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Melatonin is effective in reducing stress-induced organ damage in Wistar albino rats

Mukaddes EŞREFOĞLU[1,*], Ayşin AKINCI[2], Hülya ELBE[3], Elif TAŞLIDERE[3], Aslı ÇETİN[3], Burhan ATEŞ[4]

[1]Department of Histology and Embryology, Faculty of Medicine, Bezmialem Vakıf University, İstanbul, Turkey
[2]Doğu Fertil Tüp Bebek Merkezi, Malatya, Turkey
[3]Department of Histology and Embryology, Faculty of Medicine, İnönü University, Malatya, Turkey
[4]Department of Biochemistry, Faculty of Science and Art, İnönü University, Malatya, Turkey

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Abstract: In the present study, we tried to investigate the effects of melatonin, a novel antioxidant and a potent free radical scavenger, in stress-induced cerebral, cerebellar, cardiac, and hepatic oxidative damage using microscopic and biochemical analysis. A total of 32 male Wistar albino rats were divided into control, stress, stress + saline, and stress + melatonin groups. The rats from the stress groups were exposed to high stress conditions of starvation, immobilization, and cold exposure. The rats from the stress + melatonin group received melatonin daily at 20 mg/kg body weight intraperitoneally for 7 days. At the end of the experiment, the brain, cerebellum, heart, and liver were rapidly removed. The main histopathological damage scores (MHDSs) of the stress and stress + saline groups were higher than those of control group for all of the organs. The MHDSs of melatonin-administered group were lower than those of stress and stress + saline groups. The main tissue superoxide dismutase activities of the stress + melatonin group were even higher than those of the control group in the cerebellum and liver, and main tissue catalase activities of the stress + melatonin group were even higher than those of control group in all of the organs. As a conclusion, we found melatonin very effective in reducing stress-induced organ damage by inhibiting lipid peroxidation and supporting the cellular antioxidant defense system.

Key words: Antioxidant enzymes, organ injury, oxidative stress, melatonin

1. Introduction

Stress, either emotional or physical, disrupts the balance between free radicals and antioxidant defenses, disturbs homeostasis, and leads to cell injury. Stress-induced oxidative damage participates in the autogenesis of many diseases such as cancers and neurodegenerative and cardiovascular disorders. Oxygen is necessary for life, but, paradoxically, as a by-product of its metabolism, it produces reactive oxygen species (ROS), which are highly toxic to cells (Andersen, 2004). The heart (an obligate aerobic organ), brain, and liver have high oxygen consumption. The heart, at its resting pulse rate, consumes approximately 8–15 mL O_2 min^{-1} 100 g $tissue^{-1}$, whereas the brain consumes approximately 3 mL O_2 min^{-1} 100 g $tissue^{-1}$ (West, 1991; Braunwald, 2001). On the other hand, the liver is known to be a highly metabolically active organ, accounting for about 25% of cardiac output as well as 25% of oxygen consumption in the resting man. The mammalian heart and brain are often said to be especially sensitive to oxidative damage. Moreover, the liver, in particular, is highly susceptible to free radical-mediated insult owing to its high lipid content (Pocernich et al., 2001). ROS in oxidative stress react with almost all cellular biomolecules, such as lipids, nucleic acid, and proteins, and change their structure and functions, which leads to cell damage and even death. Malondialdehyde (MDA), a lipid-peroxidation end-product, is an important indicator of cellular injury.

Experimental stress can be induced using various methods such as immobilization, cold-restraint, and starvation. Each stress model affects different organs to different degrees (Gilgun-Sherki et al., 2002). It was reported that immobilization stress is an easy and convenient method to induce both psychological (escape reaction) and physical stress (muscle work), resulting in restricted mobility and aggression (Dhanalakshmi et al., 2007). It was reported to be the most severe type of stress in rodent models and has a comparative effect in humans (Gilgun-Sherki et al., 2002). Immobilization stress increases lipid peroxidation but decreases free radical scavenging (Liu et al., 1996). Since the rate of oxygen consumption is increased during cold stress, cold exposure results in a higher degree of oxidative

* Correspondence: drmukaddes@hotmail.com

stress (Terblance et al., 2000; Şahin and Gümüşlü, 2004). Cold exposure induces lesions such as microhemorrhages, vessel dilatation, neuronal shrinkage, pyknosis, necrosis, and edema (Kamada et al., 1995). Moreover, cold exposure results in significant cardiopulmonary stress. The subcellular changes in the myocardium, in response to external cold, are characterized by mitochondrial hypertrophy, intracellular edema, destruction of myofibrils, dilatation of the intercalated discs, and some abnormalities in the capillaries (Lin et al., 1997). Cold stress also induces prominent histopathological changes in the liver such as necrosis, hepatocyte degeneration, sinusoidal dilatation, hemorrhage, vascular congestion, and dilatation (Ateş et al., 2006).

