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DNA protective and antioxidative effects of melatonin in streptozotocin-induced diabetic rats

Selim SEKKİN[1,*], Eda Duygu İPEK[2], Murat BOYACIOĞLU[1], Cavit KUM[1], Ümit KARADEMİR[1], Hande Sultan YALINKILINÇ[1], Mehmet Onur AK[1], Hulki BAŞALOĞLU[2]

[1]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Adnan Menderes University, Aydın, Turkey
[2]Department of Anatomy, Faculty of Medicine, Adnan Menderes University, Aydın, Turkey

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Abstract: Diabetes mellitus is a chronic disease characterized by elevated blood sugar levels. In diabetic patients, oxidative stress induced by the presence of excessive free radicals is closely associated with chronic inflammation, leading to potential tissue damage. Melatonin (MEL) is a compound synthesized by the pineal gland and a scavenger of free radicals. The aim of this study was to research the effects of MEL on oxidative stress and its DNA protective effects in streptozotocin-induced diabetic rats. In total, 32 rats were equally divided into four experimental groups: control, melatonin, diabetic, and diabetic + melatonin. A single dose of streptozotocin (60 mg/kg) was given by intraperitoneal route to induce experimental diabetes. MEL (10 mg/kg daily) was administrated to rats by intraperitoneal route for 6 weeks. Oxidative stress parameters were evaluated in rat liver, kidney, brain, and pancreas tissues. Body weight, plasma glucose, and HbA_{1c} levels were studied. DNA damage was analyzed by comet assay in lymphocytes, while % tail DNA and mean tail moment parameters were evaluated. The study results indicate that the intraperitoneal administration of 10 mg/kg MEL over 6 weeks may cause amelioration in oxidative stress parameters against diabetes, leading to beneficial effects based on % tail DNA and mean tail moment parameters in rat lymphocytes.

Key words: Diabetes mellitus, comet assay, oxidative stress, melatonin, streptozotocin, DNA damage, MDA level, tail moment, % tail DNA, DNA repair

1. Introduction

Diabetes mellitus (DM) is a chronic disease characterized by elevated blood sugar levels resulting from either a lack of insulin production or a resistance to insulin. The prevalence of DM has risen to epidemic proportions worldwide. In diabetic patients, oxidative stress induced by the presence of excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS) is closely associated with chronic inflammation, leading to potential tissue damage. Thus, complications of DM (such as retinopathy, nephropathy, neuropathy, ischemic heart disease, and peripheral vasculopathy) have now become some of the most challenging health problems (Rochette et al., 2014; Prattichizzo et al., 2015; Tangvarasittichai, 2015). Radicals derived from ROS and RNS are the largest class of radical species generated in living systems. ROS are continually produced in the cell because of aerobic metabolism and are controlled by several antioxidant mechanisms. ROS and RNS are products of normal cell metabolism and have either beneficial or deleterious effects, depending on the concentration reached in the tissues (Dalle-Donne et al., 2006). Usually, the production and neutralization of ROS are balanced with antioxidants in a living system and do not cause any oxidative damage. The imbalance between these oxidants and antioxidants in the living organism system, which determine an oxidative stress state, cause damage to cellular macromolecules, such as lipids, proteins, and nucleic acids (Jangra et al., 2013; Tangvarasittichai, 2015). One of the main challenges of research in recent years has been finding ways to attenuate oxidative stress to improve diabetes. Therefore, it seems reasonable that antioxidants can play an important role in the improvement of diabetes. Many researchers reported that antioxidants have been evaluated for the management of diabetes (Rahimi et al., 2005; Shaker et al., 2009; Marrazzo et al., 2014; Rochette et al., 2014).

Melatonin (MEL) is a complex synthesized by the pineal gland in the human brain. MEL is also produced in the retina, thymus, bone marrow, respiratory epithelium, skin, lens, and intestine as well as in other sites. MEL modulates a diverse number of physiological processes (Leon et al., 2004; Tan et al., 2007). MEL acts typically through

* Correspondence: ssekkin@adu.edu.tr

the widely expressed G-protein-coupled membrane receptors MT1 and MT2. MT1 and MT2 are two well-characterized G-protein-coupled plasma membrane MEL receptors, which are activated by MEL and which regulate multiple cellular and physiological functions (Munik and Ekmekçioğlu, 2015). MEL is a major scavenger of both ROS and RNS reactive molecules. MEL provokes this effect at both physiological and pharmacological concentrations. Several of its metabolites can also detoxify free radicals and derivatives. Both physiological and pharmacological doses of MEL have been shown to increase gene expression and enzyme activities of glutathione peroxidase (GPx), glutathione reductase, superoxide dismutase (SOD), and catalase (CAT) (Leon et al., 2004; Carpentieri et al., 2012).

