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Original article

Involvement of the different extracts from roots of *Salvia miltiorrhiza* Bunge on acute hypobaric hypoxia-induced cardiovascular effects in rats – preliminary report

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Abstract

The present study was carried out to investigate the protective effects of roots of *Salvia miltiorrhiza* Bunge on hypobaric hypoxia. Two extracts of *S. miltiorrhiza* (extract 1: ethanol : water – 50 : 50; extract 2: 96% ethanol) were used. The experiments were performed after 7 consecutive days of administration of the extracts (200 mg/kg b.w., intragastrically) to male Wistar rats. Next, after placing animals for 60 min in the controlled acute hypobaric hypoxia (500 mm Hg) the systolic arterial blood pressure (SAP) in conscious rats, bioelectric heart activity in unconscious rats and analysis of oxidative stress parameters in the blood of rats: malonyldialdehyde (MDA) and lipid peroxidase (LPO) concentration, activity of superoxide dismutase (SOD) or glutathione peroxidase (GPX) were assayed. It was found out that the extract 1 augmented the lowering of SAP shown in hypoxia affected control rats. On the contrary the extract 2 reversed SAP to values obtained in control animals. Moreover, both extracts led to the normalization of hypoxia-induced tachycardia and levels of MDA, LPO and SOD. It seems that the above-mentioned effects are coupled with different active compounds content in the extracts, however more studies are needed to confirm this hypothesis.

Key words: *Salvia miltiorrhiza*, hypobaric-induced hypoxia, cardiovascular effects in rats, systolic arterial blood pressures, bioelectric of heart activity, oxidative stress parameters

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Introduction

The role of acute and chronic hypoxia in etiology of the cardiovascular diseases is evoking the great interest. One of the most important mechanisms for hypoxia induction is oxidative stress. The increase of reactive oxygen species (ROS) production takes part in the pathogenesis of many cardiovascular diseases. It is known that the increase of ROS, which is responsible for cell damage (for example cardiomiocytes), may also play a protective role in the adaptation mechanism in chronic hypoxia (Giordano 2005). On the other hand, it is known that the adaptation to hypoxia condition can increase a cardiac tolerance to acute oxygen deficiency in heart cells (Kolář and Ostadal 2004).

Salvia miltiorrhiza Bunge (family: *Lamiaceae*) is a perennial plant growing in China, Korea, Vietnam and Japan. Its roots have been used since ancient times in the traditional Chinese medicine to treat cardiovascular diseases. This herb is traditionally used as a cardioprotective agent in medicine of Far Eastern countries, but the results of many studies using extracts from the plant are equivocal (Han et al. 2008, Han et al. 2011). It is generally known that chemical constituents from *Salvia miltiorrhiza* root extracts can be divided into two major categories: water soluble compounds (mainly phenolic acids e.g. danshensu, rosmarinic acid (RA) or salvianolic acid A and B) and lipophilic diterpenoid quinones (e.g. tanshinone I (Tsh 1), tanshinone IIA (Tsh 2A), cryptotanshinone (CTsh)) (Han et al. 2008, Li et al. 2009). Numerous studies have confirmed a pharmacological activities of *S. miltiorrhiza* as a cardiovascular protective agent (Wang et al. 2005) or antioxidant (Matkowski et al. 2009). It was shown that Tsh 2A, one of the key component of *S. miltiorrhiza*, can dilate coronary arteries, increases coronary blood flow and protects the myocardium against experimental ischemia (Xu et al. 2009). There are some observations that Tsh 2A protects cardiac myocytes *in vitro* and *in vivo*, due to its antioxidative and anti-apoptotic properties (Fu et al. 2007, Gao et al. 2008). On the contrary, it was found out in some models of ischemia that danshensu is indispensable for capacity of extracts to scavenge peroxide (Chen et al. 1999). Moreover, the compound can diminish the activation of nuclear factor-κB resulting in inhibition of expression of various inflammatory mediators (Chen et al. 2011, Li et al. 2012), what is important for its protective role against microcirculatory disturbances (Han et al. 2008, Li et al. 2012). On the other hand, there are some reports that Tsh 2A mediates cytotoxicity in human endothelial cells (Yang et al. 2005), whereas salvianolate, as a highly purified aqueous extract from *Salvia miltiorrhiza* root containing mainly magnesium lithospermate B (≥85%), RA (≥10.1%) and lithospermic acid (≥1.9%), showed protective effect on myocardial ischemia in porcine closed-chest model (Han et al. 2011).