Organisms widely use glutathione, glutathione peroxidase, glutathione transferase, superoxide dismutase (SOD), catalase (CAT), and a variety of other antioxidants to protect themselves against generation of ROS (Terblance et al., 2000; Andersen, 2004; Augustyniak and Skrzydlewska, 2004). Melatonin, a unique hormone produced in the pineal gland, is a powerful antioxidant and a potent free radical scavenger. It also stimulates the activities of the enzymes of cellular defense systems (Eşrefoğlu et al., 2006, 2010, 2011). Since it is highly lipophilic, it can readily cross the blood–brain barrier and can enter the cytoplasm of neurons and glial cells when administered exogenously.

As oxidative stress is a common pathogenetic mechanism contributing to the initiation and progression of stress-induced organ injury, antioxidants represent a logical therapeutic and preventive strategy for the treatment of the stress-induced organ damage. Many natural compounds have the ability to scavenge ROS, thereby reducing oxidative stress directly, or they may offer indirect protection by activating endogenous defense systems. In fact, the induction of enzymes relevant in cell defense system seems conceivable.

The aim of the present study was to determine the potency of melatonin in improving stress-induced cerebral, cerebellar, cardiac, and hepatic damage in high-stress conditions. The severity of the injury induced by oxidative stress was evaluated by light microscopic and biochemical analysis. To our knowledge, this is the first study evaluating the effects of melatonin on oxidative stress-induced organ damage in such high-stress conditions due to cold exposure, immobilization, or/and starvation.

2. Materials and methods

2.1. Animals and experimental protocol

Thirty-two male Wistar albino rats weighing 340–360 g were used. Animals were fed with standard rat chow and received tap water ad libitum. They were maintained on a 12-h light/12-h dark cycle at 21 °C. The rats were randomly divided into 4 groups, each consisting of 8 animals. The first group represented intact controls (control group). The rats from all of the other groups were exposed to starvation for 72 h. Later, they were immobilized and kept at 4 °C for 8 h. The rats from the second group were decapitated immediately after the stress exposure (stress group). The rats from the third group received daily intraperitoneal injections of 0.5 mL of 0.9% saline for 7 days (stress + S group). The rats from the fourth group received melatonin daily at 20 mg/kg body weight intraperitoneally (Merck Chemicals, Darmstadt, Germany) (stress + Mel group). Melatonin was dissolved in absolute ethanol (99.99%) and further dilutions were made in saline with 1% final concentrations of ethanol. The rats from the third and fourth groups were decapitated after saline or melatonin treatments for 7 days.

The experiment was performed in accordance with the guidelines for animal research from the National Institutes of Health and was approved by the Committee on Animal Research at İnönü University, Malatya, Turkey (Ethics Approval Number: 2007/67).

2.2. Histological evaluations

At the end of the experiment, the brain, cerebellum, heart, and liver were rapidly removed. Tissue samples from each organ were divided into 2 portions. The first part of the samples was placed in 10% neutral formalin and prepared for routine paraffin embedding. Sections of tissues were cut at 5 μm thick, mounted on slides, and stained with hematoxylin and eosin (HE), Masson's trichrome (sections from the heart and liver), and toluidine blue (sections from the brain and cerebellum) staining methods. Samples were examined and scored by a blind observer using a Leica DFC280 light microscope and the Leica Q Win and Image Analysis System (Leica Microsystems, Cambridge, UK).

2.3. Scoring systems

Assessment of tissue alterations in 20 different fields for each section was conducted by an experienced histologist who was unaware of the treatment. Histopathological damage was evaluated by appropriate scoring methods for each organ. Brain damage was scored by grading vacuolization and perinuclear edema, interstitial and perivascular edema, necrosis, congestion, intracerebral hemorrhage, subarachnoid hemorrhage, and cell infiltration with a maximum score of 21. Cardiac damage was scored by grading vacuolization, necrosis, hemorrhage, and cell infiltration with a maximum score of 12. Finally, hepatic damage was scored by grading vacuolization, necrosis, hemorrhage, sinusoidal dilatation, and cell infiltration with a maximum score of 15. Each alteration was scored as: 0, absent or very rare; 1, mild; 2, moderate; or 3, severe.

2.4. Biochemical evaluations

The remaining tissue samples were stored at –80 °C for the determination of MDA contents and SOD and CAT enzyme activities.