Streptozotocin (STZ) is an antibiotic produced by *Streptomycetes achromogenes*. However, STZ is a widely used chemical for the induction of experimental diabetes in rodents. STZ-induced type 1 diabetes in rodents is a well-established and well-accepted practice for the studies of the pathogenesis of diabetes and its complications. The cytotoxic action of STZ is mediated by free radicals and STZ has toxic and carcinogenic effects on the pancreas, liver, brain, and kidneys. STZ diabetic animal models have been very useful in clarifying the mechanisms of diabetic pathogenesis and in screening artificial chemicals, natural products, and pharmacological agents that are potentially capable of lowering blood glucose levels (Jangra et al., 2013; Wu and Yan, 2015).

The status of oxidant-antioxidant imbalance may be one of the mechanisms leading to the DNA damage detected in the lymphocytes of diabetic patients (Garcia-Ramirez et al., 2008; Arif et al., 2010; Woo et al., 2010; Kushwaha et al., 2011). MEL, whose beneficial effects on the antioxidant status in cells of STZ-induced diabetic rats, may protect tissues from oxidative damage and reduce risk of diseases caused by free radicals. Therefore, to the best of our knowledge, no information is currently available regarding the DNA protective effects of MEL with a comet assay against STZ-induced diabetic rats. The present study was designed to research the effects of MEL on oxidative stress, as well as its DNA protective effects in STZ-induced diabetic rats.

2. Materials and methods

2.1. Animals and experimental protocol

Thirteen-week-old male Wistar albino rats were purchased from a commercial company (Kobay DHL A.S., Ankara, Turkey) and the rats were acclimatized for 3 weeks. Rats were housed in individual cages in a well-ventilated room with a 12/12 h light/dark cycle at 22 °C. Animals were fed with standard rat chow and tap water ad libitum (Adnan Menderes University Experimental Animal Production Laboratory, Aydın, Turkey). Thirty-two 16-week-old rats, weighing 430–460 g, were randomly divided into four experimental groups: control, melatonin, diabetic, and diabetic + melatonin. STZ was used for experimental diabetes (Frode and Medeiros, 2008). After 12 h of starvation, diabetes was induced by a single intraperitoneal (i.p.) injection of STZ, freshly dissolved in sodium citrate buffer (0.01 M at pH 4.5) at a dose of 60 mg/kg (Wu and Yan, 2015). At 72 h after the STZ injection, blood glucose levels of all groups were measured using reagent strips with a glucometer (Contour TS, Bayer, Basel, Switzerland) in samples obtained from the tail vein. Rats having 250 mg/dL or higher blood glucose levels were considered to be diabetic (Roy et al., 2013). The blood glucose level and the weight of the rats were measured every week until termination of the experiment. Rats with a ≥250 mg/dL blood glucose level were included in the diabetic and diabetic + melatonin groups. MEL was administrated at 10 mg/kg (i.p.) dissolved in 1 mL of 1% ethanol (Sudnikovich et al., 2007) per day to the melatonin and melatonin + diabetic group rats, and 1% ethanol was administered (1 mL) by i.p. route to the control and diabetic group rats for 6 weeks. Cardiac blood (1 mL) was taken at the end of the experiment for the quantification of glycosylated hemoglobin (HbA_{1c}) by the immune turbidimetric method (c8000 Clinical Chemistry Auto Analyzer, Abbot Architect, Abbot Laboratories, Irving, TX, USA) (Teodoro-Morrison et al. 2015). Animals were anesthetized at the end of the experiment with intramuscular injections of xylazine (Alfazyne) at 5 mg/kg and ketamine (Ketalar) at 100 mg/kg. Cardiac blood samples were taken and processed immediately, and liver, kidney, brain, and pancreas tissue specimens were collected and stored at –80 °C until analysis.

The experiments were performed in accordance with the guide for the care and the use of laboratory animals of the Animal Ethics Committee of Adnan Menderes University (2015/052).

2.2. Chemicals

STZ (S0130, Sigma-Aldrich, St. Louis, MO, USA) and MEL (M5250, Sigma-Aldrich) were used in the biological assays. The remainder of the chemicals used were also purchased from Sigma-Aldrich.

