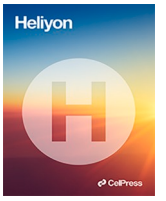
Heliyon 10 (2024) e28639

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Melatonin increases AKT and SOD gene and protein expressions in diabetic rats

Mohamed Lotfy [a,*], Aalaa Khattab [b], Mohammed Shata [a], Ahmad Alhasbani [a], Abdallah Khalaf [a], Saeed Alsaeedi [a], Mahdi Thaker [a], Hazza Said [a], Harun Tumi [a], Hassan Alzahmi [a], Omar Alblooshi [a], Mohamad Hamdan [a], Amjad Hussein [a], Biduth Kundu [a], Ernest A. Adeghate [c,d,**]

[a] *Biology Department, College of Science, United Arab Emirates University, Al Ain, United Arab Emirates*
[b] *Faculty of Dentistry, The British University in Egypt, El Sherouk City, Cairo, Egypt*
[c] *Department of Anatomy, College of Medicine & Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates*
[d] *Zayed Centre for Health Sciences, College of Science, United Arab Emirates University, Al Ain, United Arab Emirates*

ARTICLE INFO

Keywords:
Diabetes mellitus
Melatonin
Insulin signaling
SOD and PI3K/AKT

ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disease marked by hyperglycemia due to insulin deficiency or insulin resistance leading to many chronic complications. It is thus important to manage diabetes effectively in order to prevent and or delay these complications. Melatonin is produced by the pineal gland and regulates the wake-sleep circadian rhythm. Existing evidence suggests that melatonin may be effective in the management of DM. However, the evidence on the mechanism of the beneficial effect melatonin as a treatment for DM is limited. In this study, we investigated the effect of melatonin treatment on blood glucose, insulin (INS), AKT and superoxide dismutase (SOD) gene levels in diabetic rats. Non-diabetic and diabetic rats were treated orally for 4 weeks with either 25 mg or 50 mg/kg body weight of melatonin. At the end of the study, pancreatic and liver tissues morphology, glucose homeostasis, serum insulin and SOD levels, hepatic gene and protein expression of SOD as protecting antioxidant enzyme and AKT as central element involved in PI3K/AKT insulin signaling pathway were estimated. Melatonin treated diabetic rats showed reduced hyperglycemia, and increased serum insulin and SOD levels. In addition, melatonin induced an increased gene and protein expression of SOD and AKT. In conclusion, melatonin may play a role in treating diabetic rats via stimulation of insulin secretion, insulin signaling and reduction in oxidative stress.

1. Introduction

Diabetes mellitus (DM) is a chronic endocrine and metabolic disease affecting millions of people worldwide. DM is a leading global health problem, with prevalence of 537 million in 2021, and expected to reach 592 million by 2035, globally as reported by the International Diabetes Federation (IDF) [1].

* Corresponding author. Biology Department, College of Science, United Arab Emirates University, Al Ain, United Arab Emirates.
** Corresponding author. Department of Anatomy, College of Medicine & Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates.
E-mail addresses: m.lotfy@uaeu.ac.ae (M. Lotfy), eadeghate@uaeu.ac.ae (E.A. Adeghate).

https://doi.org/10.1016/j.heliyon.2024.e28639
Received 9 November 2023; Received in revised form 19 March 2024; Accepted 21 March 2024
Available online 24 March 2024

DM is a chronic metabolic disease characterized by hyperglycemia due to a lack of insulin secretion or insulin resistance. There are three types of DM: Type 1, Type 2, and Gestational Diabetes. Type 1 diabetes is an autoimmune condition where the immune system abolishes insulin secreting pancreatic beta cells. Type 2 diabetes is a condition where the body is resistant to insulin even though it has enough insulin. Gestational diabetes occurs during pregnancy and typically resolves after delivery [2].

DM is a multifactorial disease involving both genetic and environmental factors, but the main initial abnormality is hyperglycemia. DM-induced hyperglycemia leads to chronic complications such as cardiovascular disease, nephropathy with end stage of kidney failure, neuropathy causing nerve damage, retinopathy that could lead to blindness if not managed properly, foot problems, that may lead to amputation, skin infections, gastroparesis with vomiting, gingivitis causing tooth loss sexual erection dysfunction, hypertension and bone osteopenia [3]. These complications of DM occur because of chronic hyperglycemia. Hyperglycemia causes overproduction of reactive oxygen species (ROS) which results in oxidative stress. ROS damages cells but the body's protective antioxidant system, in normal condition, neutralizes ROS and other free radicals, thereby preventing oxidative stress [4].

Melatonin (5-methoxy-*N*-acetyltryptamine) is released by the pineal gland. Melatonin regulates the sleep-wake cycle along with other biological rhythms in the body. In humans melatonin production is typically highest at night and lowest during the day, which helps to promote restful sleep. In addition to its role in sleep regulation, melatonin has also been studied for its potential benefits in other areas, such as reducing jet lag, improving mood, and supporting the immune system and the body's internal clock [5].

Moreover, there is some evidence to suggest that melatonin may have a beneficial effect on blood glucose control in people with DM. Research studies have shown that melatonin can help regulate insulin secretion and sensitivity, which are key factors in maintaining a healthy blood glucose levels. In particular, melatonin has been found to increase insulin sensitivity in the liver and muscle tissue, which can help to lower blood sugar levels. In addition to its potential effects on blood glucose, melatonin may also have other beneficial effects for people with DM. For example, melatonin reduces oxidative stress, inflammation, protecting DNA from oxidative damage and other diabetic complications associated with DM [6]. In another experiment, melatonin supplementation significantly reduced DM-induced sialic acid and thiobarbituric acid-reactive substances (markers of lipid peroxidation) and was able to restore euglycemia [7]. The administration of melatonin inhibited Syk/COX-1/SERCA signaling pathways that is involved in unfolded protein response-mediated myocardial cell death in DM [8]. Endoplasmic reticulum stress is one of the causes on insulin resistance [9]. It has also been reported that melatonin, when given at a dose of 10 mg/kg body weight for one month significantly reduced the severity of diabetic complications by augmenting the AMPK/SIRT1 pathways to improve mitochondrial health [10]. However, additional studies is required to fully appreciate the potential benefits and mechanisms of action of melatonin in the treatment of people with DM.

Oxidative stress occurs when there is inequity of ROS formation and the capability of the body to get ride and repair the damage caused by them. Oxidative stress has been implicated in the development of metabolic diseases, including DM. Antioxidants are substances that help to neutralize ROS and prevent oxidative damage. One of the antioxidant enzymes involved in this process is superoxide dismutase (SOD), which converts superoxide radicals into hydrogen peroxide and oxygen, thereby reducing the amount of ROS in the body [11]. The chronic hyperglycemia associated with diabetes causes non-enzymic binding of sugars to protein and lipid molecules to form advanced glycated end products (AGEs). AGEs, which are harmful compounds, in turn induce the generation of intracellular oxidative stress, which prompt the activation of a cascade of events that will lead to diabetes complications [3]. In addition, oxidative stress can impair insulin signaling and contribute to insulin resistance, further exacerbating the development of DM. Studies have shown that SOD activity is decreased in the livers of individuals with DM, suggesting that a reduced antioxidant activity may play a role in the pathogenesis of DM [12]. An increased level of SOD reduces oxidative stress in animal models of DM and subsequently improves insulin sensitivity. Overall, increased oxidative stress contributes to the development of metabolic diseases, including DM, an antioxidant such as SOD may be useful in preventing or treating these conditions [13].

Protein kinase B (AKT) is involved in many metabolic activities of cells such as cell propagation and durability. AKT activation is induced via downstream of phosphoinositide-3-kinase (PI3K) pathway. The PI3K/AKT signaling pathway plays an important role in regulating glucose homeostasis and insulin signaling in target organs such as the liver. Abnormalities in this pathway have been implicated in the development of DM. Insulin stimulates the activation of PI3K, which then activates AKT, leading to the translocation of glucose transporter type 4 (GLUT4) to the cell membrane, resulting in increased glucose uptake by the liver cells. AKT also activates glycogen synthase, which converts glucose into glycogen for storage in the liver [14]. Moreover, AKT inhibits gluconeogenesis, which is the process by which the liver produces glucose from non-carbohydrate sources. In DM, the PI3K/AKT signaling cascade is disrupted, leading to impaired insulin signaling and decreased glucose uptake by liver cells. This results in increased blood glucose levels and the development of hyperglycemia [15,16]. In addition, an irregular stimulation of the PI3K/AKT cascade leads to increased lipogenesis and fat deposition in hepatic tissues, contributing to the development of non-alcoholic fatty liver disease and other metabolic disorders. Overall, the PI3K/AKT signaling pathway plays a crucial role in the regulation of glucose and lipid metabolism in the liver, and abnormalities in this pathway can contribute to the development of DM and other metabolic disorders [17].

It is important for people with DM to maintain euglycemia and work closely with their healthcare team to prevent and manage these complications. Treatment typically involves lifestyle changes, medication, and monitoring blood glucose levels regularly. Many drugs are currently used in treatment of DM; however, the full control of patient's hyperglycemia and other diabetic complications are still not completely achieved. Therefore, the aim of the current study was to evaluate the effect of melatonin on signs of DM in experimental diabetes and to determine whether melatonin has beneficial effect.

2. Materials and methods

2.1. Experimental animals

Male Wistar rats weight approximately 200 g were used for the experiment. The rats were obtained from the Animal House Facility of the College of Medicine and Health Sciences, United Arab Emirates University. All rats were kept in plastic cages at room temperature (25 °C) and controlled humidity with 12 h light and darkness cycle. Food and water were provided *ad libitum* [18]. Ethical clearance was received from the Animal Ethics Committee of the United Arab Emirates University (Process number ERA_2019_5931).

