Biochemistry
Research Article – 35693

Cuma Mertoğlu*, Zeynep Küskü Kiraz, Erkan Söğüt, Hüseyin Özyurt

Melatonin and a single-high dose methylprednisolone effect on the oxidant-antioxidant system in the rabbit heart tissue

Melatonin ve tek-yüksek doz metilprednizolonun tavşan kalp dokusunda oksidan-antioksidan sistem üzerine etkisi

Abstract: Objective: Glucocorticoids have been used in the treatment of a number of diseases due to their widely therapeutic effects. But they have serious side effects. Although glucocorticoid therapy is an effective measure to prevent and treat many diseases in patients, it can cause adverse effects on the cardiovascular system secondary to oxidative stress. It has been suggested that melatonin is a potent antioxidant and that melatonin supplements may protect against such age-related diseases as atherosclerosis, cancer, and Alzheimer disease. In this study, we investigated whether such effects of a single high dose steroid has adverse consequences for the oxidant-antioxidant system of rabbit heart tissue and whether melatonin treatment is protective in the rabbit. For this purpose we measurument some biochemical parameters in the rabbit heart tissue.

Methods: Level of malondialdehyde (MDA, a lipid peroxidation product), protein carbonyl (PC, a protein oxidation product) and nitric oxide (NO) were measured from heart tissue samples of all rabbits included in the study. Also antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase activities were measured from heart tissues. In the study, 20 rabbits were used. The rabbits were separated into three groups. Group I: Control group, group II: Methylprednisolone group, group III: Methylprednisolone plus melatonin group.

Results: The results of the study; PC levels significantly decreased in methylprednisolone plus melatoninin group compared to the control group (p=0.03). PC levels decreased in methlyprednisolone group compared to the control group and higher than methlyprednisolone + melatonin group but these changes aren't significant statistically. No statistical difference was determined by MDA, NO levels and SOD, GSH-Px, catalase activities of all groups.

Conclusion: According to the results of this study we can say that administration of a single high dose methylprednisolone doesn't increase oxidative stress Also, melatonine is a potent antioxidant agent in the heart tissue.

Keywords: Methylprednisolone, melatonin, oxidative stres, heart

Özet: Amaç: Glukokortikoidler günümüzde birçok hastalığın tedavisinde geniş terapotik etkinlikleri nedeniyle yaygın olarak kullanılan ilaçlardır. Ancak ciddi yan etkileri de beraberinde getirmektedirler. Glukokortikoid tedavisi birçok hastalığın tedavisinde ve önlenmesinde etkili olmasına rağmen oksidatif strese bağlı kardiyovaskuler sistemde yan etkilere neden olur. Melatoninin güçlü bir antioksidan olduğu ve melatonin takviyesinin ateroskleroz, kanser ve alzheimer hastalığı gibi yaşa bağlı hastalıklara karşı koruyucu olabileceği ileri sürülmektir. Bu çalışmada, tek-yüksek doz steroidin tavşan kalp dokusunda oksidan-antioksidan sistem üzerine yan etkileri olup

***Corresponding author: Cuma Mertoğlu:** Gaziosmanpaşa University Faculty of Medicine, Department of Medical Biochemistry, Tokat, Turkey, e-mail: drcumamert@hotmail.com

Zeynep Küskü Kiraz: Bingöl State Hospital, Biochemistry Laboratory, Bingöl, Turkey, e-mail: zeynepkusku07@gmail.com

Erkan Söğüt: Gaziosmanpaşa University Faculty of Medicine, Department of Medical Biochemistry, Tokat, Turkey, e-mail: erkanchems@yahoo.com

Hüseyin Özyurt: Gaziosmanpaşa University Faculty of Medicine, Department of Medical Biochemistry, Tokat, Turkey, e-mail: mdhuseyin@yahoo.com

olmadığını ve melatonin tedavisinin tavşanlarda koruyucu olup olmadığını araştırdık. Bu amaçla tavşan kalp dokusunda bazı biyokimyasal parametrelerin ölçümünü yaptık.

