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Melatonin prevents deterioration of erectile function in streptozotocin-induced diabetic rats via sirtuin-1 expression

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Abstract

A review of the literature indicated that sirtuin-1 expression, a regulator of nitric oxide bioavailability in erectile dysfunction (ED) after melatonin therapy, has not yet been investigated. The objective of this study was to evaluate the protective effects of melatonin for erectile function with sirtuin-1 protein expression in type 1 diabetic rat models. Fifty male Sprague Dawley rats were placed into five groups. Except for those in the control group (C), each animal received a single dose (60 mg/kg) of streptozotocin to induce diabetes. The animals were placed into the diabetes (D) group, insulin (I) group (6 U/kg/day), melatonin (Mel) group (10 mg kg^{-1} day^{-1}) and combined treatment (I + Mel) group. Ten weeks later, the serum testosterone levels, intracavernosal pressure (ICP), mean arterial pressure (MAP), malondialdehyde (MDA), cyclic guanosine monophosphate (c-GMP), 8-hydroxydeoxyguanosine (8-OHdG), nitric oxide synthase (NOS), caspase-3 activity, sirtuin-1 and endothelial nitric oxide synthase (eNOS) protein expression and histological findings were assessed. The mean ICP/MAP ratio for the D group was lower than the mean ratios for the other groups. The treatment groups, particularly the I + Mel group, exhibited lower 8-OHdG and MDA levels and caspase-3 activity than the D group. The sirtuin-1 and eNOS expression and cavernosal tissue (CT) histology seemed to have been preserved by the melatonin and/or insulin therapy. These results were indicative of a profound protective effect of melatonin by the activation of sirtuin-1 protein expression against hyperglycemia-induced oxidative CT injury.

KEYWORDS
antioxidant, erectile dysfunction, melatonin, sirtuin 1, streptozotocin, type 1 diabetes mellitus

1 | INTRODUCTION

Erectile dysfunction (ED) and diabetes mellitus (DM) are common health problems that have a significant effect on quality of life. DM is one of the leading risk factors for ED. Men with DM present with refractory and more severe ED than nondiabetic men (Walle, Lebeta, Fita, & Abdissa, 2018).

Diabetic ED has a multifactorial aetiology. It can be caused by any alteration in vascular, neural, hormonal and cavernosal tissue consistency (Thorve et al., 2011). Hyperglycemia in diabetes will finally generate reactive oxygen species (ROS) resulting in endothelial cell damage and neuropathy with the formation of advanced glycation end-products (AGEs; (Cartledge, Eardley, & Morrison, 2001; Yagihashi, Yamagishi, & Wada, 2007). Endothelial dysfunction and the

overproduction of oxygen-free radicals are essential aspects of the pathophysiology of diabetic ED (Gur, Kadowitz, & Hellstrom, 2009; Ryu et al., 2003). The reaction of ROS with nitric oxide (NO) and the impairment of endothelial and neuronal NO synthesis, as well as their expression and activity, will lead to the decrease in or impairment of the NO-dependent relaxing factors (Cartledge et al., 2001). Up-to-date several studies had proposed some pathways which can actually take role in this process (Cartledge et al., 2001). For example, one of them is Angiotensin II and Angiotensin type-1 receptor-activated oxidative stress (Kilarkaje et al., 2013). However, controlling oxidative stress in all seems to be the most crucial step (Boydens, Pauwels, Vanden Daele, & Van de Voorde, 2016; Ganz & Seftel, 2000; Paskaloglu, Sener, & Ayangolu-Dulger, 2004; Suresh & Prakash, 2011).

The early administration of an antioxidant can prevent end-organ damage from the ROS. Many antioxidant agents, such as vitamin E, Mucuna pruriens, melatonin and resveratrol, have been shown to improve and to prevent ED by decreasing the oxidative stress parameters in the corpus cavernosum (CC) of diabetic rats (Boydens, Pauwels, Vanden Daele, & Van de Voorde, 2016; Ganz & Seftel, 2000; Paskaloglu et al., 2004; Suresh & Prakash, 2011). A well-known free radical scavenger, melatonin interacts with the membrane lipids, decreases lipid peroxidation and oxidative stress, and provides the integrity of the cells (Ramis, Esteban, Miralles, Tan, & Reiter, 2015). Melatonin utilises its protective effect on erectile function (EF) in diabetic rats in various ways (Paskaloglu et al., 2004; Qiu et al., 2012; Zhang, Hui, Zhou, & Hou, 2018). Melatonin, which has been studied in various tissue models, such as those of the brain, liver and heart, has been shown to have an antioxidant effect through the receptor-dependent activation of the sirtuin-1 signal (Yang et al., 2015; Yu et al., 2015). Sirtuin-1 is a deacetylase protein that regulates the ageing and glucose metabolism processes by reducing oxidative stress, inflammation and apoptosis (Shi, Lei, Tang, Wang, & Xia, 2019). However, there is a dearth of data on the role of melatonin in diabetes-induced ED.