One of the accepted models of hypobaric hypoxia involves reduction of the barometric pressure and a corresponding drop in the atmospheric oxygen pressure. It is known that the hypoxia conditions with reduced air pressure and lower oxygen concentration occurs at high altitudes and leads to Acute Mountain Sickness (AMS). AMS is an illness that can affect mountain climbers at high altitude (typically above 5500 m above see level). There is opinion that the disorder is associated with the change of vascular permeability induced by the production of free radicals. The hypoxia-induced free radicals contribute to myasthenia, lower capillary blood flow and also to some neurological symptoms like: convulsions and swelling, including cerebral oedema (Askew 2002). Moreover, the changes in the activity of superoxide dismutase (SOD), malondialdehyde (MDA) and selenium-dependent glutathione peroxidase (GPX) are considered the indicators of ROS generation. There are only few drugs which can be of value in the treatment and alleviation of hypobaric-induced hypoxia symptoms e.g. acetazolamide and glucocorticosteroids (Coote 1995). The potential usage of plant extracts in AMS treatment has been a subject of some studies. It is known that properties of some bioactive compounds of plant origin may play a beneficial role in the adaptation to hypoxic conditions. For example, there are reports concerning the application of herbal extracts (*Panax ginseng* and *Ginkgo biloba*) in animal models of AMS (Berg 2004).

One of such promising plants is also *Salvia miltiorrhiza* Bunge, which, due to its antioxidative activity, can attenuate the acute ischemic myocardial injury in rats (You 2007, Wu et al. 2007). Therefore, the aim of this study was to investigate a possible protective effect of extracts from roots of *Salvia miltiorrhiza* Bunge in hypobaric-induced hypoxia in rats.

Materials and Methods

Extracts

Salvia miltiorrhiza was cultivated in the Garden of Medicinal Plants of Department of Botany and Agronomy of Medicinal Plants in Plewiska near Poznań. Sage roots were dried at room temperature (at 23-25°C and relative humidity of 50-55%). The powdered plant material was extracted with 50% ethanol (v/v) (extract 1) or ethanol 96% (v/v) (extract 2) using the percolation method at a 1:10 ratio of plant material to solvent. After evaporating the alcohol under reduced pressure at a temperature of 40-45°C and then freezing at -55°C, the percolate obtained was lyophilized.

Determination of active compounds content

The following parameters of extracts were determined: the loss on drying, the content of total polyphenols (expressed as rosmarinic acid equivalent), tanshinones (tanshinone I (Tsh I), tanshinone IIA (Tsh IIA), dihydrotanshinone (DTsh), cryptotanshinone (CTsh)). The loss on drying parameter was measured according to the European Pharmacopoeia 7th edition (European Pharmacopeia 2010). The total polyphenols were determined by Arnov method (Farmakopea Polska VII 2005) and expressed as rosmarinic acid (RA). For tanshinones HPLC separation method was used with some modifications (Don et al. 2006), since recently the HPLC DAD method has been developed in the Department of Quality Control of Medicinal Products and Dietary Supplements, Institute of Natural Fibres and Medicinal Plants in Poznań, to investigate multi-components in sage (Krajewska-Patan et al. 2007). The UV detection wavelength was set at 230 nm. All methods were validated in accordance with ICH guidelines for validation of analytical methods (system suitability, selectivity, linearity, regression, precision, accuracy and robustness).

