DOI: 10.4149/BLL_2019_085

Bratisl Med J 2019; 120 (7)
527 – 531

EXPERIMENTAL STUDY

Melatonin treatment prevents carbon tetrachloride-induced acute lung injury in rats by mitigating tissue antioxidant capacity and inflammatory response

Radovic M[1,2,3], Ristic L[1,2], Krtinic D[4,5], Rancic M[1,2], Nickovic V[6], Vujnovic Zivkovic ZN[7], Zivkovic JB[7], Mirkovic MV[7], Toskic DR[8], Sokolovic D[9]

Department for Internal Medicine, Faculty of Medicine, University of Niš, Niš, Serbia.
dusantsokolovic@gmail.com

ABSTRACT
AIM: Carbon tetrachloride (CCl_4) is an organic chemical that produces different tissue-damaging effects when ingested or inhaled. Present study aims to determine whether the application of exogenous melatonin, a neurohormone with numerous biological properties, can prevent disturbances in lung tissue antioxidative capacities and arginine metabolism, tissue inflammation and oxidative damage induced by exposure to CCl_4 in rats.
METHODS: The effects of melatonin on the changes occurring in rat lung tissue after an acute exposure to CCl_4 were studied by monitoring alterations in antioxidant capacities, inflammatory parameters, parameters of arginine metabolism, and lipid and protein oxidative damage.
RESULTS: The results indicated that melatonin prevents CCl_4-induced lung damage by mitigating tissue antioxidant capacity and preventing nitric oxide production through a shift from nitric oxide synthase to arginase. Also, melatonin partially prevented tissue inflammation and molecules' oxidative modification seen after exposure to CCl_4.
CONCLUSIONS: The protective activity of melatonin can be attributed to its ability to scavenge both free radicals, as well as to its potential to increase tissue antioxidant capacity. The modulation of inflammatory response through both decrease in tissue inflammatory parameters and influence on arginine-nitric oxide metabolism might be an additional mechanism of action *(Tab. 1, Fig. 2, Ref. 33)*. Text in PDF *www.elis.sk.*
KEY WORDS: lung injury, carbon tetrachloride, melatonin, tissue antioxidant capacity, arginine metabolism.

Introduction

Carbon tetrachloride (CCl_4) is an organic industrial chemical most frequently generated in chemical production facilities that produce chlorine, hydrogen, and alkali, and while its toxic potential is well known, its production and consumption for emissive uses is believed to be fully controlled (1). In humans (e.g. workers exposed to CCl_4) and in experimental animals administered with CCl_4, it causes massive damage to the liver, kidneys, respiratory system, skin, etc. (2). Since around 4 % of CCl_4 is eliminated by exhalation, one can expect that lung tissue might be a potential target organ for the damage caused by the exposure to this highly toxic chemical (3).

Shortly after its absorption, CCl_4 is metabolized by cytochrome P-450 which is predominantly localized in liver tissue. Nevertheless, its expression is not negligible in other tissues such as lungs (4), where the products of this metabolism, highly reactive trichloromethyl ($CCl_3^{\bullet}$) and trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$) induce intra-alveolar septal ruptures and interstitial cell degenerations. This sequence of events takes place due to the activity of inflammatory cells (neutrophils and macrophages) attracted to the damaged tissue (6). One could also argue that the lung tissue damage after CCl_4 application could be incurred by kidney damage, since a decrease in water excretion leads to lung oedema (7). Besides the increase in reactive oxygen species (ROS) production, CCl_4 causes a release of inflammatory cytokines (8) and suppression in antioxidant capacities (decrease in reduced glutathione (GSH), vitamin C and E, and antioxidant enzymes), as well as brings about an increase in lipid peroxidation, protein carboxylation, and DNA damage, which lead to cellular caspase-mediated apoptosis or necrosis (9).

