 4 groups as follows.

Gr.1 The hamsters from this group started drinking catholyte water 10 days before the injection with 5x10^4 *Graffi* tumor cells per hamster in the back area, and continued drinking it until the end of the experiment.

Gr.2. This group was used as a control. The hamsters from it were **s.c.** injected with the same amount of tumor cells on the 10^{th} day of the experiment as the hamsters from Gr.1, and were receiving tap water all the time.

Gr.3 consisted of healthy hamsters drinking catholyte water during the experiment.

Gr.4 consisted of healthy hamsters drinking tap water all the time.

The first two groups have to reveal the influence of the catholyte water on the appearance and growth of transplanted tumor compared to the tap water, as well as for evaluation of haematological parameters and peripheral blood cell morphology.

The last two groups were used as control for hematological research.

2.2. Measured parameters

The following parameters of tumor development are determined:

- tumor transplantability success (% of hamsters with tumor to the total number of injected ones),
- tumor size (the average diameter of tumor in mm measured with a caliper),
- survival and average survival (calculated for the respective group in days),
- lethality (% of dead animals in the group).

Animals from each group were sacrificed preserving the ethical aspects of the European convention for protection of vertebrate animals, used for experimental and other scientific purposes (OJ L 222), and approved by the National Veterinary Medical Office in Bulgaria, and blood samples have been prepared at different time periods: on days 10 and 25 after *Graffi* tumor implantation. Hematological/hematometric parameters and indices as shown in Fig.1 were measured on the automated hematological analyzer BC-2800 Vet (Mindray, China).

2.3. The WBC/LR *(White blood cells to Lymphocyte ratio and NLR (Neutrophil to lymphocyte ratio) were calculated.*

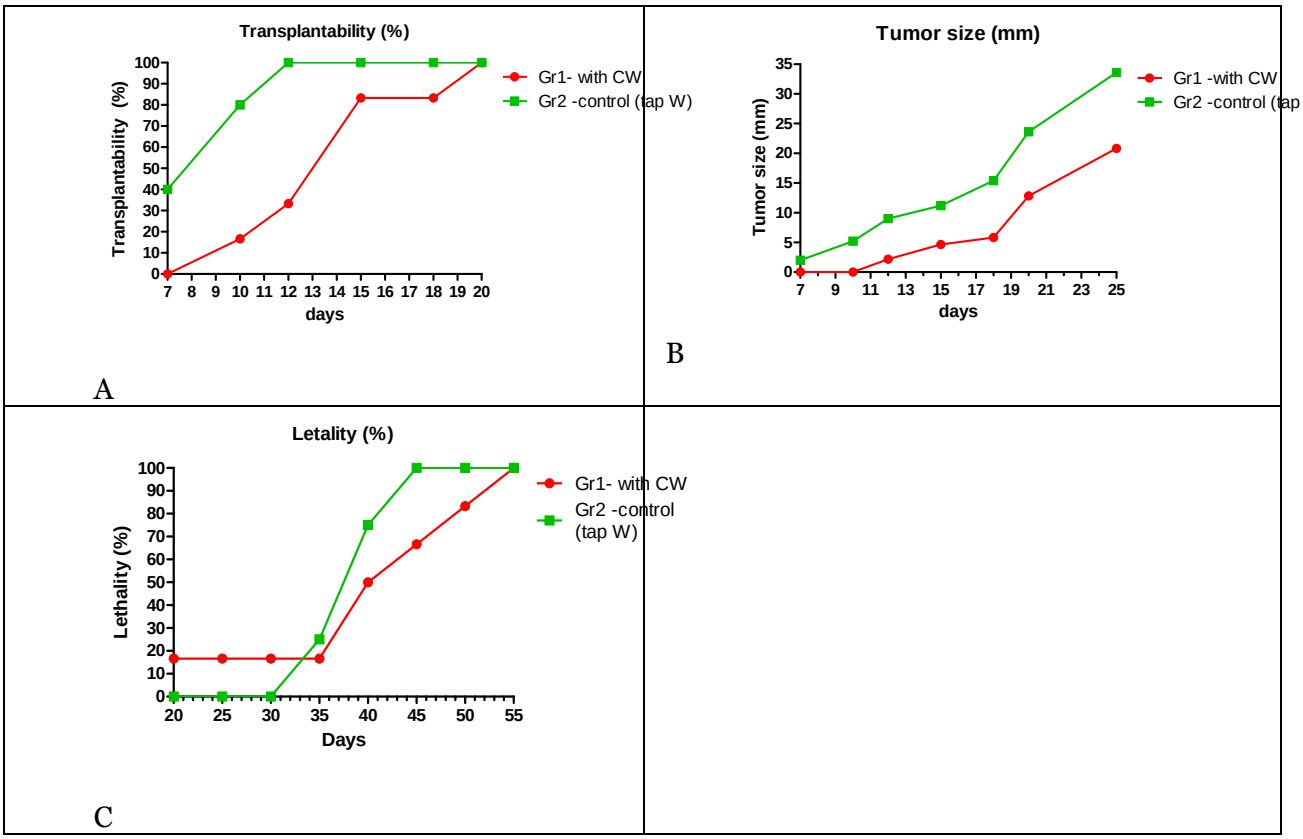
2.4. *Statistical analysis*

For the evaluation of the significance of the differences between the average values of a specific parameter *t*-test was used at levels of significance α = 0.10 and α = 0.05, respectively.

3. Results and discussion

3.1. Parameters of tumor growth for Gr.1 and Gr.2.

Catholyte water was used as experimental therapy during two months. Animals have been examined every day until tumor detection and 2 times per week until 30 days after tumor transplantation. The tumor growth parameters have been registered regularly. The differences between the two groups are shown on (Figure 1).