Methods of treatment

The experiments on rats were performed in accordance with Polish governmental regulations (Dz.U.05.33.289, 2005). The study has been approved by the Local Ethic Committee for the Use of Laboratory Animals in Poznań, Poland (no 64/2008).

Experiments were performed on male six week-old Wistar rats housed in controlled room temperature (20±0.2°C) and humidity (65-75%) under a 12h : 12h light-dark cycle (lights on 7 a.m.). Animals were kept in groups in the number of 8-10 in light plastic cages and had a free access to standard laboratory diet (pellets-Labofeed B) and to tap water in their cages. The lyophylized extracts were suspended in 0.5% methylcellulose and administered subchronically (7 x), in the dose of 200 mg/kg b.w., intragastrically. The dose of extracts was chosen according to other studies (Colombo et al. 1999). On the last day of the treatment rats were placed for 60 min in the controlled hypoxia condition (Wittner and Říha 2005). Briefly, the AMS model used in the experiment involved hypobaric hypoxia induced by a reduction of the barometric pressure and a corresponding drop in the atmospheric oxygen pressure. The rats were exposed to hypobaric hypoxia for 60 min in an experimental chamber, simulating an altitude of 5000 meters (pressure 500 mm Hg). Control experiments were performed on hypoxia-affected rats or without hypoxia-induced condition (Control rats), which were treated previously for seven days with vehiculum (0.5% methylcellulose).

Locomotor activity

Locomotor activity was evaluated using a Ugo-Basile apparatus (Varese, Italy) by placing animals in the centre of the apparatus and recording their horizontal and vertical activity (Mikołajczak et al. 2002). The data obtained was expressed as signals corresponding to spontaneous movements for 5 minutes. Locomotor activity was measured 15 minutes after the hypobaric-induced hypoxia treatment.

Motor coordination

The effect of extracts and hypoxia condition on motor impairment was assessed with "chimney" test (Borowicz et al. 2002). In this test rats were able to climb backwards up the plastic tube. Motor impairment was indicated by the inability of animals to climb backwards up the tube within 60 s. Motor coordination was measured 25 minutes after the hypobaric hypoxia induction.

Measurements of bioelectric of heart activity in unconscious rats

Measurements of bioelectric heart activity in unconscious rats was performed using method described previously (Kozłowska et al. 1987). Briefly, the rats were anaestethized with thiopental (40 mg/kg, i.p.) followed with heparine (500 j.m.). After cannulation of the trachea the animals were pithed and artificially ventilated with air (60 strokes min^{-1}) using ventilatory system. Blood pressure was measured from the right carotid artery via pressure transducer MCK-301 (Poland). Heart rate was recorded from ECG (Multicard-30, Poland) by means of intramuscular electrodes. Body temperature was kept constant at approximately 37°C using a heating table and monitored by rectal probe thermometer.

Measurement of systolic arterial blood pressure in conscious rats

Analysis of systolic arterial blood pressure (SAP) was performed on the tail of rat using TSE Blood Pressure Measuring System 9102-1-NI connected to the oscilloscope. Before measuring procedure the animals were heated for 30 min at 37°C.

Biological material isolation for biochemical analysis

After SAP measurement the rats were decapitated and the blood was collected into heparinized tubes. Then the blood was centrifuged for 10 min (700 × g) at 4°C and plasma samples were frozen at -80°C for further measurements.

Biochemical analysis of oxidative stress parameters in blood of rats

The level of oxidative stress in the blood of rats was analyzed by determination of below-mentioned parameters:

Malonyldialdehyde concentration (MDA) as a marker of lipid peroxidation – using spectrophotometric method after the reaction with tiobarbituric acid (TBARS) according to Tanaka and others method (Tanaka et al. 1994). Briefly, in the samples (100 µl) of plasma the concentration of TBARS was calculated as MDA equivalent using a commercial kit (Cayman Chemical Co., Ann Arbor, MI, USA).