Melatonin (MLT, N-acetyl-5-methoxytryptamine) is a neurohormone produced mainly by pineal gland cells, but also by other tissues/cells (10). Studies revealed that MLT is able to scavenge ox-

[1]Department for Internal Medicine, Faculty of Medicine, University of Niš, Niš, Serbia, [2]Clinic for Lung Diseases, Clinical Center Niš, Niš, Serbia, [3]Clinic for Thoracic Surgery, Clinical Center Niš, Niš, Serbia, [4]Department for Pharmacology with Toxicology, Faculty of Medicine, University of Niš, Niš, Serbia, [5]Clinic for Oncology, Clinical Center Niš, Niš, Serbia, [6]Clinical-Hospital Center, Pristina, Gracanica, Serbia, [7]Medical Faculty in Kosovska Mitrovica, University of Priština, Kosovska Mitrovica, Serbia, [8]Faculty of Sport and Physical Education Leposavic, University of Pristina, Pristina, Serbia, and [9]Institute for Biochemistry, Faculty of Medicine, University of Niš, Niš, Serbia

Address for correspondence: D. T Sokolović, Institute for Biochemistry, Faculty of Medicine, University of Niš, Zorana Dindica 81, 18000 Niš, Serbia.
Phone number: +381642136478, Fax: +381.18238770

Acknowledgements: This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. III43012).

ygen- and nitrogen-based reactants that are known to damage cell macromolecules, as well as to increase tissue antioxidant capacity (6, 10, 11). It was found that MLT acts by increasing the expression of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) mRNA, and mRNA of enzymes related to GSH synthesis (11). Melatonin was previously proven to ameliorate lung tissue injury induced by bleomycin (11), acute pancreatitis (12), aortic occlusion (13), lipopolysaccharide (14), phosgene (15), etc.

Having in mind that CCl_4 is a dangerous industrial pollutant still present in the working environments of many undeveloped countries, and that its inhalation might lead to lung tissue injury, we aimed to evaluate whether the application of MLT would prevent lung tissue damage induced by this hazardous chemical. The effects of MLT would be estimated based on its potential in preventing the disturbances in pulmonary non-enzymatic tissue antioxidant capacity (TAC; vitamin C and GSH) and enzymatic antioxidant capacities (GPx and CAT) produced by CCl_4. The effect of MLT on inflammatory cell infiltration would be estimated indirectly through the myeloperoxidase (MPO) activity, which represents an enzyme located within macrophages/neutrophils. The changes in arginine metabolism that follow CCl_4 application and the impact of MLT treatment are going to be assessed based on nitrate/nitrite and citrulline concentrations, as well as based on changes in arginase and iNOS activities. In the end, the potential of MLT in the prevention of lipid peroxidation (malondialdehyde (MDA) levels) and carbonyl protein modification induced by CCl_4 would be determined as well.

Materials and methods

Drugs and chemicals

All reagents, solvents and drugs used in this investigation were of analytical grade or better and were obtained from Sigma-Aldrich (St. Louis, MO, USA), Carl Roth (Karlsruhe,Germany) or Richter Pharma AG (Wels, Austria). On the day of the experiment, melatonin was dissolved in absolute ethanol and further diluted with a sterile saline solution (0.9 % NaCl) prior to the administration at a dose of 50 mg/kg (10).

Animals and housing

Adult male disease-free Wistar rats (n = 24) weighing 250–300 g were maintained under standard laboratory conditions: 22 ± 2 °C, 60 % humidity, and 12/12 light/dark cycle. Food and water were available *ad libitum* throughout the experiment. All experimental procedures were conducted in compliance with The European Council Directive (EU Directive of 2010; 2010/63/EU) and were approved by the local Ethics Committee.

Experimental design

The animal model of acute organ damage, in this case lung tissue damaged by CCl_4, was performed according to previous publications (2, 9). Groups of animals were treated as follows:

Group I: Animals treated once with saline containing 8 % of ethanol (10 ml/kg).

Group II: Animals treated once with MLT (50 mg/kg).

Group III: Animals received a single CCl_4 dose (1 ml/kg).

Group IV: Animals treated once with MLT (50 mg/kg) 1h after a single CCl_4 dose.

Twenty-four hours after the last treatment all animals were sacrificed with an overdose of general anaesthetic, namely ketamine, and their body weight was measured. After that, their thoracic cavity was opened, lung tissue dissected, weighted and stored in Eppendorf tubes at –80 °C until homogenization.