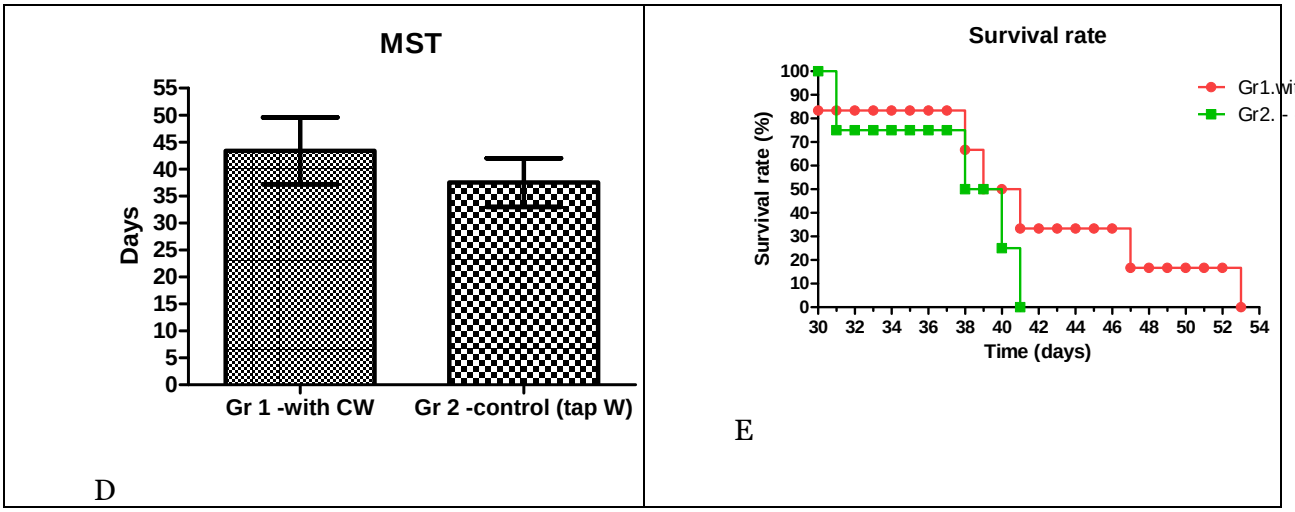


Fig. 1. Biometric parameters of tumor growth for the hamsters from Gr.1 and Gr.2: A – transplantability in %; B – tumor size in mm; C – lethality in %; D – average survival time (AST) in days; E – Survival rate

As can be seen from the graph on (Figure 1A) the tumor transplantation was delayed for the hamsters taking catholyte. While all the hamsters from Gr.2 developed tumor on the 12th day this happened only for 1/3 of hamsters from Gr.1. In the hamsters receiving catholite, tumors were detected at 100 % on day 20.

Similar effect is observed for the tumor size (Figure 1B). Until the 10th day subcutaneous firmness in the hamsters drinking catholyte was not established. This group showed an inhibition in tumor growth rate as compared to control throughout the study period.

The lethality for the hamsters from Gr.2 (Figure 1C) is increased after 35 days, compared with the hamsters from Gr.1. The control group (Gr.2) had a 100 % mortality on day 41, while the Gr.1 - on the 53-rd day of the study.

The evaluated average survival for Gr.1 is 43.4±6.9 days, while it is 37.5±4.5 days for Gr.2. Same conclusion could be taken from (Figure 1E).

These data suggest that the catholyte water slows down tumor development and as a result increases survival rate.

The illustrations on (Figure 2) give visual impresion for the tumor development in Gr. 1 and Gr. 2.

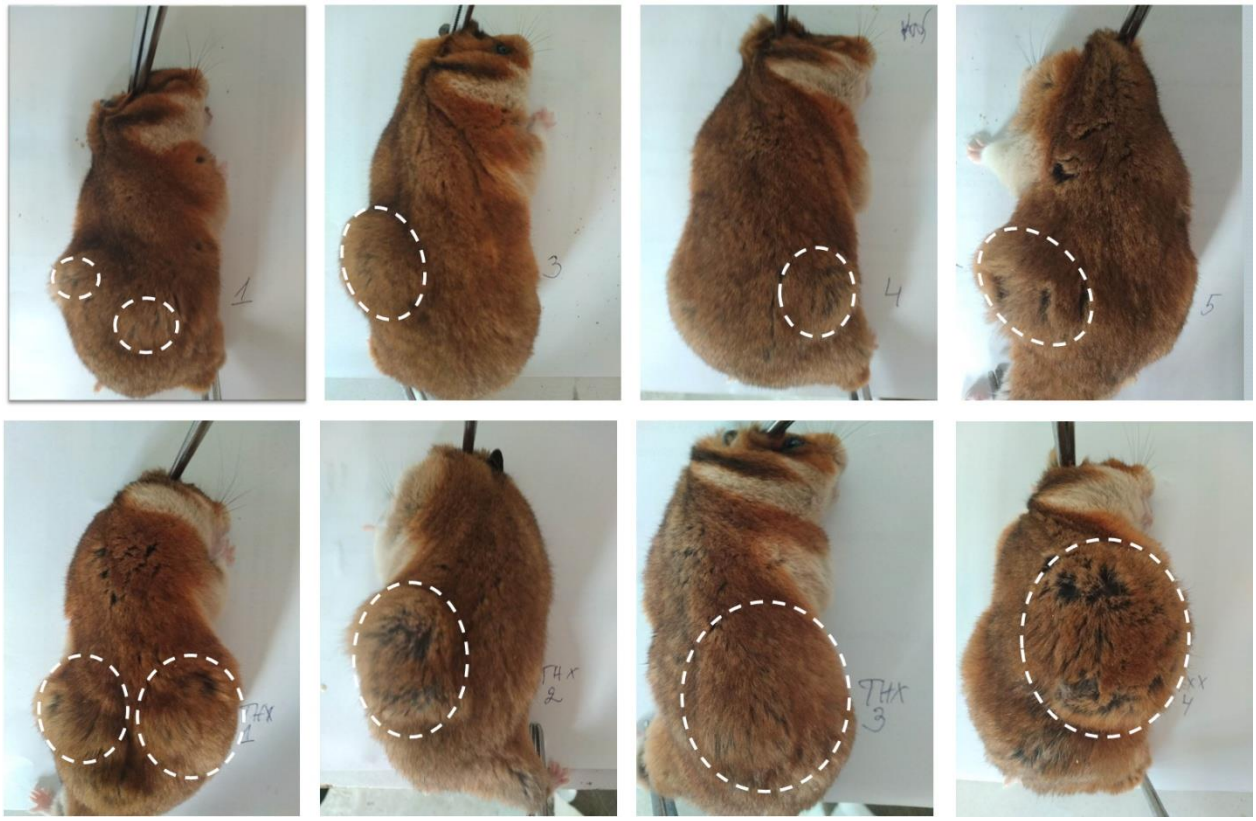


Fig. 2. Images of hamsters from Gr.1 (upper line) and Gr.2 (bottom line) taken on the 25th day after the tumor transplantation

3.2. Hematological parameters

Hamsters from the trial and control groups were euthanized after the application of deep anaesthesia on the 10th and 25th day after tumor transplanting. The obtained blood was used to report haematological parameters, for serum and preparation of blood smears, as well.