A review of the literature has revealed that to date, no study has investigated the role of sirtuin-1 expression in the cavernosal tissue in melatonin-treated ED. The present study was designed to evaluate the possible beneficial effects of melatonin treatment on EF. It also investigated the molecular mechanisms in melatonin via the expression of sirtuin-1 in streptozotocin-induced diabetic rats.

2 | MATERIALS AND METHODS

2.1 | Animals and study groups

Sprague Dawley rats (250–300 g) were supplied and prepared by the Marmara University (MU) Application and Research Center for Experimental Animals. All the rats were housed at 22°C–24°C, with a 12-hr light–dark cycle, with free access to standard rat chow and water. The MU Animal Care and Use Committee approved all the experimental protocols with Protocol Number 47-2014 mar. No screening of erectile function was performed prior to the study because all the animals were young and healthy.

2.2 | Experimental design

A total of 50 Sprague Dawley rats were randomly placed into five groups (n = 10):

- Group 1: Control (C) group
- Group 2: Streptozotocin (STZ)-induced diabetic (D) group
- Group 3: Insulin-treated (I) diabetic group
- Group 4: Melatonin-treated (Mel) diabetic group
- Group 5: Insulin and melatonin-treated (I + Mel) diabetic group

2.3 | Drugs and their administration

A single dose of 60 mg/kg body weight of STZ (Sigma) that was freshly prepared in 0.1 M citrate buffer (pH 4.5) was administered intraperitoneally to overnight-fasted rats. Two days after the administration of the STZ, a 12-hr fast was accomplished by the withdrawal of food during the preceding night. The plan was that rats with a fasting blood glucose concentration of less than 200 mg/dl would be excluded from the study. However, hyperglycemic status was achieved in all STZ-treated rats (Aksoy, Vural, Sabuncu, & Aksoy, 2003; Junod et al., 1967). After the establishment of the hyperglycemic states to ensure the existence of type 1 diabetes in the rats, the treatment regimens were begun and continued for 10 weeks. Melatonin (Sigma, St. Louis, MO, USA) was dissolved in absolute ethanol and further diluted in saline. The final concentration of ethanol was 1%. NPH insulin (Humulin N®, Lilly, Istanbul, Turkey) was dissolved in saline. Melatonin was administered intraperitoneally at a dose of 10 mg kg^{-1} day^{-1}, and NPH insulin was administered subcutaneously at a dose of 6 U/kg/day to the appropriate groups (Paskaloglu et al., 2004). The baseline (t1) and final (t2) blood glucose levels were recorded.

The rats were then anaesthetised with an intraperitoneal injection of 100 mg/kg ketamine HCl (Ketalar®; Pfizer) and 1 mg/kg chlorpromazine (Largactil®, Eczacibas; (Paskaloglu et al., 2004). Later, intracavernosal pressure (ICP) measurements were done. Later, rats were sacrificed by bleeding and hypovolemic shock. Then, blood and cavernosal tissue sample were collected.

2.4 | Main outcome measures

2.4.1 | Measurement of intracavernosal pressure

Ten weeks after STZ induction, the rats were anaesthetised, and the mean arterial pressure (MAP) in the left internal carotid artery was measured with an amplifier unit (Commat Pharmacology & Physiology Instruments), data acquisition system (MP35;

COMMAT), and software (BIOPAC Systems). The ICP in the left crus of the penis was measured after the stimulation of the cavernosal nerve with an STPTO2-A stimulator (Commat Pharmacology & Physiology Instruments). The stimulation parameters were 1.5 mA, 20 Hz, pulse width 5, and 35 ms delay at 7.5 V for 60 s each. The cavernosal nerve was stimulated, and the individual data were recorded. The maximum ICP/MAP ratio, which was calculated by dividing the highest recorded ICP and the corresponding MAP, is presented as a percentage (Mullerad, Donohue, Li, Scardino, & Mulhall, 2006). All measurements were done blindly within two consequent day time.

2.4.2 | Measurement of serum testosterone levels

The testosterone levels were measured with the sandwich enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's protocols (E0182Ra, E0179Ra, E0259Ra BT-Labs, China) using a microplate reader was used (BioTek Epoch).

2.4.3 | Measurement of tissue malondialdehyde levels

The CC tissue samples were homogenised with 150 mM ice-cold KCl. The malondialdehyde (MDA) levels were assayed as products of lipid peroxidation by the monitoring of the development of thiobarbituric acid reactive substances. The results are expressed as nmol/g tissue.

2.4.4 | Measurement of 8-hydroxydeoxyguanosine levels and caspase-3 and nitric oxide synthase activity

Measurement of 8-hydroxydeoxyguanosine levels and caspase-3 and nitric oxide synthase activity 8-hydroxydeoxyguanosine (8-OHdG) was determined in DNA isolated from CC tissue using a commercial kit (PureLink™ Genomic DNA Mini Kit Invitrogen, K182001). Isolated DNA concentration was measured with nanodrop (Nabi- UV/Vis Nano Spectrophotometer). Commercially available kits were used for evaluating the activity caspase-3 (Calbiochem), nitric oxide synthase ([NOS]; EnzyChrom, BioAssay Systems) in the CC tissue homogenates.