Activity of superoxide dismutase (SOD) as a marker of free radicals formation – using method in which the measurement of absorbance changes is coupled with spontaneous oxidation process in the presence of SOD according to Sun and Zigman method (1978). SOD activity in plasma was measured using a commercial kit (Cayman Chemical Co., Ann Arbor, MI, USA) which determines all three types of SOD (Cu/Zn, Mn, FeSOD) according to manufacturer's instructions. The plasma samples were diluted 1:5 with sample buffer (50 mM Tris-HCl, pH 8.0) before assaying the SOD activity.

Activity of glutathione peroxidase (GPX) as a marker of free radicals formation – according to Mohandas and others (Mohandas et al. 1984). Glutathione peroxidase (GPX) activity in erythrocyte lysates was measured using Cayman assay kit (Cayman Chemical Co., Ann Arbor, MI, USA). Erythrocyte lysates were prepared according to the test manufacturer instructions.

Specific lipid peroxidation (LPO) levels were determined using a commercial kit (Cayman Chemical Co., Ann Arbor, MI, USA). Plasma samples (500 µl) were deproteinated and extracted under acidic conditions with 1 ml ice-cold deoxygenated chloroform, and the chloroform extract was removed following centrifugation (1600 × g for 5 min at 0°C) for LPO determination.

Statistical analysis

The data were expressed as means ± SEM. Statistical calculations were performed using one-way analysis of variance (ANOVA) or ANOVA with replication (ANOVA II), respectively and Duncan's post hoc-test. The p-value 0.05 was considered as significant.

Results

The present study shows that extracts from roots of *Salvia miltiorrhiza* differ in their content of tanshinones (Table 1). It was found out that obtained ratios for extract 2 vs. extract 1 showed relatively large amounts of tanshinones in the extract 2 (ratios for: Tsh 1 – 18.4, Tsh IIA – 50.5, CTsh – 7.3, DTsh – 10.2, respectively). On the contrary, the content of polyphenols was at the same level in both extracts.

The results of multiple treatment with the extracts on locomotor activity (horizontal and vertical activities) and motor coordination are shown in Table 2. We found that, generally, these substances did not produce any statistically significant differences in both the horizontal activity (one-way ANOVA [$F(3,34)=0.667$; insignificant]) as well as in the vertical activity (one-way ANOVA [$F(3,33)=0.600$; insignificant]). Moreover, in the experiment testing the extracts effect on motor coordination the overall analysis of the results expressed as exit time from "chimney" did not show significant differences between the groups (one-way ANOVA [$F(3,36)=2.23$; insignificant]) (Table 2). However, using detailed analysis, we found out that the process of hypoxia shorten the exit time of rats (hypoxia-affected group vs. control, $p<0.05$).

In the experiment testing the acute effect of extracts on SAP in conscious animals the analysis of the results showed significant differences between the groups (one-way ANOVA [$F(3,33)=21.6$; $p<0.001$]) (Table 2). Using a detailed analysis we observed that the state of hypoxia lowered SAP when compared with control rats ($p<0.05$).

Concerning the effect of extracts administration on SAP, using a detailed analysis, we observed that

Table 1. The content of tanshinones and polyphenols in extracts from roots of *Salvia miltiorrhiza* Bunge.

	Loss on drying [%]	Tsh I [%]	Tsh IIA [%]	CTsh [%]	DTsh [%]	Polyphenols* [%]
Extract	11.63	0.04	0.02	0.12	0.14	0.15
Extract 2	3.03	0.74	1.01	0.85	1.43	0.19

Extract 1 – ethanol: water – 50 : 50 (v/v)
Extract 2 – 96% ethanol (v/v)
Tsh 1 – Tanshinone I
Tsh 2A – Tanshinone IIA
CTsh – Cryptotanshinone
DTsh – Dihydrotanshinone
* – calculated as rosmarinic acid

Table 2. Effect of extracts from *Salvia miltiorrhiza* roots on some behavioral parameters and SAP in hypobaric-induced hypoxia rats.