Tissue biochemical parameters

Tissue homogenization

After scarification, the lung tissue was isolated from the thoracic cavity, washed and homogenized in cold distilled water (10 %, w/v). The obtained homogenate was centrifuged at 12,000 rpm for 10 min at 4 °C in order to obtain a clear supernatant which was further used for determining the tissue biochemical parameters. The protein content in lung tissue homogenate was determined by using the Lowry's method (16), and the amount of proteins was calculated based on the bovine serum albumin standard curve. All measured biochemical parameters were calculated based on the amounts of proteins in tissue homogenates.

Determination of tissue antioxidant capacities

Lung tissue antioxidant capacity (TAC) and vitamin C were determined by using colorimetric assay kits (Abcam65329 and ab65656). The amount of GSH in lung tissue homogenates was determined using standard Ellman method which is based on the reaction of non-protein thiols with DTNB reagent (17).

The activity of GPx was determined by using a previously described spectrophotometric method that utilises GSH, H_2O_2 and DTNB reagent (5), while the catalase activity was measured spectrophotometrically after the reaction of the enzyme with H_2O_2 as a substrate and ammonium molybdate at 405 nm (10).

Inflammatory parameter determination

The activity of MPO was measured through the amount of oxidized o-phenylenediamine in a reaction which contained lung tissue homogenate supernatant and H_2O_2 (18).

Determination of biochemical parameters related to arginine metabolism

The concentration of nitrates/nitrites present in lung tissue homogenates was determined in a mixture consisting of tissue homogenate and Griess reagent (19). The amount of citrulline in lung tissue homogenate was determined according to the standard method that is based on the reaction with diacetyl monoxime and thiosemicarbazide (20). The arginase activity in tissue was determined using a colorimetric method which is based on the reaction between ornithine and ninhydrin (21). Quantitative determination of rat lung tissue iNOS was performed using CUSBIO (CSB-08325r) sandwich enzyme immunoassay kit following manufacturers' instructions.

Lipid and protein damage determination

The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric

Tab. 1. Lung tissue non-enzymatic and enzymatic antioxidant capacities determined for different experimental groups.

Biochemical parameter/Group	I (Vehicle control)	II (MLT control)	III (CCl_4 control)	IV (CCl_4 + MLT)
Total antioxidant capacity (% change)	100±1.9	112.9±6.6**	88.4±0.6*	96.1±2.1$^{\#}$
Non-enzymatic oxidative capacities				
GSH (nmol/mg of proteins)	0.25±0.007	0.27±0.004*	0.065±0.004*	0.140±0.003*$^{,\#}$
Vitamin C (nmol/g of proteins)	32.4±4.2	37.9±2.5	6.1±1.8*	27.6±14.4$^{\#}$
Enzymatic oxidative capacities				
GPx (U/mg of proteins)	1.12±0.11	1.14±0.20**	0.82±0.19*	0.93±0.05
CAT (U/g of proteins)	113.6±9.9	109.7±8.6	82.7±5.1*	99.6±2.3**$^{,\#}$
Arginine metabolism parameters				
Nitrite/nitrate (nmol/mg of proteins)	35.7±3.4	38.4±1.1	45.2±2.8*	39.1±0.8**$^{,\#}$
Citrulline (nmol/g of proteins)	73.1±9.4	67.4±5.3	89.2±4.2**	77.5±6.5$^{\#\#}$
Arginase (μmol/g of proteins)	24.7±1.5	26.5±0.2	10.7±6**	26.2±13.5
iNOS (IU/mg of proteins)	0.079±0.003	0.065±0.010	0.098±0.011*	0.081±0.013*$^{,\#}$

Values are given as means values ± SD, n = 6. One-way ANOVA followed by Tukey's test was used to compare the groups. * $p < 0.001$, ** $p < 0.05$ vs Control (group I), $^{\#}$ $p < 0.001$, $^{\#\#}$ $p < 0.05$ vs CCl_4-treated animals (group III).

acid (10). Protein carbonyl content was used for quantifying the oxidatively modified proteins and their content was determined spectrophotometrically using 2,4-dinitrophenylhydrazine as a colour reagent (22).

Statistical analysis

The obtained variables are presented as means ± SD and were compared using one-way ANOVA and appropriate post-hoc tests (GraphPad Prism, ver. 5.03; San Diego, CA). Probability values were taken to be statistically significant if the p value was lower or equal to 0.05.