2.2. Induction of diabetes in rats

Diabetes mellitus was induced in rats by a single intraperitoneally (IP) injection of alloxan (Sigma, USA) at a dose of 120 mg/kg dissolved in sterile 0.9% saline [18]. Five days after the injection of alloxan, the blood glucose level of the rats was measured from tail vein. DM was confirmed if the fasting blood glucose level was equal or more than 200 mg/dl. The rats were later divided into six groups of six rats, consisting of normal, non-diabetic controls, melatonin-treated controls and untreated diabetic and melatonin-treated diabetic rats. The rats were treated orally with equal volumes of either saline or melatonin (Sigma, USA) for four weeks as follows:

a) : Control untreated (C) rats ($n = 6$) received 1 ml of sterile 0.9% saline
b) : Control melatonin low-dose treated (CML) rats ($n = 6$) received 25 mg/kg/day
c) : Control melatonin high-dose treated (CMH) rats ($n = 6$) received 50 mg/kg/day
d) : Diabetic untreated (D) rats ($n = 6$) received 1 ml of sterile 0.9% saline
e) : Diabetic melatonin low-dose treated (DML) rats ($n = 6$) received 25 mg/kg/day
f) : Diabetic melatonin high-dose treated (DMH) rats ($n = 6$) received 50 mg/kg/day

The sample size for all groups and all experimental analyses were $n = 6$ rats per group.

The doses and duration of melatonin treatment had previously been shown to improve glucose homeostasis by restoring the vascular action of insulin [19].

At the end of the study, rats were scarified and collect blood to estimate serum insulin and serum superoxide dismutase (SOD) levels. In addition, pancreas and liver fragments were collected for histological and molecular studies.

2.3. Weight of the animals used

The total body weights of the experimental rats were measured at the end of the experiment using a 9001 Sartorius laboratory scale (Hertfordshire, UK).

2.4. Glucose tolerance test

Glucose tolerance test (GTT) was performed on the rats at the end of the four-week study. using an intraperitoneal (i.p.) injection of glucose after overnight fasting for 18 h according to a previously reported method [20]. Briefly, each rat was given an i.p. glucose load of 2 g/kg body weight followed by measurement of blood glucose level from the tail vein with a glucometer (OneTouch Ultra, LifeScan, PA, USA). The fasting blood glucose levels were measure at 0 (before glucose challenge), 30, 60, 120 and 180 min after i.p injection of glucose.

2.5. Biochemical parameters

At the end of the experiment, the animals were anaesthetized with diethyl ether for blood and tissue collection for the estimation of insulin and superoxide dismutase. Insulin concentration in serum was measured using a Merck Millipore ELISA Assay Kit (Darmstadt, Germany), according to the manufacturer's protocol. Serum superoxide dismutase (SOD) activity levels were spectrophotometrically estimated with the indirect assay method based on xanthine oxidase and a color reagent using a Sigma kit (Missouri, USA) as described according to the manufacturer protocol. Briefly, the method is based on the fact that SOD can catalyze the dismutation of the superoxide anion into hydrogen peroxide in tissue samples.

2.6. Tissue histological analysis

Rats were dissected for the collection of liver and pancreatic tissues. These tissue fragments were used for both histological and genes and protein expression estimation. Liver and pancreas tissues were cleaned in phosphate buffered saline, and then directly fixed in formalin solution. Tissue samples were dehydrated with ethanol series formed by xylene clearing and embedded in paraffin wax. Tissues were sectioned with microtome for hematoxylin and eosin staining [21]. Histological images used for analysis were 18 (3 per each rat) per group ($n = 6$).

2.7. Immunofluorescence analysis

Isolated pancreatic tissue fragments were fixed in Zamboni's fixative, embedded in paraffin wax, and processed for immunofluorescence according to a previously reported method [22]. Briefly, rabbit anti-insulin monoclonal antibody (ab181547, Abcam, MA, USA, 1:1000) conjugated to goat anti-rabbit IgG (FITC), (ab6717, Abcam, MA, USA, 1:1000) and mouse anti-glucagon monoclonal IgG antibody (sc-514592, Santa Cruz Biotechnology, TX, USA, 1:1000) conjugated to donkey anti-mouse IgG (TRITC), (ab6817, Abcam, MA, USA, 1:1000) were used to detect insulin and glucagon, respectively in the tissues. Sites of immunoreaction were viewed using Olympus fluorescence microscope (Hamburg, Germany). 18 immunofluorescence images were used for analysis (3 per each rat) per group ($n = 6$).

2.8. Quantitative real time PCR (qRT-PCR)

Rat liver tissues were used for estimation of gene expression levels of the mRNA transcripts related to insulin signal pathway and antioxidant genes via quantitative real-time (qRT-PCR). The total RNA was extracted using trizol, and cDNA was generated with the help of reverse transcriptase with random hexamer. All quantitative PCR reactions were performed on an Applied Biosystems Quant Studio™ 5 Real-Time PCR System, using the SYBR Green-based detection method of Comparative CT (ΔΔCT) method (Applied Biosystems) using specific primers sequences for the target genes. The values of average threshold cycle (Ct) were used to determine the relative differences between control, non-diabetic group and melatonin-treated group. The relative expression of the measured genes was adjusted to the internal control: glyceraldehyde 3-phosphate dehydrogenase for each gene. Relative changes in gene expression were calculated by ΔΔCt (threshold cycle) method. Fold change values were calculated using equation $2^{-\Delta\Delta}$Ct as previously described method [23]. Primer sequences used for the qRT-PCR experiment is given in Table 1.

2.9. Western blot analysis

Rats' liver tissues were homogenized in RIPA lysis buffer (Thermo Fisher Scientific, Illinois, USA) on ice using a tissue homogenizer (Omni International, GA, USA). The homogenates were subjected to centrifugation at 13,000×*g* for 20 min at 4 °C and the supernatants were decanted to measure protein concentrations by protein assay kit using Bradford method according to the manufacturer's procedures (Bio Rad, California, USA). Tissue lysates were mixed with equal amount of 2X loading buffer. Samples were separated on SDS-polyacrylamide gel. The proteins extracts were blotted and transferred to a polyvinylidene difluoride membrane (PVDF) in a transfer buffer at 4 °C for 1 h. The membrane was blocked for 1 h at room temperature with 5% nonfat milk in TBS buffer. The membrane was then incubated overnight at 4 °C with either, AKT Rabbit monoclonal antibody (ab179463, Abcam, MA, USA, 1:10000), or superoxide dismutase (SOD) rabbit recombinant antibody (ab51254, Abcam, MA, USA, 1:50000), or anti-GAPDH Rabbit Recombinant antibody as housekeeping loading control (ab181602, Abcam, MA, USA, 1:10000), followed by incubation for 1 h with horseradish peroxidase (HRP) Goat anti-rabbit IgG secondary antibody (65–6120, Invitrogen, MA, USA, 1:10000) in 1% nonfat milk. The signals were visualized and the images of the blots were obtained using the enhanced chemiluminescence system (32106, Thermo Scientific, MA, USA) with a CCD camera. The relative expression of the proteins with reference to GAPDH, the housekeeping protein was estimated with Image Lab 4.1 software [24].

2.10. Morphometric analysis

The number of pancreatic islet cells, the size of pancreatic islets and enlarged sinusoids of the liver were analyzed using Image J® (NIH, Bethesda, Maryland, USA). The counting was done blindly without a knowledge of the respective groups.

2.11. Statistical analysis

Values obtained were estimated as mean ± standard error of the mean (SEM). Statistical significance were calculated by Students t-test and (ANOVA) using Statistical Package for Social Sciences (SPSS) version 29.0 for Windows (SPSS Inc., Chicago, IL, USA). Significant differences were assessed using Duncan's Multiple Range test. Only values of $p < 0.05$ were considered as significant.

Table 1
Primer sequences used for qRT-PCR.

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
AKT	TGAGACCGACACCAGGTATTTTG	GCTGAGTAGGAGAACTGGGGAAA
SOD	CCGGTGCAGGGCGTC	TCCTGTAATCTGTCCTGACACCA
GAPDH	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA

3. Results

3.1. Effect of melatonin treatment on body weight

Four weeks of melatonin treatment on the body weight showed no significant difference between melatonin treated groups compared to either control or diabetic groups, respectively (Fig. 1).

3.2. Effect of melatonin treatment on intraperitoneal glucose tolerance test (IPGTT) and fasting blood glucose levels

The blood glucose level results of IPGTT showed that there are markedly ($P < 0.05$) reductions in blood glucose levels of normal, non-diabetic control and melatonin-treated diabetic groups compared to untreated non-diabetic control and untreated diabetic groups at 0, 60 and 120 min after glucose challenge (Fig. 2A). The reduction in the level of fasting blood glucose in the control group treated with melatonin (CML group) was significant ($P < 0.05$) when compared to untreated control group. Moreover, a significant ($P < 0.05$) reduction in fasting blood glucose was observed in both groups of diabetic groups treated with melatonin (DML, DHL) when compared to untreated diabetic group D (Fig. 2B). The area under the curve of the plot is depicted in Fig. 2C.

3.3. Effect of melatonin treatment on serum insulin and antioxidant serum superoxide dismutase (SOD) levels

Serum insulin levels in all melatonin treated groups (normal and diabetic) showed increment in comparison to the untreated control and untreated diabetic groups. The increment was most notable ($p < 0.05$) in DMH group of rats compared to that of untreated diabetic group (Fig. 3A).