2.4.1. Homogenization

Tissues were homogenized (PCV Kinematica Status Homogenizator, Lucerne, Switzerland) in ice-cold phosphate buffered saline (pH 7.4). The homogenate was sonified with an ultrasonifier (Branson Sonifier 450, VWR Int. Ltd., Poole, UK) for 3 cycles (20-s sonications and 40-s pauses on ice). The homogenate was centrifuged (15,000 × *g*, 10 min, 4 °C) and cell-free supernatant was subjected to enzyme assay immediately.

2.4.2. Catalase assay

CAT activity was measured at 37 °C by following the rate of disappearance of hydrogen peroxide (H_2O_2) at 240 nm (ε_{240} = 40 M^{-1} cm^{-1}) (Luck, 1963). One unit of catalase activity was defined as the amount of enzyme catalyzing the degradation of 1 µmol of H_2O_2 per min at 37 °C and specific activity corresponding to transformation of substrate (in µmol) (H_2O_2) per minute per milliggram protein.

2.4.3. Superoxide dismutase assay

SOD (Cu, Zn-SOD) activity in the supernatant fraction was measured using the xanthine oxidase/cytochrome c method (McCord and Fridovich, 1969), where 1 U of activity is the amount of the enzyme needed to cause a half-maximal inhibition of cytochrome c reduction. The amount of SOD in the extract was determined as U enzyme mg^{-1} protein, utilizing a commercial SOD as the standard.

2.4.4. Malondialdehyde assay

The analysis of lipid peroxidation was carried out as previously described (Buege and Aust, 1978) with minor modification. The reaction mixture was prepared by adding 250 µL of homogenate into 2 mL of reaction solution (15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N HCl, 1:1:1, w/v) and was heated at 100 °C for 15 min. The mixture was cooled to room temperature and centrifuged (10,000 × *g* for 10 min), and the absorbance of the supernatant was recorded at 532 nm. 1,1,3,3-Tetramethoxypropane was used as the MDA standard. MDA results were expressed as nmol mg^{-1} protein in the homogenate.

2.4.5. Determination of protein

Protein levels of the tissue samples were measured by the Bradford method (Bradford, 1976). The absorbance measurement was taken at 595 nm using a UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). Bovine serum albumin was used as the protein standard.

2.5. Statistical evaluation

Statistical analysis was carried out using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). All data are expressed as arithmetic mean ± standard error. For the analysis of tissue enzyme levels, Mann–Whitney U tests with the Bonferroni correction were used. $P < 0.05$, $P < 0.001$, and $P < 0.005$ were regarded as significant.

3. Results

3.1. Histopathological data

3.1.1. Brain and cerebellum

Animals form the control group presented normal organ histology. The samples obtained from the cerebellum of stress-exposed rats were also relatively normal. On the contrary, the brain specimens of the stress group showed some obvious alterations such as vacuolization, perinuclear, interstitial, and perivascular edema (Figure 1A), necrosis, congestion, intracerebral hemorrhage, subarachnoid hemorrhage (Figure 1B), and cell infiltration. In some sections, hemorrhages within the choroid plexus were also observed. Pyknosis and Nissl granule decreases were clear, especially in the toluidine blue-stained sections. The mean histopathological damage score (MHDS) of the stress