Results

Although the exposure of animals to CCl_4 led to a decrease in relative lung tissue mass, this decrease was not found to be statically significant when compared to group I (control group). Interestingly, the mean value for the relative lung tissue weight in group IV was almost identical to that in one of the control groups. Nevertheless, no significant difference was observed (data not shown).

Total antioxidant capacity in lung tissue was significantly increased in healthy animals treated with MLT, i.e. in group II (Tab. 1), while a single dose of CCl_4 led to a decrease in TAC (group III). Such a dramatic decrease in TAC was prevented by the treatment with MLT in group IV (Tab. 1). The application of MLT to healthy animals led to a significant increase in GSH concentrations and GPx activity, while having no effect on vitamin C concentrations and CAT activity (Tab 1). When compared to animals in group I, the acute administration of CCl_4 produced a significant diminution in both enzymatic and non-enzymatic antioxidant capacities ($p < 0.001$) (Tab. 1) The effects of exogenous MLT after CCl_4 were evident in almost all studied parameters related to lung tissue antioxidant capacities (Tab. 1).

When compared to untreated animals, the exposure of rats to CCl_4 led to a significant increase in lung tissue MPO activity (Fig. 1), suggesting an increase in inflammatory cells in the tissue. The treatment with MLT after exposure to CCl_4 prevented such increase in MPO activity, while MLT applied on its own had no effect on MPO activity (Fig. 1).

Compared to untreated animals, the acute application of CCl_4 significantly affected all studied participating species of arginine metabolism (enzymes and their products) (Tab. 1). The metabolism of arginine in animals treated with MLT after CCl_4 was only slightly disturbed, and in majority of parameters, MLT's effect appeared to be significant compared to the parameters obtained from animals treated with CCl_4 only (Tab. 1).

When compared to the untreated animals from group I, the applied CCl4 produced a significant increase in MDA and lung tissue concentrations of oxidized proteins ($p < 0.001$) (Figs 2A and B). The treatment with MLT taking place 1 h after CCl_4 administration prevented such a significant increase in these two parameters. However, its potential was limited since the detected concentrations of MDA and carbonylated proteins were still higher than those in group I (Figs 2A and B).

Discussion

Free radicals generated in the liver as well as in other tissues after the application of CCl_4 (4) react with polyunsaturated fatty acids present in cell and organelle membranes. Such enhancement in lipid peroxidation leads to the formation of products that can be

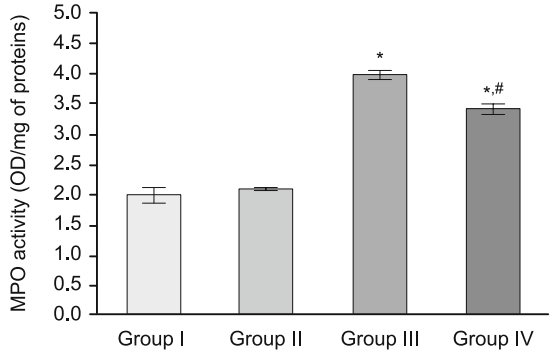

Fig. 1. Effects of MLT on CCl_4-induced inflammatory changes in lung tissue; MPO activity, data are presented as mean ± SD (n = 6). * $p < 0.001$ vs Control (group I), # $p < 0.001$ vs CCl_4 treated animals (group III).