Metod: Çalışmaya alınan tüm tavşanların kalp dokularından lipit peroksidasyon ürünü olan malondialdehit (MDA) düzeyi, protein oksidasyon ürünü olan protein karbonil (PC) düzeyi ve nitrik oksit (NO) düzeyi ölçüldü. Ayrıca, kalp dokularından antioksidan enzimler olan süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px) ve katalaz düzeyleri ölçüldü. Çalışmada 20 adet tavşan kullanıldı. Tavşanlar üç gruba ayrıldı. Grup I: Kontrol grubu, Grup II: metilprednizolon grubu, Grup III: metilprednizolon ve melatonin uygulanan grup.

Bulgular: Çalışmanın sonuçları; PC düzeyi metilprednizolon+melatoninin grubunda kontrol grubuna göre anlamlı azaldı (p=0.03). PC seviyesi metilprednizolon gubunda, control grubuna göre azalmıştır ve melatonin + metilprednizolon grubundan yüksektir fakat bu değişiklikler istatistiksel olarak anlamlı değildir. MDA, NO seviyelerinde ve SOD, GSH-Px, catalase aktivitelerinde gruplar arasında istatistiksel olarak bir fark olmadığını gösterdi.

Sonuç: Bu sonuçlara göre tek seferde yüksek doz metilprednizolon uygulamasının kalp dokusunda oksidatif stresi artırmadığı ve antioksidan enzim aktivitelerini değiştirmediği söylenebilir. Aynı zamanda melatonin kalp dokusunda güçlü bir antioksidan ajandır.

Anahtar Kelimeler: Metilprednizolon, melatonin, oksidatif stres, kalp

DOI 10.1515/tjb-2015-0019
Received August 6, 2014; accepted April 30, 2015

Introduction

The reactive oxygen species (ROS) generated in the physiological states of the body equalizes enzymatic and nonenzymatic antioxidant defense systems and provides a defense mechanism against exess ROS production. But ROS increases in numerous disease states. Previous reports suggest that ROS may attack polyunsaturated fatty acid, which is the precursor for lipid peroxide formation that results in the formation of malondialdehyde (MDA). This compound can be used as an indicator of oxidative stress load. Protein carbonyl (PC) is generated after ROS attacks the amino acids in proteins. Superoxide radicals react with nitric oxide (NO) and tissue nitrite (NO_2^-) and nitrate (NO_3^-) have been estimated as an index of NO production, which is an important mediator in tissue damage and dysfunction induced by inflammation. Excessive production of peroxynitrite can cause oxidative damage of carbohydrates, lipids, proteins, and DNA. Catalase, superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) are the main antioxidant enzymes in the body, which provide protection from the ROS [1,2].

ROS formation increases dramatically in conditions of oxidative stress induced by xenobiotics, pollutants, ionizing radiation, and ultraviolet light. Excess ROS generation can cause cellular damage directly by attacking proteins and indirectly by generating further reactive species and initiating radical chain reactions. ROS have a critical role to play in the pathogenesis of various diseases, such as cardiovascular injury associated with circulatory disturbance. Glucocorticoids have been used in the treatment of a number of disease conditions due to their anti-inflammatory and immunosuppressive effects. They are known to alter the TBA-reactant levels as well as the the antioxidant enzymes activities (AOEs) of tissue/cell [3,4].

The pineal hormone melatonin is a very effective antioxidant agent and can enter the cell and nucleus. In previous studies, melatonin has been shown to protect against nucleic acid damage via an action involving generation of oxygen based free radicals produced by the administration of the chemical carcinogen safrole and ionizing radiation [5].

Antioxidant treatment has been shown to prevent and partially restore glucocorticoid induced vascular dysfunction and hypertension [6,7]. Based on these findings, it has been proposed that oxidative stress may be the underlying mechanism mediating unwanted side-effects of glucocorticoids.

In this study, we aimed to examine high dose steroid effects on oxidant-antioxidant systems in the rabbit heart and melatonin. We examined heart tissue MDA, PC, NOlevels, SOD, GSH-Px, and catalase activity in order to evaluate the oxidative-antioxidative status.

Materials and Methods

The study was conducted upon the approval of Animal Experiments Local Ethics Committee, Gaziosmanpaşa University (approval number: 2012/10, approval date: March 01, 2012). Experimental animals were obtained from Gaziosmanpaşa University Research and Application Center of Laboratory Animals. The surgical procedu-

res, administration of drugs, and sample collection in this study were performed in the same laboratory.

