RESEARCH PAPER | Medical Science | Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

The Effects of The Administration of Quercetin, Lycium Barbarum Extract and Chitosan on Oxidative Stress Induced by Exposing Rats to Hypobaric Hypoxia

KEYWORDS: oxidative stress, hypobaric hypoxia, Quercetin, Lycium barbarum, Chitosan.

Anca DUMITROVICI
1Oncologic Institute "I. Chiricuta", Republicii st., no. 34-36, Cluj district, Zip code 400015, Cluj-Napoca, Romania

Adriana MUREŞAN
Department of Physiology, University of Medicine and Pharmacy "Iuliu Hatieganu", Clinicilor st., no.1, Cluj district, Zip code 400023, Cluj-Napoca, Romania

Ramona SIMEDREA
Department of Physiology, University of Medicine and Pharmacy "Iuliu Hatieganu", Clinicilor st., no.1, Cluj district, Zip code 400023, Cluj-Napoca, Romania

*** Irina Camelia CHIŞ**
Department of Physiology, University of Medicine and Pharmacy "Iuliu Hatieganu", Clinicilor st., no.1, Cluj district, Zip code 400023, Cluj-Napoca, Romania
* is Correspondent author

ABSTRACT *Purpose. Acute and chronic hypobaric hypoxia (HH) exposure is considered a physiological oxidative stress that induces the modification of pro-oxidants/antioxidants balance in the entire organism. The aim of this study is to evaluate the protective effects of the natural antioxidants (Quercetin, Lycium barbarum and Chitosan) administration in animals exposed to HH. Study design. 90 Wistar rats were randomly assigned into nine groups and were exposed to HH for one day or 14 days or kept in normobaric normoxia. Some of the rats were treated with natural antioxidants. The serum levels of the free radicals (malondialdehyde and carbonylated proteins) and the antioxidant status (superoxide dismutase, catalase and reduced glutathione) in the blood were measured. Results. The results show an increase oxidative stress after exposure of HH and natural antioxidants supplementation significantly ($P<0.05$) attenuated the oxidative stress in the blood of the rats. This study suggests that treatment with natural antioxidants can be beneficial in attenuating the oxidative stress associated with exposure of HH.*

INTRODUCTION

Hypobaric hypoxia (HH), encountered at a high altitude (HA), shares in common with physical exercise, pregnancy, ageing, inflammation, cardiovascular and respiratory failures, wounds and even cancer, an increased risk of "oxygen availability not coping with aerobic ATP requirements", as hypoxia has been defined. The HA hypoxia could cause increased cellular oxidative stress with consequent damage to structure and function of proteins, nucleic acids and lipids (Imray & al., 2010). The oxygen-limiting conditions favor the enhanced formation of reactive oxygen species (ROS) by mitochondria electron transport chains, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xantin oxidase/reductase, etc. and the depletion of cellular enzymatic and non-enzymatic antioxidant substrates. Low barometric pressure at HA causes higher expression of hypoxia inducible factor (HIF-1) (Romero & al., 2012). The HH induces redox imbalance which is the primary cause of pathological processes such as HA pulmonary edema, HA cerebral edema, mental dysfunction and memory deficit, motor impairment, myocardial injury, kidney and liver failure, and increased mortality. Thus, it has been advocated that supplementation with natural antioxidants may minimize the ill effects of HH (Farías & al., 2010; Vargas & al., 2011).

Quercetin is a polyphenolic compound that is present in many types of vegetables and fruits and has anti-inflammatory, antiproliferation and antioxidant effects (Boots & al., 2008). Recent studies show the Quercetin is a powerful antioxidant that exerts endothelium-independent vasodilatory effects, protective effects on nitric oxide and endothelial functions and anti-atherogenic effects in inflammatory lesions and those triggered by oxidative stress (Larson & al., 2012).