Moreover, serum SOD level increased significantly after treatment of diabetic rat with high dose of melatonin (Fig. 3B).

3.4. Effect of melatonin treatment on liver and pancreas histopathology

Liver and pancreatic tissue samples were processed for hematoxylin and eosin staining and investigated with Nikon Light microscope.to determine whether there are morphological changes.

In the non-diabetic control group (C), the architecture of the liver including the hepatic lobules was intact with cords of hepatocytes surrounding the central vein. The polygonal shaped cells and the large nuclei are organized sides by side with the neighboring hepatocytes, which are associated with sinusoids. In the untreated diabetic group (D), the sinusoids surrounding the hepatocytes were dilated. Treatment of diabetic rats with melatonin markedly improved the morphology of the liver (Fig. 4A). The number of dilated sinusoids decreased significantly after treatment with melatonin (Fig. 4B).

The structure of pancreatic islets in the control group was intact and appears normal. In the diabetic group, however, the structure and size of the islets of Langerhans increased after melatonin treatment (Fig. 5A). Treatment of normal and diabetic rats with melatonin significantly ($p < 0.05$) increased the size of islets of Langerhans (Fig. 5B).

3.5. Effect of melatonin treatment on the number of insulin- and glucagon-immuno-positive cells in the pancreas

Our results show that melatonin treatment leads to increases in the number of insulin-containing beta cells and slight decrease in the number of glucagon-containing alpha cells in the pancreatic islets of both normal, non-diabetic control and untreated diabetic rats when compared with untreated, non-diabetic control rats and untreated diabetic rats (Fig. 6A, B, C).

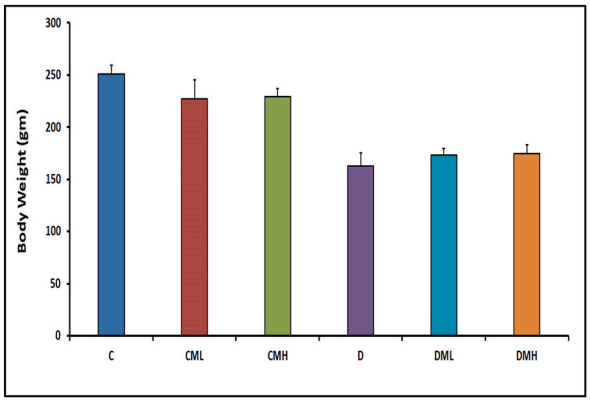


Fig. 1. No marked body weight changes were discerned between melatonin-treated and untreated control groups. Values are mean ± SEM (n = 6).

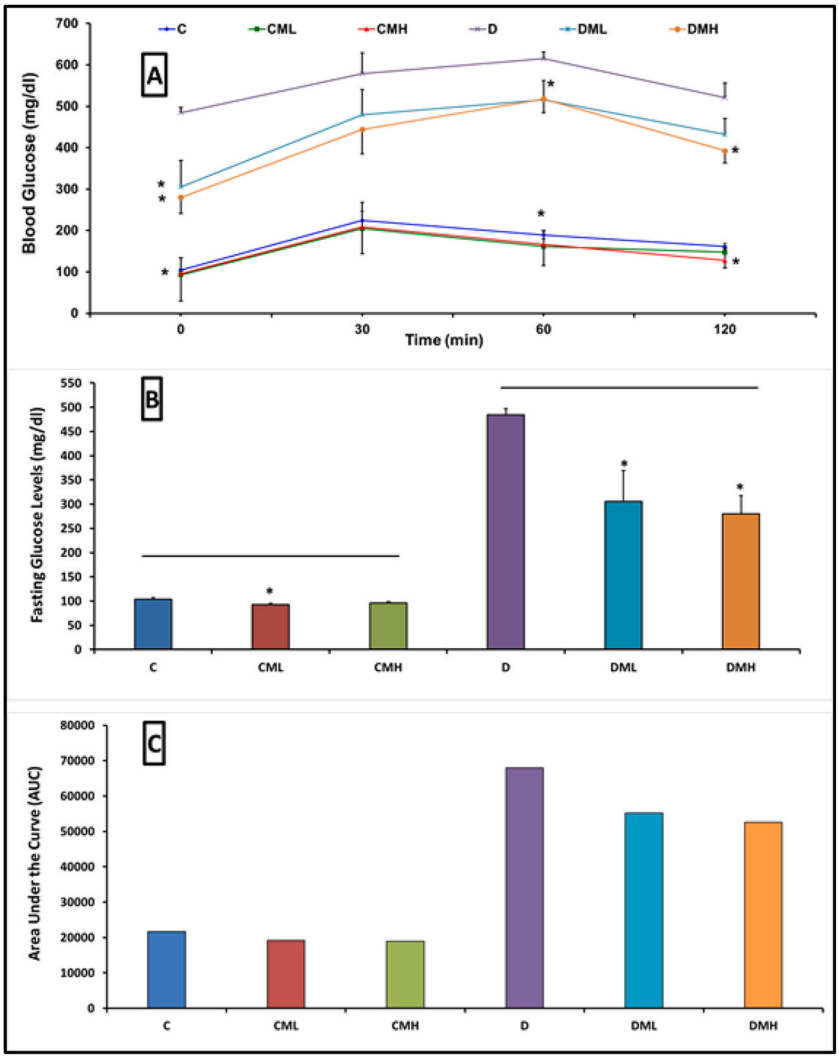


Fig. 2. Shows [A]: Blood glucose values after intraperitoneal glucose tolerance test (IPGTT) performed at the end of the experiment. Values are presented as mean ± SEM. *$P < 0.05$ versus either non-diabetic control group (C), or diabetic (D) group. Note the blood glucose-reduction effect of melatonin in control and diabetic rats. [B]: Fasting glucose levels at the end of the study (120 min after IPGTT glucose challenge). [C]: Areas under the curve for all groups of rats. Values are expressed as mean ± SE, (n = 6). *$P < 0.05$ versus the control group (C) and versus the diabetic (D) groups.

3.6. Effect of melatonin treatment on the gene expression of protein kinase B (AKT) and superoxide dismutase (SOD)

We examined the effect of melatonin treatment of the gene expression of AKT, an important signaling molecule and that of SOD a key antioxidant in the liver. Melatonin treatment, at high doses, caused significant increase ($p < 0.05$) in AKT gene expression in diabetic rats when compared to untreated diabetic rats (Fig. 7A). The gene expression of superoxide dismutase (SOD) increased significant ($p < 0.05$) in DML and DMH groups when compared to untreated diabetic group (Fig. 7B).

3.7. Effect of melatonin treatment on the protein expression of protein kinase B (AKT) and superoxide dismutase (SOD)

In addition to measuring the gene expression of AKT and SOD in the liver of all groups of rat, we also examined the protein expressions of these molecules to determine whether the increase in gene expression is associated with an increase in protein expression. The densitometric analysis results of the western blot showed significant increase ($p < 0.05$) in AKT protein expression of rats treated with low and high dose of melatonin compared to that of untreated diabetic rats. Treatment with melatonin significantly increased ($p < 0.05$) SOD protein expression in diabetic compared to untreated diabetic group (Fig. 8A, B, C, Supplement Fig, 1).

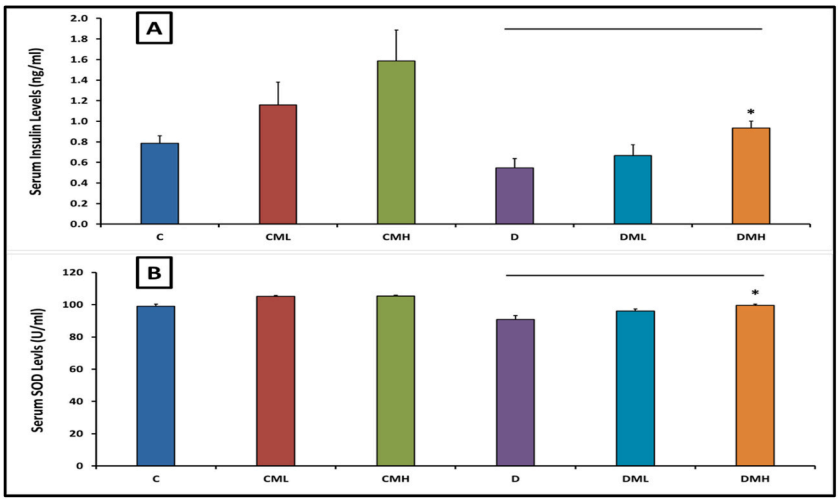


Fig. 3. [A]: Serum insulin levels of rats at the end of the four-week experiment. Values are presented as mean ± SEM. *$P < 0.05$ versus the control group (C) and versus the diabetic (D) groups. ($n = 6$). [B]: Serum superoxide dismutase (SOD) levels at the end of the study. Values are expressed as mean ± SEM. *$P < 0.05$ versus either non-diabetic control group (C) or untreated diabetic (D) groups. ($n = 6$).