Experimental animals

White male rabbits (n=20) were used in the study. The ambient temperature of the animal cages was maintained at 20–24°C and the moisture at 50–55%. The rabbits were kept in a 12-h dark and 12-h light environment and they were given water and food *ad-libitum* during the study. The animals were made tofast the night before the surgical operation.

Study groups

There were three groups of animals used in the study. The control group (Grooup I) contained 5 rabbits, the Methylprednisolone group (Group II), contained 7 rabbits,and theMethylprednisolone plus melatonin group (Group III) contained 8 rabbits. Animals in group I were injected with saline intraperitonally. Rabbits in group II were injected once with methylprednisolone 20 mg/kg body weight [8] (intramuscular). Rabbits in group III were injected every other day with melatonin 20 mg/kg body weight [9] intraperitoneally for 14 days before injected once with methylprednisolone.

Surgical procedures

Prior to the operation, 30 mg/kg ketamine hydrochloride and 5 mg/kg xylazine were administered to the rabbits and the rabbits were left to ventilate spontaneously. The chest cavity was opened and the heart tissues were taken.

Sample collection

The rabbits were sacrificed at the end of day 14 and the heart tissue samples were collected. Heart tissue samples were stored at -80°C until processed for the estimation of biochemical parameters.

Biochemical parameters

Biochemical analysis of heart tissues

After weighing the heart tissues, they were homogenized in five volumes of ice-cold tris-HCl buffer (50 mM, pH 7.4) containing 0.05% Triton X-100. The homogenization procedure was carried out for 2 min at 13.000 rpm. All procedures were performed at 4°C. Homogenate, supernatant, and extracted samples were prepared and the following determinations were made on the samples using commercial chemicals. Protein measurements were made in the samples according to the method explained by Lowry et al [10].

MDA quantitation

The tissue MDA was determined by a method, based on reaction with thiobarbituric acid (TBA) at 90–100°C[11]. In the TBA test reaction, MDA or MDA-like substances and TBA react to produce a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2–3 and 90°C for 15 min. The sample was mixed with 2 vol. of cold 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water-bath for 10 min. After cooling, the absorbance was read at 532 nm. Results were expressed as nanomoles per gram wet tissue, according to the standard graphic prepared from measurements with a standard solution (1,1,3,3-tetramethoxypropane).

Determination of NO

NO measurement is very difficult in biological specimens; therefore, tissue nitrite (NO_2^-) and nitrate (NO_3^-) were estimated as an index of NO production. Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite+nitrate) was measured by a spectrophotometer at 545 nm after conversion of nitrate to nitrite by copperized cadmium granules. A standard curve was established with a set of serial dilutions (10^{-8}–10^{-3} mol/L) of sodium nitrite. Linear regression was carried out using the peak area from the nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations. Results were expressed as mmol/g wet tissue [12].

Determination of PC content

The PC content of the samples was determined based on the principle that carbonyl groups in proteins react with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone [13]. The absorbance of the 2,4-dinitrophenylhyd-

razone formed was measured at 365 nm and the results were presented in nanomoles per gram wet tissue.

Determination of total SOD activity

Total superoxide dismutase (Cu-Zn and Mn) activity was determined according to the method of Sun et al [14]. The principle of the method is based on inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 mL of ethanol-chloroform mixture (5:3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount causing 50.0% inhibition in the NBT reduction rate. The SOD activity is expressed as U/mg protein [15].

Determination of GSH-Px activity

Glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia and Valentine [16]. The enzymatic reaction in the tube (containing NADPH, reduced glutathione, sodium azide and glutathione reductase) was initiated by addition of H_2O_2 and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity is expressed as U/g protein.

Determination of catalase activity

The catalase activity of the samples was determined by the Aebi et al. method [17]. Catalase was allowed to break down 18.0% of hydrogen peroxide in 50 mM phosphate buffer (pH 7). The activity was monitored at 240 nm and the results were presented in cat/g protein.

Statistical method

Quantitative data are expressed as mean±Standard deviation (SD). All analyses were performed with SPSS 15.0 for windows software. Distribution of the groups was analyzed with the Shapiro-Wilk test. A One-way analysis of variance (ANOVA) test was performed and post hoc multiple comparisons were done with Tukey or Tamhane's T2 tests. For assesing homogeneity of variance Levene's test was used. When there was equal variance between groups, a Tukey or a Tamhane's T2 was used. Differences were considered to be statistically significant at $p<0.05$.

