Adaptogenic effects of essential oils: prognosis in vitro and results in vivo

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Abstract. The results of a two-week in vivo experiment confirmed the adequacy of the in vitro prognosis about the possibility of oxidative balance correction. The prooxidant effects of cedar, orange and lavender essential oils (by inhalations) on the free radical production by blood phagocytes were investigated using a chemiluminescent (CL) test. The results obtained in vivo are in good agreement with those obtained in vitro models. Consequently, with the help of express CL analysis, it is possible to prognose the direction and intensity of changes in the free radical balance of the internal environment of the body under the influence of essential oils. Two conditions are necessary for such a forecast. The first, preliminary definition of the functional status of the blood phagocytes using express CL analysis is required for an individual person. Secondly, information is required about the antioxidant or prooxidant potential of essential oils, which the person has chosen when he oriented on organoleptic parameters or objective recommendations. Individual selection of essential oils as biologically active metabolic regulators will avoid the risk of unwanted side effects in aromatherapy. Using this approach, manufacturers and distributors and consumers receive a preliminary assessment of the antioxidant and prooxidant activity of essential oils.

1. Introduction
Dysfunctions of blood phagocytes are early forms of homeostasis disorder. These violations are of preclinical nature. Without adequate correction, these disorders form the base of different diseases. Such dysfunctions are reversible if early diagnosis and adequate correction will be carried out [1].

«Lazy phagocyte» syndrome was found in healthy people with a frequency of 6% [2]. This is the functional state of blood phagocytes when the rate of production of biogenic free radicals is several times lower than normal. Such dysfunction is professionally conditioned; it is most often found in IT-specialists [2]. Food adaptogens and aromatherapy are the most physiological methods for the correction of oxidative homeostasis. However, the results of the use of adaptogens are not always positive. There are many facts of food poisoning, allergies and other disorders under the influence of adaptogens [3]. The poor quality of adaptogens, their falsification, the wrong dose can be the reasons, but not only that. Great importance is the inadequate selection of adaptogens without taking into account the biological characteristics of the metabolic status of an individual consumer.

To optimize the individual selection of adaptogens, the prediction of their effectiveness in vitro can be used. For this, a modification of the Fenton model can be used, which allows extrapolating the results in vitro results to the level of the organism. The model is a reaction medium containing human
blood phagocytes as a natural source of biogenic free radicals [4]. Using chemiluminiscence (CL) analysis, one can measure the initial reactivity of blood phagocytes and its changes under the influence of an adaptogen. The method is based on the conversion of chemical energy of reactive oxygen species (ROS) into light energy and automatized counting of quantums which are formed [5]. Using this way, it will be possible to optimize the selection of individually significant substances in physiological doses and to reject substances with an undesirable effect.

Previously, using the Fenton and other chemical, subcellular and cellular experimental model systems, the antioxidant and prooxidant properties of the number essential oils were tested. Thus, the prognosis of biological effectiveness of essential oils as objects of progressive consumer demand was obtained [6]. To confirm this prediction, it is necessary to carry out the experiment in vivo.

The purpose of this work was to compare the adaptogenic ability of essential oils in vitro and in vivo. The tasks of the work included testing the survival of blood phagocytes in reaction with essential oil in vitro, prognosis the adaptogenic effects of essential oils in vitro, and evaluating the effect of essential oils on CL of human blood under the influence of essential oils in vivo.

2. Materials and methods
Commercial essential oils of orange, cedar and lavender from the company Aspera's LLC (Russia) were tested, which were obtained by hydrodistillation. The use of these oils is not dangerous to health. These substances are alimentary components and under their influence no cases of allergies have been identified [7]. Under influence these essential oils, antioxidant or prooxidant effects in the experimental models was fixed [8]. Essential oils with the most stable influence on the ROS production of blood phagocytes regardless of the experimental model were selected for in vivo research. Orange essential oil had the properties of a universal prooxidant, cedar oil was a hydrophobic pro-oxidant, lavender essential oil had a modulating effect.

Healthy volunteers (15 women, 25–45 years old) who had a hyporeactive type of redox balance of blood took part in in vivo studies. When phagocytic CL was analyzed, a blood sample was taken from a finger. The studies were conducted in compliance with the requirements of bioethics and human rights (UNESCO, 2005). Volunteers were divided into three groups (n = 5). In each group, one of the essential oils was used for 10-minute cold inhalations twice a day (in the morning and in the evening) for two weeks. When choosing a substance, the preferences of volunteers were taken into account. Two weeks later, the volunteers were retested with CL and the evaluation of the effect of the drugs in vivo was carried out. The antioxidant or prooxidant properties of the samples, as well as the blood redox balance of volunteers, were measured using the “Biochemiluminometer 3606 M” software and hardware complex. The device operates in the photon counting mode and allows studying the kinetics and parameters of oxidative reactions. The intensity of CL was estimated using the parameters of the light sum S (million impulses); peak height I (imp sec); peak time, T (sec). The kinetic parameters were automatically recorded and archived as a database. The method of analysis is described in detail [8].