Group		Horizontal activity	Vertical activity	Motor coordination	SAP
		[number of impulses /5 min]	[number of impulses /5 min]	Exit time [s]	[mm Hg]
Control	8	323.2 ± 36.9	37.7 ± 6.6	29.7 ± 6.8	126.0 ± 1.5
Hypoxia-induced	9	241.6 ± 34.0	28.3 ± 6.8	13.5 ± 2.5[a]	115.5 ± 2.2[a]
Extract 1	10	258.9 ± 52.3	38.2 ± 9.4	33.9 ± 7.2[b]	107.7 ± 1.2[a,b]
Extract 2	10	267.8 ± 36.2	26.5 ± 7.9	30.6 ± 6.7	120.0 ± 1.5[a,c]

values expressed as mean ± SEM
n – number of animals
SAP – systolic arterial pressure
a – statistically significant vs. “Control”, $p<0.05$
b – statistically significant vs. “Hypoxia-induced”, $p<0.05$
c – statistically significant vs. “Extract 1”, $p<0.05$

the extract 1 lowered SAP values when compared with hypoxia-affected rats ($p<0.05$). On the contrary, the extract 2 produced an insignificant increase of SAP (vs. hypoxia-affected), and showed a tendency towards normalizing this parameter to control values, although the difference between the extract 2 and control group reached statistical significance ($p<0.05$). Moreover, the extracts differ between each other in the effect on blood pressure changes ($p<0.05$).

In the experiment testing the extracts effect on heart activity in unconscious rats the overall analysis of the results showed significant differences between the groups (ANOVA with replication: main effect – [$F(3,19)=5.34$; $p<0.01$]; effect of time – [$F(2,38)=0.260$; insignificant]; interaction (main effect x time) – [$F(6,38)=2.46$; $p<0.05$]) (Table 3). Using a detailed analysis, it was found out that the hypobaric hypoxia condition has led to the increase of heart rate especially after 15 and 30 min from the beginning of the experiment when compared with control group ($p<0.05$). Multiple administration of both extract 1 and extract 2 protected the animals from hypoxia-induced condition because normalization of heart rate of rats when compared with control animals was found.

The results of influence of multiple treatment of the extracts on some parameters of ROS production in the blood of hypoxia-affected rats are shown in Table 4. It was found out that the analysis of the results expressed as MDA, SOD, GPX and LPO showed significant differences between the groups (MDA – one way ANOVA [$F(3,48)=21.7$; $p<0.001$]; SOD – one way ANOVA [$F(3,48)=21.9$; $p<0.001$]; GPX – one way ANOVA [$F(3,47)=6.57$; $p<0.001$]; LPO – one way ANOVA [$F(3,48)=9.44$; $p<0.001$]). A detailed post hoc analysis showed that the hypoxia-affected rats had higher values of MDA (vs. control group, $p<0.01$) and LPO (vs. control group, $p<0.01$), whereas SOD values decreased (vs. control group, $p<0.01$). Similar effect in the GPX activity was found, but the differences did not reach statistical significance.

Administration of the extracts has generally led to the normalization of obtained ROS values especially

Table 3. Effect of extracts from *Salvia miltiorrhiza* roots on heart activity in hypobaric-induced hypoxia rats.