Results

The heart tissue MDA levels, increase in methylprednisolone (7.4%), and methylprednisolone plus melatonin (1.85%) groups compared to control group, but the increases were insignificant statistically. The PC levels were found high in the control group compared to the other groups. PC levels significantly decreased in the methylprednisolone plus melatonin group compared to the control group ($p=0.031$) and were found low in the methylprednisolone group compared to the methylprednisolone plus melatonin group even though they were insignificant. The NO levels were not statistically different between the control group and the methylprednisolone group. However, NO was low in the methylprednisolone plus melatonin group compared to the other two groups.

The SOD activites were not different in all groups. The GSH-Px activites did not differ in the control group compared to the methylprednisolone group and were insignificantly high in the methylprednisolone plus melatonin group compared to the other two groups. The catalase activites were insignificantly low in the control group compared to the other groups and insignificantly high in

Table 1: MDA, PC, NO, SOD, GSH-Px and CAT values of the study groups.

Parameters	Control (C)	Methylprednisolone (M)	Methylprednisolone +melatonin(MM)
MDA (nmol/g protein)	1.62±0.44	1.74±0.45	1.65±0.35
PC (nmol/mg protein)	1.06±0.11	0.87±0.25	0.83±0.11a
NO (µmol/g protein)	0.09±0.02	0.09±0.02	0.08±0.01
SOD (U/mg protein)	0.16±0.01	0.16±0.02	0.16±0.01
GSH-Px (U/g protein)	4.01±0.58	4.02±0.69	4.42±0.37
CAT (k/g protein)	0.48±0.11	0.71±0.23	0.74±0.29

MDA: Malondialdehyde; PC: Protein Carbonyl; NO: Nitric oxide; SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase; CAT: Catalase. Values are expressed as mean+SD. ap<0.05 significant differences compared to control.

Table 2: Tests of normality.

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
CAT	.202	18	.051	.896	18	.051
SOD	.115	18	.200*	.962	18	.643
MDA	.198	18	.062	.919	18	.122
GSHPx	.135	18	.200*	.956	18	.521
Protein_carbonyl	.175	18	.150	.921	18	.132
NO	.151	18	.200*	.884	18	.052

*This is a lower bound of the true significance. [a]: Lilliefors Significance Correction.

the methylprednisolone plus melatonin group compared to the others. These results are shown in Table 1. Table 1 and Figure 1. Shapiro-Wilk test results are shown in Table 2 and Levene's test results are shown in Table 3.

Discussion

One of the most important points of this study is that the effects of a single high dose methylprednisolone researched on oxidant and antioxidant systems in the heart tissue. In the literature, several studies have examined the effects of glucocorticoids on oxidant and antioxidant systems [3,5,18,19]. But little is known about the spesific effects of glucocorticoids on oxidant and antioxidant levels in the heart. This study has shed light on this area.

In the study by Rajashree et al., the MDA levels were found to increase in heart during days 12 and 16 of administration with dexamethasone. Even after withdrawal of dexamethasone, the levels were insignificantly elevated. In the same study, activities of antioxidant enzymes catalase, SOD, and GSH-Px exhibited a significant decrease in heart during dexamethasone administration [3]. Similarly, our study showed no differences in the MDA and NO levels in the all groups like early stages of Rajashree's study. But PC levels were found to significantly decrease in the group administered methlyprednisolone and melatonin together compared to the control group. From this result we can say that melatonin had a protective effect on proteins. PC levels decreased in the methlyprednisolone group compared to the control group, but were higher than the methlyprednisolone+melatonin group. However, these changes were not statistically significant. From this result, we can say that methlyprednisolone also had a protective effect on proteins but not as much as melatonin.

In our study, activities of antioxidant enzymes catalase, SOD, and GSH-Px had no observed significant changes between all groups.

Pereira et al. investigated the action of dexamethasone on different organs. Thiobarbituric acid reactant subtances decreased in the lymph nodes, thymus, and spleen but increased gastrocnemius and soleus muscle. GSH-Px activity increased in the lymphoid organs but decreased both skeletal muscles. Cu/Zn-SOD activity decreased in all five tissues. Catalase activity decreased in the thymus and mesenteric lymph nodes but increased in other tissues [20].