2.4.5 | Measurement of cyclic guanidine monophosphate levels

The cyclic guanidine monophosphate (cGMP) levels in the tissues were determined in duplicate through the use of a commercially available ELISA kit (Enzo Life Science). The total protein was estimated by the Bradford method, with the cGMP values presented as pmol/mg protein (Bradford, 1976).

2.5 | Western blot analysis for sirtuin-1 and endothelial nitric oxide synthase protein expression

Sirtuin-1 and endothelial nitric oxide synthase (eNOS) protein expressions were measured by Western blotting. Samples of the **CC** were homogenised by a cell lysis buffer, and the protein concentrations were determined through the Bradford method (Bradford, 1976). The samples were resolved by 4%–12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride (PVDF) membrane, which was then blocked with bovine serum albumin. The membrane was incubated overnight with a primary antibody (1:500 dilution anti-sirtuin-1 sc-15404, anti-eNOS sc-136977, anti-β-actin sc-47778; Santa Cruz Biotechnology) and washed with Tris-buffered saline containing 0.1% Tween 20 (TBST). The membrane was washed and then incubated with a horseradish peroxidase-conjugated secondary antibody for 2 hr. Afterwards, the blot was developed with chemiluminescence reagents and exposed to film. The data were analysed with ImageJ optical density analysis software (NIH, USA). The signals were normalised concerning β-actin.

2.6 | Histological analysis

Histopathological analyses were performed after the cavernosal tissues were fixed in 10% formalin solution and dehydrated in a degraded ethanol series and cleared in toluene. Paraffin-embedded samples were cut at a thickness of 5 μm with a rotary microtome and stained with haematoxylin and eosin (H & E). Sections were evaluated and photographed under an Olympus BX51 light microscope (Olympus Co., Ltd).

2.7 | Statistical analysis

The statistical analysis was performed in GraphPad Prism 6.0 (GraphPad software). All the data are expressed as means ± standard deviations. Groups of data were compared through an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p < .05$ were considered significant.

3 | RESULTS

The experimental protocol was planned to proceed up to week twelve. However, two animals in the D group were lost at Week 9 because of metabolic complications. This caused the study to be ended at Week 10. The induction of STZ in all the mice resulted in hyperglycemia ($p < .01$), and insulin and/or melatonin were effective at controlling the blood glucose levels of the diabetic rats ($p < .01$; Table 1).

TABLE 1 Body weight and blood glucose in groups

	Control	D	D + I	D + Mel	D + I+Mel
Body weight					
t_1	296 ± 7.3	298 ± 6.5	299 ± 12.8	296 ± 6.4	301 ± 5.6
t_2	341 ± 4.1$^{*DI, DM}$	235 ± 6.2 ^{**DI,DM}	281 ± 7.5^{C,D,DIM}	292 ± 9.5^{C,D,DIM}	340 ± 13.8^{*DII,DM}
Blood glucose					
t_1	98 ± 0.4	324 ± 14.8^{C}	348 ± 18.9^{C}	308 ± 9.9^{C}	327 ± 9.7^{C}
t_2	91 ± 4.8^{AII}	371 ± 23.1^{AII}	126 ± 9.5^{**AII}	169 ± 11.3^{**AII}	110 ± 4.8^{**AII}

Note: C: control group, D: vehicle-treated diabetic group, I: insulin-treated diabetic group; Mel: melatonin-treated diabetic group; I + Mel: insulin + melatonin treated diabetic group. Each group consists of 10 rats. Values are represented as mean ± *SD*.

t_1: Day 2, after the induction of STZ without any other medication

t_2: Time of sacrification

Compared with initial levels of each group; $^{*}p < .05$, $^{**}p < .01$

Comparison with groups is demonstrated with the initials of each group for statistically significant difference ($p < .05$) as Control: ^{C}; Diabetes: ^{D}; Diabetes and Insulin: ^{DI}; Diabetes and Melatonin ^{DM}; Diabetes and Insulin and Melatonin: ^{DIM}

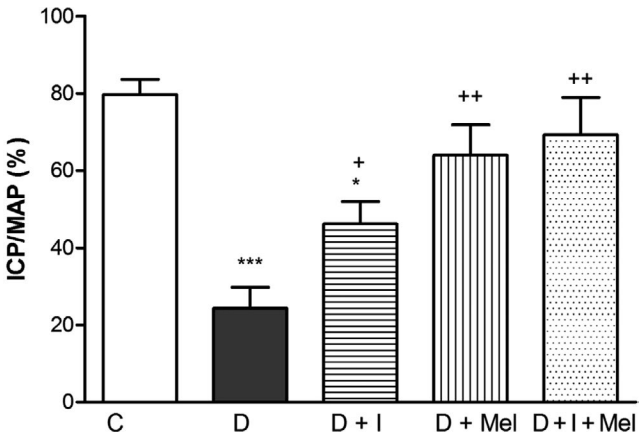

FIGURE 1 Intracavernosal pressure (ICP)/mean arterial pressure (MAP) ratio in all groups. Control (C) and diabetic group (D), Insulin (D + I), Melatonin (D + Mel) or insulin + melatonin (D + I + Mel) -treated diabetic groups. Compared with control; $^{*}p < .05$, $^{***}p < .001$. Compared with group D; $+p < .05$, $+++p < .01$

3.1 | Body weight of animals

The weight and blood glucose levels of the study groups were similar at baseline. At the end of the study, when compared with basal, mean body weights were found low in D group ($p < .01$) and high in the control and D + I + Mel groups ($p < .05$). The average body weight of the groups that were treated with only insulin or only melatonin was similar (Table 1).