2.3. Tissue homogenization and determination of antioxidant/oxidant status in tissues

Dissected liver, kidney, brain, and pancreas tissues were immediately rinsed in ice-cold phosphate-buffered saline (PBS). Tissues were homogenized (2000 rpm for 1 min, 1/10 w/v) using a stirrer (IKA Overhead Stirrer; IKA-Werke GmbH and Co. KG, Staufen, Germany) in 10% 150 mM PBS (pH 7.4) in an ice bath. The homogenate was centrifuged (Mikro 200 R, Hettich Zentrifugen, Tuttlingen, Germany) at 7000 × *g* for 10 min at 4 °C, and the supernatants were frozen at –80 °C (NU 9668E, Nuaire, Plymouth, MN, USA) until they were analyzed.

2.3.1. SOD activity

SOD activity was determined according to the method of Sun et al. (1988) and the absorbance was measured at 560 nm by a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). SOD estimation was based on the generation of superoxide radicals produced by xanthine on xanthine oxidase, which reacts with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride to form a red formazan dye. SOD activity was then measured by the degree of inhibition of this reaction and the results are shown as U/mg tissue protein.

2.3.2. CAT activity

CAT activity was determined according to the method of Aebi (1984) and was measured spectrophotometrically at 240 nm. The principle of the assay was based on the determination of the rate constant of H_2O_2 decomposition by the CAT enzyme and expressed as k/mg tissue protein, where k is the first-order rate constant.

2.3.3. Total GSH level

The amount of glutathione (GSH) in supernatants was measured according to the method described by Tietze (1969): 0.5 mL of the supernatant or standard with 0.25 mL of 1 mol/L sodium phosphate buffer (pH 6.8) and 0.5 mL of 5-5'-dithiobis (2-nitrobenzoic acid) (DNTB, 0.8 g/L in phosphate buffer) was left to stand for 5 min. Absorbance was spectrophotometrically determined at 412 nm. The results were determined by comparison with an aqueous standard solution of GSH (Sigma Chemical Co., St. Louis, MO, USA) and expressed as mg/g tissue protein.

2.3.4. MDA level

The concentrations of malondialdehyde (MDA) were determined according to the method of Ohkawa et al. (1979). The tissue homogenate was used for lipid peroxidation estimation, which was applied by measuring the formation of thiobarbituric acid reactive substances (TBARS). Absorbance was measured by using a spectrophotometer at 532 nm. The concentration of MDA was calculated by the absorbance complex (absorbance coefficient $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$) and expressed as nmol/mg tissue protein.

2.3.5. Protein concentrations

Protein concentrations in supernatants were measured by a spectrophotometer (UV-1601, Shimadzu) with biuret, using commercially available kits (Archem Diagnostic Ind. Ltd., İstanbul, Turkey), and the results are expressed as mg/mL protein.

2.4. Lymphocyte isolation and DNA analysis by comet assay

The comet assay was applied with several modifications, as previously described by Singh et al. (1988) and Collins et al. (1997). For this purpose, fresh blood samples were mixed with the PBS solution for the determination of DNA fragmentation of blood lymphocytes. Lymphocytes were isolated with Histopaque and suspended in a freezing medium. Isolated lymphocytes were slowly frozen in aliquots of 1 mL at –80 °C (NU 9668E, Nuaire).

Conventional end-frosted slides were precoated with 1% normal melting agarose, and cells were resuspended in PBS. This suspension was mixed with prewarmed (37 °C) low-melting-point agarose, and two drops of this mixture were placed on a microscope slide. A cover slip was put on the drops, and the gels were allowed to solidify at 4 °C. Once the gels had solidified, the cover slip was removed and the slides were dipped into freshly prepared lysis solution jars at 4 °C for at least 1 h. The positive control slide cells were dipped in an H_2O_2 solution for 5 min at 4 °C, then washed with cold PBS and introduced into a lysis solution in a separate jar for at least 1 h at 4 °C. Following lysis, slides were aligned in a horizontal gel electrophoresis tank (CSL-COM20, Cleaver Scientific, Warwickshire, UK) that was connected to a recirculating cooler (FL300, Julabo, Seelbach, Germany) set at 4 °C and filled with freshly made alkaline electrophoresis solution. Electrophoresis (CS-300V, Cleaver Scientific) was carried out at approximately 1 V/cm for 20 min, after which the slides were washed twice with a neutralizing buffer and then fixed with three different concentrations of ethanol. They were then allowed to dry in the dark at room temperature prior to staining with 70 µL of a 4'6-diamidino-2-phenylindole dihydrochloride (DAPI) solution (10 µg/mL).

