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RESEARCH ARTICLE

Melatonin improves liver and pancreatic tissue injuries in diabetic rats: role on antioxidant enzymes

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Abstract

Purpose Melatonin (Mel) is an indolamine mainly synthesized by the pineal gland and many other organs. It plays an important role in scavenging free radicals and stimulating antioxidant enzymes. The goal of this study was to investigate the effect of Mel and/or insulin treatment on oxidative liver and pancreas injuries in diabetic rats.

Methods Male Wistar albino rats were assigned into 5 groups. Group I: control animals. Group II: diabetes was induced via a single dose of STZ (60 mg/kg) administered intraperitoneally. Group III: diabetic rats treated with Mel (10 mg/kg/day). Group IV: diabetic rats given insulin (6U/kg) subcutaneously. Group V: diabetic rats that received insulin and Mel at the same dose and time. After 12 weeks of the experiment, the animals were decapitated, liver and pancreas tissues were collected.

Results The results indicated that reduced glutathione levels in liver and pancreatic tissue decreased, while protein carbonyl, advanced oxidized protein products and lipid peroxidation levels were elevated in diabetic group. Antioxidant enzyme activities decreased in liver tissues but increased in pancreatic tissues of the diabetic group. Administration of Mel, insulin or Mel + insulin reversed these biochemical changes in the diabetic animals.

Conclusion This work shows that in long-term oxidative stress conditions caused by STZ-induced diabetes, either Mel or Mel + insulin administration may improve the deteriorated oxidant/antioxidant system in both the liver and pancreas tissues. These results suggested that Mel alone or Mel + insulin treatments might have a significant role in protecting against liver and pancreatic damage in STZ diabetic rats via different antioxidant effects.

Keywords Diabetes · Liver · Pancreas · Insulin · Melatonin · Antioxidant enzymes

Introduction

Diabetes mellitus (DM) is a multifactorial as well as a chronic disorder that is mainly related to high blood glucose levels (known as hyperglycemia). It is characterized by an absolute or relative deficiency in the synthesis/secretion of insulin by pancreatic β-cells, or its action. Dysfunction in these cells is at the center of the onset of both type-1 and type-2 DM. In general, it is well-documented that DM easily triggers overproduction of free radicals (reactive oxygen species [ROS]), thereby resulting in oxidative stress (OS) as a consequence of chronically hyperglycemic conditions [1]. On the other hand, liver dysfunction due to chronically hyperglycemic conditions may affect over 500 metabolic processes, compromise detoxification processes, and subsequently led to the damage of other organs such as pancreas. Additionally, pancreas can be affected by high blood glucose levels, thereby leading to the occurrence of OS in

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pancreatic tissue. Thus, both liver and pancreas are prone to DM-related OS. The weakened antioxidant defense elements make the tissues highly vulnerable to ROS; thus, contributing to the further progression of diabetes-induced tissue damage through redox imbalance and OS [2].

Melatonin (Mel) is secreted by pineal gland and has been shown to be essential for healthy metabolism, by acting as synchronizer of circadian rhythm, seasonal reproduction, and also antioxidant effects [3]. Also, Mel acts as either direct or indirect scavenger of free radicals, protecting various biomolecules against the harmful effects caused by excessive ROS production. Mel can scavenge much more free radicals when compared to other antioxidants (i.e., vitamin C and GSH) with a 1:1 ratio or less free radical scavenging ability. This effect may be due to its molecular rearrangement following interaction with reactive species. More so, Mel can indirectly reduce OS by modulating the activities of antioxidant defense mechanisms and interacting synergistically with other antioxidants [4].

Based on the above information, this study was planned to examine whether Mel alone or in combination with insulin had a potential protective role on liver and pancreatic tissues of streptozotocin (STZ)-induced diabetic rats.

Materials and methods

Animals

Animals (200–250 gram) purveyed from Marmara University (MU) Experimental Animal Implementation and Research Center. The rats were provided with a 12-hour light/dark cycle at a constant temperature of 22±2 °C degrees. The rats were given standard pellet chow and as much water *ad libitum* as they wanted. All experimental protocols were performed under the approval of the MU Animal Care and Use Committee (Approval number: 2016. mar/42).

Induction of diabetes

Male Wistar Albino rats were randomly assigned into 5 groups and each group consisted of 8 rats: Control (C), diabetic (D), D + insulin treated (D + I), D + Mel treated (D + M), and D + I + M treated groups. To induce diabetes, animals received a single dose of 60 mg/kg body weight (b.w.) freshly prepared STZ (Sigma, St. Louis, MO, USA, dissolved in 0.1 M citrate buffer, pH: 4.5) intraperitoneal injection. Rats with blood glucose levels above 200 mg/dL two days after administration of STZ to rats were considered diabetic [5, 6]. Mel (Sigma, St. Louis, MO, USA) was dissolved in absolute ethanol (final concentration 1%) and further diluted in physiologic saline. Insulin (Humulin N®, Lilly, Istanbul, Turkey) was dissolved in physiologic saline. Mel (10 mg/kg b.w.) was injected with intraperitoneal route, daily for twelve weeks to D + M and D + I + M groups. Insulin (6 U/kg b.w.) was injected with subcutaneously, daily for twelve weeks to D + I and D + I + M groups [7].

Blood glucose levels and body weights were measured and recorded on the first day (day 0) and the last day (day 84) after the onset of diabetes. To assess blood glucose levels in the rats 48 h after STZ injection, the blood glucose levels were measured using a glucometer (Accu-Chek, F. Hoffman-La Roche Ltd, Basel, Switzerland). Under mild ether anesthesia, whole blood samples were collected from the orbital vein of all rats.