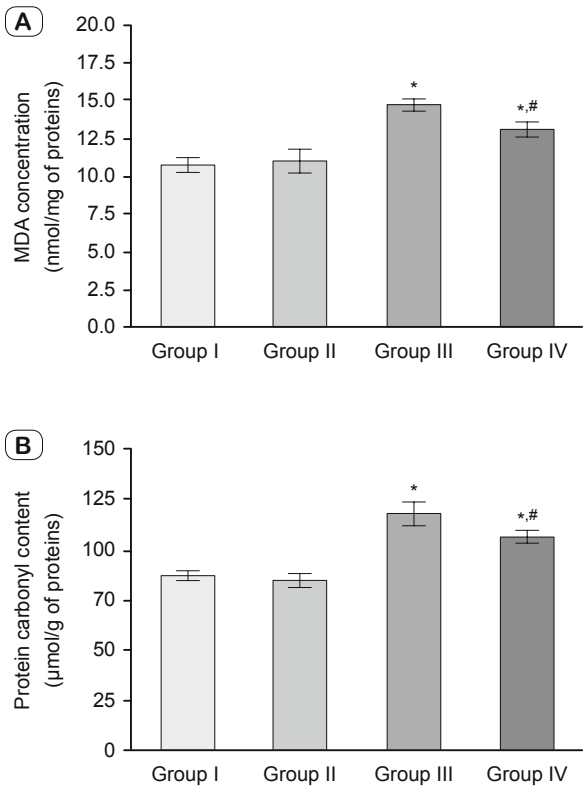

Fig. 2. Lung tissue MDA (A) and carbonylated protein (B) concentrations obtained from different experimental groups. Data are presented as mean ± SD (n = 6). * p < 0.001 vs Control (group I), # p < 0.001 vs CCl_4 treated animals (group III).

measured in the reaction with thiobarbituric acid (23). The process of lipid peroxidation is tightly connected with the activity of GPx which uses GSH to terminate this reaction. A significant increase in the amount of lipid peroxidation products (MDA and 4-hydroxy-2-nonenal) causes enzyme inhibition (GPx and others) and decreases protein synthesis (24). Generally, due to excessive ROS production after CCl_4 application, TAC is shown to be decreased in rat liver and muscle tissue (9), and it seems that lung tissue does not represent an exception (Tab. 1). The treatment of animals with MLT prevented the decrease in TAC and vitamin C concentrations, and such activity could be related to the ability of MLT to act as an antioxidant, i.e. free radical scavenger, or to increase tissue antioxidant enzyme expression (11). Additionally, MLT is known to scavenge peroxynitrite anion formed after interaction between NO and superoxide anion and shown to be highly toxic for cells while possibly contributing to the oxidative injury in the lung (25).

Besides lipid damage, the increased concentration of ROS oxidatively modifies cell proteins (forming carbonyl groups on protein side chains), thus leading to their conformational changes and impairing their function (26). This marker is suggested to be related to acute respiratory distress syndrome, where an increased concentration of carbonylated proteins are found in bronchoalveolar lavages (27). Previous studies suggest that when found in alveoli, these proteins cause a disturbance in protease/antiprotease balance and/or surfactant, or lead to mucus disorganization (27). This study is not the first one to show the potential of MLT in preventing the formation of carbonylated proteins (28). However, to the best of our knowledge, this is the first study showing the toxic potential of CCl_4 in inducing protein oxidation in lung tissue (Fig. 2B).

The results related to CAT activity in lung tissue of animals exposed to CCl_4 and treated with MLT contribute to the understanding of our previous findings. The increase in CAT activity previously reported to be seen in animals treated with MLT (11), or the preservation of its activity (spend) could lead to the expectation that the activity of GPx and GSH concentrations would be only partially affected.

Apart from the disturbances in tissue oxidative capacities, CCl_4 is known to cause tissue inflammatory response, and the lungs are not an exception (8). In the present work, we estimated lung tissue inflammation based on MPO activity and NO concentrations (Fig. 1 and Tab. 1), where a significant increase in inflammatory parameters in rat lungs was observed after CCl_4 application. As in the case of studied antioxidant capacities, exogenous MLT partially ameliorated the inflammatory reaction in rat lung tissue (Fig. 1 and Tab. 1). Previous studies showed that MLT application prevents lung tissue neutrophil infiltration estimated based on MPO activity in mice with pulmonary fibrosis induced by bleomycin (11). This partial discrepancy in the findings might be attributed to different models of lung tissue damage, as well as to a different treatment regime, which in the mentioned study was sub-chronic while in ours, it was acute. Released MPO was found to be acting as a pro-inflammatory cytokine on alveolar and bronchial epithelial cells and leading to an increase in haemoxygenase-1 expression, thus causing lung tissue oxidative damage (29). Since the effect of MLT on MPO activity was slight, it leaves a "window" for this neutrophil/macrophage-borne enzyme to act and cause cell oxidative damage.