The evaluated parameters for all groups are displayed in (Table 1).

Table 1. Blood parameters of 10th/25th day of study

Parameters	Units	Tumor (Catholyte)	Tumor (Tap water)	Healthy (Catholyte)	Healthy (Tap water)
WBC (Leukocytes)	$x10^9/L$	3.8/ 47.9	5.7/2.9	6.8/5.8	2.4/2.1
Lymph	$x10^9/L$	2.2/38.1	0.7/0.5	5.6/2.1	1.4/1.2
Mon	$x10^9/L$	0.2/2.3	0.5/0.2	0.2/0.3	0.1/0.1
Gran	$x10^9/L$	1.4/ 7.5	4.5/2.2	1.0/3.4	0.9/0.8
Lymph %	%	58.3/79.5	12.8/16.5	81.4/35.5	58.8/56.3
Mon%	%	6.0/4.9	8.5/7.3	3.3/5.2	5.7/5.1
Gran%	%	35.7/ 15.6	78.7/76.2	15.3/59.3	35.5/38.6
RBC (Erythrocytes)	$x10^{12}/L$	3.05/2.95	4.77/5.58	4.7/7.98	4.25/5.36
HGB (Haemoglobin)	g/ L	67/83	80/104	85/137	89/ 92
HCT (Hematocrit)	L/L	0.165/0.204	0.231/0.325	0.267/0.445	0.226/0.304
MCV (Mean red blood cell volume)	fl	54.2/69.2	48.5/58.3	57.0/55.8	53.4/56.9
MCH (Average HGB content in erythr)	pg	21.9/28.1	16.7/18.6	18.0/17.1	20.9/17.1
MCHC(mean conc of Hb	g/ L	406/406	346/320	318/307	393/302
RDW	%	12.6/22.9	14.1/14.0	15/11.7	12.8/16.1
PLT(Platelets)	$x10^9/L$	132/491	883/537	250/488	306/456
MPV(mean volume of platelets)	f/L	6.1/7.2	4.9/5.6	5.4/4.5	5.1/5.7
PDW	%	19.8/19.8	17.9/18.3	18.9/17.0	17.7/19.3
PCT	%	0.080/ 0.353	0.432/.300	0.135/0.219	0.156/0.259

The development of experimental Graffi myloid tumor in hamsters influenced diversely the two main WBC subpopulations – neutrophil granulocytes and lymphocytes (column 4). Significantly elevated WBC, granulocyte count and percent, as well as significant reduction of lymphocyte count and percent were observed in the Graffi myeloid tumor-bearing hamsters (Gr. 2, column 4) and (Figure 3). These effects are well expressed on the 10-th day, and are profound on the 25-th day. The treatment of tumor bearing animals with catholyte as drinking water improved the values of same parameters during the investigation (Gr.1, column 3) and (Figure 3).

Results from the comparison of the blood parameters for which significant difference between Gr.1 and Gr.2 was obtained are shown in (Table 2).

Table 2. Significant difference between blood parameters of Gr.1 and Gr.2

Parameter	Lymp%	Mon%	Gran%	RBC	MCHC	PDW
α	0.05	0.10	0.05	0.05	0.05	0.05

Based on haematological values the WBCs/LR (White blood cells to Lymphocyte ratio) and NLR (Neutrophil to Lymphocyte ratio) hematometric indices were calculated. Both WBCs/LR and NLR indices are strongly elevated in tumor hamsters taking tap water and highly reduced in hamsters taking catholyte water. The values are similar to healthy hamsters (Figure 3).

Differences in some of the hematological parameters (WBCs, Ly) and WBCs/LR, and NLR hematometric index for groups are shown in (Figure 3).

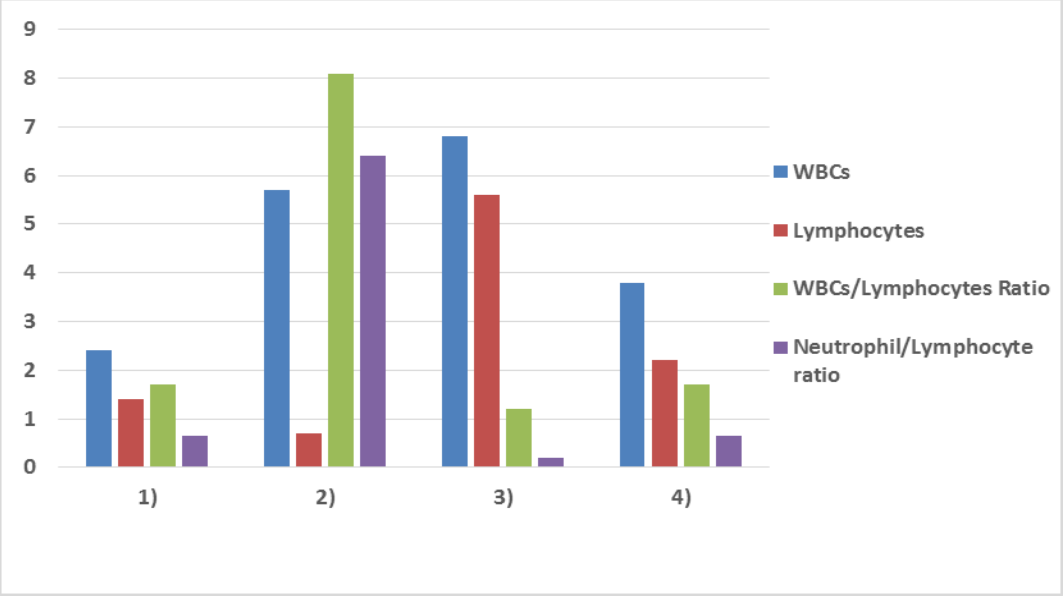


Fig. 3. WBCs (leukocytes) and Lymphocytes total count (x 10^9 /L), and WBCs/Lymphocytes Ratio (at the 10^{th} day); 1) Controls; 2) Untreated tumor-bearing hamsters; 3) Healthy hamsters drinking catholyte; 4) Tumor-bearing hamsters drinking catholyte

Significantly elevated WBC count and total granulocyte/neutrophil ratio were obtained in the untreated Graffi myeloid tumor-bearing hamsters (Gr. 2).

