

## PROTECTIVE EFFECT OF ADAPTOGENS IN OXIDIZING STRESS

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As the title implies the article describes an experimental investigation of vegetable adaptogens influence of Euphorbia Fischer extract and tincture on hemostasis, endothelium dysfunction, lipid peroxidation and antioxidant protection activity, the investigation of lymphocytic thrombocytic adhesion in normobaric hypercapnic hypoxia, normoxia and experimental hyperhomocysteinemia. The experiment included 200 white laboratory rats (mean weight  $16 \pm 20$  gr.) engaged in the trial were divided into 9 groups. Medicines were injected intraperitoneally during 7 days, half of animals were subjected to hypoxia influence. The data obtained testify a marked antioxidant properties of medications under discussion from Fischeriana root. Euphorbia Fischer root medications inhibit lymphocytic thrombocytic adhesion and therefore they regulate aggregative thrombocytic properties. In hyperhomocysteinemia and hypoxia the most significant effect was observed in Euphorbia Fischeriana elixir intervention and it was more than 2 times higher than in Euphorbia Fischeriana tincture.

**Keywords:** adaptogens, Euphorbia Fischer, hemostasis, endothelium dysfunction, lipid peroxidation, antioxidant protection activity, the investigation of lymphocytic thrombocytic adhesion, normobaric hypoxia, hyperhomocysteinemia

One of the most significant mechanisms promoting ischemic heart diseases is endothelium dysfunction playing a causal role [9]. In modern cardiology oxidizing stress is a key process included in intracellular accumulation of free radicals strictly affecting endotheliocytes integrity and function [15]. Thrombocytes play a key role in inflammatory reactions, immunity and atherosclerosis development [6]. This process is realized by releasing proinflammatory mediators and expressing surface molecules that may contact with other blood cells and endothelium. Substances secreted and expressed by thrombocytes have a wide range of factors affecting intercellular adhesion, cells migration through vessels endothelium and are known to play an important role of specific signals for target cells [11, 14]. Adhesion interaction between thrombocytes and leucocytes ensures leucocytes discharge to foci of inflammatory and immune reactions. Different factors may initiate these processes including hyperhomocysteinemia and hypoxia [4].

Despite numerous studies in this field general principals of endothelium dysfunction correction were not defined and medications removing the results of oxidizing stress were not found [2, 3, 8]. An increasing demand for these medicinal plants and herbs is mainly associated with the fact that chemical nature of these plants and herbs is close to the human organism and they are easily included into biochemical processes of a human being acting gently and safely continuously regulating all vital processes of a man.

The aim of our study was to find out perspective medications from plants and herbs to correct endothelium dysfunction in experimental normobaric hypoxia and experimental hyperhomocysteinemia. In our experimental work we carried out complex evaluation of their effect on hemostasis level, lipid peroxidation processes and antioxidant protection,

endothelium function the investigation of lymphocytic thrombocytic adhesion in normoxia, hypoxia and experimental hyperhomocysteinemia. From all vegetable plants we selected remedies on the basis of Euphorbia Fischeriana Stend. of Zabaikalia. Euphorbia Fischeriana is used in the treatment of oncological diseases, tuberculosis, furunculosis, burn disease, chronic lung diseases, impotency, prostate adenoma, anemia of any genesis [1].

### Materials and methods of research

Euphorbia Fischeriana elixir and tincture were obtained from preliminary purified raw material from resin. Euphorbia Fischeriana elixir was obtained by a 4 stepwise manner of heat chloroform spirit elimination treatment (Krivosheeva E.M. et al., 2009). Euphorbia Fischeriana tincture was obtained by spirit elimination from vegetable raw material without heating and extract removal according to state Pharmacopoeia IX (1990).