Tissue preparation and homogenization

At the end of experimental period (after 12 weeks), rats were decapitated, and liver and pancreatic tissue samples collected. Thereafter, 10% (weight/volume) tissue homogenates were prepared using cold physiologic saline solution. Clear supernatants were separated from tissue homogenates by centrifuging at 10.000 x *g* for 10 min at 4°C and thereafter the clear supernatants were pooled out for biochemical analysis of oxidative injury.

Determination of reduced glutathione (GSH) levels

GSH levels in liver and pancreas tissues were determined according to Beutler's [8] method. The process is based on the measurement of intense yellow 5-thio-2-nitrobenzoic acid formed by the reaction of GSH with 5,5′-dithiobis (2-nitrobenzoic acid) at 412 nm with the help of a spectrophotometer (Shimadzu UV-Mini-1240, Kyoto, Japan). Results were expressed as *n*mol GSH/mg protein.

Determination of lipid peroxidation (LPO) levels

LPO levels in both tissues were estimated by the methods proposed by Ledwozyw et al. [9]. Briefly, tissue homogenate and trichloroacetic acid (TCA, 1.22 M) solution were mixed and incubated at room temperature (15 min). The thiobarbituric acid solution was pipetted to each tube and then incubated at 95 °C for 30 min. After cooling, the mixture was mixed with the n-butanol solution so as to extract organic phase. The absorbance of the organic phase was then measured (at 532 nm) in the spectrophotometer in terms of tissue malondialdehyde (MDA, an index of LPO). Results are reported as *n*mol MDA/mg protein in the figure.

Determination of protein carbonyl (PC) levels

PC levels in liver tissues were done according to Levine et al. [10]. In brief, clear tissue homogenates (0.5 ml) were mixed with 2 ml 2,4-dinitrophenyl hydrazine (10 mM) and incubated in dark at room temperature for 1 h and mixed gently every 15 min. 2.5 ml 20% TCA were added to each test tubes and they were kept at 0 °C for 5 min. The clear supernatants separating from the reaction medium by centrifuging were poured out. The precipitates were washed 3 times using 2 ml of prepared ethanol and ethyl acetate (1:1) solution. Thereafter, they were added with 1 ml of guanidine.HCl (6 M) to the each tubes and incubated at 37 °C for 10 min. The absorbances of test tubes were recorded at 370 nm, and the results given in *n*mol/mg protein.

Determination of advanced oxidized protein products (AOPP) levels

To evaluate AOPP levels in pancreas samples, the method developed by Witko-Sarsat et al. [11] was used. Appropriate volumes of samples, phosphate buffer (20 mM, pH 7.4), and potassium iodide (1.16 M) were mixed and allowed to stand in dark at room temperature for 2 min. Finally, the resultant mixture was mixed with glacial acetic acid solution, thereafter absorbance of the resultant products was read at 340 nm. The results were reported as *p*mol/mg protein.

Determination of catalase (CAT) activity

CAT activities of liver and pancreatic tissues of rats were assessed by the method of Aebi [12]. In brief, appropriate volumes of tissue sample and substrate solution (30 mM H_2O_2 in phosphate buffer, pH 7.0) were mixed, and decrease in absorbance (between 0 and 1 min) as a consequence of reduction of H_2O_2 to H_2O in the presence of CAT was read spectrophotometrically at 240 nm. Physiologic saline solution was used as blank instead of tissue sample. The results were defined as U/mg protein.

Determination of superoxide dismutase (SOD) activity

Liver and pancreatic SOD activities were determined using the method described by Mylroie et al. [13]. In reaction protocol, phosphate buffer (50 mM, containing 0.1 mM EDTA, pH 7.8), *o*-dianisidine.2HCl (6 mM), and vitamin B_2 (in 10 mM phosphate buffer, pH 7.5) were used. The catalytic activity of the SOD enzyme is determined by measuring the ability of riboflavin-sensitized *o*-dianisidine to increase the photooxidation rate under UV light. The superoxide radical formed by riboflavin under the influence of UV light is converted into H_2O_2 by the catalytic effect of the SOD. The first absorbance value of the reaction initiated by the addition of riboflavin was measured in a spectrophotometer at 460 nm. The second absorbance of the samples, which were kept under UV light for 8 min, was measured again. The difference between the absorbance values obtained at 0 and 8 min was taken, and the results were expressed as U/g protein.

Determination of glutathione peroxidase (GPx) activity

GPx activities in both tissue homogenates were estimated by the kinetic reaction protocol developed by Paglia and Valentine [14] and modified by Wendel [15]. GPx catalyzes the oxidation of GSH in the presence of H_2O_2. As a result, oxidized glutathione (GSSG) and H_2O are formed. The resulting GSSG is reduced back to GSH by a reaction catalyzed by glutathione reductase (GR) using $NADPH + H^+$ as a reducing coenzyme. During these reactions, the oxidation of the coenzyme leads to a decrease in the absorbance value. GPx activity is calculated by measuring the change in absorbance at a wavelength of 366 nm per minute for 5 min with a spectrophotometer. GPx activity was expressed as U/g protein.

Determination of glutathione reductase (GR) activity

The activities of GR of liver and pancreatic tissues of rats were estimated by the kinetic reaction protocol developed by Beutler [16]. Briefly, reaction mixture containing Tris-HCl buffer (50 mM, pH 8.0), $NADPH + H^+$ (2 mM), and GSSG (20 mM) were mixed with appropriate volume of tissue samples. The decline in absorbance was recorded in a spectrophotometer at 340 nm per minute for 5 min. GR activity was expressed as U/g protein.

