Regulatory effect of essential oils on the production of primary and secondary biogenic free radicals in vitro

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Abstract. Using molecular enhancers of chemiluminescence, the regulatory effects of several essential oils on the production of primary and secondary biogenic free radicals in vitro were investigated. For the more correct extrapolation of the results, the human blood phagocytes as biogenic source of free radicals was used in an experimental model during latex stimulation and without it. The antioxidant activity of pine essential oil against primary and secondary biogenic radicals was identified, and the prooxidant activity of eucalyptus essential oil against primary and secondary biogenic radicals was determined. Unlike both of these substrates, the modulating effect of lavender essential oil was identified. Under the influence of this substance luminol-dependent chemiluminescence increased, while lucigenin-dependent chemiluminescence decreased without changing the basal activity of phagocytes. Essential oils of lemon and peppermint acted as radical-specific antioxidants. Under the influence of peppermint essential oil, the production of primary not secondary radicals decreased significantly. Under the influence of lemon essential oil, the production of secondary radicals decreased while production of superoxide did not change significantly. Based on the results are obtained, it is possible to develop or adjust the schemes of health-improving and preventive procedures, as well as to optimize the choice of adaptogens chemical forms.

1. Introduction
The influence of natural adaptogens on the body varies widely. Not only the sanitizing and bactericidal effects, allergenic ones can also be observed [1]. In order to effectively use adaptogens, it is necessary to obtain more information about their reactions with ROS. Biogenic radicals are formed in a certain sequence. The primary type of ROS is the superoxide anion radical (O$_2^-$, superoxide).
oxygen molecule with an unpaired electron. All other ROS are its derivatives (figure 2), these include hydrogen peroxide ($\text{H}_2\text{O}_2$), hydroxyl radical (OH•) and singlet oxygen ($^1\text{O}_2$). Among the secondary ROS, the hydroxyl radical dominates quantitatively. It’s the product of spontaneous and enzymatic superoxide transformations. In the ROS series, lyophilic properties increase from superoxide to its derivatives [3]. So it’s necessary to obtain information about selective adaptogens taking into account their lyophilic properties.

Figure 1. Levels of homeostasis regulation.

Figure 2. ROS hierarchy and CL-enhancers targets.

Unlike most other adaptogens, essential oils enter the body in two ways, alimentary and respiratory. According to the marketing company BusinesStat [4], among the products with the highest consumer demand in Russia are essential oils of pine, eucalyptus, lavender, lemon and mint. At the same time, information on the effect of essential oils on the production of ROS in the blood is fragmentary and contradictory. On the one hand, the bactericidal effects of some essential oils are explained by their prooxidant action, although these effects are not highly resistant and are poorly reproduced in the experiment [5]. On the other hand, some essential oils have an antioxidant effect [6]. Perhaps this is the result of using irrelevant models as well as a deficiency of information about the selective reaction of substrates with ROS. So such information is necessary.

Redox balance is one of the causes of allergy [7]. The study of the molecular mechanisms of interaction of essential oils with biogenic ROS has a high relevance and practical significance. Therefore, it is necessary to identify the targets of such an interaction and establish its prooxidant or antioxidant character. Essential oils which are modulate production of superoxide may be promising redox regulators in a hydrophobic environment (air). In contrast, modulators of secondary ROS will be inert in a hydrophobic medium, but may be hight active reagents in hydrophilic medium (biological fluids).

Differentiation of essential oils according to these properties is necessary to do reasonably choose some of them for the sanation of the air environment of the home and others of them as functional food and profilactic drugs. The way to solve this problem is to use selective molecular CL enhancers: lucigenin (selectively reacts with superoxide) and luminol (selectively reacts with secondary ROS, primarily with a hydroxyl radical) (figure 2). During the reaction of ROS with CL-enhancers, chemical energy is transformed into light energy. By registering the light sum, one can separately estimate the dynamics of the production of primary and secondary free radicals. The purpose of this work was to distinguish between essential oils according to their selectivity and direction of influence on the production of primary and secondary ROS by human blood phagocytes.
2. Materials and methods

Commercial essential oils from «Aspera» (Russia) was used. Substances was obtained by hydrodistillation from the following substrates: Pinus sylvestris, Eucalyptus globulus, Lavandula vera, Citrus limon, Mentha piperita. To study the antioxidant and prooxidant activity of essential oils, a modified method of chemiluminescence analysis according to Tono-Oka e.a. (1983) was applied. To provide extrapolation of the results obtained in vitro to the level in vivo, the blood phagocytes of practically healthy people as biogenic source of ROS was used in the experimental model in vitro. The rate of ROS production by phagocytes was analyzed both under latex stimulation (load condition) and without it (basal condition). The measurements were carried out using the “Biochemi Luminometer 3606 M” soft and hardware complex, which operates in the photon counting mode to estimate the lightsum of oxidative reactions. The method of analysis is described in detail [8].