Dexamethasone administration is tissue specific and catalase, GSH-Px, and GSH decrease in liver but manga-

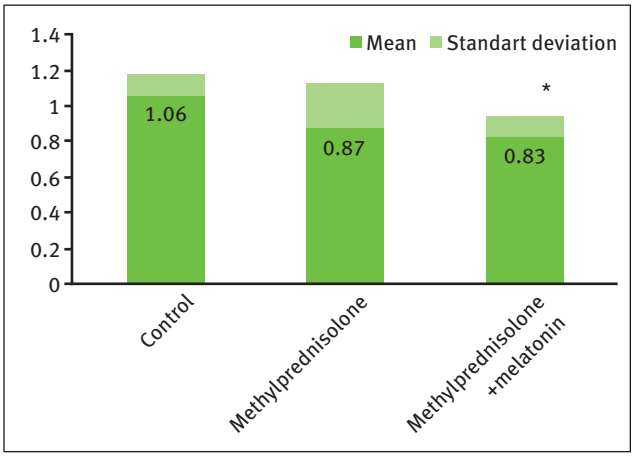


Figure 1: PC (nmol/mg protein), *p=0.031 compared to control group.

Table 3: Test of homogeneity of variances.

	Levene Statistic	df1	df2	Sig.
CAT	4.447	2	17	.028
SOD	.830	2	17	.453
MDA	.886	2	17	.431
GSHPx	1.143	2	17	.342
Protein_carbonyl	2.252	2	15	.140
NO	.741	2	17	.491

nese-SOD (Mn-SOD) increases in the brain and adipose tissue [21].

Orzechowskı et al. suggested dexamethasone is harmful directly or indirectly to antioxidants. In addition, old rats may be more vulnerable to the catabolic action of dexamethasone, whereas young rats are more susceptible to the oxidative stres induced by dexamethasone [18].

Camm et al. showed that postnatal glucocorticoid treatment in human clinically relevant doses reduced the total brain volume and induced oxidative stress in the developing brain in their study. Combined glucocorticoid and antioxidant treatment may improve some of the adverse effects of postnatal glucocorticoid therapy on the developing brain [4].

Arima et al. investigated dexamethasone administration effects in fetal and neonatal rat lung and between groups. There were no significant differences in Cu-Zn SOD, Mn-SOD, GSH-Px, and catalase levels similar to our study [22].

Dexamethasone administration during pregnancy increased mRNA expression of several aortic antioxidant enzymes in cubs of monkeys [23]. Schafer et al. found suppressed in serum levels of NO_2^-/NO_3^- by dexamethasone [24]. Melatonin enhances synthesis of antioxidant enzymes superoxide dismutase (SOD), GSH, catalase glutathione reductase, and glutathione peroxidase (GSH-Px) [25,26].

As understood from the above studies, the effects of glucocorticoids on oxidant-antioxidant systems were not a consensus subject. Some studies suggest that glucocorticoids increase oxidant markers but some increase antioxidant enzymes in the some organs.

According to the results of this study, we can say that administration of methylprednisolone once has no statistical effect on MDA, NO levels and SOD, GSH-Px, and catalase activity in the heart tissue. PC levels decreased in methylprednisolone plus melatonin group compared to the control group because of the strong antioxidant effects of melatonin. Consequentially, administration of a single high dose methylprednisolone doesn't increase oxidative stress and doesn't change antioxidant enzymes activity in the heart tissue. Also, melatonin is a potent antioxidant agent in the heart tissue.

Conflict of Interest: The authors have no conflict of interest.

References

[1] Alaçam H, Karli R, Alici O, Avci B, Güzel A, et al. The effects of α-tocopherol on oxidative damage and serum levels of Clara cell protein 16 in aspiration pneumonitis induced by bile acids. Hum Exp Toxicol 2013; 32(1):53–61.

[2] Alaçam H, Avcı B, Salıs O, Dilek A, Kozan A, et al. Does ADMA affect the oxidant/antioxidant balance in rats? Turk J Med Sci 2013; 43:405–10.

[3] Rajashree S, Puvanakrishnan R. Dexamethasone induced alterations in enzymatic and nonenzymatic antioxidant status in heart and kidney of rats. Mol Cell Biochem 1998; 181(1-2):77–85.

[4] Camm EJ, Tijsseling D, Richter HG, Adler A, Hansell JA, et al. Oxidative stress in the developing brain: effects of postnatal glucocorticoid therapy and antioxidants in the rat. PLoS One 2011; 6(6):21142.