Determination of glutathione-S-transferase (GST) activity

GST activities of both homogenates were done according to the method proposed by Habig and Jakoby [17]. Briefly, potassium phosphate buffer (20 mM, pH 6.6), appropriate volume of homogenate, GSH (60 mM), 1-chloro-2,4-dinitrobenzene (CDNB, 60 mM, in absolute ethanol), and distilled water were mixed. The change in absorbance of the mixture was observed at room temperature at a wavelength of 340 nm for 3 min. GR activity was expressed as U/g protein.

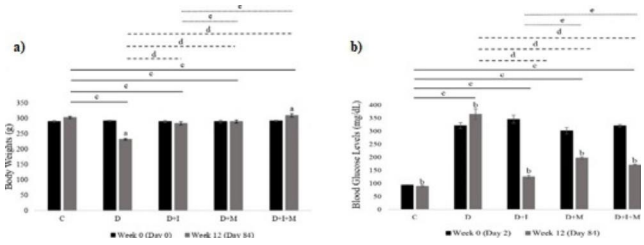

Fig. 1 Body weights and blood glucose levels of the groups at the beginning and at the end of the experiment [19]
[a]$p < 0.05$ compared with Week 0 (Day 0); [b]$p < 0.05$ compared with Week 0 (Day 2); [c]$p < 0.05$ compared with group C; [d]$p < 0.05$ compared with group D; [e]$p < 0.05$ compared with group D + I; [f]$p < 0.05$ compared with group D + I + M.

Determination of total protein levels

Total protein levels in tissue homogenates were quantified with the use of Folin-Ciocalteu reagent as described in the study of Lowry et al. [18]. In brief, proteins in tissues were reacted with Cu^{2+} ions in alkaline medium (2% Na_2CO_3 in 0.1 N NaOH) and reduced by the Folin-Ciocalteu reagent. The absorbance of the blue-colored product whose color intensity is proportional to the amount of protein in the sample was measured using a spectrophotometer at 500 nm. Bovine serum albumin was used to obtain calibration curve. Thereafter, the amount of total protein in both tissues were calculated as 100% mg protein per ml of tissue.

Statistical analysis

The GraphPad Prism 6.0 (GraphPad Software, San Diego, California, USA) instant statistical program was used to evaluate all biochemical data, which were expressed as means ± standard deviation (SD). The mean values were subjected to an unpaired *t-test* and "One-Way Analysis of Variance" (One-Way ANOVA) and post-hoc Tukey's multiple comparison tests were used to interpret the differences. The limit of statistical significance was taken as $p < 0.05$ which was regarded as significant. The principal component analysis (PCA) method was used to show the biochemical parameters studied in liver and pancreas tissues. PCA was performed via OriginPro 2022b (v9.95) (OriginLab Data Analysis and Graphing Software, Northampton, Massachusetts, USA).

Results

Body weights and fasting blood glucose levels

Body weights and fasting blood glucose levels stated in our study were reported in the publication examining the effects of Mel on the brains of diabetic rats [19]. Body weights remarkably elevated in control (4.48%, $p < 0.05$) and insulin plus Mel treated diabetic groups (6.13%, $p < 0.05$) whereas notably diminished in diabetic group (20.55%) at the end of the 12 weeks when compared with the initial body weights ($p < 0.05$). Insulin administration alone meaningfully increased (22.41%, $p < 0.05$) body weights in comparison with diabetic group at day 84. When compared with diabetic group, Mel administration alone (25%, $p < 0.05$) or plus insulin (33.58%, $p < 0.05$) also sharply increased the body weights (Fig. 1a). Blood glucose levels significantly increased in diabetic group (341,49% for day 2 and 410.11% for day 84) compared with control group and decreased in insulin (65.75%), Mel (46.03%), and insulin + Mel (53.42%) administered groups compared with diabetic group at the end of 84 days ($p < 0.05$) (Fig. 1b).

Effects of Mel on liver and pancreatic GSH levels

Liver and pancreatic GSH levels are depicted in Fig. 2a and d. Induction of diabetes caused a notable drop in the GSH levels in both liver ($p < 0.0001$) and pancreas ($p < 0.05$) tissues when compared to control group. The administration of insulin to diabetic rats caused a significant increase of GSH levels in liver tissue ($p < 0.0001$), the increase was insignificant in pancreas. Whereas, either Mel or Mel plus insulin administration caused a significant rise in GSH levels in both tissues ($p < 0.001$, $p < 0.05$, and $p < 0.001$, respectively) (Fig. 2a and d).

Effects of Mel on liver and pancreatic LPO levels

Liver and pancreatic LPO levels are given in Fig. 2b and e. MDA content, as a biomarker of LPO, was found to be higher in liver and pancreas tissues ($p < 0.01$) of diabetic group as compared to control group. By contrast, insulin ($p < 0.001$), Mel ($p < 0.05$ and $p < 0.001$), and insulin plus Mel ($p < 0.0001$ and $p < 0.001$) administration to diabetic rats brought about total recovery of MDA levels with respect to diabetic group (Fig. 2b and e).

Effects of Mel on liver PC and pancreatic AOPP levels

Liver PC and pancreatic AOPP levels are presented in Fig. 2c and f. Induction of diabetes resulted in a significant elevation in liver PC ($p < 0.0001$) and pancreatic AOPP levels ($p < 0.01$) as compared with the control group . Conversely, a remarkable reduction in PC and AOPP levels as a consequence of the administration of insulin or Mel and their combination in the diabetic group ($p < 0.0001$) was observed (Fig. 2c and f).