For the visualization of DNA damage, the slides were examined under a fluorescence microscope (DM3000, Leica, Wetzlar, Germany). Measurements of the tail intensity and tail moment of comets were made for 100 randomly selected cells, i.e. 50 cells from each of two gels from each sample, using a computer-based image analysis system (Comet Assay IV, Perceptive Instruments, Bury St. Edmunds, UK). The mean value of the % tail DNA and mean tail moment parameters was calculated and used to assess the DNA damage.

2.5. Statistical analysis

The data were compared among groups using the Kruskal–Wallis analysis of variance (ANOVA) or one-way ANOVA. Post hoc multiple comparisons were performed using the Mann–Whitney U test with the Bonferroni corrected or Duncan test (IBM SPSS Version 21.0, IBM Corp., Armonk, NY, USA). Differences were considered statistically significant if $P < 0.05$. All data were expressed as mean and standard error.

3. Results

3.1. Body weight, blood glucose, and HbA_{1c} levels

The mean initial body weights were similar in the control and the other groups ($P > 0.05$). Diabetic rat weights were less than those of the control and melatonin group rats

during the 6-week period ($P < 0.001$). MEL administration did not change the body weight of the rats ($P > 0.05$) (Table 1). The blood glucose levels of the rats were similar in the beginning of the experiment. At 72 h following the i.p. STZ injection, blood glucose levels were increased in the diabetic and diabetic + melatonin groups compared with the control and melatonin groups ($P < 0.001$). During the experiment, MEL did not change the blood glucose levels ($P > 0.05$) (Table 2). The serum HbA_{1c} levels of the diabetic (6.40 ± 0.28) and diabetic + melatonin (6.20 ± 0.30) groups were higher than those of the control (3.23 ± 0.04) and melatonin (3.31 ± 0.02) groups ($P < 0.001$). MEL administration was not able to decrease the serum HbA_{1c} levels ($P > 0.05$). Similarly, there was no significant difference between the serum HbA_{1c} levels of the diabetic and diabetic + melatonin groups ($P > 0.05$).

3.2. Antioxidant/oxidant status in tissues

Among the groups, the mean SOD activities of the control group were found to be higher in brain tissue. The SOD activities of the diabetic group were lower, except for those of the kidney and pancreas tissues ($P < 0.01$), because MEL administration ameliorated SOD activities in the diabetic + melatonin group's liver and brain tissues ($P < 0.001$) (Figure 1A). CAT activities of the liver and kidney tissues were lower ($P < 0.05$ and $P < 0.001$, respectively), while brain and pancreas tissue CAT activities were not significantly different in the diabetic group. CAT activity was higher in the kidney tissue ($P < 0.001$) of the melatonin group and the MEL administration improved the CAT activities in the kidney tissue of the diabetic + melatonin group (Figure 1B). GSH levels of all tissues were significantly lower in the diabetic rats compared with the control and melatonin group rats (except brain tissue), but MEL administration enhanced the GSH levels only in the pancreas tissue of the diabetic + melatonin group ($P < 0.01$) (Figure 1C). MDA levels were significantly higher in all tissues of the diabetic group. MEL reduced the MDA levels of diabetes in the liver ($P < 0.001$), kidney ($P < 0.001$), and brain ($P < 0.01$) tissues, except the pancreas tissue (Figure 1D).

Table 1. Effects of MEL administration on body weight in STZ - induced diabetic rats (n = 8 per group).

Groups	Weight (g)					
	1st week	2nd week	3rd week	4th week	5th week	6th week
Control	497.00 ± 25.21 [a]	502.83 ± 24.53 [a]	510.70 ± 22.79 [a]	521.35 ± 24.51 [a]	526.97 ± 25.36 [a]	532.62 ± 25.50 [a]
Melatonin	490.33 ± 21.26 [a,b]	491.02 ± 19.55 [a]	476.00 ± 20.33 [a]	484.62 ± 21.66 [a]	486.26 ± 20.84 [a]	486.36 ± 19.87 [a]
Diabetic	429.62 ± 11.56 [c]	385.65 ± 13.67 [b]	369.58 ± 14.45 [b]	344.08 ± 12.36 [b]	330.73 ± 12.01 [b]	291.20 ± 6.93 [b]
Diabetic + melatonin	441.10 ± 9.40 [b,c]	389.81 ± 8.11 [b]	369.31 ± 7.72 [b]	353.98 ± 7.39 [b]	336.35 ± 6.66 [b]	284.36 ± 8.16 [b]
P	*	***	***	***	***	***

[a, b, c]: Different letters indicate statistically significant differences in the same column.
*: $P < 0.05$, ***: $P < 0.001$.