3.2 | Assessment of erectile function

The mean ICP/MAP ratio for the D group was lower than those for the other groups ($p < .05$). However, the administration of melatonin alone or in combination with insulin resulted in a mean ICP/MAP ratio that was similar to that for the C group. These findings regarding EF are indicative of the superiority of melatonin over insulin or the combination of melatonin and insulin (Figure 1).

3.3 | Assessment of sex hormones

The mean serum testosterone levels of the rats in the D group were significantly lower than those of the rats in the C group ($p < .001$). The mean serum testosterone levels in the groups treated with either insulin or melatonin or a combination of these two agents were found to be higher than those in the D group ($p < .05$ and $p < .001$). However, the mean serum testosterone levels of the diabetic animals treated with either melatonin or insulin or a combination of the two agents were similar to those in the C group. These findings revealed that either melatonin or insulin or a combination of these two agents was similarly efficacious for testosterone production (Figure 2a). Blood samples (2 ml each sample) were taken from the heart and centrifuged at 3,500 rpm for 10 min just before sacrification.

3.4 | Malondialdehyde, 8-hydroxydeoxyguanosine levels and caspase-3 activity in the cavernosum tissue

The diabetic rats were found to have significantly higher MDA and 8-OHdG levels and caspase-3 activity than the C group rats if they did not receive any treatment ($p < .05$, $p < .01$, and $p < .01$ respectively). Melatonin treatment alone or in combination with insulin reduced the higher MDA and 8-OHdG levels and caspase-3 activity in the cavernosum tissue to the levels observed in the C group. In addition, the treatment groups had significantly less oxidative stress than the D group ($p < .01$–$.05$). The I group had similar MDA and 8-OHdG tissue levels as those observed for the D and C groups. However, the caspase activity in the I group rats was also lower than that in the D group rats. These findings suggest that insulin facilitated partial caspase activity; however, melatonin was more efficacious for reducing oxidative stress (Figure 2d–f).