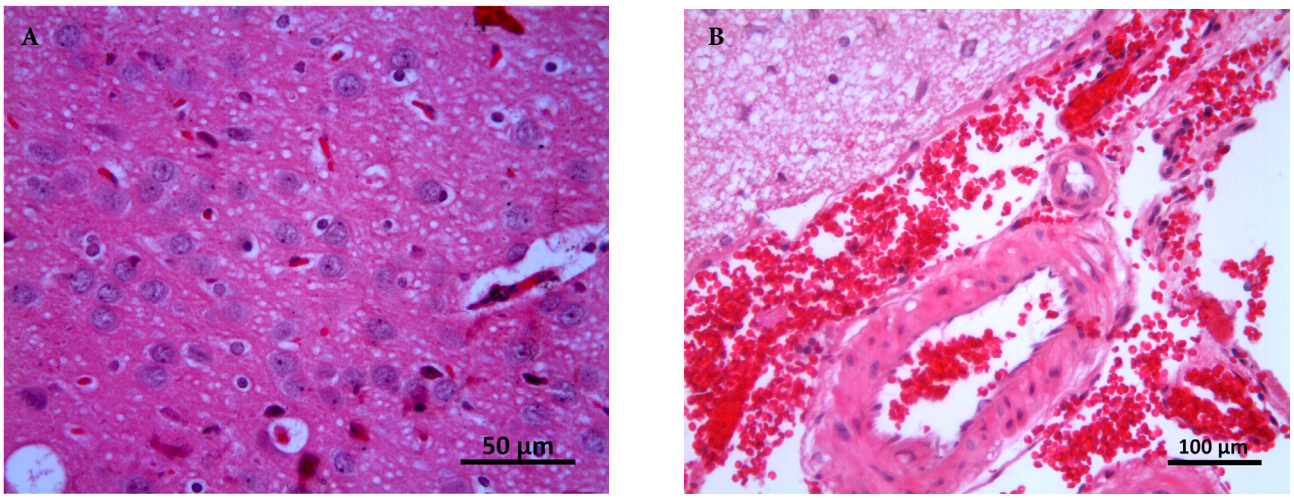

Figure 1. Microscopic appearances of brain specimens of rats from the stress group. **A)** Vacuolization and perinuclear, interstitial, and perivascular edemas are obvious, HE, 100×. **B)** Subarachnoid hemorrhage is observed, HE, 40×.

group was significantly higher than those of the control and stress + S groups ($P < 0.005$ for both). In the stress + Mel group, histopathological evidence of tissue damage was significantly reduced. The MHDS of the stress + Mel group was significantly lower than those of the stress and stress + S groups ($P < 0.005$ and $P < 0.05$, respectively). MHDSs of all groups are summarized in Figure 2.

3.1.2. Heart

The control group revealed normal cardiac histology. The heart specimens of the stress group showed prominent alterations such as vacuolization, necrosis (Figure 3), hemorrhage, and cell infiltration. Nuclear pyknosis and karyolysis were evident. Intercellular edema was clear in some areas. MHDSs of the stress and stress + S groups were significantly higher than that of control group ($P < 0.001$ and $P < 0.01$, respectively). The MHDS of the stress + Mel group was clearly lower than those of stress and stress + S groups. MHDSs of all groups are summarized in Figure 2.

3.1.3. Liver

Liver samples from the control group were normal in histological appearance. Histopathological changes such as vacuolization, necrosis, hemorrhage (Figure 4), cell infiltration, and sinusoidal dilatation were observed in the stress group. There were many pyknotic nuclei throughout the parenchyma. The MHDS of the stress group was significantly higher than that of the control group ($P < 0.001$). The damage score of the stress + Mel group was lower than those of the stress ($P < 0.05$) and stress + S groups. MHDSs of all groups are summarized in Figure 2.

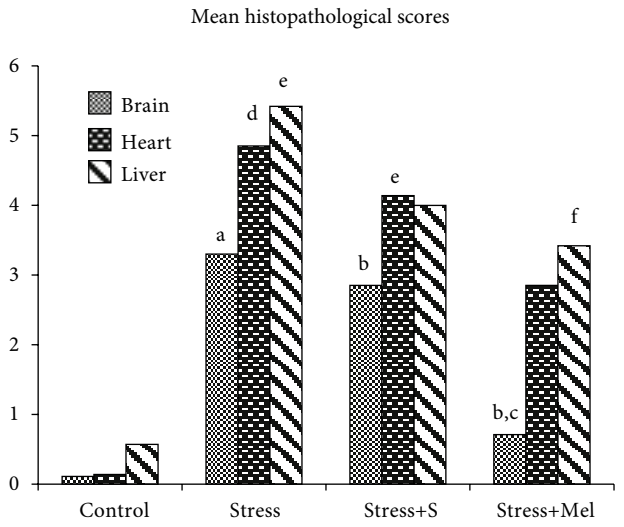

Figure 2. Mean histopathological damage scores of all groups. [a]: $P < 0.005$ vs. control, [b]: $P < 0.005$ vs. stress, [c]: $P < 0.05$ vs. stress + S, [d]: $P < 0.001$ vs. control, [e]: $P < 0.01$ vs. control, [f]: $P < 0.05$ vs. stress.

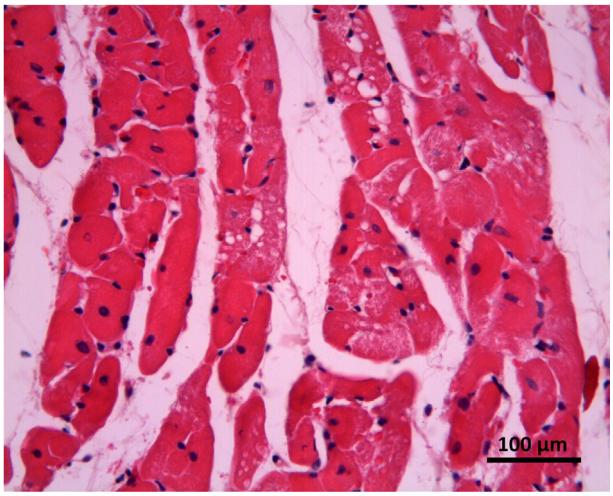

Figure 3. Microscopic appearance of a heart specimen of a rat from the stress group. Intensive nuclear pyknosis, vacuolization, and necrosis are evident. HE, 40×.