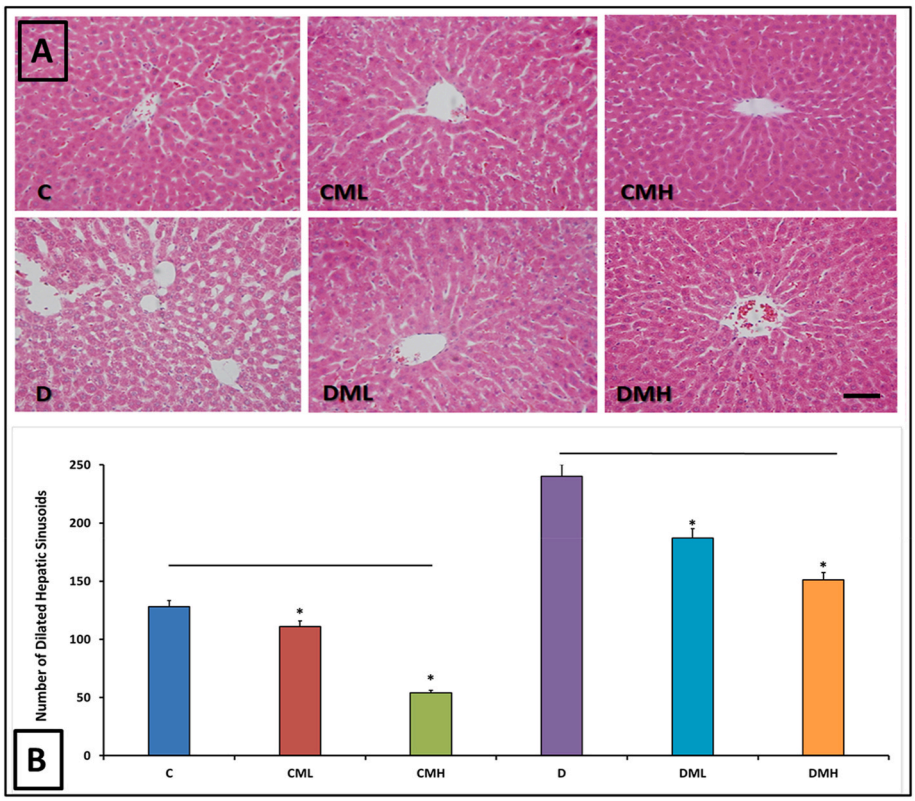


Fig. 4. [A] Histological study of liver sections from different rat groups. Section from (C) control untreated control group showing normal liver architecture of hepatic cords and central vein with normal size of relatively narrow spaces of hepatic sinusoids. In CML and CMH sections of normal control melatonin treated groups showing also normal liver architecture with normal spaces of hepatic sinusoids. In section (D) from untreated diabetic group showing general deteriorative histological liver changes with much dilated sizes of hepatic sinusoids. In DML and DMH sections from diabetic group treated with melatonin showing near normal liver architecture restoring the normal size of hepatic sinusoids. The number of dilated sinusoids pe field was significantly reduced after treatment with melatonin [B]. Scale bar = 2 μm; C = non-diabetic control rats, CML = control rats treated with low dose of melatonin, CMH = control rats treated with high dose of melatonin, D = diabetic control, DML = diabetic rats treated with low dose of melatonin, DMH = diabetic rats treated with high dose of melatonin. Values are mean ± SEM ($n = 6$). *p < 0.05.

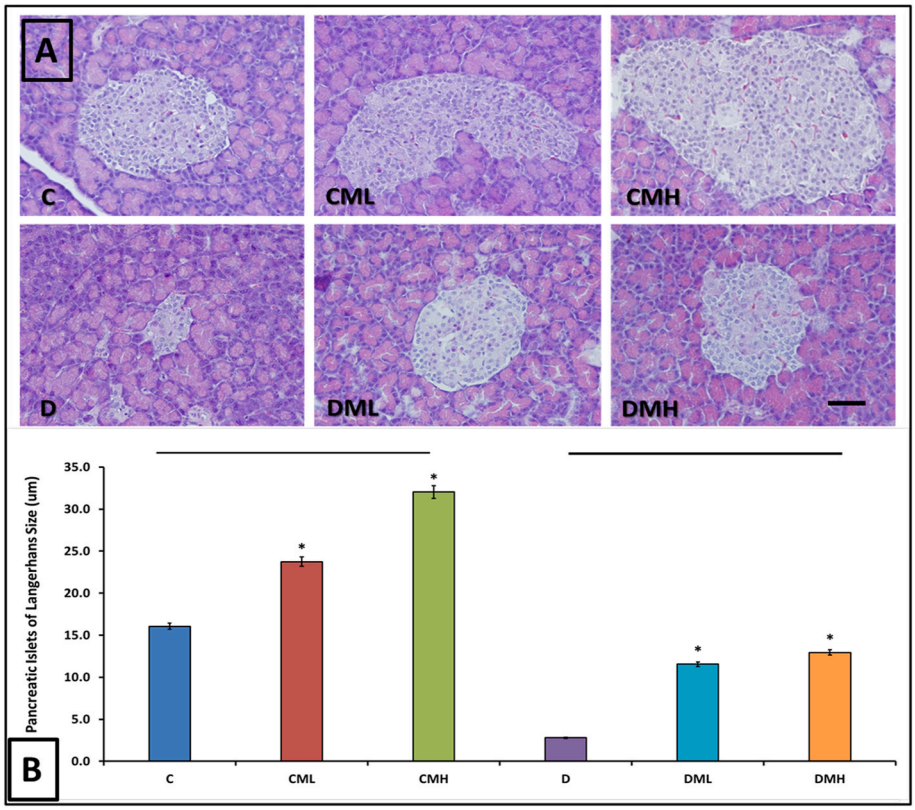


Fig. 5. [A]: Histological (H & E stain) study of pancreas from different rat groups. Section from untreated normal control group (C) shows normal architecture of islet of Langerhans. Sections from melatonin treated normal control groups (CML and CMH) also revealed normal pancreases architecture with slightly bigger size of islets of Langerhans. Section from untreated diabetic group (D), shows a significantly smaller islet of Langerhans compared to those from melatonin-treated rats [B]. Sections from diabetic groups treated with melatonin (DML and DMH) shows a near normal islet size and architecture. Scale bar = 2 μm. C = non-diabetic control rats, CML = control rats treated with low dose of melatonin, CMH = control rats treated with high dose of melatonin, D = diabetic control, DML = diabetic rats treated with low dose of melatonin, DMH = diabetic rats treated with high dose of melatonin. Values are mean ± SEM ($n = 6$). *p < 0.05.

4. Discussion

The prevalence of DM continues to be high. DM currently affects about 1 out of 10 people globally and it considered as the third most common disease affecting human being worldwide [25]. Therefore, it is essential to increase our knowledge about DM, its progression and to find out more effective and prominent treatment for a complete cure.

In the current study, we examine the effects of melatonin on the metabolic and gene expression of key molecules in experimental diabetes mellitus. Our study shows that the body weight was not significantly altered after treatment of normal and diabetic rats with melatonin. This observation is in contrast to those of previous studies [26,27] but in accordance to recent reports [28–30]. The differences between observed may be due to duration of study, doses used and the type of animal used in the study. Our study however confirmed that melatonin had no effect on body weight in all melatonin treated groups.

A previous study reported that diabetic rats have a low blood melatonin level [31], and because of that it was recommended that it should be included in antidiabetic blood glucose lowering therapies [32]. Indeed, insulin resistance established in diabetic obese mice correlated with a reduced melatonin synthesis and melatonin administration promoted the glucose homeostasis [33]. A similar low melatonin levels was detected in diabetic patients and melatonin administration improved the glucose tolerance test [34,35] and decreased the glycated hemoglobin percentage [36]. Moreover, melatonin treatment for obese persons enhanced insulin sensitivity [37]. Similarly, in a recent report, melatonin improved insulin sensitivity and reduced fasting blood glucose [38]. In yet another study, melatonin treatment in diabetic rats increased insulin level and improved glucose homeostasis [39]. Diabetic complications are closely related to over production of free radicals and appearance of oxidative stress condition [40], which is accompanied with insulin resistance and a decline in antioxidant enzymes such as SOD, catalase, glutathione peroxidase that cause cells and tissues injuries [41,42]. Moreover, melatonin treatment in diabetic rats, increased the antioxidant enzyme such as SOD and decreased free radicals and oxidative stress [43].

All of the previous studies were in accordance with our study, where melatonin treatment improved glucose handling and lowers blood glucose levels in both non-diabetic normal control and untreated diabetic groups as recorded in IPGTT. Melatonin also reduced