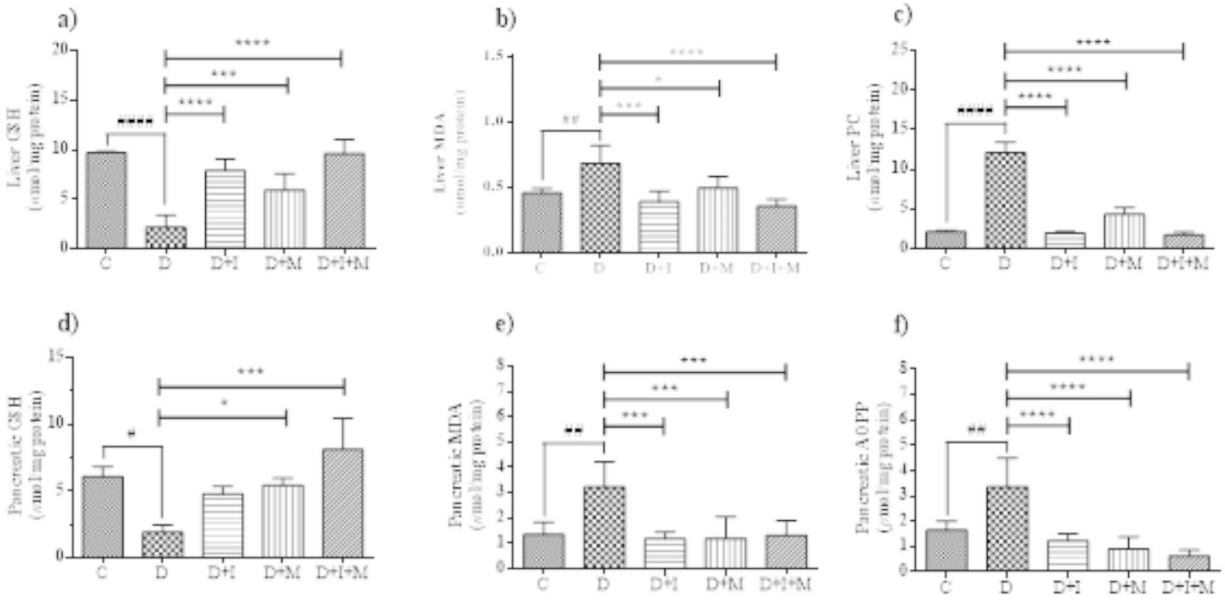

Fig. 2 The effect of melatonin on liver GSH (a), LPO (b), and PC (c) and pancreatic GSH (d), LPO (e), and AOPP (f) levels of all groups of rats. All data are presented as mean ± SD. ####p < 0.0001, ##p < 0.01, #p < 0.05 versus control; ****p < 0.0001, ***p < 0.001, *p < 0.05 versus diabetic

Effects of Mel on liver and pancreatic CAT activities

CAT activities in liver and pancreatic tissues of all groups are shown in Fig. 3a and c. The liver CAT activities were found to be reduced in the diabetic group when compared to the control group ($p < 0.0001$), while a remarkable elevation of CAT activities in pancreas of diabetic rats in comparison with control rats was observed ($p < 0.0001$). A notable increment in liver CAT activities and reduction in pancreatic CAT activities of diabetic rats was seen as a result of treatment with insulin ($p < 0.05$ and $p < 0.0001$) or Mel ($p < 0.0001$) and their combination ($p < 0.0001$) (Fig. 3a and c).

Effects of Mel on liver and pancreatic SOD activities

SOD activities in liver and pancreatic tissues of all groups are depicted in Fig. 3b and d. A pronounced decrease in liver of SOD activities were detected in diabetic rats as compared to control rats ($p < 0.0001$), while a notable increase was observed in pancreatic tissues ($p < 0.001$). Administration of insulin or Mel to the diabetic group or treatment with their combination gave rise to a statistically significant increase of SOD activities in the liver ($p < 0.0001$), and diminished in pancreas ($p < 0.0001$) (Fig. 3b and d).

Effects of Mel on liver and pancreatic GPx activities

GPx activities in liver and pancreatic tissues of all groups are given in Fig. 4a and d. Liver GPx activities of diabetic groups decreased significantly ($p < 0.05$). A remarkable elevation of GPx activities were found in pancreas tissues of diabetic rats with respect to control rats ($p < 0.0001$). Administration of insulin or Mel to the diabetic group or treatment with their combination led to a statistically significant reversal of liver and pancreatic GPx activities ($p < 0.0001$ and $p < 0.001$, respectively) (Fig. 4a and d).

Effects of Mel on liver and pancreatic GR activities

GR activities in liver and pancreatic tissues of all groups are presented in Fig. 4b and e. When compared with control rats, GR activities remarkably diminished ($p < 0.05$) in liver tissue but elevated in pancreas tissues of diabetic group ($p < 0.001$). A remarkable increase of GR activities in liver tissues and decline in GR activities in pancreas tissues of diabetic rats were seen when insulin ($p < 0.0001$ and $p < 0.01$) or Mel ($p < 0.01$ and $p < 0.001$) and insulin plus Mel ($p < 0.0001$ and $p < 0.05$) were administered to diabetic rats (Fig. 4b and e).