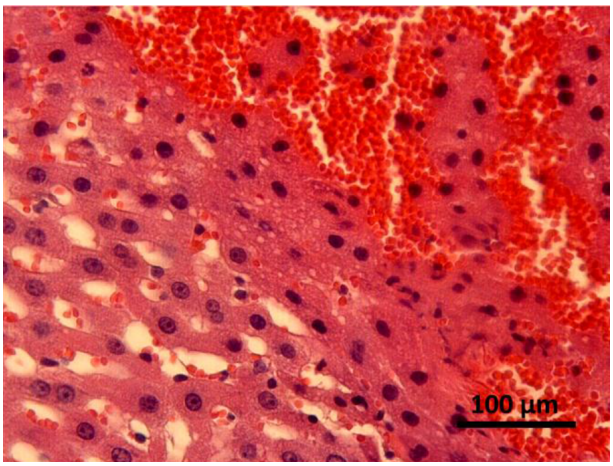

Figure 4. Microscopic appearance of a liver specimen of a rat from the stress group. Vacuolization, pyknosis, necrosis, and massive hemorrhage are observed. HE, 40×.

3.2. Biochemical data

3.2.1. Brain and cerebellum

3.2.1.1. Mean tissue MDA levels

The highest mean cerebral MDA levels were detected in the stress and stress + S groups, respectively. The mean cerebral MDA level of the stress group was significantly higher than that of the control group and of the stress + Mel group ($P < 0.05$ for both). Nevertheless, the MDA level of the stress + Mel group was very similar to that of the control group; additionally, it was lower than that of the stress + S group.

3.2.1.2. Mean tissue CAT activities

The mean cerebral CAT activity of the stress group was significantly higher than those of the control group ($P < 0.005$) and the stress + S group ($P < 0.05$). CAT activity of the stress + Mel group was lower than that of the stress group ($P < 0.005$). Moreover, the mean tissue CAT level of the stress + Mel group was higher than that of the control group.

The mean cerebellar CAT activities of the stress and stress + S groups were higher than those of the control group ($P < 0.05$). Additionally, CAT activity of the stress group was higher than that of the stress + S and stress + Mel groups ($P < 0.05$ for both). The CAT activity of the stress + Mel group was also higher than that of the control group.

3.2.1.3. Mean tissue SOD activities

The mean cerebral SOD activity of the stress group was higher than that of the control group. SOD activity of the stress + S group was significantly lower than those of the control and stress groups ($P < 0.05$). SOD activity of the stress + Mel group was higher than that of the stress + S group but lower than those of the other groups. On the other hand, the main cerebellar SOD activities of the stress and stress + S groups were lower than that of the control group. Mean tissue SOD activity of the stress + Mel group was significantly higher than those of control, stress, and stress + S groups ($P < 0.05$, $P < 0.005$, and $P < 0.005$, respectively).

3.2.2. Heart

3.2.2.1. Mean tissue MDA levels

The highest mean cardiac MDA levels were detected in the stress group. Statistical relevance was detected between the control and stress + Mel groups ($P < 0.05$). MDA level of the stress + Mel group was lower than that of the stress + S group.

3.2.2.2. Mean tissue CAT activities

The mean cardiac CAT activity of the stress group was significantly higher than those of control ($P < 0.005$), stress + S ($P < 0.005$), and stress + Mel ($P < 0.05$) groups. CAT activity of the stress + Mel group was higher than those of control ($P < 0.05$) and stress + S groups.

3.2.2.3. Mean tissue SOD activities

The highest tissue SOD activity was detected in the control group. SOD activity of the stress + S group was lower than that of the control group ($P < 0.005$). SOD activity of the stress + Mel group was higher than that of the stress + S group ($P < 0.05$). Additionally, SOD activity of the stress + Mel group was similar to that of the control group.

3.2.3. Liver

3.2.3.1. Mean tissue MDA levels

The mean hepatic MDA level of the control group was lower than those of all other groups. Tissue MDA levels of the stress and stress + S groups were higher than that of control group ($P < 0.005$). MDA level of the stress + Mel group was significantly lower than those of the stress and stress + S groups ($P < 0.05$ and $P < 0.005$, respectively).

3.2.3.2. Main tissue CAT activities

The lowest CAT activity was detected in the control group. Mean tissue CAT activities of the stress + Mel group were higher than those of the control group.