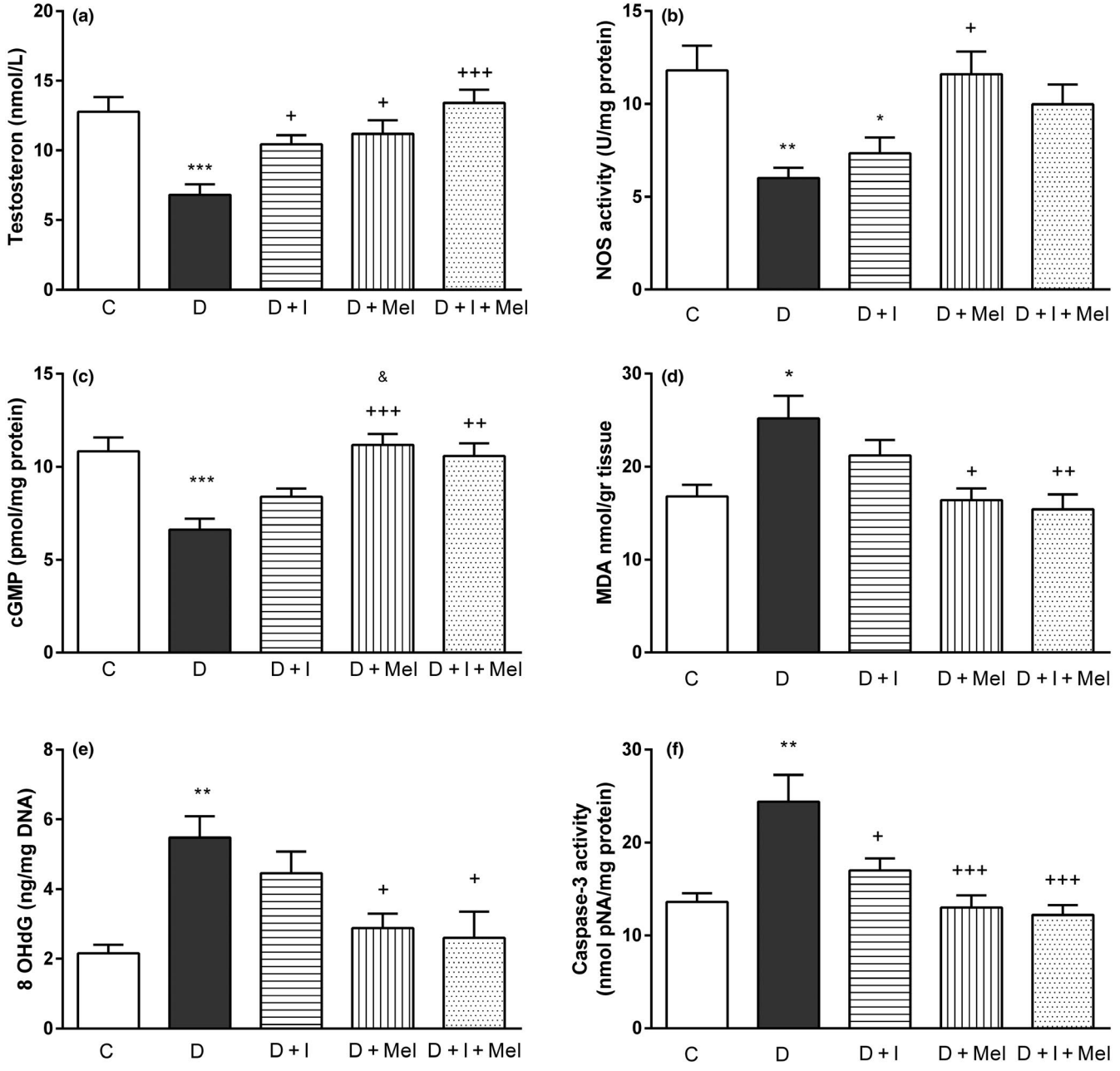

FIGURE 2 (a) Serum testosterone levels in all groups, (b) Nitric oxide synthase (NOS) activity and (c) c-GMP levels in the corpus cavernosum tissues, (d) Malondialdehyde (MDA), (e) 8-OHdg levels and (f) caspase 3 activity in the corpus cavernosum tissues of control. Control (C) and diabetic group (D), Insulin (D + I), Melatonin (D + Mel) or insulin + melatonin (D + I + Mel) -treated diabetic groups. Compared with control; ** $p < .01$, ***$p < .001$. Compared with group D; + $p < .05$, ++ $p < .01$, +++ $p < .001$. Compared with group D + I; & $p < .05$

3.5 | Nitric oxide synthase activity and cyclic guanidine monophosphate levels in the cavernosum tissue

The diabetic rats were found to have significantly less NOS activity and lower cGMP levels than those in the C group. Insulin treatment alone could not fully protect the NOS activity in the diabetic rats. However, melatonin treatment alone could effectively maintain the NOS activity and cGMP levels at those observed in the C group. Similarly, the combination of melatonin and insulin protected the cGMP levels but not the NOS activity (Figure 2b,c).

3.6 | Sirtuin-1 and endothelial nitric oxide synthase expression in the cavernosal tissue

The diabetic rats were found to have significantly less sirtuin-1/beta-actin and lower eNOS/beta-actin levels than those observed in the C group. Any treatment modality seemed to improve the sirtuin-1/

beta-actin levels. However, the best improvement was seen with melatonin alone, and the least improvement was seen with insulin alone. Interestingly, this was not true for the eNOS/beta-actin levels. Melatonin alone or the combination therapy seemed to be similarly efficacious for restoring the eNOS/beta-actin levels. Unfortunately, insulin alone was not as efficacious as melatonin alone for restoring the eNOS/beta-actin levels (Figure 3).

3.7 | Histopathological evaluation of cavernosal tissues with haematoxylin and eosin staining

An assessment of the normal sinusoidal morphology of the CC in the C group rats (Figure 4a) showed that diabetes accompanied by vehicle treatment led to degeneration, such as vascular congestion, diffuse sinusoidal damage and cytoplasmic vacuolisation in the endothelial cells (Figure 4b). Either the insulin or the melatonin treatment or a combination of both reduced vascular congestion and preserved the sinusoidal architecture (Figure 4c,e). Overall, the best histological preservation was observed in the treatment group in which a combination of insulin and melatonin was used (Figure 4e).

4 | DISCUSSION

After the first week, the blood glucose levels of the rats that were treated with insulin, melatonin, or a combination of the two agents seemed to return to those of the controls. Interestingly, melatonin was found to regulate the blood glucose levels in a manner similar to that observed for insulin therapy in earlier studies (Andersson & Sandler, 2001; Khorsand, Akmali, & Akhzari, 2019; Montilla et al., 1998). The increase in the MDA and 8-OHdG levels and caspase-3 activity, in addition to the concomitant decrease in the cGMP levels and NOS activity, suggested the presence of hyperglycemia-induced oxidative stress on the CC. An important factor for EF and sirtuin-1 in diabetic rats, the eNOS protein was found to have lower expression levels. However, the melatonin treatment