To test the survival of blood phagocytes under the influence of substances, selective molecular probes luminol and lucigenin and a suspension of pine essential oil were used (load test, in vivo was not used). Each sample was analyzed at least three times. Statistical processing was carried out using Wilcoxon T-criterion for related samples (the distribution of sample data was normal; the variances were comparable) with a confidence of 0.05.

3. Results and discussion
3.1. Phagocyte survival testing
All immunocompetent cells are highly sensitive to chemical exposure. Therefore, the survival of hyporeactive phagocytes under the influence of essential oils was preliminarily tested. For this, a sample with unstimulated phagocytes after registration of the CL kinetics under the influence of pine essential oil was incubated for 90 minutes with a stimulating agent (latex). In the event that
phagocytes lost vitality, they would be incapable of a “respiratory explosion” under stimulation and if you repeat the CL analysis, the light sum would be sharply reduced. On the contrary, if the functional potential of phagocytes is not depleted under the influence of essential oil, the light sum will increase. The results of the experiment are shown in figure 1.

In presence two molecular probes (lucigenin or luminol) it is possible to control the production of primary and secondary (superoxide or hydroxyl radical, respectively) ROS. As the results showed, the cells retain their viability and generate both primary and secondary ROS in spontaneous and enzymatic processes. In the presence of lucigenin, the production of primary ROS by previously unstimulated phagocytes (CL₂) was higher than the CL₃ level. Thus, the influence of the essential oil does not break the functional and metabolic processes of the phagocyte, which produces radicals. Radical-directed effects of essential oils are not artifacts, but reflect the adaptive compensatory and adaptive responses of the phagocytes.

3.2. Adaptogenic effects of orange, cedar and lavender essential oils in vitro
Under the influence of orange, lavender and cedar essential oils, the production of ROS by hyporeactive blood phagocytes in vitro increased (figure 2).

Ascending prooxidant action, the essential oils were arranged in the following order: lavender – cedar – orange. The effect increased inversely with the dose. According to these results, these substances were selected for the experiment in vivo.

3.3. Adaptogenic effects of orange, cedar and lavender essential oil in vivo
Under the influence of orange essential oil, the strongest correction of the function of hyporeactive phagocytes was observed (figure 3). The background activity of phagocytes in these probands corresponded to the “presence” status according to the classification of M. Magrisso [9].

This status corresponds to the ability to generate ROS in processes when free radical metabolites are formed primarily extracellularly, although phagocytic activity is generally insufficient. Under these conditions, the threat of destruction of the cell membranes of other cells is formed.
Under the influence of orange oil inhalation, the nature of the kinetics changed significantly. The peak height increased 2.4 times and came near the lower level of the norm which was established for healthy people (2500...3500 impulses /sec). Symmetry of kinetics was corrected. On the starting kinetogram, the peak of the activated chemiluminescence was shifted to the left so strongly that only its final phase was recorded (figure 3, curve 1). This reflects dysregulation of phagocytic function. Under the influence of orange essential oil, the peak time was corrected (T = 39 min). The fact indicate balance of the production and elimination of free radicals began to correct. This balance is the base of an oxidative homeostasis.

The literature contains dissonant data on the effect of essential oils on the production of biogenic free radicals by blood phagocytes [10–12]. The results are agreeing with the fact that citrus fruits (but not essential oil) can be cause of food allergies [13]. Orange essential oil was a prooxidant regardless of the lyophilicity of the medium. Under the influence of it, the production of both primary and secondary radicals increased. Excessive stimulation of phagocytes does not form a defense, but endogenous aggression. But in the case of low activity of phagocytes, their function can be adjusted with essential oils. Thus, adaptogenic properties of EM orange appear only with the correct use of the substance.

Similar changes in the redox balance of phagocytes were revealed under the influence of cedar and lavender essential oils (figure 4, 5).

As results shown, the CL response kinetics, not the intensity, was optimized. The height of the peak increased in 4 (lavender) – 8 (cedar) times, but did not reach the normal level yet. Apparently, a two-week inhalation period in this case was not enough to get significant change in blood redox status.
4. Conclusion
Under the influence of the essential oils the functional and metabolic processes of the phagocyte producing free radicals does not break. Using express CL analysis, it is possible to predict the direction and intensity of changes in the free radical balance of the internal environment of the body under the influence of essential oils.

The prognosis in vitro of the adaptogenic effects of the essential oils of orange, cedar and lavender was confirmed in vivo experiment. For a successful forecast, two conditions are necessary. First of them is a preliminary determination of the phagocytic functional and metabolic status. Secondary, information about antioxidant or prooxidant potential of essential oils is need. It's also important to take in consideration the organoleptic preferences of consumer. Individual selection of biologically active metabolic regulators such as essential oils will avoid the risk of unwanted side effects in aromatherapy. Using the described approach, manufacturers and distributors can pre-evaluate the antioxidant and prooxidant properties of essential oils.

This allows us to recommend such adaptogens with a high guarantee of success. In the other hand, consumers will be able to choose and use adequate adaptogens, taking into account the individual state of free radical balance of the internal environment of the body to prevent decrease of adaptation potential. The results can be useful for the medicine and prophylactic departments, as well as in domestic and industrial premises.

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