3.2.3.3. Main tissue SOD activities

Mean tissue SOD activity of the stress group was higher than that of the control group ($P < 0.05$). CAT activities of the stress + S and stress + Mel groups were higher than that of the control group.

Mean tissue MDA levels and CAT and SOD activities of all groups are summarized in Figures 5, 6, and 7, respectively.

4. Discussion

Stress can disrupt the balance in an oxidant/antioxidant system and can cause oxidative damage to several tissues by altering antioxidant status, protein oxidation, and lipid peroxidation (Liu et al., 1996; Şahin and Gümüşlü, 2004). These alterations are explained as a mechanism resisting the negative effects of ROS (Selman et al., 2000). In the present study, we used immobilization, cold restraint, and starvation methods in order to imitate intense oxidative stress conditions. Oxidative stress is important in either the primary or the secondary pathophysiological mechanisms underlying acute or chronic organ injury. The reduction in the endogenous antioxidant defense system may contribute to oxidative stress evolution. Therefore, the discovery and development of potent antioxidant agents has been one of the most interesting

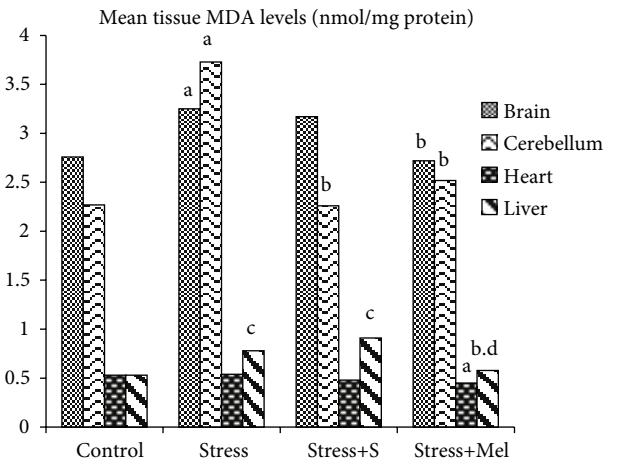

Figure 5. Mean tissue MDA levels of all groups. [a]: $P < 0.05$ vs. control, [b]: $P < 0.05$ vs. stress, [c]: $P < 0.005$ vs. control, [d]: $P < 0.005$ vs. stress + S.

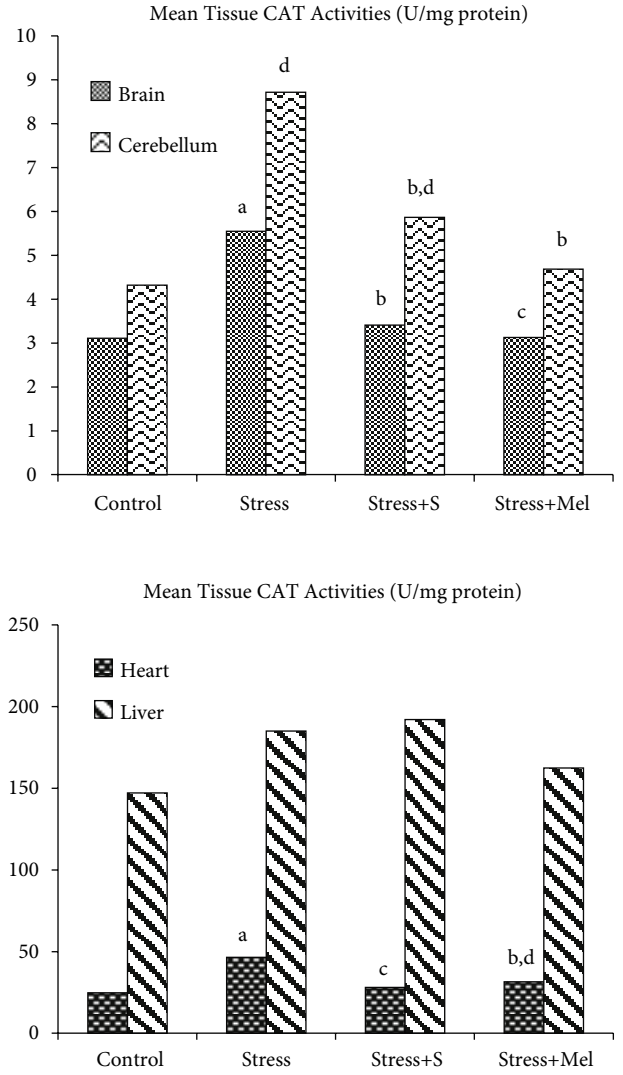

Figure 6. Mean tissue CAT activities of all groups. [a]: $P < 0.005$ vs. control, [b]: $P < 0.05$ vs. stress, [c]: $P < 0.005$ vs. stress, [d]: $P < 0.05$ vs. control.