[5] Cayli SR, Kocak A, Yilmaz U, Tekiner A, Erbil M, et al. Effect of combined treatment with melatonin and methylprednisolone on neurological recovery after experimental spinal cord injury. Eur Spine J 2004; 13(8):724–32.

[6] Iuchi T, Akaike M, Mitsui T, Ohshima Y, Shintani Y, et al. Glucocorticoid excess induces superoxide production in vascular endothelial cells and elicits vascular endothelial dysfunction. Circ Res 2003; 92(1):81–7.

[7] Zhang Y, Croft KD, Mori TA, Schyvens CG, McKenzie KU, et al. The antioxidant tempol prevents and partially reverses dexamethasone-induced hypertension in the rat. Am J Hypertens 2004; 17(3):260–5.

[8] Kuribayashi M, Fujioka M, Takahashi KA, Arai Y, Ishida M, et al. Vitamin E prevents steroid-induced osteonecrosis in rabbits. Acta Orthop 2010; 81(1):154–60.

[9] Spreer A, Gerber J, Baake D, Hanssen M, Huether G, et al. Antiinflammatory but no neuroprotective effects of melatonin under clinical treatment conditions in rabbit models of bacterial meningitis. J Neurosci Res 2006; 84(7):1575–9.

[10] Lowry Oh, Rosebrough Nj, Farr Al, Randall Rj. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193(1):265–75.

[11] Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol 1990; 186:407–21.

[12] Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem 1990; 36(8 Pt 1):1440–3.

[13] Levine RL, Garland D, Oliver CN, Amici A, Climent I, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990; 186:464–78.

[14] Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34(3):497–500.

[15] Durak I, Yurtarslanl Z, Canbolat O, Akyol O. A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. Clin Chim Acta 1993; 214(1):103–4.

[16] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70(1):158–69.

[17] Aebi H, Wyss SR, Scherz B, Skvaril F. Heterogeneity of erythrocyte catalase II. Isolation and characterization of normal and variant erythrocyte catalase and their subunits. Eur J Biochem. 1974; 48(1):137–45.

[18] Orzechowski A, Ostaszewski P, Wilczak J, Jank M, Bałasińska B, et al. Rats with a glucocorticoid-induced catabolic state show symptoms of oxidative stress and spleen atrophy: the effects

of age and recovery. J Vet Med A Physiol Pathol Clin Med 2002; 49(5):256–63.
[19] Karlidağ T, Yalçin S, Oztürk A, Ustündağ B, Gök U, et al. The role of free oxygen radicals in noise induced hearing loss: effects of melatonin and methylprednisolone. Auris Nasus Larynx 2002; 29(2):147–52.
[20] Pereira B, Bechara EJ, Mendonça JR, Curi R. Superoxide dismutase, catalase and glutathione peroxidase activities in the lymphoid organs and skeletal muscles of rats treated with dexamethasone. Cell Biochem Funct 1999; 17(1):15–9.
[21] Petrovic VM, Saicic ZS, Spasic M, Radojicic R, Buzadzic B. Hormones and AO defence. In: OF Nygaard, AC Upton (eds). Proc Int Conf Anticarcinog Radiat Prot 2; 1989. Plenum, New York 1991; 405–13.
[22] Arima M, Kumai T, Asoh K, Takeba Y, Murano K, et al. Effects of antenatal dexamethasone on antioxidant enzymes and nitric oxide synthase in the rat lung. J Pharmacol Sci 2008; 106(2):242–8.
[23] Atanasova S, Wieland E, Schlumbohm C, Korecka M, Shaw L, et al. Prenatal dexamethasone exposure in the common marmoset monkey enhances gene expression of antioxidant enzymes in the aorta of adult offspring. Stress 2009; 12(3):215–24.
[24] Schäfer SC, Wallerath T, Closs EI, Schmidt C, Schwarz PM, et al. Dexamethasone suppresses eNOS and CAT-1 and induces oxidative stress in mouse resistance arterioles. Am J Physiol Heart Circ Physiol 2005; 288(1):H436–44.
[25] Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. J Biomed Sci 2000; 7(6):444–58.
[26] Menendez-Pelaez A, Poeggeler B, Reiter RJ, Barlow-Walden L, Pablos MI, et al. Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. J Cell Biochem 1993; 53(4):373–82.