In the pathophysiological response of lungs to CCl_4, macrophages seem to play an important role since they release numerous different lytic enzymes (MPO) and inflammatory signalling molecules (NO, TNF-α and IL-1 β) (8). An increase in these signalling molecules is known to be tightly connected to the increase in calcium-independent iNOS activity and NO which increases tissue blood flow by causing profound vasodilation (30, 31). An application of MLT in rats with damaged lung tissue effectively inhibited NO production and iNOS activity which is in accordance with a previously observed decrease in NO production by inhibition of iNOS expression via inhibition p300 histone acetyltransferase in macrophages (32). The hereby obtained results of the lung tissue arginase activity in rats treated with CCl_4 and MLT are in accordance with previous studies, where possibly due to an increase in arginine concentrations following iNOS inhibition, the substrate upregulates the activity of the enzyme (arginase) by itself (33).

The activity of MLT in CCl_4-mediated lung tissue damage model can occur in consequence of several factors. Having in mind that MLT was administered after intoxication with CCl_4 in a single dose of 50 mg/kg, we can conclude that MLT most certainly possesses a significant protective potential. Since the application of CCl_4 is causing multiorgan damage via a very similar, one may say identical mechanism, it is unrealistic to expect that a single dose of MLT would completely prevent multiorgan damage. Generally

speaking, MLT predominantly improved tissue antioxidant capacities, or prevented its decline, and decreased oxidative tissue damage that was estimated through the levels of MDA and carbonyl-modified proteins. Also, MLT inhibited the inflammatory response by mitigating the increase in MPO activity produced by the application of CCl_4.

References

1. Sherry D, McCulloch A, Liang Q, Reimann S, Newman PA. Current sources of carbon tetrachloride (CCl_4) in our atmosphere. Environ Res Lett 2018; 13: 024004.

2. Radulović NS, Randjelović PJ, Stojanović NM, Ilić IR, Miltojević AB. Influence of methyl and isopropyl N-methyl anthranilates on carbon tetrachloride-induced changes in rat liver morphology and function. FU Phys Chem Technol 2013; 11 (1): 67–73.

3. Al-Dbass AM, Al-Daihan SK, Bhat RS. *Agaricus blazeimurill* as an efficient hepatoprotective and antioxidant agent against CCl_4-induced liver injury in rats. Saudi J Biol Sci 2012; 19 (3): 303–309.

4. Slater TF, Cheeseman KH, Ingold KU. Carbon tetrachloride toxicity as a model for studying free-radical mediated liver injury. Philos Trans R Soc Lond B Biol Sci 1985; 31 (1152): 633–645.

5. Popović D, Đukić D, Katić V, Jović Z, Jović M, Lalić J et al. Antioxidant and proapoptotic effects of anthocyanins from bilberry extract in rats exposed to hepatotoxic effects of carbon tetrachloride. Life Sci 2016; 157: 168–177.

6. Ahmad B, Khan MR, Shah NA. Amelioration of carbon tetrachloride-induced pulmonary toxicity with *Oxalis corniculata*. Toxicol Ind Health 2015; 31 (12): 1243–1251.

7. Agency for toxic substances and disease registry (ATSDR) Toxicological Profile for Carbon Tetrachloride, 2005.

8. Kurt A, Tumkaya L, Yuce S, Turut H, Cure MC, Sehitoglu I et al. The protective effect of infliximab against carbon tetrachloride-induced acute lung injury. Iran J Basic Med Sci 2016; 19 (6): 685–691.

9. Xie J, Liu J, Chen TM, Lan Q, Zhang QY, Liu B et al. Dihydromyricetin alleviates carbon tetrachlorideinduced acute liver injury via JNK-dependent mechanism in mice. World J Gastroenterol 2015; 21 (18): 5473–5481.

10. Djordjevic B, Sokolovic D, Kocic G, Veljkovic A, Despotovic M, Basic J et al. The effect of melatonin on the liver of rats exposed to microwave radiation. Bratisl Lek Listy 2015; 116 (2): 96–100.

11. Genovese T, Di Paola R, Mazzon E, Muià C, Caputi AP, Cuzzocrea, S. Melatonin limits lung injury in bleomycin treated mice. J Pineal Res 2005; 39 (2): 105–112.

12. Huai JP, Sun XC, Chen MJ, Jin Y, Ye XH, Wu JS, Huang ZM. Melatonin attenuates acute pancreatitis-associated lung injury in rats by modulating interleukin 22. World J Gastroenterol 2012; 18 (36): 5122–5128.

13. Okutan H, Savas C, Delibas N. The antioxidant effect of melatonin in lung injury after aortic occlusion-reperfusion. Interact Cardiovasc Thorac Surg 2004; 3 (3): 519–522.