200 white experimental rats (mean weight  $167 \pm 20$  gr.) engaged in the trial were divided into 9 groups to evaluate hemostasis, endothelium dysfunction, lipid peroxidation and antioxidant protection activity in normal state, in hypercapnic hypoxia and experimental hyperhomocysteinemia. The experimental hyperhomocysteinemia was obtained by intraperitoneal injection of homocystein solution of 0,001 mg/ml that is 0,1 ml of the total blood circulating volume. Hyperhomocysteinemia was confirmed by highly effective liquid chromatography. All experimental animals received the medications under study for 7 days. The doses of euphorbia Pallasii elixir and tincture were preliminary defined by acute toxicity and comprised 0,1 ml/100 gr of body weight intraperitoneally. The control animals were given the same quantity of isotonic sodium chloride solution. Hypercapnic normobaric hypoxia was designed by Kovalyev G.V., (1990) in pressurized camera [5].

The following techniques were used in this trial: theobarbituric acid test by Andreeva L.I., et al., (1988); chemiluminescence reaction by Vladimirov Yu.A. (1972); serum NO by Golikov V.V., (2004). Hemostasis activity was evaluated by unified techniques: – partly activated thrombin time [Larrien M.J., Weiland C., 1977]; prothrombin time [quick A., 1943]; thrombin time [Syrmai E., 1957]. Lymphocytic thrombocytic adhesion was determined in the following way: fresh heparinized blood

of the experimental animals formed layers on gradient urographin-phycol (density – 1,077) and discharged lymphocytes, was washed by phosphate buffer pH – 7,4), then calculated the number of lymphocytic thrombocytic coaggregators by 100 cells in Goryaeva camera in microscopy.

Statistical calculation of the data obtained was performed by Fisher-Student method using Statistica 5.5.

### Results of research and their discussion

Change in NO concentration is one of the markers of endothelium damage. NO level was evaluated by NO<sub>3</sub> quantity [2] as NO is chemically unstable compound existing only some seconds. In rats all substances under study caused the elevated NO<sub>3</sub> level in normoxia, the Euphorbia Fischeriana tincture raised NO level by 57%.

In hypoxia NO marked reduction was observed and was confirmed by 7 times decrease of NO<sub>3</sub> concentration. Euphorbia Fischeriana tincture in hypoxia demonstrated 13 times elevation of NO discharge. Euphorbia Fischeriana elixir did not effect endothelium function in hypoxia.

In hypohomocysteinemia there was 3,4 times NO elevation. In hypohomocysteinemia in normoxia condition Euphorbia Fischeriana tincture and elixir had a modulatory effect which resulted in NO reduction to the initial levels.

When evaluating hemostasis Euphorbia Fischeriana tincture and elixir were established to cause the extension of prothrombinase formation in intact rats. In hypoxia and experimental hypohomocysteinemia we observed the development of hypercoagulation. Under these conditions Euphorbia Fischeriana elixir returned all results of coagulogram to normal state but Euphorbia Fischeriana tincture statistically elevated thrombin time and partly activated thrombin time but they were not comparable to their norm.

The study of activity changes in system lipid peroxidation – antioxidant defense showed that Euphorbia Fischeriana tincture and elixir statistically decreased theobarbituric acid active products level in serum in normoxia. More manifested efficacy of the medications under discussion was determined in hypoxia. Thus, Euphorbia Fischeriana tincture reduced theobarbituric acid active products concentration by 86%, elixir by 71% compared with control. The decrease of theobarbituric acid active products hyperhomocysteinemia correlated with elevated antioxidant defense activity. So, according to chemiluminogram antioxidant activity elevated by 89% when injected Euphorbia Fischeriana tincture, elixir by 67% compared with control. And in a group of rats with hyperhomocysteinemia by 4-th day theobarbituric acid active products level in erythrocytes statisti-