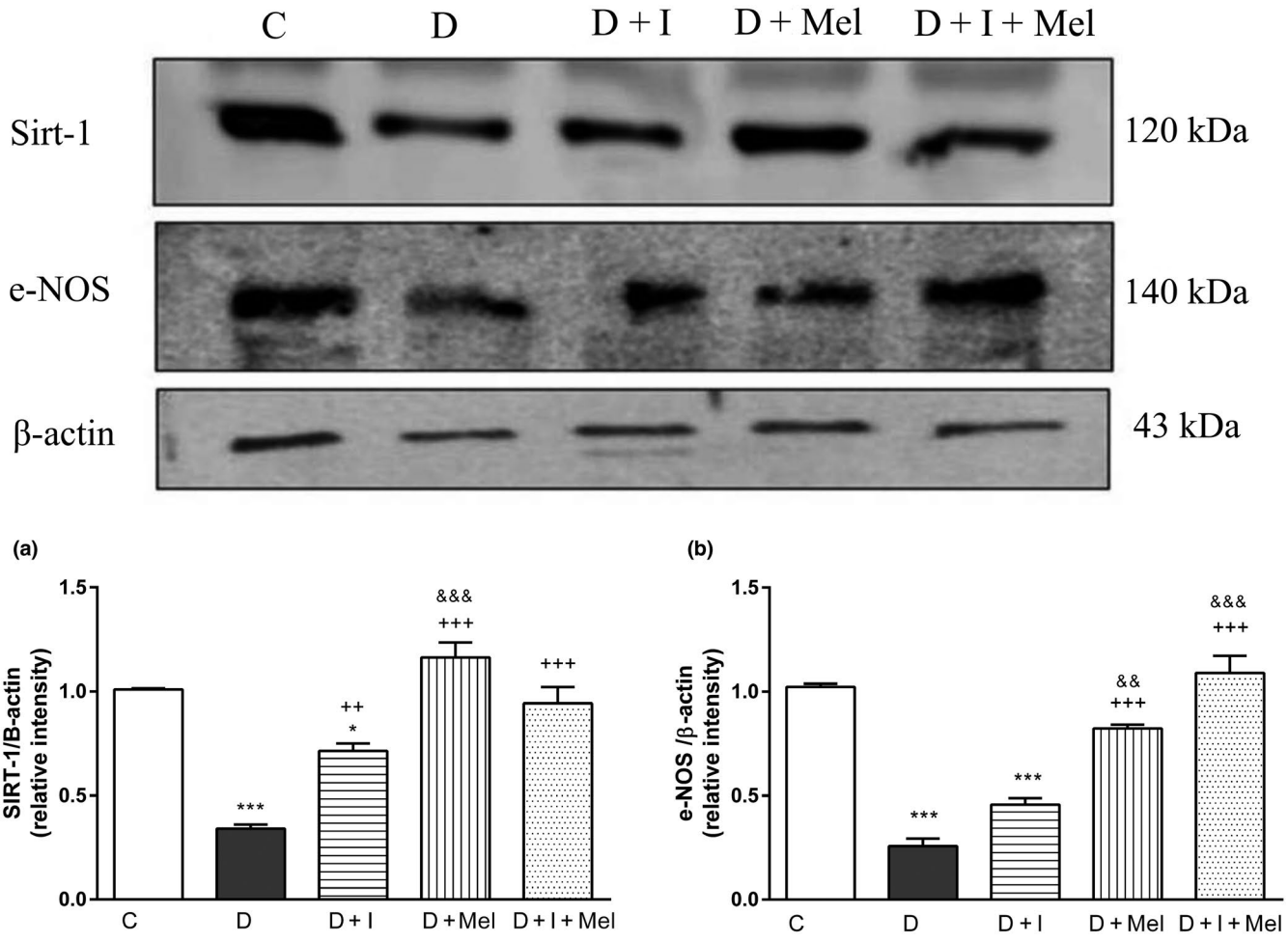

FIGURE 3 (a) Representative images of each protein in Western blot are provided for all groups with Sirtuin-1 (b) and e-NOS protein expression (c) in the corpus cavernosum tissues. Control (C) and diabetic group (D), Insulin (D + I), Melatonin (D + Mel) or insulin + melatonin (D + I + Mel) -treated diabetic groups. Compared with control; *$p < .05$, ***$p < .001$. Compared with group D; ++$p < .01$, +++$p < .001$. Compared with group D + I; &&$p < .01$, &&&$p < .001$