Simultaneously, a significant reduction of the lymphocyte number was evaluated in the same animals.

Additionally, we obtained that catolyte water influenced (elevated) some main PLT-hematometric values in both – control and experimental animals (Table 1, PLT, MPV and and PDW). The hematometrical results obtained were confirmed by our cytological studies on PLTs in the peripheral blood smears of hamsters where one could see clusters of activated thrombocytes – more pronounced in the blood of tumor-bearing animals (Fig. 4 -i).

3.3. Cytological study

Images from blood smears are shown on (Figure 4).

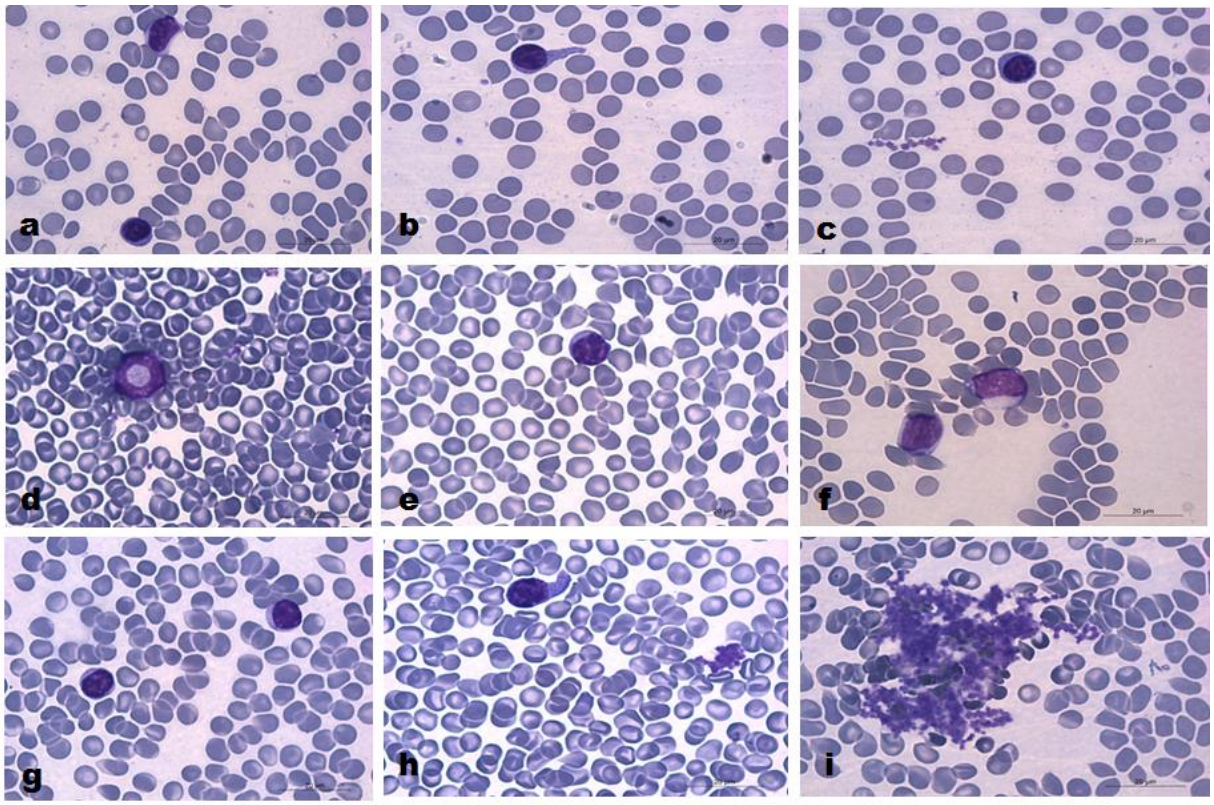


Fig. 4. Images of blood smears as follows: upper line - healthy hamster treated with catholyte, middle line – tumor-bearing hamster (control) drinking tap water, and bottom line –tumor – bearing hamster treated with catholyte. (May Gruenwald Giemza staining. Objective X 100).

In the peripheral blood of healthy hamster, taking electrolyzed alkaline water (catholyte) activated lymphocytes with a large cytoplasmic pseudopode (ptotrusion), monocytes and platelets were observed (Figure 4 –a, b, c) upper line.

Atypical myeloid cells and blast-like cells from the peripheral blood of Graffi myeloid tumor-bearing hamsters at the day 10th from tumor inoculation were observed. One could see atypical immature granulocyte with peripherally localized ring-shaped nucleus and eosinophile granules – in the central part of cytoplasm (Figure 4 – d, e, f) middle line.

In Graffi tumor bearing hamsters, treated with catholyte atypical activated lymphocytes and cluster of PLTs in the peripheral blood smear were noticed. Lymphocytes from these hamsters, although atypical, show characteristic signs of activation as in healthy ones (Figure 4 – g, h, i) bottom line.

4. Conclusion

The influence of catholyte water on the development of *Graffi* tumor implanted in hamsters was assessed. Some delay in tumor growth and increased survival rate were observed. Significant differences in some of the blood parameters were noticed.

We obtained activated (small and medium-size) lymphocytes in the peripheral blood smears of healthy hamsters – treated with catholyte, instead of tap-water (Figure 4 – a, b, c). The same biological phenomenon was also evaluated partially in the peripheral blood of tumor-bearing animals, under the influence of catholyte (Figure 4-g, h). But in comparison to the activated immunocytes in healthy hamsters, the tumor-infiltration cells (TILs) in the tumor-bearing animals are soon atypical and insufficiently activated (or deactivated – in the preapoptotic or apoptotic states).

The appearance of the so-called blast-like cells (Figure 4 – f) has been related to dissemination of the neoplastic disease and could be earlier obtained in the peripheral blood

smears of untreated tumor-bearing animals (Zvetkova et al., 2006, 2007). The results correlated also with changes in WBCs/LR and NLR hematometric indices obtained in the two experimental groups of treated and untreated tumor-bearing hamsters.