cally elevated (5 times increase) compared with control ( $8,7 \pm 0,6$  mkmol/mg lipids) and became  $43,5 \pm 0,6$  mkmol/mg l ( $p = 0,001$ ) but by 6-th day reduced to  $6,7 \pm 1,3$  mkmol/mg l. When investigating theobarbituric acid active products blood serum the similar situation was noticed: by the 4-th day of experimental hyperhomocysteinemia their concentration significantly elevated to  $4,0 \pm 0,4$  mkmol/mg l ( $p = 0,034$ ) but by 6-th day reduced to control and composed  $1,7 \pm 0,4$  mkmol/mg l. But rate of serum elevation in theobarbituric acid active products is less emphasized compared with elevation of theobarbituric acid active products in erythrocytes. When analyzing active oxidant protection the following results were obtained. General antioxidizing serum activity by the 4-th day is statistically elevated to  $77,0 \pm 2,7\%$  ( $p = 0,038$ ) and by the 6-th day remains the same elevated level of  $78,3 \pm 1,0\%$  ( $p = 0,001$ ). In hyperhomocysteinemia elixir effect was more significant. Euphorbia Fischeriana tincture did not greatly change this level. Lymphocytic thrombocytic adhesion intensity in healthy intact rats was 11,2%. In 2 days of homocystein injections in experimental hyperhomocysteinemia lymphocytic thrombocytic adhesion index statistically lowered to 9,33%, on the 5-th day of it elevated to 78%, on the 7-th day it reached 93,5%. In hypercapnic normobaric hypoxia mean increase of lymphocytic thrombocytic adhesion index was by 10%. Drugs of Euphorbia Fisher roots effected unidirectionally both in intact rats and in hyperhomocysteinemia. The most significant effect was shown by Euphorbia Fisher extract. Thus, it lowered rosella formation by 46,5% (Euphorbia Fisher tincture reduced lymphocytic thrombocytic adhesion level by 26,5%), in hypoxia by 56,8% (tincture by 32,7%), in experimental hyperhomocysteinemia by 71% (tincture by 52%).

Reduced lymphocytic thrombocytic adhesion activity in normobaric hypercapnic hypoxia may be caused by decreased NO concentration which is known to inhibit lymphocytic thrombocytic adhesion [2]. Earlier we established that in normobaric hypercapnic hypoxia NO reduction takes place but in hypoxia under the influence of Euphorbia Fisher extract and tincture NO concentration has 15-13 increase respectively.

T-helpers and natural killers are known to form rosella with thrombocytes therefore the maximum lymphocytic thrombocytic adhesion intensity may be not more than 60% but in modulating hyperhomocysteinemia we received higher numbers. It might be explained by damaged homocystein effect on cells or nonspecific receptors expression for

thrombocytes by another lymphocytes sub-population.

Thus, the data obtained testify a marked antioxidant properties of medications under discussion from *Euphorbia Fischeriana* root. *Euphorbia Fischeriana* root medications inhibit lymphocytic thrombocytic adhesion and therefore they regulate aggregative thrombocytes properties. In hyperhomocysteinemia and hypoxia the most significant effect was observed in *Euphorbia Fischeriana* elixir intervention and it was more than 2 times higher than in *Euphorbia Fischeriana* tincture.

All the medications studied blocked lipid peroxidation processes and activated antioxidant protection. The medications from *Euphorbia Fischeriana* root appear to have antioxidant properties due to a high level of antioxidants. Thus, according to Teletjev V.V., *Euphorbia Fischeriana* roots contain flavonoids, saponins, glycosides, selkenium, traces of anthracenederived substances, ascorbic acid [1, 7]. Our studies suppose antioxidant mechanism of flavonoids to be bases on ability to defense capillary walls from radicals damage by neutralization of oxygen active forms and break of free radicals reaction chains according to [13]. Besides flavonoids selenium effects significantly antioxidant activity and is a constituent element of selenium dependent glutathione-peroxidase inactivating oxygen active forms. Hence, hemostasis and endothelium function reduction in hypoxia and hyperhomocysteinemia by medications under study may account for decrease of damaged effect of oxidizing stress on endotheliocytes [10, 12, 15]. *Euphorbia Fischeriana* roots have the most significant protective efficacy.

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