Group	n	Heart beating [number/min]		
		0 min	15 min	30 min
Control	6	352.8 ± 21.4	318.0 ± 12.6	317.7 ± 29.3
Hypoxia-induced	6	381.2 ± 23.5	421.2 ± 23.0[a]	431.8 ± 19.9[a]
Extract 1	6	322.3 ± 22.5	311.0 ± 7.0[b]	316.5 ± 7.4[b]
Extract 2	6	364.3 ± 17.8	352.8 ± 21.4	360.7 ± 24.1

values expressed as mean ± SEM
n – number of animals
a – statistically significant vs. "Control", $p<0.05$
b – statistically significant vs. "Hypoxia-induced", $p<0.05$

Table 4. Effect of extracts from roots of *Salvia miltiorrhiza* Bunge on some parameters of reactive oxygen species (ROS) production in blood of hypobaric-induced hypoxia rats.

Group	n	MDA [μmol/L]	SOD [U/ml]	LPO [μM]	GPX [nmol/min/g Hg]
		in plasma	in plasma	in plasma	in erytrocytes
Control	17	8.2 ± 0.6	9.76 ± 0.05	4.22 ± 0.36	233 ± 24
Hypoxia-induced	15	22.5 ± 2.2[a]	8.87 ± 0.15[a]	7.92 ± 0.69[a]	203 ± 31
Extract 1	10	15.1 ± 0.7[b]	9.86 ± 0.10[b]	4.77 ± 0.57[b]	287 ± 42
Extract 2	10	14.0 ± 0.8[b]	9.84 ± 0.05[b]	4.85 ± 0.71[b]	400 ± 38[a,b,c]

MDA – malonyldialdehyde concentration
SOD – activity of superoxide dismutase
GPX – activity of glutatione peroxidase
LPO – specific lipid peroxidation
values expressed as mean ± SEM
n – number of animals
a – statistically significant vs. "Control", $p<0.01$
b – statistically significant vs. "Hypoxia-induced", $p<0.01$
c – statistically significant vs. "Extract 1", $p<0.05$

for SOD and LPO (Table 4). It was found out that after 7-day treatment with both extracts the obtained values of SOD and LPO did not differ statistically significantly when compared with control rats. Similar effect was measured for MDA, although the protective action of extracts was not complete, since there were significant differences between the values for the extract-treated rats and control animals ($p<0.01$). In the case of GPX the extract 1 did not produce significant effect when compared with control rats, whereas the extract 2 showed statistically significant increase of the values both in comparison to hypoxia-affected and control animals ($p<0.01$). Moreover, the levels of GPX values for extract 2 was also significantly different when compared with the extract 1 ($p<0.05$).

Discussion

It was found out that the extracts from roots of *Salvia miltiorrhiza* differ in their content of tanshinones, since in the extract 2, which was obtained using more lipophilic solvent (96% ethanol), the relatively large amounts of tanshinones were shown. The obtained results are in agreement with the suggestions that a high concentration of alcohol is preferred for extracting the lipophilic diterpenoids (Zhong et al. 2001, Han et al. 2008, Li et al. 2009).

When analyzing locomotor activity of rats it was found out that hypobaric hypoxia did not affect this parameter. Simultaneously, the state of hypoxia shortened the exit time of rats. It means that the hypobaric hypoxia condition produces stimulative effects on motor coordination in rats. It is rather unexpected observation since the acute exposures to severe or moderate hypoxia are known to impair muscular coordination (McLennan et al. 1983) and postural stability (Cymerman et al. 2001) in humans. On the other hand, under certain conditions, a short episode of hypoxia exerts beneficial action on the central nervous system, as shown by the protective role of hypoxia preconditioning (Gidday et al. 1994, Cantagrel et al. 2003). Due to the fact that the rat brain might be less sensitive to hypoxia than the human brain

(Shukitt-Hale et al. 1996) and a duration of hypoxia condition in the experiment was relatively short, therefore the negative effect of hypobaric hypoxia on motor coordination of rats can be excluded. Moreover, the administration of both extracts has led to normalization to control values of this parameter. Summarizing this part of the study it seems that our results showed rather positive effects, because we observed protective aspects of activity of the extracts in "chimney" test without changing the basic activities of rats shown in locomotor activity test.