The elevated thrombocytes count could be unfavorable predictor in cancer patients, having in view high risk of thrombogenesis and embolism. On the other hand, the catholyte water could be useful in cases with thrombocytopenia, but not in thrombocytoses, when application of electrolyzed water would be not recommended.

This study also further strengthens the role of WBC-hematometric indices in diagnosis and prognosis of cancer.

Catholyte water (investigated *in vivo* - in our experimental model of Graffi tumor-bearing hamsters), could improve TILs cellular immunity (immunomodulating, immunostimulating influence).

The first conclusion is that the developing experimental Graffi myloid tumor in hamsters influenced diversely the two main WBC subpopulations (predominantly neutrophils) and lymphocytes. These diverse effects – well expressed on the 10th day, are profound on the 25th day.

Our experimental results suggest that in this model the treatment of tumor-bearing animals with catholyte, as drinking water, improves the hematometric indices to the normal values.

Thus, our second conclusion is that the catholyte water – employed instead of tap water in our experimental model with tumor-bearing hamsters, has a positive impact on the main hematometric indices e.g. WBCs/LR and NLR - neutrophil to lymphocyte ratio on day 10 (Table 1) was: for WBCs/LR- 1.71 for a healthy hamster, 1.72 for a tumor-bearing, treated with catholyte and 8.14 for a tumor-bearing, untreated animal, and for NRL - 0.64 for a healthy hamster, 0.64 for a tumor-bearing, treated with catholyte and 6.42 for a tumor-bearing, untreated animal, respectively.

All these points at a favorable influence of catholyte on the hematopoiesis both in case of tumor–bearing animals, and healthy ones.

The obtained results lead to the general conclusion that catholyte could be used as a supporting non-invasive therapy to other cancer therapies as radiotherapy and chemotherapy. However, our pioneer study in this field needs further experimental and clinical confirmation.

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Traditional Herbal Therapy for Some Cardiovascular Diseases in Bulgaria

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Abstract

Traditional medicine is a cultural heritage. Herbs are the basic remedy in Bulgarian traditional medicine, the empirical knowledge of medicinal plants being passed down from generation to generation primarily by word of mouth. Most of the plants are used in Bulgarian traditional medicine (BTM) on the grounds of the experience gained over the centuries. As for the treatment of diseases of the cardiovascular system, the data contained in some Bulgarian handwritten remedy books published in the late 19th and early 20th century show that our people empirically used medicinal plants the pharmacological activity of which is proven today through experimental and clinical studies. Thus far, science has not discovered the mechanism of pharmacological action of a lot of the herbs that people used. The researches in this direction are still going on. Bulgarian herbal therapy makes its contribution to world science.

Keywords: Bulgarian traditional medicine, traditional use of herbs, heart disease.

1. Introduction

Traditional medicine is a cultural heritage under the Cultural Heritage Law (prom. in State Gazette No. 19 of 13.03.2009). There is a multitude of synonyms and definitions used for characterizing traditional medicine experience (folk medicine, ethnomedicine, traditional medicine). The published strategy of the World Health Organization (WHO, 2002-2005) specifies that traditional (folk) medicine makes use of experience acquired over the centuries. According to the Bulgarian people, traditional herbal medicine "is the knowledge, experience and practice of a large number of people who have studied the properties of a lot of herbs in the past and have bequeathed them to their descendants" (Karamitrev, 1934). According to some authors (Modern phytotherapy, 1982), what is characteristic of traditional medicine is that the empirical knowledge of medicinal plants and other remedies and methods used has been passed down from generation to generation primarily by word of mouth, a small part thereof being preserved in written sources (handwritten remedy books). Herbs are the basic remedy in Bulgarian traditional medicine (Modern phytotherapy, 1982). People have used them for treatment of all diseases known thereto (Materials for Bulgarian Botanical Guide, 1939), including heart diseases. Currently, in our country there is a renewal of the interest in the use of plant products (Bachev et al., 2018; Bachev et al., 2018a).

The diseases of the blood circulation organs (BCOs): ischemic heart disease, including acute myocardial infarction, other forms of ischemic heart disease; other heart diseases; cerebrovascular diseases (NSI) have been the leading causes of death in Bulgaria for decades. CVDs* are regarded as

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* Cardiovascular diseases

a serious public health problem due to the high morbidity and mortality rates (WHO, 2007). In North America (Canada) it is natural health products that are mainly used in the treatment of heart diseases (Basu, et al., 2007). Today the experimental and clinical studies provide data regarding the efficiency of using herbs in cardiovascular diseases (Sara et al., 2006).

Because of the importance of BCOs diseases as socially significant ones, the purpose of this publication is the study of the treatment of cardiovascular diseases with herbs in BTM as presented in several handwritten remedy books published in the late 19th and early 20th century.

2. Materials and methods

2.1. The method used is a documentary one – exploration of Bulgarian handwritten remedy books published in the late 19th and early 20th century which deal with the treatment of some CVDs with herbs in BTM.

3. Results and discussion

In certain handwritten Bulgarian herbal remedy books* published in the late 19th and early 20th century, some of those cardiovascular diseases which our people used to treat with herbs are referred to as: heart diseases (vitium cordis, heart valve problems), heart palpitation, weak heart, atherosclerosis, hypertension. Depending on the pharmacological activity of the plants used by our people for the treatment of CVDs, the diseases they were used for can be presumed. Some of the herbal prescriptions published in the remedy books specify the composition and weight of the plant ingredients used and the way of preparing the herbal drug, and give instructions on its dosage. Other prescriptions specify the herbal collection only. In most cases herbs were prepared in the form of decoction and were drunk cold (Urumov, 1926; Pan Noev, 1932; Kalchev, 1992), before or after a meal; they were also taken internally in the form of tinctures (Pan Noev, 1932) and infusions (Pan Noev, 1932; Materials for Bulgarian Botanical Guide, 1939). Our people knew the poisonous plants and recommended that they be used in strictly fixed doses, the handwritten remedy books specifying that a certain plant is poisonous (100 medicinal herbs; Pan Noev, 1932; Materials for Bulgarian Botanical Guide, 1939).

