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The Vasugan Swamp peats (Tomsk region) as a promising source of organic matter with antioxidant properties

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Abstract

A review of studies which evaluate intensity and mechanisms affecting the antioxidant activity (AOA) of humic acids (HAs) was conducted. Based on colorimetric assessment of HAs, high antiradical activity (ARA) of AO has been detected. The mechanism of this action is most likely related to the ability of HAs to act as free radical “traps”. According to EPR-spectroscopy results HAs contain stable semi-quinone type radicals in the matrix, which are “traps” for free short-living and stable radicals. The novel results of the nitro-blue tetrosol test (NBT-test) have revealed a direct antiradical effect of HAs on superoxide anion radical in a wide range of concentrations, which enables to recommend a specific biological test making use of NBT with non-enzymatic generation of superoxide anion radical for AOA evaluation in HAs. For all HAs samples, high levels of AOA were determined by the voltammetric method as well.

Keywords: Humic acids, antioxidants, peat, antiradical activity;

1. Introduction

The existence of all living organisms is associated with molecular oxygen absorption and utilization by the mitochondrial respiratory chain [16, 13]. This is the inherent stage of different substances metabolic transformations. During the sequential addition of an electron to oxygen molecular form (triplet), a pool of intermediates is being formed: superoxide anion radical, hydroperoxide, hydrogen peroxide and hydroxyl radical, which are united by a common term — reactive oxygen species (ROS). On the one hand, they can act as triggers of many physiological processes such as an intracellular signal transmission or the biologically active substances formation, etc. On the other hand, under extremal conditions of ROS concentrations exponential growth, essential substances such as lipids and carbohydrates can turn into targets of their action at the cellular level. This effect causes chemical modification and destruction of biomolecules. The imbalance between rates of synthesis and utilization in ROS leads to "oxidative stress" [16, 13]. For the pharmacological correction of causes and effects of oxidative stress natural and synthetic antioxidants (AO) of both direct and indirect action are widely used.

The detection of the new substances capable of AO activity is a relevant area of preclinical and clinical studies, since they maintain the integrity and activity of molecular lipid and protein assemblies in the body. Their cytoprotective action results in many pharmacological effects: cardiovascular and hepatoprotective, adaptogenic, antisclerotic, antidiabetic etc. In addition, AOs can effect on neurodegenerative and aging processes. Therefore, the evaluation of AO-activity of any active pharmaceutical substance should be an integral part of every pharmacological study due to leading role of AO-activity as a source of many mechanisms of biological activity implementation.

The historical significance of oxidative stress pharmacotherapy with drugs of natural origin has reduced only recently due to the advent of highly effective synthetic drugs. Consequently, the problem of iatrogenic diseases directly caused by side effects of synthetic drugs on the body became acute several decades ago. Meanwhile, drug preparations of natural origin are ontologically more tropic to the human body; subsequently, they hardly manifest any xenobiotic properties and, due to this fact are much more tolerable, when high doses are being used.

Natural products of humification (peat, sapropel, mummy, leonardite) are widely used to develop drugs for various purposes. Peat is an organic soil formed as a result of incomplete decay and humufication of bog plants (and humification) in high moisture conditions. The organic matter in peat soils is composed of humic substances (HB) (up to 40%), lignin, polysaccharides, lipids, pectins, hemicellulose and cellulose [12].

Considering the fact that the Vasyugan swamp, which contains Russia's second-largest peat deposit, is located in Tomsk Oblast, there are significant prospects for conducting studies on pharmacological effects of HAs and their practical applications. The issue is relevant from economical and ecological point of view, since HAs have a great positive impact on living systems, and are currently studied as an interdisciplinary field within the scope of pharmacy, medicine, chemistry, agriculture.

Thus, the purpose of the study was to analyze the potential of Vasyugan peats as a source of innovative drug with high AO properties for medical use.

2. AOs classification

Over the past decades, many substances capable of AO-activity which revealed their properties through various mechanisms of anti-radical action have been isolated and described, thus making a systematic AO classification necessary. Nowadays, there is no uniform classification of AOs. However, among those proposed the most interesting are the following.

All AOs were classified into preparations of indirect and direct action. In addition, AOs were divided by their origin into two groups of enzymatic and non-enzymatic. Non-enzymatic AOs are endogenous substances (coenzyme Q10, glutathione, α-lipoic acid, etc.) and exogenous - vitamins A, C, E, carotenoids, polyphenols (flavonoids) with their synthetic analogs (low molecular weight compounds (ubiquinone, glutathione)), trace elements (selenium) [4, 6].