The fruit of the plant Lycium barbarum (LBG), belong to the Solanaceae family, contains a full spectrum of antioxidants, one of the best sources of carotenoids, and is also heavily used in traditional Chinese medicine in ameliorating obesity and diabetes (Devalaraja & al., 2011; Potterat, 2010; Yu & al., 2006). Lycium barbarum represent a rich source of vitamins C, A, B_1, B_6; carotenoids; flavonoids; iron; selenium and germanium; and contains 18 types of amino acids and twenty-one minerals. Recent research shows the biologically beneficial effects of LBG extract and its specific anti-aging properties, hypotensive effects, anti-apoptotic activity, anti-tumor and cytoprotective properties, immunostimulatory effects, etc. Eyesight improvement, blood pressure control, cholesterol level lowering, a good adjunct to combat the adverse effects of chemotherapy and radiotherapy in various tumors, etc. have also been reported (Gan & al., 2004; Luo & al., 2004). The recent studies has demonstrated cardioprotective and neuroprotective effects of LBG extract (Dumitrovici & al., 2013; Chan & al., 2007; Li & al., 2013).

Chitosan is a α-(1-4)-D-glucosamine polymer, a natural polysaccharide present in shellfish, clams, krill, oysters, fungi, etc. Recent research has demonstrated the antilipidemic, antiulcerogenic, anti-aging, membrane-stabilizing and antioxidant properties of Chitosan (Anandan & al., 2012, 2013). The recent studies showed the cardioprotective effect of Chitosan on the myocardial defense system in a myocardial infarction is due to its antioxidant, hypolipidemic and membrane stabilizing properties (Anandan & al., 2012).

The aim of this study was to evaluate the efficiency of Quercetin, LBG extract and Chitosan supplementation in the lowering of oxidative stress induced through acute and chronic exposure to HH.

MATERIALS AND METHODS

Drugs and chemicals

The Chitosan used in this experiment were purchased from Sigma Chemical Company Inc., UK. The chemicals were of analytical grade. Quercetin and Lycium barbarum (LBG) were extracted, dosed and encapsulated at the "PROPLANTA" Applied Vegetal Biotechnologies Center in Cluj-Napoca, Romania. Quercetin, LBG extract and Chitosan were dissolved in physiological NaCl solution (PS).

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

Animals
The study involved 90 albino Wistar male rats (weight 180-200 g at 12 weeks old). All the animals were obtained from the Biobase of the "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca. The animals were cared for in the Biobase of the Physiology Department of the same University. They were isolated for 10 days prior to their introduction in the study for acclimatization. The animals received a standard diet and their access to water was not restricted. The experimental protocol has been approved by the Ethics Committee of the University of Medicine and Pharmacy Cluj-Napoca and conforms to the Guide for the care and use of laboratory animals NIH publication No. 85-23, revised 1996.

The animals were randomly subdivided into nine experimental groups (n=10): 1st Group (control group)- rats maintained in acute hypobaric hypoxia (AHH) conditions and treated with PS (AHH+PS); 2nd Group- rats maintained in AHH conditions and treated with Quercetin (AHH+Que); 3rd Group- rats maintained in AHH conditions and treated with LBG extract (AHH+LBG); 4th Group- rats maintained in AHH conditions and treated with Chitosan (AHH+Chi); 5th Group (control group)- rats maintained in normobaric normoxia (Nx) conditions (760 mmHg, 21% O_2 and 79%) for 14 consecutive days and treated with PS (Nx+PS); 6th Group- rats maintained in chronic HH (CHH) conditions and treated with LBG extract (CHH+LBG); 7th Group- rats maintained in CHH conditions and treated with Quercetin (CHH+Que); 8th Group- rats maintained in CHH conditions and treated with Chitosan (CHH+Chi) and 9th Group- CHH rats treated with PS (control group)- rats maintained in CHH conditions and treated with PS (CHH+PS);

The animals were exposed to a simulated altitude (380 mmHg, 12% O_2 and 88% N_2) of 5500 m in a barochamber, where temperature and humidity were maintained at 28°C and 55-60%, respectively, for one day (AHH) and for 14 consecutive days (CHH). The rats were taken out of the hypoxic chamber once after every 23 hours exposure for 1 hour, for receiving food and water.