Table 2. Effects of MEL administration on glucose levels in STZ-induced diabetic rats (n = 8 per group).

Groups	Glucose (mg/dL)					
	1st week	2nd week	3rd week	4th week	5th week	6th week
Control	99.50 ± 2.58 [b]	105.62 ± 2.21 [c]	105.62 ± 2.76 [b]	105.87 ± 2.14 [b]	104.87 ± 3.02 [b]	104.37 ± 3.59 [b]
Melatonin	109.50 ± 3.20 [b]	108.50 ± 3.41 [c]	105.87 ± 5.53 [b]	105.12 ± 2.51 [b]	108.00 ± 3.11 [b]	106.37 ± 2.82 [b]
Diabetic	379.37 ± 18.22 [a]	365.50 ± 33.01 [b]	450.62 ± 33.01 [a]	418.12 ± 38.71 [a]	502.75 ± 33.61 [a]	559.75 ± 6.98 [a]
Diabetic + melatonin	396.25 ± 26.11 [a]	430.25 ± 20.71 [a]	457.50 ± 20.84 [a]	487.87 ± 29.69 [a]	511.25 ± 23.03 [a]	563.00 ± 6.84 [a]
P	***	***	***	***	***	***

[a, b, c]: Different letters indicate statistically significant differences in the same column.
***: $P < 0.001$.