AOs of indirect action show their activity *in vivo* and they are ineffective *in vitro*. They demonstrate the ability to stimulate an antioxidant system and are able to reduce the intensity of free-radical oxidation (FRO). They can be characterized by the following mechanisms: stimulation of activity or reactivation of antioxidant system enzymes, the inhibition of processes that lead to the accumulation of ROS, the shift of FRO processes towards the formation of less reactive compounds, etc. [15].

Directly acting AOs have pronounced antiradical properties which can be determined by *in vitro* tests. Most drugs with antioxidant effect belong to this group [15].

The most universal classification is based on the AOs chemical structure, according to which all AOs are divided into 5 main categories: proton donors, polyenes, catalysts, radical "traps" and complexing agents [8]. To the first group of AOs (proton donors) belong substances that have a mobile hydrogen atom. They neutralize free radicals by the following reaction: $AN + X\bullet \rightarrow A\bullet$, where AN is an AO with a mobile hydrogen atom and $X\bullet$ is a radical initiator or FRO intermediate product. Their AO action is due peroxo- (ROO *) and alkoxy radical (RO *) reduction. The second group of AOs comprises polyenes containing several multiple bonds in the structure; when they enter the medium, they become substrates for the ROS oxidation, thereby preventing damage to biomolecules. The third group of AOs includes complexing agents which cause binding of metal cations (which lead to ROS formation and biomolecule alteration). The fourth group of AOs is catalysts (or the "enzymes imitators"). They increase the activity of endogenous antioxidant systems (glutathione peroxidase and superoxide dismutase), provided glutathione or vitamin C are present to stimulate their catalytic activity. AOs in the fifth group are radical “traps”. They can be accumulated selectively in the matrix of mitochondria with effectively reduction by the mitochondrial enzymes.

3. Brief description of natural AOs

As noted above, plant species are considered as the most promising sources of AOs [10]. The overwhelming majority of natural AOs from plant sources are phenol compounds by their chemical nature. Currently, several thousands of phenolic compounds have been isolated, among which vitamins E and K, ubiquinones, tryptophan and phenanaline, as well as the majority of plant (flavonoids, phenocarboxylic acids) and animal fragments have a pronounced antioxidant effect [1]. Due to the ability to easily donate and capture electrons phenolic antioxidants can be referred to as “reducing agents”.

Among such promising candidate drugs of plant origin capable of AO-activity there are high-molecular compounds of caustobioliths, in particular, peat HAs.

4. Brief description of humic acids

HAs are natural high-molecular amphiphilic amphoteric organic nitrogen-containing randomized redox heteropolymers of arylglycoprotein nature, characterized by colloid properties and inconstancy of the chemical composition, different molecular weights. These are polyfunctional polyampholytes, which are acid-base complex with pronounced reducing properties [3].

Peat HA is an acid-base complex with properties of anions, cations, protons and electrons donors and acceptors. They can participate in various redox reactions, enzymatic-substrate interactions and detoxification of xenobiotics, osmotic pressure affection. They also take part in the formation of complex compounds (including chelated ones) with some biophilic elements and in other processes [14]. The listed features determine not only a wide variety of chemical properties of these acids, but also a wide range of their biological activity. Despite the presence of a significant amount of common elements in the structure, the biological activity of HAs obtained from different peat deposits may vary significantly. That can be explained by the high-degree polymorphism of their chemical structure [11].