The experimental model was the water solution which included Hanks unpainted medium (1 ml), human blood suspension (100 μl, heparin 20 units / ml), essential oil suspension in dimethyl sulfoxide, DMSO (50 μl) in differ doses, latex suspension (20 μl) or equival volume distill water and CL-enhancer (luminol or lucigenin, 20 μl). Phagocytes were not isolated from whole blood because their quota in the blood is over 70% of the total number of circulating leukocytes and they are the exclusive source of free radicals in the blood.

The control was the level of chemiluminescent (CL) activity of stimulated or unstimulated phagocytes without the addition of essential oil. Antioxidant or prooxidant effect in presence of the sample was estimated by the magnitude and direction of lightsum change (millions impuls in second). The kinetic parameters were automatically recorded and archived as a database. Each sample was analyzed at least three times. Statistical processing was carried out using Student's criterion (the distribution of sample data was normal; the variances were comparable) at a confidence level of 0.95.

3. Regulatory effect of various essential oils on the biogen free radical production in vitro

3.1. Antioxidant effect of pine essential oil

Under the influence of pine essential oil, the decrease of the number of free radicals in the presence of both CL-enhancers in the reaction mix was obtained (figure 3).

![Figure 3. Influence of pine essential oil to activated (a) and basal (b) reaction under selective CL enhancer; *p < 0.05 under the control of Student's criterion.](image-url)

The production of both primary and secondary free radicals stimulated by phagocytes (CL_a) was inhibited. The basal activity of basal (CL_b) did not significantly change, either when using lucigenin or luminol. A significant decrease in luminol-dependent CL under the influence of pine essential oil on activated phagocytes was established. The effect increased in inverse proportion to the dose. Consequently, the components of the essential oil of pine have a higher chemical affinity for secondary ROS, primarily for the hydroxyl radical. This metabolite is much more chemically aggressive than superoxide radical. Since lucigenin-dependent CL was less inhibited to a compare with luminol-dependent one, it can be assumed that aerobic inhalations with pine essential oil will
have more physiological effect on the body's oxidative homeostasis. On the contrary, in the composition of the lyophilic compositions, the essential oil of pine may have a stronger antioxidant effect on the body, which is not always required. So, it can be concluded that pine essential oil has a stable antioxidant effect on the production of primary and secondary biogenic free radicals by phagocytes under activated, not basal condition.

3.2. Prooxidant effect of eucalyptus essential oil
Under the influence of eucalyptus essential oil, the increase of the number of free radicals in the presence of both CL-enhancers in the reaction mix was obtained (figure 4).

![Figure 4. Influence of eucalyptus essential oil to activated (a) and basal (b) reaction under selective CL enhancer; *p < 0.05 under the control of Student's criterion.]

The production of both primary and secondary free radicals by blood phagocytes (CLa) was accelerated. It is important that the prooxidant phenomenon was manifested not only under phagocytic stimulation, but also without it. It was previously established that some functional states and professional dysfunctions of the human organism are associated with the syndrome of "lazy phagocytes" [9]. Therefore, the discovered phenomenon shows that eucalyptus essential oil may be an effective adaptogen for increase the functional and metabolic reserve of phagocytes in basal conditions. This substance is effective in relation to both primary and secondary radicals. Prooxidant effects were observed throughout the entire dose range using both enhancers, but lucigenin-dependent CL was most expressed. Judging by the data obtained, superoxide is the more affinable target of this substance. Superoxide is the least aggressive biogenic radical which are formed not only in water, but also in air [10]. The data on the high reactivity of the essential oil of eucalyptus and superoxide is agreed with the facts of the high microbicidal property of this substance, as well as the well-known phenomenon of the general health-improving effect of eucalyptus forests [11].