Effects of Mel on liver and pancreatic GST activities

Liver and pancreatic GST activities of all groups of rats are shown in Fig. 4c and f. As compared to control rats, GST activities declined significantly ($p < 0.0001$) in liver but increased ($p < 0.01$) in pancreatic tissues of diabetic rats. The administration of insulin or Mel to the diabetic group or treatment with their combination resulted in a statistically

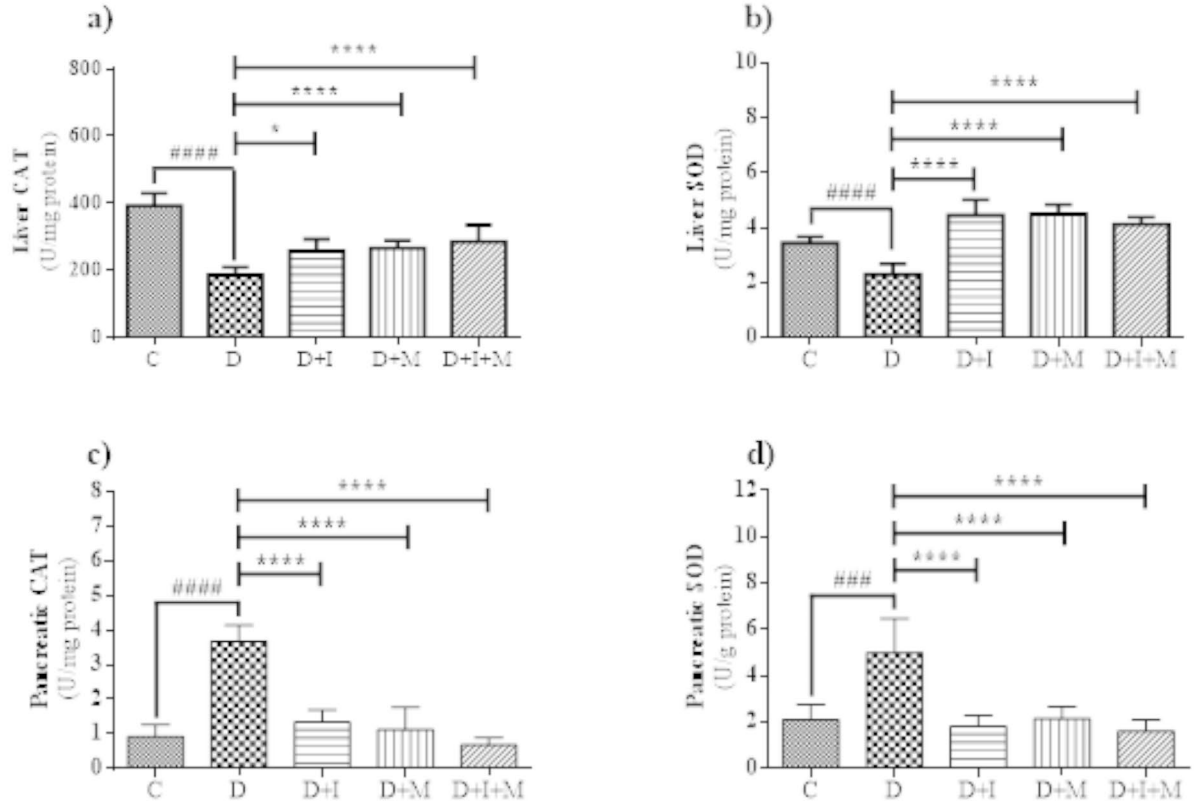

Fig. 3 The effect of melatonin on liver CAT (a), SOD (b) and pancreatic CAT (c) and SOD (d) activities of all groups of rats. All data are presented as mean ± SD. ####p < 0.0001, ###p < 0.001 versus control; *p < 0.05, ****p < 0.0001, ***p < 0.001 versus diabetic