14. Zhang Y, Li X, Grailer JJ, Wang N, Wang M, Yao J et al. Melatonin alleviates acute lung injury through inhibiting the NLRP3 inflammasome. J Pineal Res 2016; 60 (4): 405-414.

15. Zhang L, Zhang F, He D, Xu D, Zhong Z, Shen J. Melatonin attenuates phosgene-induced acute lung injury via the upregulation Wnt/β-catenin pathway. Int J Clin Exp Pathol 2017; 10 (11): 11281–11287.

16. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193 (1): 265–275.

17. Ellman G. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82 (1): 70–77.

18. Radulović, N.S., Zlatković DB, Mitić KV, Randjelović PJ, Stojanović NM. Synthesis, spectral characterization, cytotoxicity and enzyme-inhibiting activity of new ferroceneeindole hybrids. Polyhedron 2014; 80: 134–141.

19. Radulović NR, Randjelovic PJ, Stojanović NM, Cakić ND, Bogdanović GA, Živanović AV. Aboriginal bush foods: a major phloroglucinol from Crimson Bottlebrush flowers (*Callistemon citrinus*, Myrtaceae) displays strong antinociceptive and antiinflammatory activity. Food Res Internat 2015; 77 (2); 280–289.

20. Boyde TR, Rahmatullah M. Optimization of conditions for the colorimetric determination of citrulline, using diacetyl monoxime. Ann Biochem 1980; 107 (2): 424–431.

21. Porembska Z, Kedra M. Early diagnosis of myocardial infarction by arginase activity determination. Clin Chim Acta 1975; 60 (3): 355–361.

22. Levine RL, Williams JA, Stadtman ER, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 1994; 233: 346–357.

23. Bicer M, Akil M, Baltaci AK, Mogulkoc R, Sivrikaya A, Gunay M, Akkus H. Protective effect of melatonin on lipid peroxidation in various tissues of diabetic rats subjected to an acute swimming exercise. Bratisl Med J 2012; 113 (12): 698–701.

24. Szymonik-Lesiuk S, Czechowska G, Stryjecka-Zimmer M, Słomka M, Madro A, Celiński K et al. Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. J Hepatobiliary Pancreat Surg 2003; 10 (4): 309–315.

25. Ischiropoulos H, Al-Mehdi AB, Fisher AB. Reactive species in ischemic rat lung injury (contribution of peroxynitrite). Am J Physiol 1995; 269 (2 Pt 1): 158–164.

26. Jung T, Bader N, Grune T. Oxidized proteins: intracellular distribution and recognition by the proteasome. Arch Biochem Biophys 2007; 462 (2): 231–237.

27. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta 2003; 329 (1–2): 23–38.

28. Dkhar P, Sharma R. Attenuation of age-related increase of protein carbonylation in the liver of mice by melatonin and curcumin. Mol Cell Biochem 2013; 380 (1–2): 153–160.

29. Haegens A, Vernooy JH, Heeringa P, Mossman BT, Wouters EF. Myeloperoxidase modulates lung epithelial responses to pro-inflammatory agents. Eur Respir J 2008; 31 (2): 252–260.

30. Pedoto A, Caruso JE, Nandi J, Oler A, Hoffmann SP, Tassiopoulos AK et al. Acidosis stimulates nitric oxide production and lung damage in rats. Am J Respir Crit Care Med 1999; 159 (2): 397–402.

31. Tanaka N, Tanaka K, Nagashima Y, Kondo M, Sekihara H. Nitric oxide increases hepatic arterial blood flow in rats with carbon tetrachloride-induced acute hepatic injury. Gastroenterology 1999; 117 (1): 173–180.

32. Deng WG, Tang ST, Tseng HP,Wu, K K. Melatonin suppresses macrophage cyclooxygenase-2 and inducible nitric oxide synthase expression by inhibiting p52 acetylation and binding. Blood 2006; 108 (2): 518–524.

33. Aydogdu N, Erbas H, Atmaca G, Erten O, Kaymak K. Melatonin reduces nitric oxide via increasing arginase in rhabdomyolysis-induced acute renal failure in rats. Ren Fail 2006; 28 (5): 435–440.

Received February 24, 2019.
Accepted April 8, 2019.