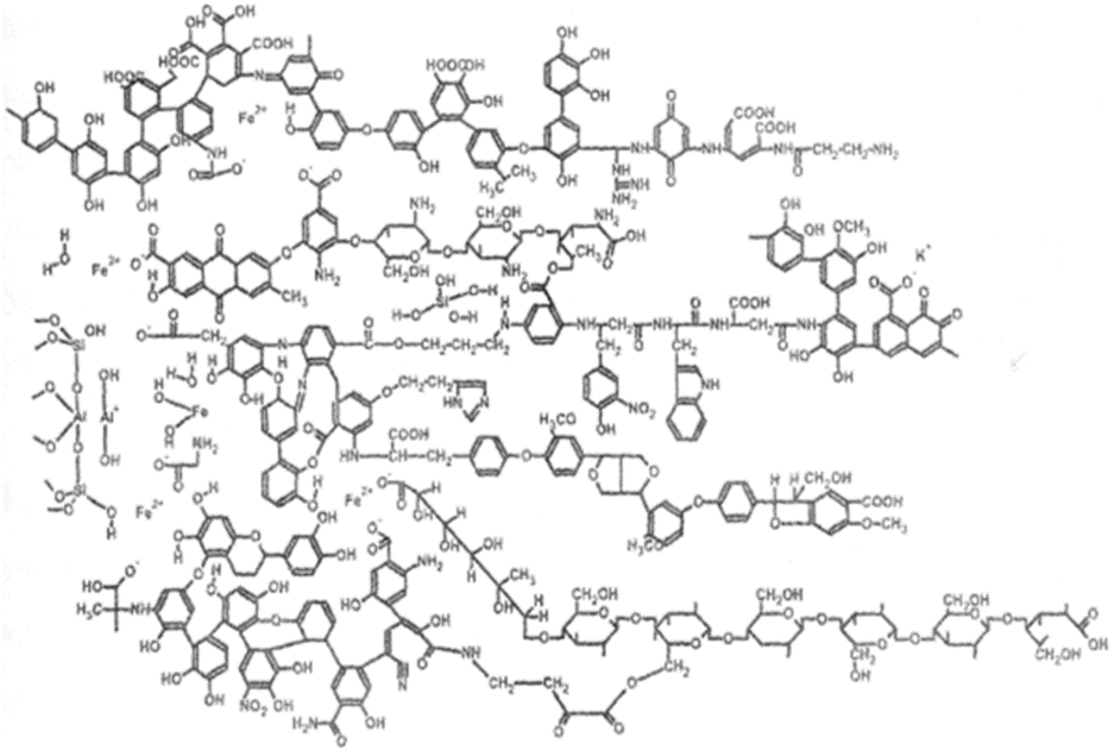

Fig. 1. Hypothetical structural fragment of soil HAs according to Kleinhempel.

5. Review of conducted studies on AO properties

Antioxidant action is one of the important properties of humic substances since it acts as the mediating mechanism in many of their biological effects. AO action of humic substances is been realized due to their reducing properties [17], ability to capture the free radicals directly, participate in the endogenous antioxidant reduction and iron-chelating effect.

There are a large number of methods to study AO-activity, which differ in the type of oxidation source and compound, as well as the method of the oxidized compound measuring. For example, there are spectroscopic and photometric (electron paramagnetic resonance, colorimetry, etc.), electrochemical (voltammetry using different electrodes), fluorimetric, chemiluminescent, volumetric and specific biological tests (evaluation of the antioxidant enzymes and malonic dialdehyde activity). Most of the methods are based on model oxidation reaction that proceeds according to a free radical mechanism with subsequent detection of the test substance effect on reaction behavior.

Colorimetric determination with a stable free radical

In 2018, Zykova et al. presented the results of the colorimetric assessment of AO-activity in humic acids. A colorimetric evaluation of antiradical activity in HAs over in time was carried out based on the reaction of interaction with the free stable diphenylpycrylhydrazyl (DPPH) radical. A series of experiments has shown that all the studied HAs have pronounced ARA, and their radical-binding activity (as a percentage of the DPPH radicals "death") is close to levels of reference preparation (dihydroquercetin). In general, it can be noted that all the studied samples of peat HAs have ARA. The mechanism of this action is most likely determined by the ability of HAs to act as free radical "traps" [18].

EPR spectroscopy

Another method for antiradical activity evaluation is the quantitative determination of PMCs (paramagnetic centers) using the EPR spectroscopy. This method allows to accurately register the number of free radicals. In particular, HAs contain stable semi-quinone type radicals in the matrix which are “traps” for free short-living and stable radicals. The same authors showed that all absorption spectra have a relatively symmetric singlet line with the g-factor of their spectroscopic splitting close to the semi-quinone type radical g-factor and indicating the existence of a strongly delocalized molecular that is a characteristic feature of HAs [5].

NBT-test

In recently published materials (2019) we presented the results of the specific biological test using nitro-blue tetrosol (NBT) with non-enzymatic generation of superoxide anion radical, which was not previously described in the literature sources for the assessment of HAs AO activity.

The study of antiradical activity in response to superoxide anion radical, with NBT being reduced to formazan, has shown that HAs interacted with the superoxide anion radical in a wide range of concentrations. Thus, we were the first who adapted the NBT-test method to the products of a peat group. At the same time, the NBT-test results indicate that the studied HAs interact with the superoxide anion radical in a non-enzymatic model system. It has also been detected that HAs had a direct antiradical effect on superoxide anion radical in a wide range of concentrations, and a medium-effective HAs concentration has been determined for each sample of peat. Thus, the obtained results make it possible to recommend a specific biological test, which uses nitro-blue tetrosol (NBT) with a superoxide anion radical of non-enzymatic generation for the evaluation of AO-activity in HAs [9].

Voltammetric method in AOA

In 2018, we also presented the results of the voltammetric method applied to determine AO-activity in HAs. This method evaluates the AO-activity of natural compounds and takes into account their effect on the electrochemical O_2 reduction (ER O_2) kinetics. Therefore, it enables to suggest the mechanisms of ROS interaction with various biologically active substances in model systems similar to oxygen reduction in a living cell.