3.3. Modulating effect of lavender essential oil
Under the influence of lavender essential oil, the modulating effect on the production of free radicals in the presence of both CL-enhancers in the reaction mix was obtained (figure 5).

![Figure 5. Influence of lavender essential oil to activated (a) and basal (b) reaction]
under selective CL enhancer. *$p < 0.05$ under the control of Student’s criterion.

The effect increased inversely with the dose. That fact confirms the high biological activity and regulatory capacity of the substance. It is evident from the data obtained that under the influence of lavender essential oil, luminol-dependent CL is increasing and lucigenin-dependent CL is decreasing. Based on the results obtained, the greatest physiological effect can be prognosis using the substance in hydrophobic mixtures as an anti-inflammatory agent. On the contrary, under hydrophilic conditions, this substance can play a prooxidant role, enhancing the microbicide activity of blood phagocytes while maintaining the level of basal activity.

There is information in the literature about the antioxidant properties of lavender essential oil. [12]. The data obtained in discussed study showed that the effect of this substance on the redox balance has more complex mechanism, including the ability to adaptively modulate the balance of biogenic radicals, which are formed by blood phagocytes. Lavender essential oil can be used as a component of functional nutrition in hydrophilic mixtures.

3.4. Selective hydroxyl-oriented antioxidant effect of lemon essential oil
Under the influence of a lemon essential oil, the selective antioxidant effect on secondary free radicals was found, since a decrease in the light sum of only luminol-, non-lucigenin-dependent CL was observed throughout the entire dose range (figure 6).

![Figure 6](image)

**Figure 6.** Influence of lemon essential oil to activated (a) and basal (b) reaction under selective CL enhancer. *$p < 0.05$ under the control of Student’s criterion.

Decreasing of luminol-dependent CL did not depend on the rate of production of ROS, it was observed during the stimulated and basal reactions. When lucigenin was used, fluctuations in the light sum were not statistically significant. Thus, lemon essential oil is a hydrophilic antioxidant and preferentially reacts with secondary ROS, but not superoxide radicals. The maximum biological effect can prognosis in case when the substance will be used for the prevention and / or reduction of inflammatory processes in the composition of hydrophilic mixtures.

3.5. Selective superoxide-oriented antioxidant effect of peppermint essential oil
Under the influence of peppermint essential oil, a selective antioxidant effect was found on primary, not secondary, free radicals, unlike lemon (figure 7).

![Figure 7](image)

**Figure 7.** Influence of peppermint essential oil to activated (a) and basal (b) reaction
under selective CL enhancer; *p < 0.05 under the control of Student's criterion.

Lightsum of CL reduced in 3...6 times under lucigenin only, not luminol over the entire dose range. This parameter decreased in the course of both stimulated and basal reactions, effect did not depend on the rate of ROS production by blood phagocytes.

When luminol used, the changes were not statistically significant. Thus, peppermint essential oil has a high chemical affinity for the superoxide radical, but not for its hydrophilic metabolites. Since peppermint essential oil selectively interacts with superoxide, the substance can be used as a hydrophobic antioxidant. The maximum biological effect can be prognosed when substance use in the composition of hydrophobic mixtures or spray to reduce the excess activity of blood phagocytes and to prevent inflammation as allergy stage [10].

4. Conclusion
Using molecular enhancers of chemiluminescence, the selectivity and nature of the influence of essential oils on the production of primary and secondary ROS by human phagocytes in vitro was detected. The antioxidant properties of pine essential oil in relation to both primary and secondary biogenic free radicals have been identified. In the contrast, the eucalyptus essential oil was characterized by a prooxidant effect, selective to primary hydrophobic superoxide anion radicals. Under the influence of lavender essential oil, luminol-dependent CL increased, while lucigenin-dependent CL decreased without changing the basal activity of phagocytes. This means that the substance has a modulating effect on the redox balance of the blood. Lemon and peppermint essential oils manifested radical-specific antioxidant effects. Under the influence of peppermint essential oil, the production of primary radicals (superoxide) decreased and the production of secondary radicals did not change significantly. On the contrary, under the influence of lemon essential oil, the production of secondary radicals decreased and the production of superoxide did not change significantly. Based on the results that were obtained, schemes of health and preventive procedures can be developed or adjusted, and the selection of adaptogens and their chemical forms can be optimized.

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