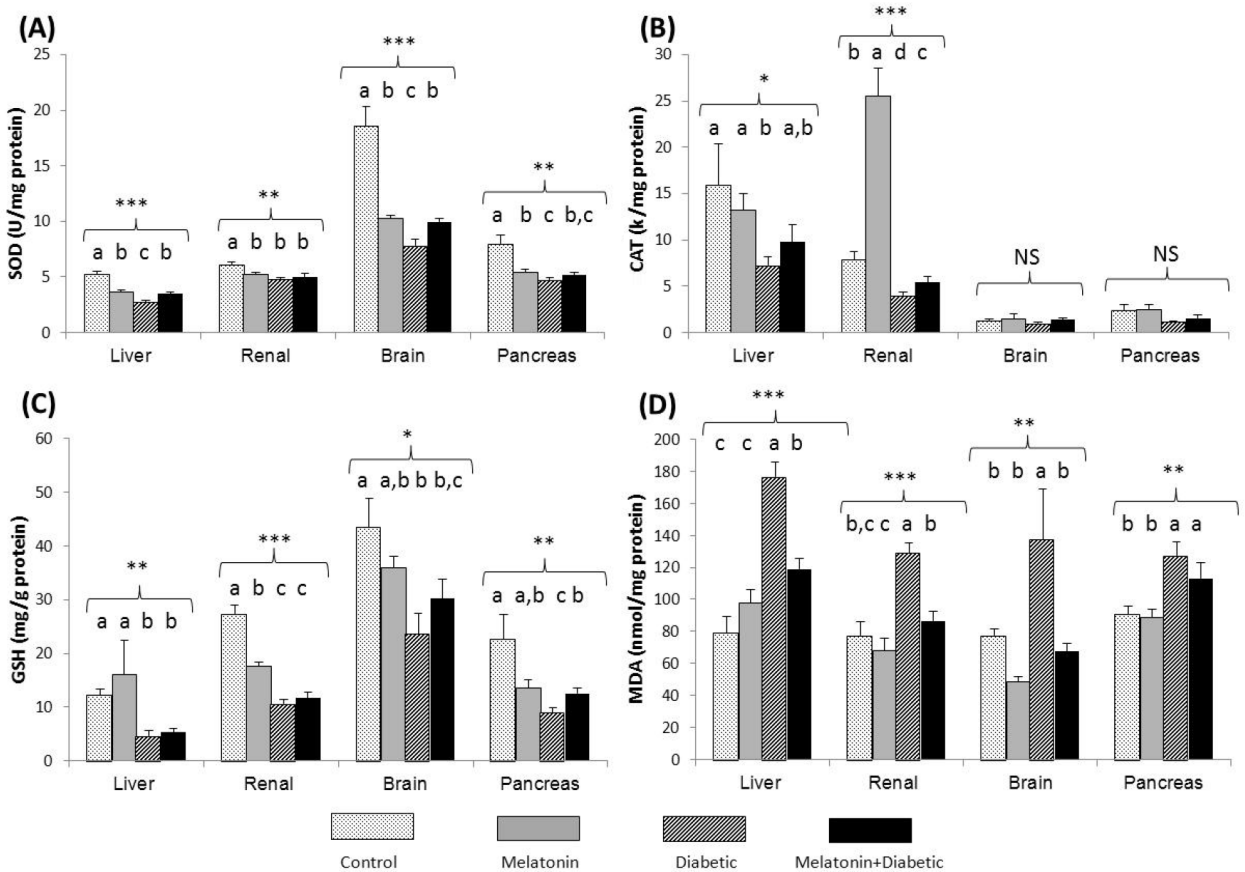

Figure 1. Effects of melatonin administration on the SOD (A) and CAT (B) activities as well as the GSH (C) and MDA (D) levels in liver, kidney, brain, and pancreas tissues (n = 8 per group). [a, b, c, d]: Different letters indicate statistically significant differences in the columns. NS: Not significant. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

3.3 DNA analysis (% tail DNA and mean tail moment parameters)

The effects of MEL administration on % tail DNA and mean tail moment parameters in STZ-induced diabetic rats are presented in Table 3. The lowest parameter values represent minimum DNA damage at the isolated lymphocytes in the control group. The highest values of DNA damage were found in the diabetic group ($P < 0.001$). MEL administration to the diabetic + melatonin group reduced the % tail DNA and mean tail moment parameters compared with the diabetic group ($P < 0.001$).

4. Discussion

The present research reports the effects of MEL on the oxidative damage induced by STZ in rats and its possible role in ameliorating damaged DNA and the development of diabetes. The most important result of the present research was the demonstration of a decrease in the oxidative stress parameters, leading to a decrease in DNA damage by MEL administered to STZ-induced diabetic rats over 6 weeks.

Diabetes has emerged as a major threat to health worldwide. In diabetic patients, oxidative stress induced by the presence of excessive ROS and RNS is closely associated with chronic inflammation, leading to potential tissue damage. Many research studies in recent years aim to weaken oxidative stress to improve diabetes (Oršolić et al., 2013; Marrazzo et al., 2014; Rochette et al., 2014; Xu et al., 2014).

At the beginning, all the experimental groups had similar body weights, but body weights had increased only in the control group by the end of the 6 weeks. Body weight loss is generally observed during short- and long-term experimental diabetes due to the overcatabolism of tissue proteins because of hyperglycemia. In the present study, an i.p. administration of MEL at 10 mg/kg was incapable of stopping the body weight loss in diabetic rats ($P > 0.05$) and this finding supports previous research studies (Cam et al., 2003; Sudnikovich et al., 2007; Oršolić et al., 2011; Elbe et al., 2015; Gleissner, 2015). MEL is capable of initiating weight loss in adult rats and plays an extra role in

Table 3. Effects of MEL administration on % tail DNA and mean tail moment parameters in STZ-induced diabetic rats (n = 8 per group).

Groups	Parameters	
	% tail DNA	Mean tail moment
Control	12.99 ± 1.43 [c]	2.29 ± 0.23 [c]
Melatonin	18.26 ± 1.13 [b]	5.52 ± 0.55 [b]
Diabetic	41.55 ± 2.03 [a]	17.98 ± 1.46 [a]
Diabetic + melatonin	17.68 ± 1.00 [b,c]	5.65 ± 0.56 [b]
P	***	***

[a, b, c]: Different letters indicate statistically significant differences in the same column.
*** $P < 0.001$.

the regulation of body weight through the stimulation of brown adipose tissue metabolism (Korkmaz et al., 2012). However, weight loss in the present study of the melatonin group rats was not significant.