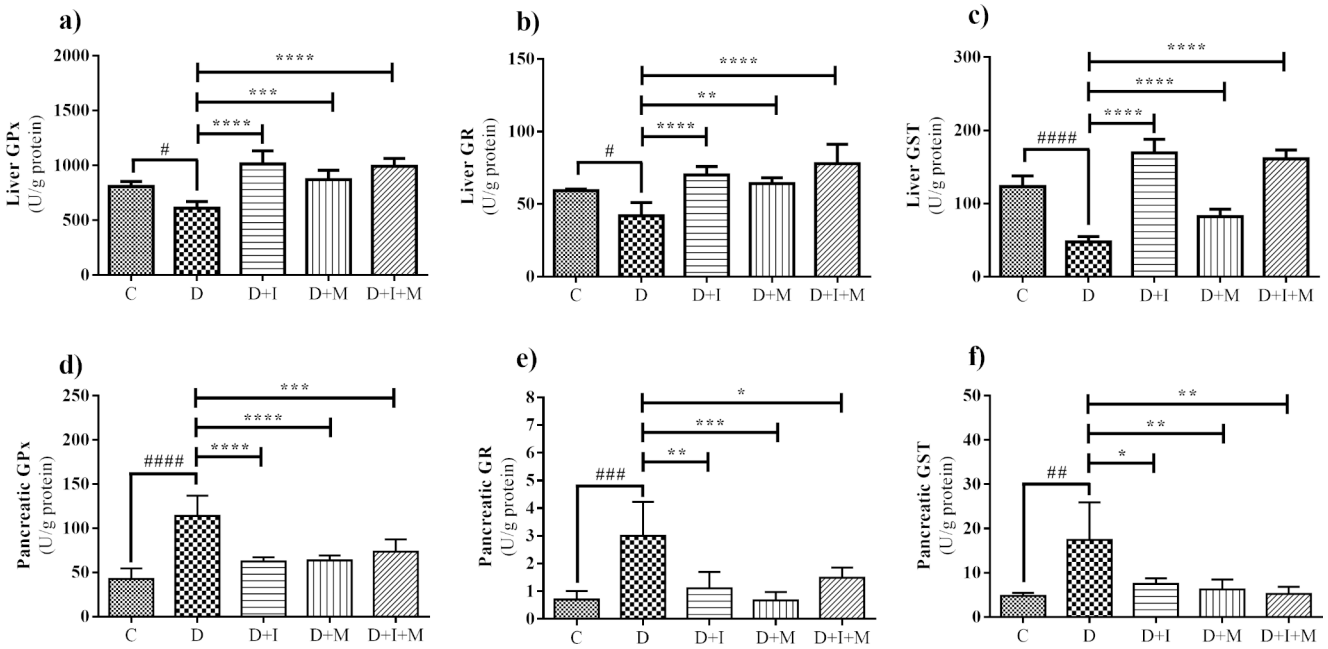

Fig. 4 The effect of melatonin on liver GPx (a), GR (b), and GST (c) and pancreatic GPx (d), GR (e), and GST (f) activities of all groups of rats. All data are presented as mean ± SD. #p < 0.05, ####p < 0.0001, ###p < 0.001, ##p < 0.01 versus control; ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05 versus diabetic

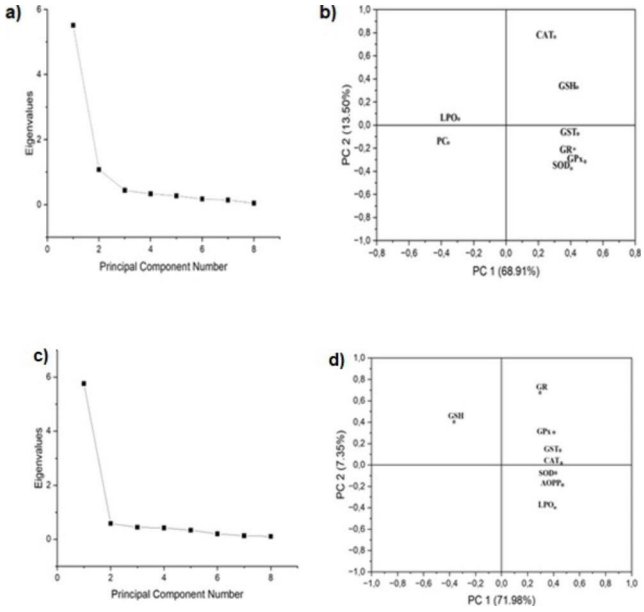

Fig. 5 Principal component analysis of liver biochemical parameters plot showing (a). All biochemical results of liver tissues indicator plotted as a function of two first components, explaining together 82.41% of information in the obtained dataset (b). Principal component analysis of pancreas biochemical parameters plot showing (c). All biochemical results of liver tissues indicator plotted as a function of two first components, explaining together 79.33% of information in the obtained dataset (d)

significant reversal of liver ($p < 0.0001$) and pancreatic GST activities ($p < 0.05$ and $p < 0.01$), respectively (Fig. 4c and f).

Principal component analysis for liver and pancreatic tissues of all groups

PCA is a method that aids in the interpretation of data and simplifies the complexity of high-dimensional data. It does this by converting the data into fewer dimensions that act like a summary of the properties. This method combines highly correlated variables to create a smaller set of artificial variables called "principal components" that generate the most variation in the obtained data. That is why analysts often use PCA as a tool for data analysis and for building predictive models. Each principal component is a linear combination of the variables that went into it and principal component 1 (PC1) is the one that extracts the maximum variance, principal component 2 (PC2) is the one that extracts the maximum variance from what is left [20].

PCA of liver and pancreatic tissues are depicted in Fig. 5a, b, c and d, respectively. The PCA method was used to prove and show the relationship between the results of the biochemical parameters studied in liver and pancreas tissues. PCA analysis showed that the first two components detailed around 82.41% of the total variation in the experimental data in liver (PC1: 68.91%, PC2: 13.50%). CAT, GSH, GST, GR, GPx, and SOD data clustered together in the first component. These clusters were importantly negatively correlated with LPO and PC (Fig. 5a and b). Also, in pancreatic tissues, the first two components detailed around 79.33% of the total variation in the experimental data in liver (PC1: 71.98%, PC2: 7.35%). In the first component, GSH, CAT, SOD, GPx, GR, and GST data clustered together in liver tissues. These clusters were considerably negatively correlated with LPO and PC (Fig. 5c and d).

Discussion

DM is a multifactorial, chronic, progressive disorder that triggers the formation of ROS, causes OS in all tissues, and can lead to diabetic complications [3].

Mel has strong endogenous antioxidant capacity to neutralize extravagant levels of ROS owing to its ability to donate electron or hydrogen atom. Additionally, Mel protects pancreatic β-cells having low antioxidant potential as compared to liver against noxious effects of ROS. Also, Mel (or its co-administration with insulin) has been revealed to alter the activities of antioxidant enzymes in diabetic animals. Although this alteration mostly indicates a decrease in activities of these enzymes that are often increased in diabetes [21, 22]. Contrary to these, other studies show opposing results [5, 23].