Some of the rats received Quercetin (30 mg/kg/day) or LBG extract (30 mg/kg/day) via an intragastric tube (0.6 ml/rat) or Chitosan (0,30-0,35 microg/animal/day) by intra-peritoneal injections for one day or 14 consecutive days prior to HH exposure and 30 minutes before to every HH exposure respectively. The control groups were treated with PS (0.6 ml/rat) via an intragastric tube.

At the end of the experiment all rats were deeply anesthetized (with sodium pentobarbital, 60 mg/rat ip) and sacrificed by cervical decapitation.

Biochemical analysis
After the last treatment, following the sedation and inhalation of etilic alcohol, venous blood samples were collected with an anticoagulant (EDTA) from the rats' retro-orbital sinuses for the purpose of determining the parameters of oxidative stress.

The serum levels of oxidative stress were estimated by the measuring of free radical production: lipid peroxides and carbonylated proteins.

Lipid peroxidation was estimated by measuring the malondialdehyde (MDA) levels (by fluorescein dosage, Conti method) (Conti & al., 1991). The results were expressed in [nmol/ml].

Carbonylated proteins (CP) as products of the reaction between the reactive oxygen species and proteins were determined in serum, using the hydrochloric guanidine method (Reznick & al., 1994). The results were expressed in [nmol/mg protein].

The erythrocitaires antioxidant enzymes were estimated by measuring the level of: superoxide dismutase and catalase in the haemolysates of RBCs and non-enzymatic status were estimated by measuring the level of serum reduced glutathione.

The serum reduced glutathione (GSH) level was measured using the Hu method (Hu, 1994) and expressed in [nmol/ml].

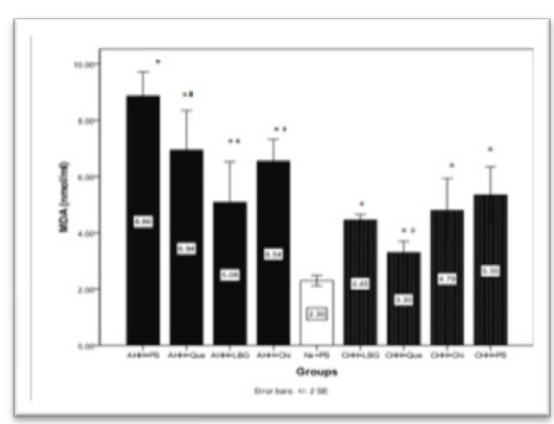
The activity of superoxide dismutase (SOD) was assayed as described by Flohe et al. (Flohe & al., 1990). A unit of the enzyme activity was defined as the enzyme reaction giving 50% inhibition of NBT reduction in 1 min under the assay conditions and expressed as specific activity in U/mg of protein.

The catalase (CAT) activity was determined according to Pippenger (Pippenger & al., 1998) and was expressed in U/mg of protein.

Statistical analysis
Results were expressed as the mean values±SEM (standard error of means). Significance level accepted at P<0.05. Data were analyzed using Statistical Package for the Social Sciences version 17.0 (SPSS-17). All experimental parameters measured were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey´s multiple comparisons post-test.

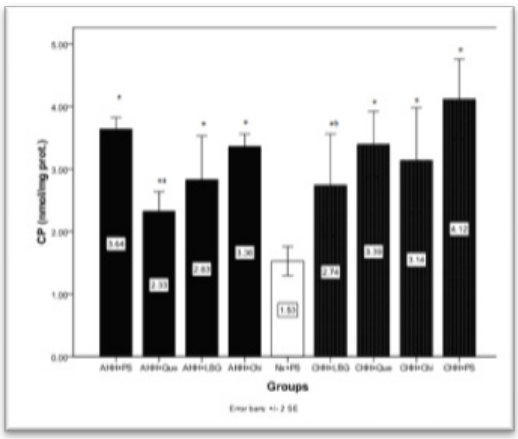
RESULTS
The effect of antioxidant treatment on decreased oxidative stress in the serum of rats exposed to acute or chronic hypobaric hypoxia
Lipid Peroxidation. The serum MDA level of the rats exposed to acute or chronic hypobaric hypoxia was significantly higher (P<0.05) than that of the control rats (Nx+PS) [Figure 1 (a)]. The rats exposed to AHH and treated with Quercetin (AHH+Que), LBG (AHH+LBG) and Chitosan (AHH+Chi) respectively presented a significantly lower (P<0.05) of the MDA levels compared with the control rats (AHH+PS). The results show that LBG more efficiently brought about a decrease in the MDA level than Quercetin or Chitosan after AHH exposure [Figure 1 (a)]. In regards to the rats exposed to CHH, significantly lower (P<0.05) MDA levels were observed only after treatment with Quercetin (CHH+Que) whereas the results were not significantly after treatment with LBG (CHH+LBG) or Chitosan (CHH+Chi) [Figure 1 (a)].