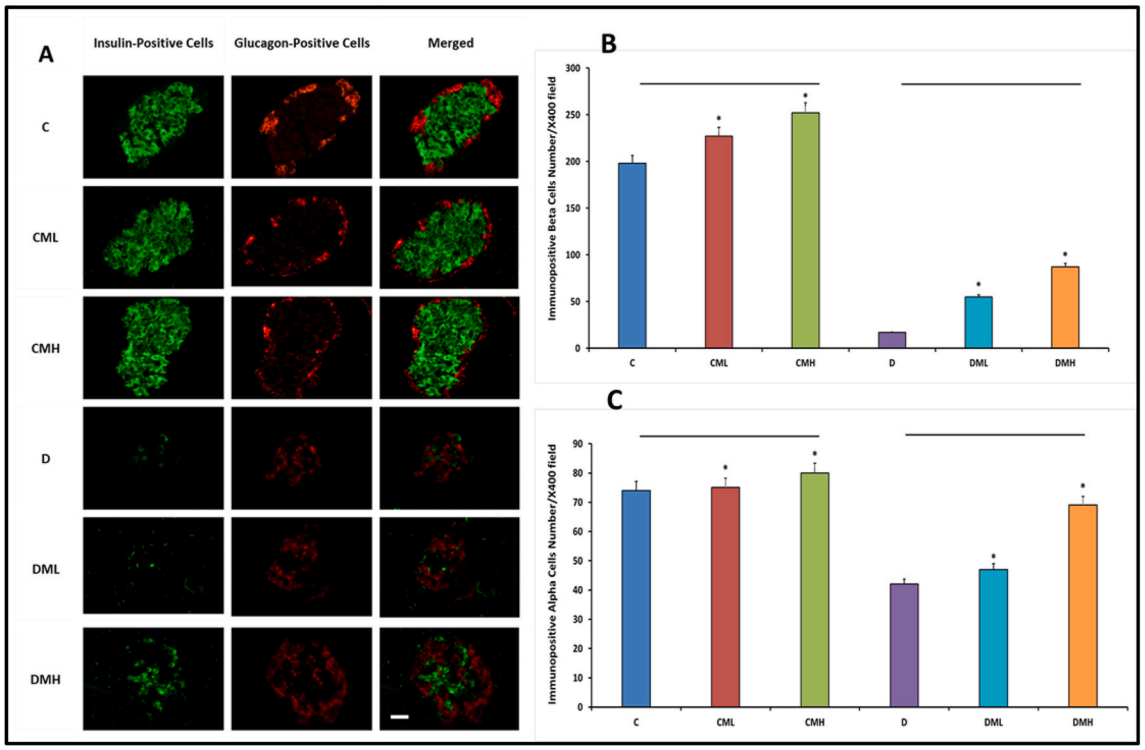


Fig. 6. [A]. Immunofluorescence double labeled images showing the pattern of distribution of insulin-containing beta cells (green fist column) and glucagon-containing alpha cells (red second column) and merged images for all groups (third column), in pancreatic islets of normal and diabetic rats. Rats' pancreas tissues were immunofluorescent stained and examined under fluorescence microscope at X400 magnification. C = non-diabetic control rats, CML = control rats treated with low dose of melatonin, CMH = control rats treated with high dose of melatonin, D = diabetic control, DML = diabetic rats treated with low dose of melatonin, DMH = diabetic rats treated with high dose of melatonin. Scale bar = 2 μm. Morphometric analysis of the immunofluorescence images showed significant increases in the number of pancreatic beta [B] and alpha [C] cells ($n = 6$). Figures [B] and [C] shows histograms of the number of insulin and glucagon immunoreactive cells, respectively, after melatonin treatment. Note that the number of insulin-, and glucagon-positive cells has significantly increased after melatonin treatment. Values are mean ± SEM ($n = 6$). *$p < 0.05$.

the fasting blood glucose, increased both serum insulin and serum SOD levels indicating that melatonin has hypoglycemic action, secretagogue effect of enhancing insulin secretion and promoting SOD activity. In addition, melatonin itself acts as a protective antioxidant agent [30].

DM has been reported to cause liver histological degenerative hepatocytes shrinkage and necrotic impairment with wide demolished sinusoids vascularization and disoriented linear hepatic cords as reported in previous study [44]. Diabetic complications with over-production of free radicals lead to hepatocellular destruction [45]. Other studies on diabetic mice had revealed liver hypertrophy with distended hepatocyte nuclei and cytoplasmic distortion with less glycogen granules [46]. Diminished hepatocytes glycogen synthesis because of insulin deficiency in diabetic rat was alleviated by melatonin treatment which induces the glycogenesis from the glucose content in the liver cells [47]. In our study, melatonin treatment, recovered the deteriorative liver tissue in diabetic rats. Melatonin reduced hepatocyte vascularization and necrosis with increment of glycogen cytoplasmic granulation and restoring near normal liver tissue histology. Our finding was in agreement with previous study [45], where the structure of the liver is restored. The ability of melatonin to keep liver architecture intact could due to its inhibitory action on hepatotoxic free radicals through enhancing the antioxidant beneficial activity of glutathione peroxidase and SOD as recorded in previous study [48]. In addition, elevation of lipid peroxidation in the liver tissues of diabetic rats was reduced because of the antioxidant activity of melatonin [49]. These finding are in agreement with our observations in the current study on histological analysis of liver tissues after melatonin treatment. Accordingly, melatonin could play a significant protective role on hepatocytes damage as a potent antioxidant scavenger, in addition to its promoting action on many antioxidant enzymes such as SOD in opposing the insulting damage of elevated free radicals in diabetic rats [50].

DM causes necrosis of the islets of Langerhans resulting in islets with a very small size [51–53]. These findings could be due to oxidative stress, accelerated cell death and inflammatory progression [54]. Our study showed melatonin treatment caused significant improvement in islet size of Langerhans of diabetic in comparison to that of untreated diabetic rats. In the current study, melatonin treatment improved pancreatic beta cells proliferation as shown in our melatonin treated rats in contrast to untreated control and diabetic groups. The increase in number and neogenesis of newly added beta cells is probably associated with elevated serum insulin secretion in melatonin treated groups coupled with a reduction in oxidative stress level. Our finding was in concordance with those of

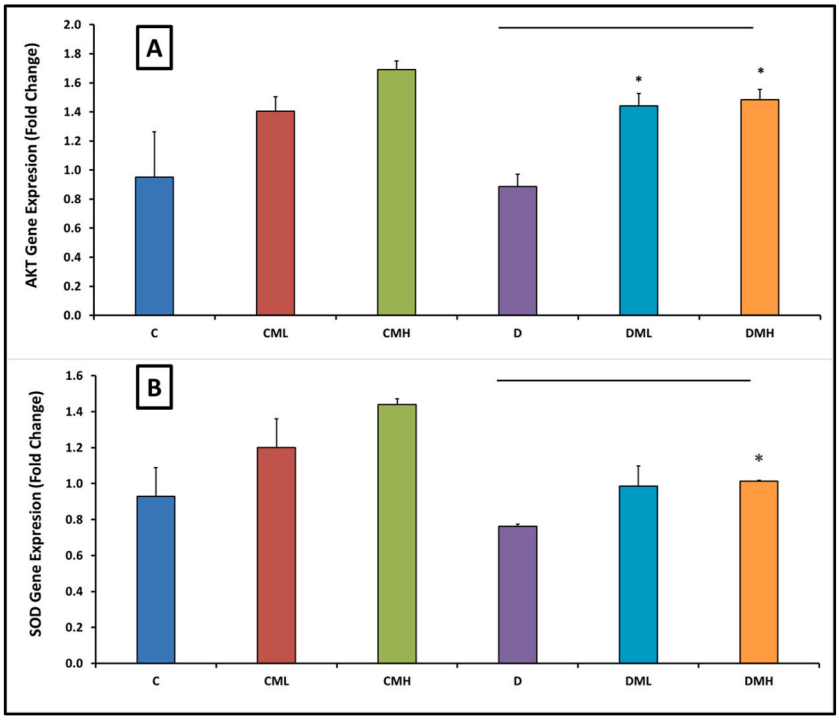


Fig. 7. Gene expression fold-change values derived from qPCR data. The changes in expression of [A] protein kinase B (AKT) and [B] superoxide dismutase (SOD) genes in rats' liver were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) the housekeeping gene and plotted with respect to equivalent tissues from control and untreated rats. C = non-diabetic control rats, CML = control rats treated with low dose of melatonin, CMH = control rats treated with high dose of melatonin, D = diabetic control, DML = diabetic rats treated with low dose of melatonin, DMH = diabetic rats treated with high dose of melatonin. Values are mean ± SEM ($n = 6$). *$p < 0.05$.

previous studies [30, 55]. For example, in one study, melatonin treatment in mice recovered the pancreatic beta cell function and improved the insulin sensitivity and glucose metabolism. These results are in concurrent with previous studies that reported that melatonin administration reduced the oxidative stress via enhancing antioxidant activity and improving the pancreatic tissues morphology through anti-inflammatory action of melatonin [56,57]. The improvement of the pancreatic histology shown in the current study is associated with melatonin reducing glucose level and increasing insulin secretion. This protective effect of melatonin could be established via the hypoglycemic and antioxidant action of melatonin [58].

The PI3K/AKT insulin signaling pathway has a major role in liver glucose uptake, and in other tissues [59,60]. Impairment of PI3K/AKT signaling promotes insulin resistance in liver cells accompanied mainly with diabetes. Insulin binding to the insulin receptors on hepatocyte membrane induces intracellular activation of insulin receptor substrate that stimulates PI3K which activates AKT that upregulates many downstream beneficial biological actions including glucose uptake and glycogenesis [61–63].

One of the major consequences of diabetic complications is the development of oxidative stress, a damaging condition that cause overproduction of free radicals such as reactive oxygen species (ROS) [42]. DM-induced oxidative stress in could lead to cell membrane distortion, cell death, tissue injury that ultimately, intensify more free radical production [64]. In addition, hyperglycemia is strongly accompanied with elevated levels of ROS production in mitochondria during ATP production [65]. The protective body system against free radicals includes many endogenous antioxidants such as SOD, glutathione, and glutathione peroxidase [66]. SOD levels have been recorded to be decreased in the liver of diabetic rats with elevated cell apoptosis [67,68]. Studies have shown that melatonin supplementation has a beneficial effect on the antioxidant defense system in the liver of diabetic rats. In diabetes, there is an increase in oxidative stress due to the production of reactive oxygen species (ROS), which can damage tissues and lead to complications [69]. In one study, diabetic rats were treated with melatonin and the liver tissue was analyzed. The results showed that melatonin supplementation increased the activity of SOD in the liver, which helped to reduce oxidative stress and prevent damage to liver cells [70]. Furthermore, melatonin was found to reduce lipid peroxidation, a process by which free radicals attack and damage the lipids in cell membranes, leading to cell damage and death. Melatonin was also found to increase the levels of glutathione, a key antioxidant molecule that helps to protect cells from oxidative stress [71]. Additionally, melatonin itself can act as an antioxidant by scavenging free radicals and reducing oxidative stress. This can help to protect cells and tissues from damage and reduce the risk of various diseases associated with oxidative stress, such as cardiovascular disease, cancer, and neurodegenerative disorders [72]. Moreover, melatonin can prevent DNA oxidative damage and protect against cancer development [73]. Overall, these previous studies were in agreement with our result of the melatonin antioxidant activity by increasing SOD gene and protein expressions in diabetic rats. This would suggest that melatonin could help to improve the antioxidant defense system in the liver of diabetic rats. Additionally,