In the current study, the biochemical effects of 10 mg/kg of Mel administration on liver and pancreatic tissues of normal and STZ-induced diabetic Wistar rats were investigated. Due to the high levels of ROS resulting from chronic complications of diabetes, all cells are vulnerable to detrimental effects of ROS [1]. Intracellular GSH (its reduced form) neutralizes diverse reactive substances (e.g., ROS, lipid hydroperoxides, and heavy metals), thereby maintaining pro-oxidant/antioxidant balance through regulation of thiol/disulfide formation in the cell [24]. An imbalance between production and elimination of ROS owing to diabetic complications can negatively affect cellular GSH levels [25]. In the current study, GSH levels in both tissues of diabetic rats were statistically lower than those of control rats. The decrease might have occurred due to depletion of GSH reserves as a result of OS. The findings are in line with the reports of Albazal et al. [26] and Gilani et al. [27]. Accumulating evidence reveals that depletion of the intracellular GSH pool is associated with high levels of OS resulting from uncontrolled ROS generation [28]. On the other hand, decreased GSH levels in diabetic rats were reversed upon treatment of the experimental animals with insulin and/or Mel or their combination. When administered alone, the restoring effect of Mel on GSH levels in pancreatic tissue was stronger than that of insulin as opposed to liver. These

outcomes indicate that Mel could restore GSH levels in both tissues of diabetic rats. This effect may be attributed to not only Mel's powerful radical mopping action, but also its stimulatory effects on GSH restoration [21]. Similar outcomes were reported by John et al. [28] and Winiarska et al. [29].

The high levels of MDA (one of the end-products of LPO) is considered an indicator of oxidative damage [30]. Under hyperglycemic conditions, elevated MDA levels disrupt cellular membrane, thus initiating LPO processes that brings about the formation of reactive aldehydes (e.g., MDA) [31]. Similar to previous findings by Turkyilmaz et al. [32] and which demonstrated that a declined GSH contents were accompanied by a rise in the levels of LPO, diabetic conditions in the current study led to elevation of LPO levels. The elevated LPO levels along with declined GSH contents in the diabetic group revealed that oxidative damage in both liver and pancreatic cells may play an important role in consequences of the prolonged diabetic condition. However, administration of insulin, Mel, or their combination to the diabetic groups reverted these alterations. The radical mopping effect of insulin, Mel or their combination were found to confer almost same protective effect against LPO chain reaction in both tissues. Mel effect on lowering LPO levels may be related to its ease of crossing cellular barriers due to its lipophilic structure and protecting cells against oxidative damage by reducing ROS accumulation [33]. Thus, Mel alone or in combination with insulin may have been effective in eliminating increased OS scenario as a consequence of this effect. The present outcomes are in accordance with previous research that demonstrated that Mel mops up ROS due to electron rich indole ring structure [22].

Protein carbonylation is a post-translational modification process that alters protein structure. This can involve attaching carbonyl groups to proteins and in addition, metal-catalyzed oxidation can directly lead to the carbonylation of certain amino acid side chains. Modification of amino acids occurs when free radicals such as chlorinated oxidants attack proteins, thus forming cross-linked protein products containing mono- or dichlorotyrosine known as AOPPs. Therefore, PC and AOPPs are accepted as promising biomarkers of OS-mediated protein damage [34]. While AOPP was preferred in the experimental procedure to determine protein oxidation in pancreatic tissues because less tissue is required, PC was used to assess oxidative protein damage in liver tissue. PC levels in diabetic rats have been reported to be meaningfully raised when compared with that of both control groups and diabetic + Mel (10 mg/kg/day) groups [35]. It has been proven that AOPP usually occurs due to prolonged OS conditions, and also distorted myeloperoxidase activities [36]. Also, AOPP levels have been shown to increase in both serum of diabetic patients [37, 38] and several tissues of diabetic animals [39, 40]. In the current study, increased PC levels in liver, and elevated AOPP levels in pancreatic tissues of diabetic rats were detected. This is an indication that structural changes (generation of carbonylated, cross-linked proteins) in liver and pancreatic cells have occurred due to augmentation in chlorinated oxidants. A correlation between the duration of diabetes and high AOPP levels was observed which was similar to the previous report of Heidari et al. [37]. The present findings were in harmony with the results of Turkyilmaz et al. [41]. On the other hand, Mel, insulin, or Mel + insulin administration brought about a significant diminishment of both PC and AOPP levels. The PC diminishing ability of Mel was less effective in the liver, but its AOPP lowering effect was more potent than that of insulin. The present results were consistent with findings of Djordjevic et al. [42].

DM induces alterations in the activities of primary enzymes (i.e., SOD, CAT, and GPx, GR, and GST) that directly neutralize ROS [43]. Several reports indicate that Mel prevents OS via its antioxidant potential in diverse experimental animal models including diabetes [19, 21, 27]. More so, administration of Mel (10 mg/kg) to experimental rats have been reported to be effective in the restoration of the enzymatic antioxidant activities [44, 45]. Thus, decrease or increase in their activities may be accepted as useful tools for OS-mediated diseases.

SOD is a robust and first antioxidant defense enzyme that converts superoxide anion ($\bullet O^{2-}$) to H_2O_2. However, expression or activity of SOD may be altered (decrease or increase) in DM due to higher OS levels [46]. The current study demonstrated that induction of diabetes was accompanied by a notable decline in SOD activity in liver but a remarkable rise in pancreas. This finding might be another indicator of diabetes-mediated OS. It has also been proposed that the accumulation of PC and AOPPs triggers β-cell destruction and gives rise to NADPH oxidase-dependent $\bullet O^{2-}$ generation, which may cause elevation of SOD activity in diabetic rats [47]. The present outcomes were in harmony with previously published reports indicating hyperglycemia led to a decrease or an increase in SOD activities on a tissue-by-tissue basis [21, 48–50]. Mel, insulin or Mel + insulin administration to diabetic rats led to mitigation of altered SOD activity. However, when combined with insulin, these alterations were more pronounced than the effect of Mel alone. This may be owing to the robust radical mopping effect of Mel.