Protein carbonylation. The serum CP level of the rats exposed to acute or chronic hypobaric hypoxia was significantly higher (P<0.05) than that of the control rats (Nx+PS) [Figure 1 (b)]. In contrast to the rats exposed to AHH (AHH+PS), the rats exposed to CHH (CHH+PS) presented a not significantly increase in the CP levels. The rats exposed to AHH and treated with Quercetin (AHH+Que) presented a significantly lower (P<0.05) of the CP level compared to the control rats (AHH+PS). The rats exposed to AHH and treated with LBG (AHH+LBG) or Chitosan (AHH+Chi) presented a not significantly decrease of the CP level compared to the control rats (AHH+PS). A significantly lower (P<0.05) of the CP level was observed in the rats exposed to CHH and treated with LBG (CHH+LBG) and a not significantly lower was observed after treatment with Quercetin (CHH+Quercetin) and Chitosan (CHH+Chi) respectively [Figure 1 (b)].

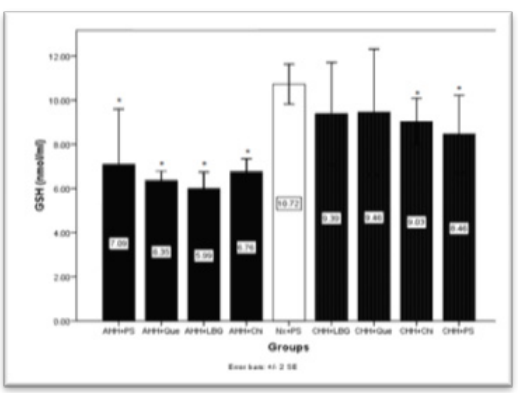


(a)

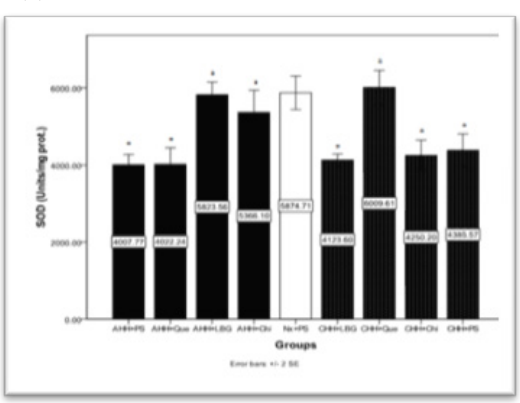
Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X



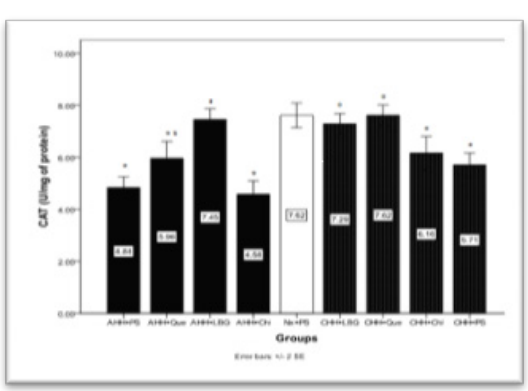
(b)



(c)



(d)



(e)