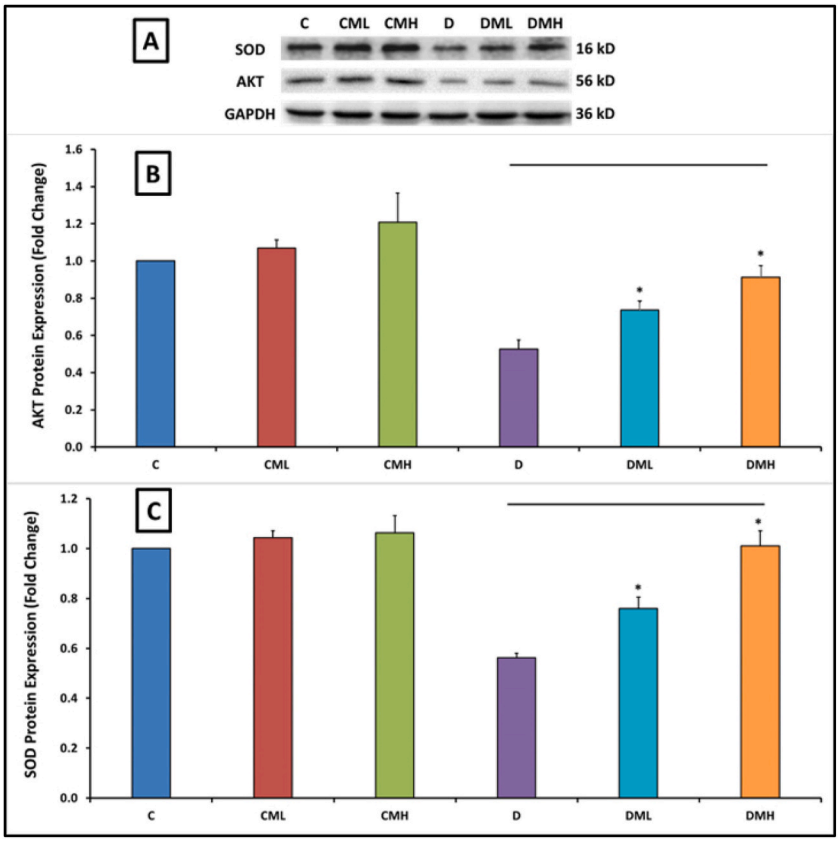


Fig. 8. [A]. Western blot analysis of protein kinase B (AKT) and superoxide dismutase (SOD) in the liver of normal, non-diabetic control and diabetic rats treated with melatonin. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping protein was used for protein normalization. The calculated protein expression of AKT [B] and SOD [C] is given. The C = non-diabetic control rats, CML = control rats treated with low dose of melatonin, CMH = control rats treated with high dose of melatonin, D = diabetic control rats, DML = diabetic rats treated with low dose of melatonin, DMH = diabetic treated rats with high dose of melatonin. The raw blots of Fig. 8A is provided in Supplement Fig, 1. Values are means ± SEM ($n = 6$). *p < 0.05.

melatonin treatment has been found to reduce the expression of genes involved in inflammation and oxidative stress, which are known to contribute to the development of DM. This suggests that melatonin may also have both anti-inflammatory and antioxidant effects that can help to protect the liver from damage caused by high blood sugar levels [74].

Therefore, the use of melatonin treatment in diabetic rats may to have a positive effect in the regulation of expression of genes responsible for glucose uptake in the liver, and may be a useful therapeutic strategy for the management of diabetes and in preventing diabetic complications associated with oxidative stress.

The reduction in hepatic insulin PI3K/AKT signaling pathway may reduce the release and transport of glucose transporter-2 (GLUT-2) molecules in the hepatocyte vesicles leading to a reduced liver glucose uptake [75]. Moreover, a reduction in AKT could inhibit liver glycogenesis leading to a decrease in liver glycogen synthesis in diabetic rats [76]. AKT impairment may lead to marked elevation in blood glucose level, coupled with insulin resistance in diabetes [77], because of the inability of AKT to stimulate glycogen synthesis. Melatonin has a potential therapeutic benefit for diabetes management. It has been reported that melatonin exerts its effects through the modulation of glucose metabolism in the liver, a major organ involved in glucose homeostasis [78]. The PI3K/AKT signaling pathway is a key pathway involved in glucose metabolism and insulin signaling in the liver. It has been shown in previous study that melatonin treatment can modulate the activity of this pathway in diabetic rats, leading to improved glucose metabolism and insulin sensitivity [77]. Melatonin treatment has also been shown to activate the PI3K/AKT signaling pathway in the liver of diabetic rats, culminating in marked phosphorylation of AKT and downstream targets such as GSK-3β and FoxO1. Our results on the ability of melatonin to improve blood glucose level is in accordance with [79]. The PI3K/AKT signaling pathway is activated by insulin and is responsible for the activation of glucose transporters in the liver, which in turn leads to an increase in glucose uptake and utilization. Melatonin treatment has been shown to stimulate the PI3K/AKT signaling pathway and increase the expression of glucose transporters in the liver which is responsible for glucose uptake. This can help to lower blood sugar levels and improve glucose tolerance and insulin sensitivity in diabetic rats [80]. In addition, melatonin treatment has also been shown to decrease the expression of certain genes involved in glucose production, such as gluconeogenic enzymes, which further improves glucose metabolism in the liver [81].

In the current study we studied the mechanism of action of melatonin on PI3K/AKT insulin signaling pathway via estimation of

gene and protein expression of AKT. AKT gene and protein expression levels were reduced in untreated diabetic rats. Melatonin treated diabetic groups showed elevation in AKT gene and protein expressions that would potentially reduce the impairment of insulin resistance in liver tissues. This suggests that melatonin could potentially be used as a therapeutic agent for diabetes management.

5. Limitations of the study

The major limitation of the study is the model used, the alloxan-induced diabetes rodent model, which is similar to human type 1 diabetes. In alloxan and streptozotocin murine models, there is a significant reduction in insulin level because of loss of pancreatic beta cell [82,83]. A model of type 2 DM, such Goto-Kakizaki, Zucker diabetic, or Wistar rat fed on high-fat diet would have had a more common parameters when compared to human type 2 DM [84]. In spite of this shortcomings, hyperglycemia is a common denominator in both type 1 and type 2 DM, where chronically high blood glucose level can stimulate the formation of AGEs. The AGEs will then bind to their receptors on the plasma membrane of several cells to induce ROS generation and oxidative stress [3,10,85]. The other limitations are the duration of the study and the dosage of alloxan and melatonin. A longer duration of more than four weeks and a higher dosage of melatonin (>50 mg/kg body weight) or alloxan (120 mg/kg body weight) may have resulted in a different outcome. The other limitations of this study is the absence of sleep evaluation even though the sleep cycle in rats is polyphasic (~12 h) in contrast to monophasic sleep (~8 h) pattern in humans [86,87]. Since melatonin is known to be a sleep enhancer [88], would been an additional supplemental data that would have supported other findings in this study. Additional limitations are the lack of assessment of skeletal and adipose tissues. These tissues play important role in glucose uptake [89,90], and their assessment would have provided additional insight into how melatonin mitigates the signs of experimental diabetes.

6. Conclusion

Melatonin has the potential to improving blood glucose homeostasis in diabetic rats. It is tempting to speculate that one mechanism by which this may occur is through the modulation of AKT and SOD gene expressions in hepatocytes. Studies have found that melatonin treatment can lead to increased expression of genes regulating glucose uptake and metabolism, and decreased expression of genes involved in glucose production. Additionally, melatonin improves insulin sensitivity and reduce oxidative stress in hepatic tissue, which can also contribute to its beneficial effects on glucose metabolism. Overall, melatonin treatment may have a positive impact on the expression of gene regulating glucose in diabetic rats and could be a promising drug that could be added to the arsenal of diabetes treatment.

Data availability

All data have been made available in the manuscript and no hidden data was used in the article.

Ethical approval

The study was approved by the Animal Research Ethics Committee, United Arab Emirates University, Al Ain, United Arab Emirates (Approval number: ERA-20/5931).

CRediT authorship contribution statement

Mohamed Lotfy: Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Aalaa Khattab:** Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Mohammed Shata:** Validation, Resources, Methodology, Investigation, Data curation, Conceptualization. **Ahmad Alhasbani:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Abdallah Khalaf:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Saeed Alsaeedi:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mahdi Thaker:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hazza Said:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Harun Tumi:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hassan Alzahmi:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Omar Alblooshi:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mohamad Hamdan:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Amjad Hussein:** Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Biduth Kundu:** Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ernest A. Adeghate:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This SURE PLUS Grant – 2022 was funded by Research Affairs, UAE University, United Arab Emirates (Grant Code: G00003900).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28639.