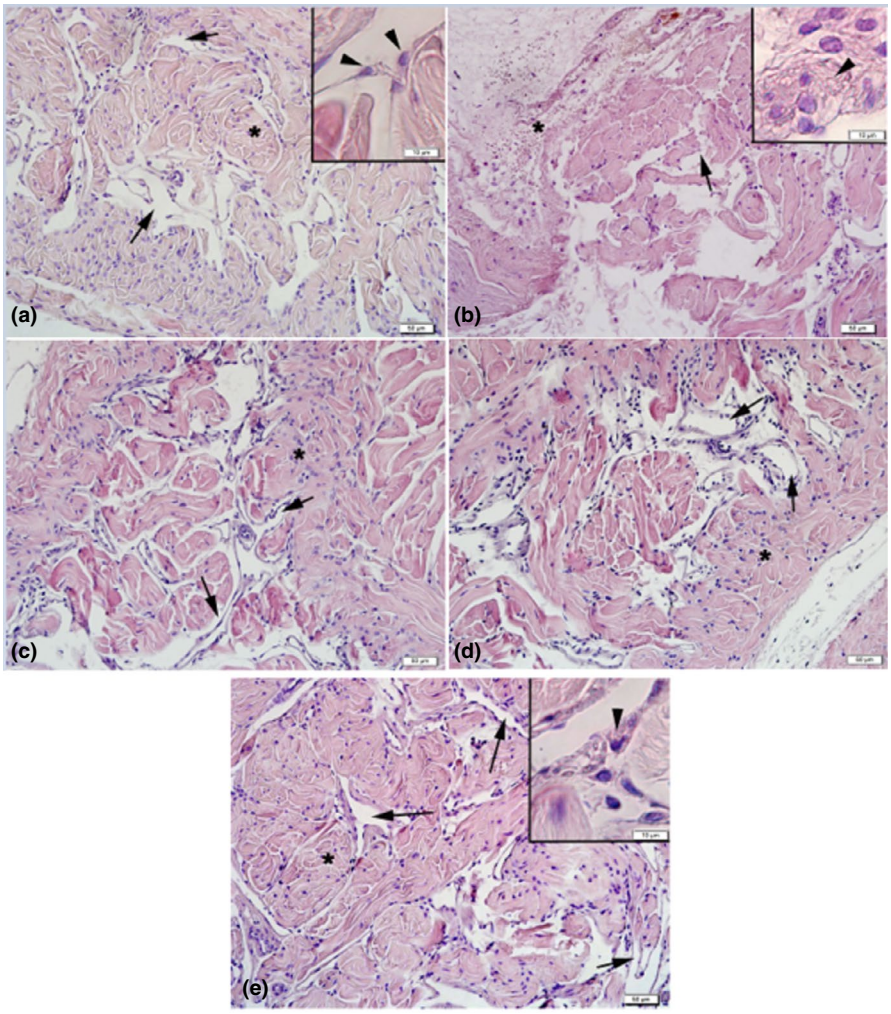

FIGURE 4 Histopathological sections of cavernosal tissue samples prepared with haematoxylin and eosin (H & E) staining are shown for each group (X200). a) Control group: regular endothelial lining of vein (arrow) and collagenous, elastic and smooth muscle fibres (*), regular endothelial cells (arrowheads) b) Streptozotocin (STZ) induced diabetic group (D); congestion in the veins (*) and endothelium (arrow), collagenous, elastic and smooth muscle fibres (*) c) insulin-treated diabetic group (I): reduced congestion (*) and regular endothelium (arrow) d) melatonin-treated diabetic group (Mel): reduced congestion (*), and regular endothelium (arrows), e) insulin and melatonin-treated diabetic group (I + Mel): reduced congestion (*), regular endothelium (arrows), regular endothelial cells (arrowheads)

protected the eNOS and sirtuin-1 expression levels of eNOS and the ICP/MAP ratio. These two also suggest that melatonin has a significant effect on EF. Histopathological studies have shown that melatonin can be a preventive agent against diabetes-induced architectural damage.

4.1 | Oxidative stress indicators

Currently, a great deal of effort is being made to identify the effective antioxidant agents for preventing or treating free-radical mediated tissue damage. Previous studies have shown that hyperglycemia can lead to oxidative stress in the cavernosal tissue and organs, such as the heart, kidney and liver (Aksoy, Vural, Sabuncu, & Aksoy, 2003; Qiu et al., 2012; Zhang et al., 2018). Melatonin is secreted by the pineal gland. This amine hormone has neural protective, anti-inflammatory and antioxidant functions (Aksoy et al., 2003; Sailaja Devi, Suresh, & Das, 2000). As a free radical scavenger, melatonin can enter the tissue space directly to protect the cytoplasmic proteins and the nuclear DNA. It can also stimulate gene expression to employ an antioxidant effect by increasing the antioxidant enzyme activity (Sailaja Devi et al., 2000). Melatonin's antioxidant effect on the oxidative damage caused by diabetes is not yet fully understood. However, melatonin has been shown to reverse this damage via its antioxidant effects, and it has been found to decrease cell proliferation and to increase apoptosis (Xu, Zhao, Liu, Wang, & Lu, 2019).

MDA levels are an indicator of endogenous lipid peroxidation that increases with hyperglycemia (Aksoy et al., 2003). The chief marker of free radical-induced oxidative damage in the mitochondrial and nuclear DNA has been reported to be 8-OHdG. Therefore, it has been used extensively as a biomarker in several studies (Korkmaz, Uzun, Cakatay, & Aydin, 2012). Several recent studies have shown oxidative stress and that free oxygen radicals fundamental role in apoptosis and some anti-oxidants can delay this apoptosis (Sehirli et al., 2013). In the present study, a decrease in caspase-3 activity and 8-OHdG and MDA levels was observed after melatonin treatment. Thus, as was found in previous studies, melatonin seemed to improve cavernous tissue integrity and to sustain the EF by limiting oxidative stress in the cavernosal tissue (Aksoy et al., 2003; Korkmaz et al., 2012; Sehirli et al., 2013).