Figure 1. The effect of acute and chronic hypobaric hypoxia and antioxidant treatment on: (a) lipid peroxidation (MDA) (nmol/ml), (b) protein carbonylation (CP) (nmol/mg protein), (c) reduced glutathione (GSH) (nmol/ml), (d) superoxide dismutase (SOD) activity (U/mg of protein) and (e) catalase (CAT) activity (U/mg of protein). Rats were submitted to acute (1day) or chronic (14 consecutive days) hypobaric hypoxia (AHH or CHH) or normobaric normoxia (Nx) condition, with treatment of physiological NaCl solution (PS), Quercetin (Que), Lycium barbarum (LBG) extract or Chitosan (Chi). Results are the mean±standard error for ten animals in each group. Significant differences *P< 0.05 (AHH and CHH vs Nx+PS); [a]P< 0.05 (AHH+ Que, AHH+ LBG, AHH+ Chi vs AHH+PS); [b]P< 0.05 (CHH+ Que, CHH+ LBG, CHH+ Chi vs CHH+PS).

The effect of antioxidant treatment in increased enzymatic and non-enzymatic antioxidants in the blood of rats exposed to acute or chronic hypobaric hypoxia

The activity of GSH in serum after exposure to acute hypobaric hypoxia was significantly lower (P<0.05) compared to the control rats (Nx+PS) [Figure 1 (c)]. The rats exposed to AHH and treated with Quercetin (AHH+Que), LBG (AHH+LBG) or Chitosan (AHH+Chi) presented a not significantly lower of the serum GSH activity comparated to the control rats (AHH+PS). The serum activity of GSH after exposure to chronic hypobaric hypoxia was significantly lower (P<0.05) compared to the control rats (Nx+PS) and increased not significantly after treatment with Quercetin (CHH+Que), LBG (CHH+LBG) or Chitosan (CHH+Chi) [Figure 1 (c)].

SOD and CAT activity was significantly lower (P<0.05) after exposure to acute or hypobaric hypoxia compared to the control rats (Nx+PS) [Figure 1 (d) and (e)]. The rats exposed to AHH and treated with LBG (AHH+LBG) presented a significantly higher (P<0.05) of the SOD and CAT activity comparated to the control rats (AHH+PS). SOD activity was also significantly higher (P<0.05) for the rats exposed to AHH and treated with Chitosan (AHH+Chi), but CAT activity was significantly higher (P<0.05) in the rats exposed to AHH and treated with Quercetin (AHH+Que). The rats exposed to CHH and treated with Quercetin (CHH+Que) presented a significantly higher (P<0.05) of the SOD and CAT activity compared to the control rats (CHH+PS). CAT activity was significantly higher (P<0.05) for the rats exposed to CHH and treated with LBG (CHH+LBG) compared to the control rats (CHH+PS) [Figure 1 (d) and (e)].

DISCUSSIONS

The protective role of Quercetin, LBG extract and Chitosan against oxidative stress in rats exposed to acute or chronic HH was analyzed in this study. Oxidative stress can be triggered by a series of endogenous and exogenous factors, exposure to HH being one of them (Farias & al., 2010). Exposure to HA is associated with an increase in the production of ROS, which are generated during the phase of reoxygenation of HH and contributes to the physiological responses. The cause of oxidative stress is lower availability of oxygen as it converts to H_2O by cytochrome oxidase. Hypoxia appears to affect enzymatic and non-enzymatic antioxidants such as superoxide dismutase, catalase and reduced glutathione, which are usually reduced. Recently, studies have demonstrated Quercetin, LBG and Chitosan interactions with many physiological processes and diseases.

The present study demonstrated that acute or chronic HH exposure induces increase lipid peroxidation and protein carbonylation in the serum. This suggests an increase in the level of oxidative stress and is in accordance with results obtained by other researchers (Imray & al., 2010). After exposure to acute HH, the MDA levels in the serum increased more significantly (P<0.05), as against to exposure to chronic HH. The serum level of CP did not present a significant different between the exposure to acute and chronic HH. The rats treated with Quercetin and exposed to HH demonstrated