References

[1] K. Kupai, T. Várkonyi, S. Török, V. Gáti, Z. Czimmerer, L.G. Puskás, G.J. Szebeni, Recent progress in the diagnosis and management of type 2 diabetes mellitus in the era of COVID-19 and single cell multi-omics technologies, Life 12 (8) (2022 Aug 8) 1205.
[2] C. Solis-Herrera, C. Triplitt, C. Reasner, R.A. DeFronzo, E. Cersosimo, Classification of Diabetes Mellitus, Endotext, 2018 Feb 24 [Internet].
[3] M. Lotfy, J. Adeghate, H. Kalasz, J. Singh, E. Adeghate, Chronic complications of diabetes mellitus: a mini review, Curr. Diabetes Rev. 13 (1) (2017 Feb 1) 3–10.
[4] C.C. Lo, S.H. Lin, J.S. Chang, Y.W. Chien, Effects of melatonin on glucose homeostasis, antioxidant ability, and adipokine secretion in ICR mice with NA/STZ-induced hyperglycemia, Nutrients 9 (11) (2017 Oct 29) 1187.
[5] G. Mannino, C. Pernici, G. Serio, C. Gentile, C.M. Bertea, Melatonin and phytomelatonin: chemistry, biosynthesis, metabolism, distribution and bioactivity in plants and animals—an overview, Int. J. Mol. Sci. 22 (18) (2021 Sep 16) 9996.
[6] J. Arendt, A. Aulinas, Physiology of the Pineal Gland and Melatonin. InEndotext, MDText. com, Inc., 2022 Oct 30 [Internet].
[7] F.M. Görgün, Z. Oztürk, M.K. Gümüştaş, E. Kökogu, Melatonin administration affects plasma total sialic acid and lipid peroxidation levels in streptozotocin induced diabetic rats, J. Toxicol. Environ. Health 65 (10) (2002 May 24) 695–700.
[8] H. Zhou, Y. Yue, J. Wang, Q. Ma, Y. Chen, Melatonin therapy for diabetic cardiomyopathy: a mechanism involving Syk-mitochondrial complex I-SERCA pathway, Cell. Signal. 47 (2018 Jul) 88–100.
[9] M.O. Mahgoub, I.I. Ali, J.O. Adeghate, K. Tekes, H. Kalász, E.A. Adeghate, An update on the molecular and cellular basis of pharmacotherapy in type 2 diabetes mellitus, Int. J. Mol. Sci. 24 (11) (2023 May 26) 9328.
[10] J. Siddhi, B. Sherkhane, A.K. Kalavala, V. Arruri, R. Velayutham, A. Kumar, Melatonin prevents diabetes-induced nephropathy by modulating the AMPK/SIRT1 axis: focus on autophagy and mitochondrial dysfunction, Cell Biol. Int. 46 (12) (2022 Dec) 2142–2157, https://doi.org/10.1002/cbin.11899. Epub 2022 Sep 10. PMID: 36086947.
[11] A.A. Shah, A. Gupta, Antioxidants in health and disease with their capability to defend pathogens that attack apple species of Kashmir, Plant Antioxidants and Health (2020) 1–26.
[12] JS Bhatti, A Sehrawat, J Mishra, IS Sidhu, U Navik, N Khullar, S Kumar, GK Bhatti, PH. Reddy, Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives, Free Radic Biol Med. 184 (2022) 114–134.
[13] S. Tangvarasittichai, Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus, World J. Diabetes 6 (3) (2015 Apr 4) 456.
[14] B.A. Hemmings, D.F. Restuccia, Pi3k-pkb/akt pathway, Cold Spring Harbor Perspect. Biol. 4 (9) (2012 Sep 1) a011189.
[15] P.M. Titchenell, W.J. Quinn, M. Lu, Q. Chu, W. Lu, C. Li, H. Chen, B.R. Monks, J. Chen, J.D. Rabinowitz, M.J. Birnbaum, Direct hepatocyte insulin signaling is required for lipogenesis but is dispensable for the suppression of glucose production, Cell Metabol. 23 (6) (2016 Jun 14) 1154–1166.
[16] N. Kubota, T. Kubota, S. Itoh, H. Kumagai, H. Kozono, I. Takamoto, T. Mineyama, H. Ogata, K. Tokuyama, M. Ohsugi, T. Sasako, Dynamic functional relay between insulin receptor substrate 1 and 2 in hepatic insulin signaling during fasting and feeding, Cell Metabol. 8 (1) (2008 Jul 2) 49–64.
[17] X. Huang, G. Liu, J. Guo, Z. Su, The PI3K/AKT pathway in obesity and type 2 diabetes, Int. J. Biol. Sci. 14 (11) (2018) 1483.
[18] R.R. Cheekati, A.S. Rao, R. Vijayaraghavan, A histological study of alloxan-induced diabetes on experimental male Wistar rats, Natl. J. Physiol. Pharm. Pharmacol. 7 (12) (2017) 1329–1334.
[19] J.I. Heo, D.W. Yoon, J.H. Yu, N.H. Kim, H.J. Yoo, J.A. Seo, S.G. Kim, K.M. Choi, S.H. Baik, D.S. Choi, N.H. Kim, Melatonin improves insulin resistance and hepatic steatosis through attenuation of alpha-2-HS-glycoprotein, J. Pineal Res. 65 (2) (2018 Sep) e12493, https://doi.org/10.1111/jpi.12493. Epub 2018 Apr 21. PMID: 29607540.
[20] M. Lotfy, J. Singh, H. Rashed, S. Tariq, E. Zilahi, E. Adeghate, The effect of glucagon-like peptide-1 in the management of diabetes mellitus: cellular and molecular mechanisms, Cell Tissue Res. 358 (2014 Nov) 343–358.
[21] M. Lotfy, J. Singh, H. Rashed, S. Tariq, E. Zilahi, E. Adeghate, Mechanism of the beneficial and protective effects of exenatide in diabetic rats, J. Endocrinol. 220 (3) (2014 Feb 10) 291–304, https://doi.org/10.1530/JOE-13-0426. PMID: 24353307.
[22] X. Wan, X. He, Q. Liu, X. Wang, X. Ding, H. Li, Frequent and mild scrotal heat stress in mice epigenetically alters glucose metabolism in the male offspring, Am. J. Physiol. Endocrinol. Metabol. 319 (2) (2020 Aug 1) E291–E304.
[23] Z.M. Zhang, Z.H. Liu, Q. Nie, X.M. Zhang, L.Q. Yang, C. Wang, L.L. Yang, G.Y. Song, Metformin improves high-fat diet-induced insulin resistance in mice by downregulating the expression of long noncoding RNA NONMMUT031874. 2, Exp. Ther. Med. 23 (5) (2022 May 1) 1–21.
[24] G. Sadi, M.C. Baloğlu, M.B. Pektaş, Differential gene expression in liver tissues of streptozotocin-induced diabetic rats in response to resveratrol treatment, PLoS One 10 (4) (2015 Apr 23) e0124968.
[25] S. Gautam, M. Banerjee, The macrophage Ox-LDL receptor, CD36 and its association with type II diabetes mellitus, Mol. Genet. Metabol. 102 (4) (2011 Apr 1) 389–398.
[26] M.P. Terrón, J. Delgado-Adámez, J.A. Pariente, C. Barriga, S.D. Paredes, A.B. Rodríguez, Melatonin reduces body weight gain and increases nocturnal activity in male Wistar rats, Physiol. Behav. 118 (2013 Jun 13) 8–13.
[27] S.S. Puchalski, J.N. Green, D.D. Rasmussen, Melatonin effect on rat body weight regulation in response to high-fat diet at middle age, Endocrine 21 (2003 Jul) 163–167.
[28] M.E. Montano, V. Molpeceres, J.L. Mauriz, E. Garzo, I.B. Cruz, P. González, J.P. Barrio, Effect of melatonin supplementation on food and water intake in streptozotocin-diabetic and non-diabetic male Wistar rats, Nutr. Hosp. 25 (6) (2010) 931–938.
[29] H. Elbe, N.İ. Vardi, M.U. Esrefoglu, B. Ates, S. Yologlu, C. Taskapan, Amelioration of streptozotocin-induced diabetic nephropathy by melatonin, quercetin, and resveratrol in rats, Hum. Exp. Toxicol. 34 (1) (2015 Jan) 100–113.
[30] A. Farid, P. Moussa, M. Youssef, M. Haytham, A. Shamy, G. Safwat, Melatonin relieves diabetic complications and regenerates pancreatic beta cells by the reduction in NF-kB expression in streptozotocin induced diabetic rats, Saudi J. Biol. Sci. 29 (7) (2022 Jul 1) 103313.
[31] T. Frese, A.G. Bach, E. Mühlbauer, K. Pönicke, H.J. Brömme, A. Welp, E. Peschke, Pineal melatonin synthesis is decreased in type 2 diabetic Goto–Kakizaki rats, Life Sci. 85 (13–14) (2009 Sep 23) 526–533.
[32] W. Balduini, M.D. Weiss, S. Carloni, M. Rocchi, L. Sura, C. Rossignol, M. Longini, F. Bazzini, S. Perrone, D. Ott, R. Wadhawan, Melatonin pharmacokinetics and dose extrapolation after enteral infusion in neonates subjected to hypothermia, J. Pineal Res. 66 (4) (2019 May) e12565.
[33] C. Sartori, P. Dessen, C. Mathieu, A. Monney, J. Bloch, P. Nicod, U. Scherrer, H. Duplain, Melatonin improves glucose homeostasis and endothelial vascular function in high-fat diet-fed insulin-resistant mice, Endocrinology 150 (12) (2009 Dec 1) 5311–5317.