Blood glucose concentrations of diabetic rats were more than 250 mg/dL 3 days after the STZ injection, which showed the existence of DM. Blood glucose concentrations were not affected by MEL treatment in this experiment. Examined blood glucose levels were not significantly changed at the end of the treatment among the untreated diabetic rats and the MEL-treated diabetic rats. This finding is in agreement with previous research studies (Andersson and Sandler, 2001; Vural et al., 2001; Aksoy et al., 2003), although the blood glucose levels of the present study were different from those of some published studies (Bibak et al., 2014; Elbe et al., 2015). Gorgun et al. (2002) reported that the administration of MEL prior to or after STZ treatment decreases plasma glucose and HbA_{1c} levels. Moreover, Andersson and Sandler (2001) reported that DM induced by STZ (140 mg/kg intravenously) in rats was prevented by the administration of MEL (100 mg/kg i.p.) 30 min before STZ injection. As expected, the serum HbA_{1c} levels of diabetic rats were higher than those of the nondiabetic groups ($P < 0.001$). Likewise, there was no significant difference between the serum HbA_{1c} levels of the diabetic and diabetic + melatonin groups ($P > 0.05$). Some previous studies (Montilla et al., 1998; Sudnikovich et al., 2007) reported similar HbA_{1c} alterations in rats. Parallel to the blood glucose levels, the results of this study indicate that MEL administration was unable to decrease the serum HbA_{1c} levels in STZ-induced diabetic rats ($P > 0.05$).

ROS and RNS are products of cellular metabolism and have either deleterious or beneficial effects, depending on the concentration reached in the tissues. In DM, permanent hyperglycemia had been shown to lead to an excess production of ROS. A reduced antioxidant capacity and augmented production of ROS are the main shared mechanisms that lead to increased oxidative stress in DM; thus, tissue damage is facilitated. The electron transport chain in the mitochondrial, peroxisome, and cytochrome P450 systems are the most important sources of ROS production, such as superoxide anion ($O_2\bullet^-$). In addition, various enzymes can accelerate ROS production, such as cyclooxygenases, xanthine oxidase, uncoupled nitric oxide synthases (NOS), and nicotinamide adenine dinucleotide phosphate oxidases. Oxidative stress has been associated with the major complications of DM. Cellular contents, such as lipids, proteins, carbohydrates, and nucleic acids, are affected by alterations of the oxidant and antioxidant equilibrium (Agil et al., 2013; Rochette et al., 2014; Tangvarasittichai, 2015). As oxidative stress is the principal reason for diabetic complications, the management of antioxidants appears to be one of the most rational restorative approaches. Studies indicate that diabetic complications might be diminished by the administration of various antioxidants (Rahimi et al., 2005; Di Naso et al., 2011; Marrazzo et al., 2014; Rochette et al., 2014; Xu et al., 2014).

In the present study, oxidative stress parameters were evaluated based on the rats' liver, kidney, brain, and pancreas tissues; SOD and CAT activities; and MDA and GSH levels. MDA was accepted as a marker of lipid oxidation. The mechanism of lipid and protein metabolism is diminished in the tissues of diabetic rats. The enhanced generation of ROS, lipid peroxidation, and diminished tissue concentrations of SOD, CAT, and GSH are reported in both clinical and experimental diabetes (Montilla et al., 1998; Gorgun et al., 2002; Aksoy et al., 2003; Shaker et al., 2009; Kushwaha et al., 2011). Sudnikovich et al. (2007) suggested that MEL might affect glucose metabolism, thus restoring the tissue redox balance and nitric oxide

bioavailability. Our results about MEL administration corroborate these observations. SOD reduces intracellular levels of superoxide radicals and converts $O_2\bullet^-$ into H_2O_2, which then decomposes into water via CAT and glutathione peroxidase. The highest SOD activity was found in the control group rat brain tissue and the lowest activity was found in diabetic rat tissues (except kidney and pancreas tissues), while MEL administration ameliorated the SOD activities in diabetic rat liver and brain tissues. CAT is located in peroxisomes and converts H_2O_2 to water and oxygen. MEL significantly enhanced the CAT activities of the melatonin group rats, as well as in the diabetic + melatonin group in the kidney tissues. GSH function via GSH peroxidase is also located in the mitochondria for the detoxification of H_2O_2. Increased GSH level might have been sustained to counteract fast-generating oxygen radicals or might have protected the cells from reactive free radicals and peroxides. In the present study, all the tissues of GSH levels were significantly lower in the diabetic and diabetic + melatonin groups when compared with the control group. However, MEL administration enhanced the GSH levels only in the pancreas tissue of the diabetic + melatonin group rats. Oxidative damage can induce lipid peroxidation, depending on the increase of ROS. Lipid peroxidation, protein oxidation, and mitochondrial DNA mutations can be consequences of the oxidative damage to mitochondrial components (Munik and Ekmekçioğlu, 2015). MDA levels were significantly higher in all tissues of the diabetic rats, but MEL administration reduced the MDA levels in the liver, kidney, and brain tissues, except for the pancreas tissues. Comparable results were also obtained by some researchers (Vural et al. 2001; Aksoy et al., 2003; Eşrefoğlu et al. 2014; Elbe et al., 2015).