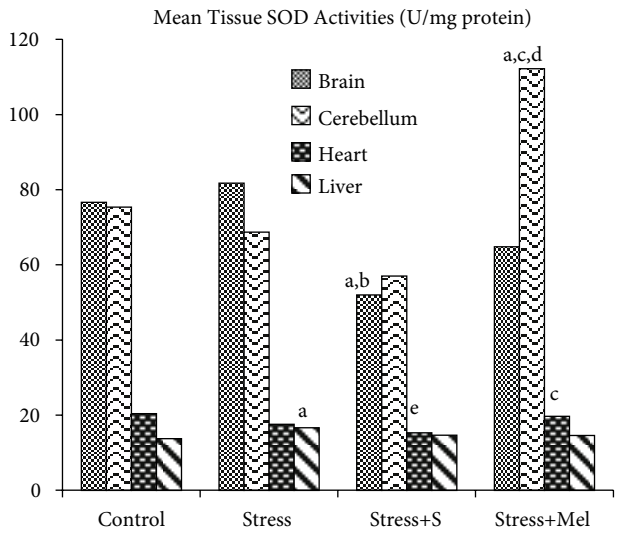

Figure 7. Mean tissue SOD activities of all groups. [a]: $P < 0.05$ vs. control, [b]: $P < 0.05$ vs. stress, [c]: $P < 0.005$ vs. stress, [d]: $P < 0.005$ vs. stress + S, [e]: $P < 0.005$ vs. control.

and promising approaches in the search for treatment and prevention of oxidative stress-induced organ damage. Herein, we investigated the potency of melatonin on stress-induced microscopic and biochemical alterations in the brain, cerebellum, heart, and liver by creating high-stress conditions using immobilization, cold exposure, and starvation all together.

In the present study, mean tissue MDA levels of all organs were increased in the stress groups. Statistically significant differences were detected in the brain, cerebellum, and liver. Although the heart has one of the highest mass-specific oxygen consumption rates in the body, therefore coping with high rates of oxidant formation and stress (Liu et al., 2000), we did not detect an important increase in the heart. Şahin and Gümüşlü (2007) found the most increased MDA levels in the liver, brain, and stomach, respectively. In our study, the P-value for the difference between the control and the stress groups was most significant in the liver. MDA levels were generally decreased in the stress + S group (except in the liver), but significant differences were observed only in cerebellum. Relatively low levels of MDA in the stress + S group may be related to the recovery capabilities of the tissues in the course of time (as we mentioned before, we sacrificed this group 7 days after the end of the stress induction), although high regenerative capacity is not expected from the cerebellum, nor is low regenerative capacity expected from the liver. This may be also related to the tissue-specific availability of cellular antioxidant enzymes and the degree of oxidant-induced degeneration during tissue damage. It is a fact that tissue MDA levels and MHDSs were correlated with each other. MHDSs of all organs from the stress group were significantly higher than those of the control and higher than those of the stress + S group. The reduction of the damage scores and tissue MDA levels might represent a time-dependent recovery of tissue damage.

The first cellular response against increased production of ROS may be a temporary increase in the level or activity of cellular antioxidant enzymes. However, when the synthesis mechanisms are destroyed by ROS, their cellular level decreases; thus, oxidative damage occurs. In the present study, stress resulted in increases in cerebral and hepatic SOD activities and cerebral, cerebellar, cardiac, and hepatic CAT activities. Şahin and Gümüşlü (2007) found SOD activities increased in the brain and liver, as