4.2 | Nitric oxide

NO is a vital mediator in the smooth muscle relaxation and vasculature of cavernosal tissue (Cartledge et al., 2001; Thorve

et al., 2011). The main mediators of the NO/cGMP signalling pathway in the cavernosal tissue have been suggested to be cGMP and total NOS activity. In hyperglycemia, the AGEs react with the superoxide anions and NO; consequently, the NO bioavailability is altered because of peroxynitrite (ONOO) production (Cartledge et al., 2001). The NO that is generated by nNOS might have an important effect on the immediate relaxation of the cavernosal tissue. However, the NO in eNOS seemed to be crucial for maintaining relaxation (Andersson, 2003). Reductions in the eNOS and nNOS levels could alter the circulatory and structural functions of the cavernosal tissue, and this could cause ED (Andersson, 2003). The diabetic rats in the present study had significantly less total NOS activity and low cGMP levels; thus, their ED was the result of the altered NO mechanism. However, melatonin therapy raised the total NOS, eNOS and cGMP levels in the treatment groups so that they were close to those in the C group. It also improved the NO/cGMP signalling pathway. This was confirmed by the ICP measurements. Either the insulin or the melatonin treatment improved EF; however, the combination of insulin and melatonin provided greater protection. The ICP/MAP ratios were 24%, 62%, 76% and 85% in the D, I, Mel and D + Mel groups respectively.

4.3 | Sex hormones

Lower testosterone levels are a characteristic of diabetic animals. Low testosterone levels could cause a decrease in libido and, thus, lead to ED (Cap, 2012). De costa reported that hyperglycemia increased oxidative stress in testicular cells. Melatonin administration to diabetic rats prevents the drop in serum testosterone levels and avoids sperm motility alterations by reducing the oxidative damage. Melatonin improves Leydig cell testosterone production (da Costa, Gobbo, Tab oga, Pinto-Fochi, & Goes, 2016). (Kataoka, Hotta, Maeda, & Kimura, 2014) reported that testosterone replacement improved the endothelial and EFs in diabetic rats. Oliveira et al. (2018) showed that diabetes could cause a mass reduction in the epididymis and testis. Melatonin treatment in combination with insulin preserved the testis and epididymis mass but not the testosterone levels (Oliveira et al., 2018). Likewise, the present study found that the combination of melatonin and insulin could protect the testosterone levels. This might be one of the possible protective mechanisms against ED in diabetic rats.

4.4 | Sirtulin-1

The sirtuin-1 protein deacetylase facilitates many effects on the metabolic pathways and the organismal lifespan (Mattagajasingh et al., 2007). The sirtuin gene family plays an important role in DNA recombination and repair mechanisms, apoptosis, cellular response to stress, insulin secretion, fat mobilisation from human cells, axonal protection and ageing (Ramis et al., 2015; Yu et al., 2014). Sirtuin-1 has a regulatory role in the bioavailability of NO through the eNOS cascade. The nNOS and eNOS are isoenzymes. They play critical roles during an erection and act differently in the production of NO. At the nerves to the penis, NO is derived from nNOS and helps to initiate the erection. In contrast, the NO derived from the endothelium with the help of the eNOS helps to maintain a full erection (Tomada, Tomada, Almeida, & Neves, 2013). Blocking the sirtuin-1 protein expression stimulates endothelial-dependent vasodilatation by directing the eNOS for deacetylation. This leads to enhanced sirtuin-1 function and, thus, decreases the NO bioavailability. As a result, vasorelaxation through the endothelium is blocked (Mattagajasingh et al., 2007). These effects have been shown to be reversed by the activation of sirtuin-1 protein expression through the administration of resveratrol and melatonin.

A review of the literature indicates that this is the first study to investigate the role of sirtuin-1 expression in the cavernosal tissue in melatonin-treated ED. Sirtuin-1 protein expression was found to be high in the groups treated with melatonin and/or insulin. However, it was significantly low in the animal group with STZ-induced diabetes.

4.5 | Erectile function

This functional study of melatonin therapy is the first to report the sirtuin-1 induced protection of EF in a type 1 diabetic rat model. Because the melatonin was started shortly after the STZ, the observed effects can be considered to be only protective. The reversal effects of the various treatments following STZ administration cannot be fully explained.

4.6 | Limitations of the study

This study has some major limitations. The first is the conclusion of the study two weeks earlier than had been planned. Although the STZ dosage was determined on the basis of previous studies, serious metabolic problems and severe weight loss in the D group led to early termination being the only option. The second limitation is the lack of a test, such as a TUNEL assay, for determining the apoptotic cell population. The third limitation is the use of the STZ-induced type 1 diabetic rat model, which might not represent the pathologic processes of all types of diabetes. Interestingly, some studies have suggested that melatonin signalling is a risk factor in the animal models of type 2 diabetes. Thus, caution is needed in the potential translation of the findings (Tuomi et al., 2016). The histological assessment would have been strengthened by the presentation of quantitative data regarding the smooth muscle/collagen ratio and microscopical alterations. A wash-off period is not preferred in the current study before the assessments as some of the medications cannot be withdrawn. Thus, one must keep in mind that some of the effects observed could be directly related to the medications' effect.

5 | CONCLUSION

Melatonin treatment significantly protects the cavernosal tissue against hyperglycemia-induced oxidative injury in type 1 diabetic rats. The main protective effect seemed to be attributable to the activation of sirtuin-1 protein expression, which promoted apoptosis inhibition and provided oxidative stress resistance. Unfortunately, the present study provides information on the protective effects only. If the current findings are supported by further clinical studies, melatonin could become a supplementary treatment option for ED.

CONFLICT OF INTEREST

None.

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