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

a significant decrease in oxidative stress. Our results are in concordance with other reports where Quercetin had a protective role in cardiomyocytes and neurons of rats subjected to HH (Annapurna & al., 2009; Chen & al., 2013; Sarkar & al., 2012). The present results showed that Quercetin had a protective effect on serum. The LBG extract had a protective effect against oxidative stress which was primarily observed through the significant lowering of the serum level of MDA after exposure to acute HH and through the significant lowering of the serum level of CP after exposure to chronic HH. These results are in concordance with other reports which demonstrated anti-oxidative actions of LBG, especially cardio- and neuroprotective effects after HH exposure (Anandan & al., 2012; Li & al., 2013). In the present study, we observed that rats exposed to acute or chronic HH and treated with LBG extract presented a protective effect in serum but in a more moderate way than with Quercetin; this is a result of its antioxidant properties. In contrast, the rats treated with Chitosan demonstrated a lowering in the serum level of MDA after acute HH exposure. Our study shows that Chitosan administration in rats exposed to HH presented protective effect in an inferior way to that of Quercetin.

Our results indicated that GSH activity in serum decreased significantly after acute or chronic HH exposure and rats treated with antioxidants did not present any changes in GSH expression. However, the GSH activity was significantly restored in animals subjected to HH and treated with antioxidants suggesting that these compounds could activate the powerful endogenous antioxidant defenses. These effects were especially noticeable with Quercetin treatment.

CONCLUSION

In conclusion, our data demonstrates that acute and chronic hypobaric hypoxia exposure induces the growth of oxidative stress and Quercetin, Lycium barbarum and Chitosan supplementation before and during exposure to hypoxia may be beneficial to counteract alterations resulting from oxidative stress.

ACKNOWLEDGMENTS

We express our appreciation to Mr. Remus Moldovan for his support in the achievement of the experimental model. We also thank Mrs. Prof. Carmen Socaciu (MD, PhD) and "PRO-PLANTA" Applied Vegetal Biotechnologies Center in Cluj-Napoca for furnishing the Quercetin and LBG extract.