3.1. Heart palpitation

Our people has defined heart palpitation as a separate nosological entity (Kalchev, 1992). It is explained by plethora (hypertension), nervousness, drinking coffee, tea, spirits (Kalchev, 1992) as a disease of the nervous system (Materials for Bulgarian Botanical Guide, 1939). According to contemporary science, heart palpitation is an anomaly of cardiac activity. In most cases heart palpitation is due to a heart disease – cardiac insufficiency, myocardial ischemia; other diseases – diseases of the thyroid gland, hypoglycaemia, anaemia, fever; dehydratation; use of stimulants – caffeine, nicotine, alcohol; intensive physical activity; stress, strain, fear; pregnancy; menopause; heart palpitation after a meal (Alijaniha, et al., 2015; What can be the causes of heart palpitation? ARS MEDICA.bg). Our people have empirically, yet rightly, oriented itself in the causes of heart palpitation and has used medicinal plants giving the respective pharmacological effect. The medicinal plants used by our people in heart palpitation can be divided into several groups on the basis of the biologically active substances contained therein:

3.1.1. Medicinal plants used in heart palpitation caused by cardiac insufficiency

Our people used lily-of-the-valley (Convallaria majalis L.) (Kalchev, 1992) and pheasant's eye (Adonis vernalis L.) in heart palpitation which is most probably caused by chronic cardiac insufficiency, this being due to the fact that both plants contain cardiac glycosides (Asenov, Nikolov, 1988). The tincture of the lily-of-the-valley blossom is recommendable in "nervous heart palpitation" (Pan Noev, 1932).

3.1.2. Medicinal plants used in heart palpitation caused by stress, strain, fear

Most probably, in "heart palpitation as a disease of the nervous system" our people used medicinal plants with a sedative action (Modern phytotherapy, 1982) – roots and rootage of valerian (Valeriana officinalis L.), (Kalchev, N., 1992; Materials for Bulgarian Botanical Guide, 1939), decoction of motherwort (Leonurus cardiaca L.), (Materials for Bulgarian Botanical Guide, 1939), lemon balm (Melissa officinalis L.), "decoction of the seed capsules of field poppy" (Papaver rhoeas L.); tea from the leaves of rue (Ruta graveolens L.), sweet woodruff (Asperula odorata L.),

* The authors do not claim to have covered all the CVDs and herbs used for them in BTM

(Materials for Bulgarian Botanical Guide, 1939). In the course of their clinical studies, some authors have established that lyophilized water extract of lemon balm can be used for the treatment of benign palpitation, as lemon balm slows down cardiac activity (Alijaniha et al., 2015). An extract and an alkaloid fraction of rue (Ruta graveolens L.) produce a potential antiarrhythmic effect in the treatment of supra ventricular tachyarrhythmia in experimental studies (Khori et al., 2008).

3.1.3. Medicinal plants used in heart palpitation after a meal.

Medicinal plants with gas-gone action (Modern phytotherapy, 1982) are applied by our people, most probably, in heart palpitation caused by problems of the digestive system – summer savory (Satureja hortensis L.), lovage (Levisticum officinale Koch.) (Materials for Bulgarian Botanical Guide, 1939), peppermint (Mentha piperita L) "in frequent heart palpitation caused by improper condition of abdominal organs" (Materials for Bulgarian Botanical Guide, 1939; Pan Noev, 1932). Apart from its sedative action, valerian also has its favourable effect in meteorism (Modern phytotherapy, 1982).

3.1.4. Medicinal plants used in heart palpitation caused by anaemia.

In this case our people applied plants containing the microelement iron – decoction of the root or leaves of dwarf nettle (Urtica urens L.) (Materials for Bulgarian Botanical Guide, 1939). Experimental data related to the application of common nettle (Urtica dioica L.) in heart palpitation show that it lowers the heart rate in non-cholinergic and non-adrenergic ways (Legssyer et al., 2002).

3.1.5. Medicinal plants used in heart palpitation in the period of menopause.

In BTM, infusion of leaves and blossoms of garden sage (Salvia officinalis L.) is used in heart palpitation (Materials for Bulgarian Botanical Guide, 1939). Authors confirm that garden sage (S. officinalis) is traditionally used for relieving the symptoms occurring in the period of menopause, such as hot waves, insomnia, night sweats, dizziness, headache and heart palpitation (Bommer et al., 2011).

3.1.6. Other medicinal plants and herbal collections used in BTM for treatment of heart palpitation.

The following are also used in BTM in heart palpitation: decoction of the bark of young branches of aspen (Populus tremula L.) (Materials for Bulgarian Botanical Guide, 1939; Urumov, 1926); herbal collection of common mallow (Malva sylvestris L.), wormwood (Artemisia absintium L.) and valerian (Valeriana officinalis L.) (Modern phytotherapy, 1982; Urumov, 1926). Common mallow has spasmolytic action (Modern phytotherapy, 1982), wormwood is used in meteorism (Modern phytotherapy, 1982), while valerian has sedative action and produces favourable effect in meteorism (Modern phytotherapy, 1982), which provides grounds for presuming that the said herbal collection is mainly used in our traditional medicine in "heart palpitation after a meal".

3.2. Heart diseases

Most handwritten remedy books under consideration do not specify heart diseases as separate nosological entities. One of the remedy books we deal with sets out that heart diseases relate to heart valve problems (vitium cordis) (Urumov, 1926).

3.2.1. Heart valve problems

Heart valve problems can affect one or all of the four valves of the heart. They emerge in the process of aging, however, they might be caused by congenital anomalies or specific diseases or physiological processes, including rheumatic heart diseases and pregnancy (Nkomo et al., 2006; Kovacs et al., 2008). The symptoms depend on the type and the extent to which the valve apparatus of the heart is affected. In heart valve problems (Urumov, 1926), heart diseases (Pan Noev, 1932) our people applied a mixture of the roots of valerian and rue (Ruta graveolens L), which have a sedative effect; nettle roots (Urtica dioica L.), (Urumov, 1926), herbal collection of lemon balm and mullein (Verbascum phlomoides L.), (Urumov, 1926; Pan Noev, 1932). Valerian also produces favourable antistenocardiac effect in cardiospasm, while rue shows hypotensive action in experimental models of animals (Modern phytotherapy, 1982). In experimental studies, water extract of nettle has hypotensive action, lowers the heart rate, influences vascular contractility and provokes aortic vasoconstriction, which is due to the activation of alpha1-adrenergic receptors (Legssyer et, al., 2002). According to authors' data, lemon balm has an antiarrhythmic and protective effect upon the heart (Akhondali et al., 2015; Joukar et al., 2016).