did we; however, they found decreased SOD activities in the heart in a cold stress model. Interestingly, in one of our recent studies, we found tissue CAT and SOD activities to be increased in an acute cold stress model (Ateş et al., 2006). Şahin and Gümüşlü (2007) found CAT activities increased in the brain, liver, and heart. Popovic et al. (2009) also found increased cerebral activity of CAT in immobilization and cold-induced stress. Kaushik and Kaur (2003) reported that CAT activity was decreased in the heart and liver due to cold stress. On the other hand, Zaidi and Banu (2004) reported decreases in SOD and CAT activities in the rat brain under restraint-induced stress. When we compared CAT activities in different tissues, the highest percentage of increased activity was observed in the brain and heart. This result may suggest that the H_2O_2 concentration is higher in the brain and heart of rats in the stress group than in the control animals, because CAT functions in a state of high H_2O_2 concentration (Liu et al., 1996; Abe and Saito, 1998). H_2O_2 has been reported to be especially toxic to brain neurons (Abe and Saito, 1998). The liver was the tissue that had the highest CAT activity and the brain was the one that had the highest SOD activity in the normal state. The most significant increase in SOD activity, induced by stress, was detected in the liver in our study. Increase in SOD activity suggests that cold stress may increase the rate of O^- generation and subsequently H_2O_2 formation (Emirbekov et al., 1998; Liu et al., 2000). All parts of the nervous system contain SODs: enzymes that remove superoxides by catalyzing their dismutations with one superoxide being reduced to H_2O_2 and another oxidized to O_2 (Liochev and Fridovich, 2005). SODs must work together with enzymes to remove H_2O_2 (Turrens, 2003). It has been reported that increased SOD activity in the brain reduces the development of vasogenic brain edema and infarction, since superoxide radicals play an important role in the pathogenesis of these lesions in the cold-traumatized brain (Chan et al., 1991). CAT is not important to the brain and is not present in brain mitochondria, where much of the superoxide is generated (Turrens, 2003). Indeed, cerebral and cerebellar CAT activities of control group were lower than those in the heart and liver. The levels or activities of antioxidant enzymes and stress-induced changes are tissue-specific.

Melatonin has been shown to reduce tissue MDA levels in the course of various pathological conditions via its antioxidant and free radical scavenging actions. In the present study, melatonin supplementation resulted in decreases in tissue MDA levels of the stress and stress + S groups in the brain, cerebellum, heart, and liver. The potent antioxidant effect of melatonin on cerebral, cerebellar, and hepatic tissue MDA levels represents its beneficial function against stress-induced cellular and histopathological damage within the brain, heart, and liver. As expected, antioxidant supplementation affected MHDSs. Compatible with the decreases in tissue MDA levels, decreases in MHDSs were detected in the stress + Mel group. Melatonin significantly reduced cerebral and hepatic MHDSs versus the stress group and cerebral MHDS versus the stress + S group. While the differences between the stress and stress + Mel groups are important, the differences between the stress + S and stress + Mel groups are far more important. The latter differences represent the degree of capability of the antioxidant agent to improve tissue healing. From this point of view, we can conclude that melatonin is highly effective in reducing stress-induced brain damage. In terms of tissue MDA levels, melatonin seems to be highly effective at reducing lipid peroxidation levels in the liver.

Melatonin, known as the 'hormone of darkness', is secreted into the blood by the pineal gland (Challet, 2007). It has a particular role in the protection of nuclear and mitochondrial DNA (Reiter et al., 2001). Melatonin is a highly ubiquitous free radical scavenger and is indirectly an antioxidant. It can easily enter the central nervous system by penetrating the brain–blood barrier (Menendez-Peleaz and Reiter, 1993). It has been shown to exert neuroprotection in a variety of oxidative stress-associated neuropathologies, including brain and spinal cord trauma, cerebral ischemia, neurotoxicity, and models of Parkinson and Alzheimer diseases (Beni et al., 2004). Melatonin has been frequently shown to be superior to vitamins C and E in protecting from oxidative damage and in scavenging free radicals (Lopez-Burillo et al., 2003). In the present study, melatonin was found to be very effective in decreasing MHDSs in the brain and liver and in decreasing MDA levels in all of the organs. The MHDS of the stress + Mel group was lower than that of the stress + S group in the brain, as were the MDA levels in the liver. These differences, representing the supportive effect of melatonin on tissue recovery potentials, are especially important. On the other hand, as mentioned before, melatonin increased tissue antioxidant enzyme levels or activities. Tissue SOD activities in the brain, cerebellum, and heart were higher in the stress + Mel group than in the stress + S group, and tissue CAT activities were higher in the stress + Mel group than even those of the control group in all of these organs. The main tissue SOD activities of the stress + Mel group being higher than those of the control group for the cerebellum and liver and the main tissue CAT activities of the stress + Mel group being higher than those of the control group for all of the organs shows us the potent supportive effect of melatonin on cellular antioxidant enzyme systems.

In conclusion, our results show that the same stress-inducing factors affect various organs to different degrees. The cellular and biochemical reactions of some physically

close organs, like the brain and cerebellum, might be different from each other. Additionally, our results show that exogenously administered melatonin improves stress-induced cellular damage and antioxidant enzyme systems on different levels for different organs. However, even in high-stress conditions, we found melatonin very effective in reducing stress-induced organ damage by inhibiting lipid peroxidation and supporting the cellular antioxidant defense system. Thus, the consumption of melatonin might be most useful in daily mild-stress conditions in order to protect organs from stress-induced cellular damage.

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