CAT, predominantly found in peroxisomes of mammalian cells, catalyzes the disproportionation of H_2O_2 into H_2O and gaseous O_2 [51]. The present outcomes revealed that diabetic conditions gave rise to a decrease in CAT activities in the liver, whereas a rise in its activity in pancreatic tissues of rats was observed. Furthermore, CAT overexpression

seen in pancreatic β-cells after STZ injection has been associated with protection against the diabetogenic effect of STZ [52]. The present outcomes revealed that diabetic condition resulted in an elevation of CAT activities. This may be related to the increased SOD activity concomitantly with the diabetes-induced increase in $\bullet O^{2-}$; which could explain the overproduction of H_2O_2 and thus the increase in CAT activity. This was in accordance with the previous reports indicating overproduction of ROS in DM [32, 53]. A remarkable recovery of the activities of CAT was observed when Mel, insulin or their combination were administered to diabetic rats. The possible reason for changes in CAT activities either in the liver or pancreatic tissues after Mel treatment may be associated with the stimulatory effect of Mel on the regulation and expression of this enzyme. In addition to this, Mel has been proposed to have regulatory effects on catalytic subunits of antioxidant enzymes, hence reducing SOD activity in a dose-dependent manner to Mel [54]. Therefore, this study demonstrated that CAT activity decreased through the antioxidant properties of Mel, which contributed to the restoration of oxidative balance in the cell. The results obtained are consistent with findings of Aksoy et al. [5] who reported that 10 mg/kg Mel administration to diabetic rats (6 weeks after STZ injection) increased SOD and GPx activities in liver, heart, and kidney tissues. In DM-induced nephropathy, Mel has also been shown to alleviate oxidative damage by increasing GSH levels and decreasing CAT and SOD activities [23].

GPx plays crucial role in the detoxification of lipid peroxides to their corresponding alcohols, and H_2O_2 to water as well. When GPx catalyzes the decomposition of H_2O_2, GSH is converted into its oxidized form, which in turn is reduced back to GSH by GR [55]. GST is responsible for the conjugation of GSH to hydrophobic toxic compounds. It utilizes GSH in detoxifying organic hydroperoxides and other electrophiles derived as by-products from LPO [56]. Any alteration in the levels of these enzymes makes cells more susceptible to OS/damage. This is because of their intrinsically low expression in pancreatic β-cells [57].

In present study, GPx, GR, and GST activities were remarkably decreased in liver tissues of diabetic rats. This is in harmony with the results of Rai et al. [50]. On the other hand, several research groups revealed that diabetes tends to decrease the activities of antioxidant enzymes because of increased LPO levels [58, 59]. Contrarily, elevated activities of these enzymes were observed upon induction of DM with STZ injection to rats. The results were in line with the findings of Bandeira et al. [60] and Ozsoy et al. [61]. Similarly, Winiarska et al. [29] reported that DM-induced OS led to an increase in both GPx and GR activities in liver tissues but decreased in the kidney tissues of rabbits. These controversial outcomes may be owing to persistent diabetic conditions (observed in the current study with a duration of 12 weeks) or probably an adaptive response to overproduction of ROS. Hence, may possibly reduce or elevate the activities of these enzymes to neutralize high levels of ROS [60]. In the current study, treatment of diabetic rats with insulin, Mel, or their combination led to normal levels of antioxidant enzyme activities. Restoration of these activities after administration of insulin, Mel, or their combination may be associated with restored low levels of glucose by insulin, and direct potential to neutralize ROS, as well as the ability to stimulate antioxidant enzymes by Mel [62]. It is also possible that Mel exerted its protective effect by maintaining GSH levels (as substrate for GSH-dependent enzymes) and/or by its direct antioxidant properties.

There are studies showing that melatonin has a protective effect on tissues in the liver and pancreas inflammation and also in diabetes. It can be considered as an alternative treatment method for diabetic people, especially because of the curative effect of melatonin on pancreatic β-cells [63], its successful results in animal experiments, the fact that melatonin levels in T1D are reported to be significantly lower compared to the control group [64], and it has protective properties against oxidative stress [65]. In addition, although the short-term effect of acute melatonin treatment reduces insulin secretion, it is known to reduce HbA1c levels without affecting glucose and lipid metabolism in T2D models with long-term use [66]. Considering all these factors, it is thought that the clinical application of melatonin may reflect a new perspective in clinical research on diabetes and may have a beneficial option as a therapeutic agent since it has minimal toxicity and a wide dose range.

Conclusion

Consequently, STZ-induced diabetes (duration of 12 weeks) brought about OS that triggered excessive ROS formation. This was observed not only by depletion of GSH levels but also elevation of LPO, PC (in liver), and AOPP (in pancreas) levels. Additionally, activities of antioxidant enzymes were dropped in the liver but rose in pancreatic tissues of diabetic rats. On the other hand, administration of Mel, insulin, or combination of both led to a decline in LPO, PC, and AOPP levels, along with an increase in GSH levels. These effects may be attributed to Mel's robust radical mopping potential. More so, it was observed that there is a difference in the activities of antioxidant enzymes in liver and pancreatic tissues of diabetic rats. The effects of Mel on all assessed enzymatic parameters were generally more effective than that of insulin. Whereas, combined administration of Mel and insulin was more effective than single treatments. Altogether,

Mel showed a protective action by reducing OS via a robust antioxidant effect as observed in this study.

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Declarations

Conflict of interest The authors declare no conflict of interests.

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