3.2.2. Heart diseases

In cases of heart diseases (Pan Noev, 1932) that were most probably related to chronic cardiac insufficiency, BTM applied tinctures of lily-of-the-valley blossom (Pan Noev, 1932) and rue stalks and leaves (weak dose). The handwritten remedy books specify that rue is poisonous (100 Medicinal Herbs). Studies confirm that in Taiwan the overground part of rue is applied in traditional medicine for treatment of circulatory disorders (Miguel, 2003). In heart diseases, BTM also applies decoction of the blossom of hawthorn (Crataegus monogyna Jacq) (Materials for Bulgarian Botanical Guide, 1939). According to experimental and clinical data, hawthorn has diverse effects on the cardiovascular system – positive inotropic effect, anti-inflammatory effect, anticardiac remodelling effect, coronary dilatation effect, endothelial protective effect, antiarrhythmic effect, lipid lowering effect, hypotensive effect; a very good effect has been established in the treatment of patients with myocardial lesion, coronary insufficiency and heart block, the mechanisms being most probably due to a flavonoid mixture contained in hawthorn (Modern phytotherapy, 1982; Wang et al., 2013, Chang, Zuo, 2002). According to a clinical study, hawthorn preparations are effective in the early stages of congestive cardiac insufficiency (Wang et al., 2013). According to some authors, the positive inotropic effect of hawthorn extract is related to the cAMP*-independent mechanism. Probably, hawthorn extract increases the force of contraction by inhibition of the sodium pump (Schwinger et al., 2000). It is established that hawthorn extracts also have an anticardiac remodelling effect (Frey, Olson, 2003). In addition, according to some authors, the vasodilating effect of hawthorn extracts relates to the induction of the NO†-ergic mediator system (Brixius et al., 2006). According to other authors, the vasodilating action of hawthorn on both the coronary circulation and the peripheral vasculature might be due to the inhibition of the angiotensin converting enzyme (ACE) (Miller, 1998). Preliminary research evidences the cardioprotective effects of hawthorn in the in vivo models of ischemia/reperfusion (Veveris et al., 2004). Experimental studies confirm the antiarrhythmic activity of hawthorn (Makdessi et al., 1999) and its lipid-reduction action (Wang et al., 2013); clinical studies evidence the hypotensive activity of hawthorn extract (Belz et al., 2002).

3.3. Weak heart

Probably, what our people meant by "weak heart" was chronic cardiac insufficiency (CCI).

Cardiac insufficiency develops when, as a result of a decrease in the cardiac output, the heart is unable to provide the required amount of blood to all the organs in accordance with their needs (Lambev, 2010). Depending on the swiftness of its occurrence, cardiac insufficiency (CI) might be acute or chronic. CCI develops over a long period of time and there are several types of it (Cardiology, 2010), however, it is highly probable that our people could not tell them apart. Here are some of the symptoms of chronic cardiac insufficiency: fatigue, shortness of breath as a result of exertion, nighttime cough, heart palpitation, swelling of the ankles in the evening which disappears in the morning, and, in later stages, hepatomegaly, pleural effusion (Cardiology, 2010). The causes of CI might result from a number of health conditions, which affect the cardiovascular system and increase the risk of its occurrence – coronary artery disease, valve conditions, risk factors such as smoking, diabetes mellitus, arterial hypertension, familial history (Cardiology, 2010). In this case it is fully explicable to apply medicinal plants "for strengthening the activity of the heart (a cardiac)", (Materials for Bulgarian Botanical Guide, 1939), these plants being rich in cardiac glycosides (Asenov, Nikolov, S., 1988). Cardiac glycosides produce a positive inotropic effect, a negative chronotropic effect, a positive bathmotropic and a negative dromotropic effect as well as certain diuretic action, and have a narrow therapeutic window (Lambev, 2010). Those plants used by our people in cases of weak heart which are rich in cardiac glycosides are: decoction of the rootage of lily-of-the-valley (Convallaria majalis L.), (Materials for Bulgarian Botanical Guide, 1939), infusion of pheasant's eye (Adonis vernalis L), (Pan Noev, 1932). Purple foxglove (Digitalis purpurea L), as well as the other Digitalis species, which constitute basic raw stuff for the manufacture of cardiac glycosides in pharmaceutical industry, were also used by our people as cardiacs. For instance, the infusion of their leaves "was used for strengthening and regulating the activity of the heart as well as a potent diuretic agent and also against shortness of breath". In the remedy books our people pointed out that these plants are poisonous (Materials for Bulgarian

* Cyclic adenosine monophosphate
† Nitric oxide

Botanical Guide, 1939). Our people also used infusion of the leaves and blossoms of mallow (Malva silvestris L.) for strengthening the activity of the heart (Materials for Bulgarian Botanical Guide, 1939). Today's experimental studies show the cardioprotective effect of mallow (Malva sylvestris L.) on myocardial ischemia/reperfusion in rats (Zuo et al., 2007). In cases of weak heart, BTM also applied Iceland moss (Cetraria islandica L.), (Urumov, 1926). According to some authors, Iceland moss (Cetraria islandica L.) has an influence upon symptoms such as heart or chess pain (Pieroni, et al., 2014). In cases of weak heart, BTM recommends that raw garlic (Allium sativum L.) is eaten or garlic juice is drunk (Pan Noev, 1932). Experimental and clinical studies confirm the ancient experience of people regarding the role of garlic in the prevention of cardiovascular diseases. Garlic reduces cardiovascular risk, decreases low-density lipoproteins (LDL), inhibits thrombocyte aggregation and has hypotensive, antioxidant and antibacterial effects (Ginter, Simko, 2010; Modern phytotherapy, 1982; Banerjee et al., 2002; Mousa, Mousa, 2007). The cardioprotective action of garlic in the form of food is presumed to be mediated via the generation of hydrogen sulphide (H2S). Experimental studies confirm the effect of garlic as an inhibitor of the rate of progression of coronary calcification (Ginter, Simko, 2010).