To the author's knowledge, the present research reports the first DNA protective effects of MEL demonstrated by comet assay, probably by decreasing oxidative stress in STZ-induced diabetic rats. We studied the DNA damage and protection in isolated rat lymphocytes with the comet assay. The comet assay is a reliable, simple, sensitive, and rapid method for assessing DNA damage and repair in the cells (Dhawan et al., 2009) and it may be used for the purposes of evaluating the antioxidant status of the cells (Collins, 2014). Parameters of % tail DNA and mean tail moment were used for the evaluation of DNA damage, and these parameters were altered by MEL administration.

We found that the DNA damage was significantly higher in the lymphocytes of diabetic group rats compared with the other groups. In contrast, the parameters were significantly decreased in the diabetic + melatonin group rats when compared with the diabetic group rats. MEL treatment prevented STZ-induced DNA damage and increased the DNA repair capacity in the lymphocytes of the rats. Many researchers (Andersson and Sandler, 2001; Kushwaha et al., 2011; Oršolić et al., 2011, 2013; Tangvarasittichai, 2015) reported that STZ-induced diabetes caused DNA damage. A similar result was also observed in the present study. DNA damage was significantly increased in STZ-induced diabetic rats. DNA damage could be explained by increased levels of ROS production in oxidative stress among hyperglycemic rats. ROS inhibit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) through a mechanism involving the activation of enzyme poly-ADP-ribose polymerase-1 (PARP-1). This enzyme is involved in DNA repair and apoptotic pathways. Normally, PARP resides in the nucleus in an inactive form, waiting for DNA damage to activate it. When increased intracellular glucose generates increased ROS in the mitochondria, free radicals induce DNA strand breaks, thereby activating PARP. ROS cause strand breaks in nuclear DNA, which activates PARP-1. PARP-1 activation results in inhibition of GAPDH by poly-ADP-ribosylation. GAPDH plays a critical role in DNA repairs (Brownlee, 2005; Giacco and Brownlee, 2010). Interestingly, we found that isolated lymphocyte DNA damage of melatonin group rats was slightly higher compared with that of control group rats. Likewise, Cemeli et al. (2009) reported that MEL on its own (0.1–1 mM) might generate a slight increase in DNA damage in mammalian cells in vitro.

The DNA-protecting effect of MEL against STZ-induced lipid peroxidation might be due to the free radical scavenging property of N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK). AFMK is one of the most important MEL metabolites. AFMK and structurally different metabolites of MEL are sequentially interacting with ROS/RNS, referred to as a scavenging cascade reaction of MEL (Manda et al., 2007; Zhang and Zhang, 2014). Tan et al. (2007) reported that this cascade makes MEL highly effective as a free radical scavenger and antioxidant, e.g., a MEL molecule might be able to scavenge up to 10 ROS/RNS. In addition to the direct effects of MEL and its metabolites with ROS, Tan et al. (2007) stated that AFMK and N-acetyl-5-methoxykynuramine might minimize prooxidative and proinflammatory enzymes, as well as accomplish free radical prevention functions. However, we have not analyzed the MEL and AFMK statuses of the rats in the present study. Moreover, MEL is reported for its protective effect related to interference with DNA damage and PARP activation by increasing cleavage of PARP protein in cancer, methotrexate-induced intestinal damage, and STZ-induced β-cell damage (Jangra et al., 2013). Furthermore, MEL might regulate the antioxidant enzyme activity at cellular mRNA levels for GPx, SOD, and CAT, both under physiological conditions and conditions of elevated oxidative stress. MEL might also regulate its precursor, serotonin, which is reported to be a lipid peroxidation inhibitor (Gorgun et al., 2002; Cemeli

et al., 2009; Munik and Ekmekçioğlu, 2015). The examined parameters of the present research (except mRNA expression levels) corroborate with this phenomenon. However, the role of neutralizing $O_2^{\bullet -}$ radical by MEL is unclear in vivo. Proper functioning of mitochondria requires sufficient ATP for virtually all functions including repair of damage caused by ROS (Zephy and Ahmad, 2015).

In conclusion, the i.p. administration of 10 mg/kg of MEL over 6 weeks might ameliorate oxidative stress parameters against diabetes, thus producing beneficial effects on % tail DNA and mean tail moment parameters in rat lymphocytes. Therefore, the results of the study showed that MEL might play an important role in preventing oxidative DNA damage by scavenging excessive ROS that were generated in hyperglycemic conditions, such as DM.

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