It was shown that all the studied HAs samples can be characterized by the same voltammograms form. They show a manifestation of catalytic activity in response to the ER O_2 process. Thus, it can be noted that the presented results of the HAs catalytic activity assessment are similar to the EPR spectroscopy data. Differences in AO properties of HAs do not depend on the peat etiology significantly [2,5].

For all HAs samples high levels of AO-activity have been determined. A possible mechanism of AO-activity in HAs can be associated with the ability of quinoid group to initiate the ER O_2 process due to the presence of a large number of phenolic groups [5].

6. Discussion

Molecular oxygen absorption and transformation are necessary for activity of all living organisms. However, cellular respiration is associated with the ROS formation that performs different functions in living systems. The endogenous ROS inactivation leads to “oxidative stress”, which is the most important pathogenetic factor in many acute and chronic diseases.

With respect to all the mentioned above, the detection of new substances with AO-activity is of particular relevance. The determination of AO-action mechanisms is a key stage in AO-based synthesis of any pharmaceutical substance.

It is generally recognized that the pharmacologically active substances of plant origin have a number of hardly overestimated advantages over synthetic ones due to a more pronounced tropism to the human body. One of the most promising groups of such candidate substances in the new AO drugs development is peat HAs. The geographical location of the Vasyugan swamp in Tomsk Oblast provides favourable conditions for studying AO activity of the accumulated HAs, which, in turn, determines the economic importance of such work.

HAs are a class of natural high-molecular amphiphilic amphoteric organic nitrogen-containing compounds with the properties of colloids. They are also characterized by complex acid-base and reducing properties, which determines a wide range of their biological activity.

In recent years, many studies have been conducted mainly to trace the presence and determine the mechanisms of HAs specific AO-activity using modern physicochemical, electrochemical and other methods.

Based on colorimetric evaluation of HAs antiradical activity, the experiments conducted in 2018 by Zykova et al. showed that the levels of AO-activity are comparable with those of dihydrodroquercetin. Dihydrodroquercetin is one of the most important drugs within the group of antioxidants and antihypoxants. Chemically, this is a bioflavonoid and it has a registration certificate valid in the territory of the Russian Federation. Dihydroquercetin is being used as a reference preparation (comparator) in evaluating AO properties of pharmaceutical substances in model systems. In addition, positive results were obtained by experiments using the EPR spectroscopy method. The emphasis should be laid on the indicated ability of the HAs to act as “traps“ for free short-living radicals. Over the recent years, many researchers have shown that up to 2/3 of DNA damage in human cells is caused by free radicals that exist for a short period of time: nanoseconds, microseconds [7]. Such radicals are responsible, in particular, for tumor suppressor genes damage, and their action makes a significant contribution to carcinogenesis.

Aseries of studies has shown the specific AO-activity of HAs in response to the superoxide anion radical of (obtained in a) non-enzymatic origin. It is known that superoxide anion radical is a key active form of oxygen and is generated by the attachment of an electron to an oxygen molecule in the ground state. From a physiological point of view, the ability of superoxide anion radical to interact with cell proteins containing iron-sulfur clusters (aconitase, dehydrogenase succinate, etc.) with their subsequent damage and the formation of more reactive radicals is a significant negative feature. The ability of HAs to inactivate superoxide anion radical in a wide range of concentrations was revealed in the experiments.

In addition, the obtained results were confirmed by recent studies on the HAs AO-activity using the voltammetric method, which is recognized as one of the most effective and highly sensitive. Specific OA-activity was shown for peroxide radicals. An important result of this study was detection of one possible mechanisms of anti-radical activity due to the ability of quinoid groups in the HAs structure to initiate the ER O_2 process. Quinones are the only class of compounds capable to interrupt the chain process of free radical oxidation not only due to interaction with free oxygen radicals, but also by binding alkyl radicals. This finding indicates that AO-activity in HAs is possible in the course of free radical oxidative processes under conditions of reduced oxygen.

Thus, the results of AO activity studies make evident the great potential of HAs as candidate substances for developing new drugs within the antioxidant group for medical uses. Despite the fact that earlier in the USSR, HAs-based drugs were widely and efficiently used in medical practice (biogenic stimulators: Gumisol, Torfot, FIBS, Peloidistillate), today there are no registered HAs-based drugs in the Russian Federation. That shows emphasizes the opportunities and prospects for further research in this area. The current situation can probably occur due to obvious problems with the standardization scheme for this class of substances owing to the complexity of their structure. It is also necessary to conduct further experiments to detect AO mechanisms of HAs action, as well as their possible toxic effects on model systems *in vitro* and *in vivo*. All the mentioned above indicates the relevance of studying HAs properties and reveals a wide area for both fundamental and applied research.

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