3.4. Hypertension

Hypertension is an important risk factor for CVDs. High arterial pressure (AP) is ranked first among the 26 most common risk factors as a cause of death in all regions of the world (Torbova et al.). Elevated blood pressure is the most frequent, easily detectable and reversible risk factor for myocardial infarction, apoplexy, cardiac insufficiency, auricular fibrillation, aortic dissection, atherosclerosis (Freedman, Cohen, 2016; Daskalov, et al., 2008). Hypertension is said to be present in cases of systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥90 mmHg (Tabassum, Ahmad, 2011). Elevated blood pressure is categorized by types: primary (essential) and secondary hypertension, which is due to identifiable causes such as diabetes and lesion of the kidneys, etc. (Sara et al, 2016).

In hypertension, garlic (Allium sativum L) is used as food (Pan Noev, 1932) in BTM. The therapeutic effects of garlic have been known for centuries in different cultures (Qidwai, Ashfaq, 2013, Frishman et al., 2009). The hypotensive effects of garlic are related to several mechanisms (Shouk et al., 2014). They are grounded on the biologically active substances contained in garlic – allicin, S-allylcysteine, diallyl disulfides, diallyl trisulfides, methyl thiosulfonate (Ried et al., 2013, Banerjee et al., 2002; Qidwai, Ashfaq, 2013). The hypotensive effect of garlic is explained by some authors as resulting from the influence upon the NO-ergic mediator system (NO is a potent vasodilator) (Banerjee et al., 2002; Mousa, Mousa, 2007), and the inhibition of ACE* activity (Mousa, Mousa, 2007; Sendl, et al., 1992). In BTM, decoction of common horsetail (Equisetum arvense L.) was regarded as a good remedy for high blood pressure (Materials for Bulgarian Botanical Guide, 1939). It is for the same purpose that our people also applied decoction of wallpepper (Sedum acre L.), its hypotensive effect being proven in experimental studies (Modern phytotherapy, 1982).

The favourable effect of medicinal plants in hypertension, atherosclerosis and other vascular complications is connected with their antioxidant activity (Montezano, Touyz, 2014).

3.5. Atherosclerosis

Atherosclerosis is a disease of medium and large arteries, which occurs under the action of numerous factors – genetic, behavioural, environmental. It is clinically manifested as coronary (ischemic) disease of the heart, cerebrovascular and peripheral vascular disease. In atherosclerosis (General Practitioners' Guide..., 2006) the elevated cholesterol is deposited in the connective tissue of arterial walls. In BTM, decoction of wallpepper (Sedum acre L.) was applied against atherosclerosis (Materials for Bulgarian Botanical Guide, 1939). Some authors confirm that woll pepper was used in atherosclerosis by other people as well (Duke, 2002). In BTM, decoction of common horsetail (Equisetum arvense L.) was regarded as a good remedy for atherosclerosis (Materials for Bulgarian Botanical Guide, 1939). According to some authors, common horsetail has antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory, antidiabetic, antitumor, cytotoxic and anticonvulsant effects (Asgarpanah, Roohi, 2012). In BTM, raw garlic (Allium sativum L) in the form of food is also recommendable in atherosclerosis (Pan Noev, 1932). Garlic decreases low-density lipoproteins (LDL) and has a favourable effect in atherosclerosis (Ginter,

* Angiotensin converting enzyme

Simko, 2010). In BTM, rue (100 medicinal herbs); decoction of hawthorn blossoms (Crataegus monogyna Jacq); woodsorrel (Oxalis acetosella L.); mistletoe (Viscum album L.) (1—2 grains three times a day because of its toxic action) (Materials for Bulgarian Botanical Guide, 1939) were also applied in atherosclerosis.

3.6. Pains in the heart – most probably, they relate to acute coronary syndrome (Alexander et al., 2007; Alexander et al., 2007), arrhythmias.

In cases of pains in the heart, BTM recommended drinking decoction of wormwood (Artemisia absintium, L), rosemary (Rosmarnus officinalis L.), common horsetail (Equisetum arvense L.) (Materials for Bulgarian Botanical Guide, 1939).

3.7. Oedema (Swelling).

A disease in which liquid is gathered and accumulated in tissues and body cavities (Materials for Bulgarian Botanical Guide, 1939), one of the symptoms of chronic cardiac insufficiency (CCI) (Cardiology, 2010).In BTM, the following plants with diuretic effect were used in oedema (Modern phytotherapy, 1982): silver birch (Betula pendula L), (Karamitrev, 1934; 100 Medicinal Herbs), wild strawberry (Fragaria vesca complex), common juniper (Juniperis communis L.), (100 medicinal herbs), common horsetail, decoction of the root, leaves and blossoms of asparagus (Asparagus officinalis L.), decoction of sweet woodruff (Asperula odorata L.), kidney beans (Phaseolus vulgaris L), common dandelion (Taraxacum officinale Web) (Materials for Bulgarian Botanical Guide, 1939), pheasant's eye (Adonis vernalis L) (Pan Noev, 1932), common speedwell (Veronica officinalis L.), elecampane (Inula helenium L), live-ever (Helichrysum arenarium DC.), rosemary (Materials for Bulgarian Botanical Guide, 1939).

3.8. Medicinal plants BTM used in inflammation of veins

In phlebitis, BTM applied tinctures of crushed fruits of horse chestnut (Aesculus hippocastanum L.) in alcohol (Materials for Bulgarian Botanical Guide, 1939). Obviously, it is the seeds of horse chestnut and not its fruits that are envisaged here. In contemporary phytotherapy, horse chestnut is applied in varicose symptom complex (Modern phytotherapy, 1982).

3.9. Pirin tea (ironwort), (Sideritis scardica Griseb) is an endemic plant in the Balkan Peninsula. It is applied in stenocardia (Modern phytotherapy, 1982). According to contemporary published data, the plant has an antioxidant effect (Koleva, 2007; Kratchanova et al., 2010), which is attributable to the polyphenol compounds contained therein (Kratchanova et al., 2010).

4. Conclusion

In traditional medicine, herbs are applied on the basis of centuries-long human experience in the treatment and prophylaxis of diseases. As for the treatment of diseases of the cardiovascular system, the people empirically used medicinal plants the pharmacological activity of which is proven today through experimental and clinical studies. Thus far, science has not discovered the mechanism of pharmacological action of a lot of the herbs that people used. The researches in this direction are still going on. Bulgarian herbal therapy makes its contribution to world cultural heritage.

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