REFERENCE

1. Anandan, R., Ganesan, B., Obulesu, T., Mathew, S., Kumar, R.S., Lakshmanan, P.T. & Zynudheen, AA. (2012). Dietary chitosan supplementation attenuates isoprenaline-induced oxidative stress in rat myocardium. Int. J. Biol. Macromol., 51(5), 783-787. | 2. Anandan, R., Ganesan, B., Obulesu, T., Mathew, S., Asha, K.K., Lakshmanan, P.T. & Zynudheen, A.A. (2013). Antiaging effect of dietary chitosan supplementation on glutathione-dependent antioxidant system in young and aged rats. Cell Stress and Chaperones, 18 (1), 121-125. | 3. Annapurna, A., Reddy, C.S., Akondi, R.B. & Rao, S.R. (2009). Cardioprotective actions of two bioflavonoids, quercetin and rutin, in experimental myocardial infarction in both normal and streptozotocin-induced type I diabetic rats. J. Pharm. Pharmacol., 61(10), 1365-1374. | 4. Boots, A.W., Haenen, G.R. & Bast, A. (2008). Health effects of quercetin: from antioxidant to nutraceutical. Eur. J. Pharmacol., 585 (2-3), 325-337. | 5. Chen, Y.W., Chou, H.C., Lin, S.T., Chen, Y.H., Chang, Y.J., Chen, L. & Chan, H.L. (2013). Cardioprotective effects of quercetin in cardiomyocyte under ischemia/reperfusion injury. Evidence-based Complementary and Alternative Medicine. Article ID 364519, 16 pages. | 6. Chan, H.C., Chang, R.C., Koon-Ching, Ip.A., Chiu, K., Yuen, W.H., Zee, S.Y. & So, K.F. (2007). Neuroprotective effects of Lycium barbarum Lynn on protecting retinal ganglion cells in an ocular hypertension model of glaucoma. Experimental Neurology, 203(1), 269-273. | 7. Conti, M., Morand, P.C., Levillain, P. & al. (1991). Improved Fluoromeric Determination of Malonaldehyde. Clin Chem, 37(7), 1273-1275. | 8. Devalaraja, S, Jain, S. & Yadav, H. (2011). Exotic Fruits as Therapeutic Complements for Diabetes, Obesity and Metabolic Syndrome. Food Res. Int., 44 (7), 1856-1865. | 9. Dumitrovici, A., Chiş, I.C., Mureşan, A., Marton, A., Moldovan, R., Vlad, D., Borza, G. & Pompei, F.B. (2013). Quercetin, Lycium barbarum and Chitosan reverse the effects of hypobaric hypoxia and exert cardioprotective effects in rats. Fiziologia (Physiology), 23, 1 (77), 18-22. | 10. Farías, J.G., Puebla, M., Acevedo, A., Tapia, P., Gutiérrez, E., Zepeda, A., Juantok, C., Calaf, G. & Reyes, J. (2010). Oxidative Stress in Testis and Epididymis Under Intermittent Hypobaric Hypoxia in Rats: Protective Role Role of Antioxidant Supplementation. Journal of Andrology, 31(3), 314-321. | 11. Flohe, L., Becker, R., Brigelius, R. & al. (1990). Convenient assays for superoxide dismutase. In: CRC Handbook of Free Radicals and Antioxidants in Biomedicine, 3, 287- 293. | 12. Gan, L., Hua, Z.S., Liang, Y.X., & Bi, X.H. (2004). Immunomodulation and antitumor activity by a polysaccharide-protein complex from Lycium barbarum. Int. Immunopharmacol., 4 (4), 563-569. | 13. Hu, M.L. (1994). Measurement of protein thiol groups and glutathione in plasma. În: Methods in Enzymology vol. 233, Academic Press Inc., 380-384. | 14. Larson, A.J., Symons, J.D. & Jalili, T. (2012). Therapeutic Potential of Quercetin to Decrease Blood Pressure: Review of Efficacy and Mechanisms. Adv. Nutr., 3 (1), 39-46. | 15. Li, H., Liang, Y., Chiu, K., Yuan, Q., Lin, B., Chang, R.C. & So, K.F. (2013). Lycium barbarum (wolfberry) reduces secondary degeneration and oxidative stress, and inhibits JNK pathway in retina after partial optic nerve transection. PLoS One, 8 (7): e68881. doi: 10.1371/journal.pone.0068881. | 16. Luo, Q., Cai, Y., Yan, J., Sun, M. & Corke, H. (2004). Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from Lycium barbarum. Life Sci., 76 (2), 137-149. | 17. Potterat, O. (2010). Goji (Lycium barbarum and L. chinense): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Med., 76 (1), 7-19. | 18. Pippenger, C.E., Browne, R.W. & Armstrong, D. Regulatory antioxidant enzymes. In vol.: Methods in Molecular Biology, vol.108: Free Radicals and Antioxidant Protocols D. Armstrong (ed.). Humana Press Inc. Totowa NJ 1998; 108: 299-309. | 19. Reznick, A.Z. & Packer, L. (1994). Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. Methods Enyymol, 233, 347-357. | 20. Romero, R.M., Canuelo, A., Siles, E., Oliver, F.J. & Lara, M,E. (2012). Nitric oxide modulates hypoxia-inducible factor-1 and poly (ADP-ribose) polymerase-1 cross talk in response to hypobaric hypoxia. J. Appl. Physiol., 112, 816-823. | 21. Imray, C., Wright, A., Subudhi, A. & Roach, R. (2010). Acute mountain sickness: pathophysiology, prevention, and treatment. Prog Cardiovasc Dis., 52, 467-484. | 22. Vargas, A., Bustos-Obregón, E. & Hartley, R. (2011). Effects of hypoxia on epididymal sperm parameters and protective role of ibuprofen and melatonin. Biol Res., 44, 161-167. | 23. Sarkar, A., Angeline, M.S., Anand, K., Ambasta, R.K. & Kumar, P. (2012). Naringenin and quercetin reverse the effect of hypobaric hypoxia and elicit neuroprotective response in the murine model. Brain. Res., 24 (1481), 59-70. | 24. Yu, M.S., Ho, Y.S., So, K.F., Yuen, W.H. & Chang, R.C.C. (2006). Cytoprotective effects of Lycium barbarum against reducing stress on endoplasmic reticulum. International Journal of Molecular Medicine. 17 (6), 1157-1161.