In the experiment testing the effect on SAP in conscious animals it was found that the state of hypoxia lowered values of this parameter. It is in line with the observation found especially in spontaneously hypertensive rat where adult rats exposed to acute hypoxia in a hypobaric chamber showed significant attenuation of SAP (Henley et al. 1989, Henley and Bellush 1989). Similar effects were observed in subjects after rapid ascent to high altitude in whom the autonomic nervous activities were suppressed and sympathetic activity was relatively predominant (Chen et al. 2008). We found out at the state of hypoxia induced the increase of heart beating. It is in agreement with observations of the others that the acute hypobaric stress produced by high altitude resulted in significant increases in heart rate in conscious dogs (Saltz et al. 1976); similar effects were found in nonacclimatized human subjects (Schirlo et al. 1997).

Administration of extracts has led to different effect on SAP of rats, since the extract 1 lowered SAP values whereas the extract 2 produced an insignificant increase of SAP and showed a tendency to normalizing this parameter to control values. On the contrary, both extract 1 and extract 2 protected the animals from hypoxia-induced effect, because the normalization of heart rate of rats was found. It means that, probably, the effects on SAP are coupled with different active compounds content, whereas the normalization of heart beating after the extracts administration to rats with hypobaric-induced hypoxia is independent of the concentration of tanshinones. Explanation of these differences is difficult since the real mechanism of interaction between tanshinones and hypoxia of rats is still not known.

Analyzing parameters of ROS production in the blood of rats, it was shown that rats with hypobaric hypoxia had higher values of MDA and LPO, whereas SOD values decreased. Generally, it is in line with the observations of other authors who suggested that the lack of oxygen in relation to hypoxia goes along with an increased generation of ROS (Behn et al. 2007). For example, the increase in MDA production directly correlates with acute mountain sickness intensity at an altitude of 5000 m in human (Araneda et al. 2005). Mostly after acute hypoxia exposure a tendency to produce oxidative stress-related indicators is observed (Row et al. 2003, Magalhaes et al. 2004, Shukla et al. 2009, Sharma et al. 2011), although no significant differences in ROS production is sometimes mentioned (Esteva et al. 2010).

Administration of the two extracts has generally led to the normalization of obtained ROS values especially for SOD and LPO. The latter effect is produced probably due to the presence of tanshinones in the extracts, since the compounds can inhibit elevated LPO levels in some models of microcirculatory disturbance and reperfusion injury (Han et al. 2008). There are opinions resulting from *in vitro* studies, that both lipophilic and water-soluble compounds from extracts of *Salvia miltiorrhiza* root are capable of attenuating microcirculatory disturbances and protection from multiple-organ injury by exerting various actions, such as inhibition of the production of peroxides (Han et al. 2008). Thus, it can be speculated that both water-soluble and lipophilic compounds of the two extracts appear to improve hypoxia-induced changes synergically. On the contrary, there are some differences after their administration to hypoxia-affected rats when measuring GPX, since the extract 2 induced increase of the GPX values, whereas the extract 1 did not produce significant effect. It means that the extracts show their protective role and can probably change the antioxidant status similarly as it was shown by aqueous extract of roots of *Salvia miltiorrhiza* (Danshen) for improvement of adriamycin-induced cardiac toxicity in rats (You et al. 2007)**,** whereas observed differences can be explained by the different tanshinones' content in the extracts. It is in line with the suggestions that the two types of constituents of *Salvia miltiorrhiza*, the hydrophilic phenols (e.g. RA) and the lipophilic tanshinones have been proven responsible for the polyvalent activities of this herb (Han et al. 2008, Li et al. 2009).

In conclusion, it seems that there are differences in the pharmacological profile of the extracts and, probably, the obtained protective effects against experimentally induced hypobaric hypoxia condition are coupled with different active compounds content in the extracts, however more detailed studies are needed to confirm this hypothesis.

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