[34] J.-I. Heo, D.W. Yoon, J.H. Yu, N.H. Kim, H.J. Yoo, J.A. Seo, S.G. Kim, K.M. Choi, S.H. Baik, D.S. Choi, N.H. Kim, Melatonin improves insulin resistance and hepatic steatosis through attenuation of alpha-2-HS-glycoprotein, J. Pineal Res. 65 (2) (2018) e12493.
[35] J. Xu, H. Gao, L. Zhang, S. Rong, W. Yang, C. Ma, M. Chen, Q. Huang, Q. Deng, F. Huang, Melatonin alleviates cognition impairment by antagonizing brain insulin resistance in aged rats fed a high-fat diet, J. Pineal Res. 67 (2) (2019 Sep) e12584.
[36] D. Garfinkel, M. Zorin, J. Wainstein, Z. Matas, M. Laudon, N. Zisapel, Efficacy and safety of prolonged-release melatonin in insomnia patients with diabetes: a randomized, double-blind, crossover study, Diabetes, Metab. Syndrome Obes. Targets Ther. 2 (2011 Aug) 307–313.
[37] H. Sun, X. Wang, J. Chen, A.M. Gusdon, K. Song, L. Li, S. Qu, Melatonin treatment improves insulin resistance and pigmentation in obese patients with Acanthosis nigricans, International Journal of Endocrinology 12 (2018 Mar) 2018.
[38] M.S. Albreiki, B. Middleton, S.M. Hampton, The effect of melatonin on glucose tolerance, insulin sensitivity and lipid profiles after a late evening meal in healthy young males, J. Pineal Res. 71 (4) (2021 Dec) e12770, https://doi.org/10.1111/jpi.12770. Epub 2021 Oct 9. PMID: 34582575; PMCID: PMC9285903.
[39] A. Agil, I. Rosado, R. Ruiz, A. Figueroa, N. Zen, G. Fernández-Vázquez, Melatonin improves glucose homeostasis in young Zucker diabetic fatty rats, J. Pineal Res. 52 (2) (2012 Mar) 203–210.
[40] L.A. Pham-Huy, H. He, C. Pham-Huy, Free radicals, antioxidants in disease and health, International journal of biomedical science: IJBS 4 (2) (2008 Jun) 89.
[41] A.C. Maritim, A. Sanders, Iii JB. Watkins, Diabetes, oxidative stress, and antioxidants: a review, J. Biochem. Mol. Toxicol. 17 (1) (2003) 24–38.
[42] B. Lipinski, Pathophysiology of oxidative stress in diabetes mellitus, J. Diabetes Complicat. 15 (4) (2001 Jul 1) 203–210.
[43] S.M. Kadry, M.H. El-Dakdoky, N.Z. Haggag, L.A. Rashed, M.T. Hassen, Melatonin improves the therapeutic role of mesenchymal stem cells in diabetic rats, Toxicol. Mech. Methods 28 (7) (2018 Sep 2) 529–538.
[44] Y.A. Hajam, S. Rai, Melatonin supplementation revives diabetic induced biochemical, histological and hematological impairments in rats, Heliyon 6 (4) (2020 Apr 1) e03770.
[45] E.T. Godam, M.O. Samaila, A.O. Ibegbu, W.O. Hamman, Histological and biochemical effects of Azadirachta indica and melatonin in streptozotocin-induced diabetic wistar rats, Annals of Experimental Biology 2 (2) (2014) 9–22.
[46] A. Guven, O. Yavuz, M. Cam, F. Ercan, N. Bukan, C. Comunoglu, F. Gokce, Effects of melatonin on streptozotocin-induced diabetic liver injury in rats, Acta Histochem. 108 (2) (2006 Jul 10) 85–93.
[47] J.M. Fernández-Novell, J. Ariño, J.J. Guinovart, Effects of glucose on the activation and translocation of glycogen synthase in diabetic rat hepatocytes, Eur. J. Biochem. 226 (2) (1994 Dec) 665–671.
[48] G. Sener, O. Tosun, A. Kaçmaz, S. Arbak, Y. Ersoy, G. Ayanoğlu-Dülger, Melatonin and N-acetylcysteine have beneficial effects during hepatic ischemia and reperfusion, Life Sci. 72 (24) (2003 May 2) 2707–2718.
[49] A.C. Maritim, B.H. Moore, R.A. Sanders, I.I.I.J.B. Watkins, Effects of melatonin on oxidative stress in streptozotocin-induced diabetic rats, Int. J. Toxicol. 18 (3) (1999 Apr) 161–166.
[50] M. Khorsand, M. Akmali, M. Akhzari, Efficacy of melatonin in restoring the antioxidant status in the lens of diabetic rats induced by streptozotocin, J. Diabetes Metab. Disord. 18 (2019 Dec) 543–549.
[51] E. Adeghate, T. Donath, Morphological findings in long-term pancreatic tissue transplants in the anterior eye chamber of rats, Pancreas 5 (3) (1990 May 1) 298–305.
[52] K.S. Balamash, H.M. Alkreathy, E.H. Al Gahdali, S.O. Khoja, A. Ahmad, Comparative biochemical and histopathological studies on the efficacy of metformin and virgin olive oil against streptozotocin-induced diabetes in Sprague-Dawley rats, J. Diabetes Res. (2018 Nov 18) 2018.
[53] S. Nurdiana, Y.M. Goh, H. Ahmad, S.M. Dom, N. Syimal'ain Azmi, N.S. Noor Mohamad Zin, M. Ebrahimi, Changes in pancreatic histology, insulin secretion and oxidative status in diabetic rats following treatment with Ficus deltoidea and vitexin, BMC Compl. Alternative Med. 17 (1) (2017 Dec) 1–7.
[54] L.M. Shawky, A.A. Morsi, E. El Bana, S.M. Hanafy, The biological impacts of sitagliptin on the pancreas of a rat model of type 2 diabetes mellitus: drug interactions with metformin, Biology 9 (1) (2019 Dec 25) 6.
[55] M.C. Kahya, M. Naziroğlu, Melatonin reduces lens oxidative stress level in STZ-induced diabetic rats through supporting glutathione peroxidase and reduced glutathione values, Journal of Cellular Neuroscience and Oxidative Stress. 8 (2) (2016 Jul 1) 588–594.
[56] C. Carrasco, A.M. Marchena, M.S. Holguín-Arévalo, G. Martín-Partido, A.B. Rodríguez, S.D. Paredes, J.A. Pariente, Anti-inflammatory effects of melatonin in a rat model of caerulein-induced acute pancreatitis, Cell Biochem. Funct. 31 (7) (2013 Oct) 585–590.
[57] C. Carrasco, A.B. Rodríguez, J.A. Pariente, Effects of melatonin on the oxidative damage and pancreatic antioxidant defenses in cerulein-induced acute pancreatitis in rats, Hepatobiliary Pancreat. Dis. Int. 13 (4) (2014 Aug 15) 442–446.
[58] S. Rai, M. Basheer, H. Ghosh, S. Singh, Protective role of melatonin in streptozotocin induced pancreatic damages in diabetic wistar rat, Pakistan J. Biol. Sci.: PJBS 21 (9) (2018 Jan 1) 423–431.
[59] N. Wang, T. Li, P. Han, The effect of tianmai xiaoke pian on insulin resistance through PI3-K/AKT signal pathway, J. Diabetes Res. 2016 (2016 Oct).
[60] B. Duan, Z. Zhao, L. Lin, J. Jin, L. Zhang, H. Xiong, N. Ta, T. Gao, Z. Mei, Antidiabetic effect of Tibetan medicine Tang-Kang-Fu-San on high-fat diet and streptozotocin-induced type 2 diabetic rats, Evid. base Compl. Alternative Med. (2017 Jan 1) 2017.
[61] H. Wang, Q. Zhang, Q. Wen, Y. Zheng, P. Lazarovici, H. Jiang, J. Lin, W. Zheng, Proline-rich Akt substrate of 40 kDa (PRAS40): a novel downstream target of PI3k/Akt signaling pathway, Cell. Signal. 24 (1) (2012 Jan 1) 17–24.
[62] S.G. Ward, J. Westwick, S. Harris, Sat-Nav for T cells: role of PI3K isoforms and lipid phosphatases in migration of T lymphocytes, Immunol. Lett. 138 (1) (2011 Jul 1) 15–18.
[63] H. Breitbart, T. Rotman, S. Rubinstein, N. Etkovitz, Role and regulation of PI3K in sperm capacitation and the acrosome reaction, Mol. Cell. Endocrinol. 314 (2) (2010 Jan 27) 234–238.
[64] T. Lao-ong, W. Chatuphonprasert, N. Nemoto, K. Jarukamjorn, Alteration of hepatic glutathione peroxidase and superoxide dismutase expression in streptozotocin-induced diabetic mice by berberine, Pharmaceut. Biol. 50 (8) (2012 Aug 1) 1007–1012.
[65] L. Piconi, L. Quagliaro, A. Ceriello, Oxidative stress in diabetes, Clin. Chem. Lab. Med. 41 (2003) 1144–1149.
[66] I.N. Zelko, T.J. Mariani, R.J. Folz, Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression, Free Radic. Biol. Med. 33 (3) (2002 Aug 1) 337–349.
[67] R.P. Robertson, Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes, J. Biol. Chem. 279 (41) (2004 Oct 8) 42351–42354.
[68] E. Adeghate, C.M. D'Souza, Z. Saeed, S. Al Jaberi, S. Tariq, H. Kalász, K. Tekes, E.A. Adeghate, Nociceptin increases antioxidant expression in the kidney, liver and brain of diabetic rats, Biology 10 (7) (2021 Jul 3) 621.
[69] Z.A. Yakut, G.B. Akcay, O. Cevik, G. Sener, Ameliorative effects of melatonin on intestinal oxidative damage in streptozotocin-induced diabetic rats, J. Fac. Pharm. Istanb. Univ. 50 (3) (2020 Dec 1) 160–168.
[70] C. Rodriguez, J.C. Mayo, R.M. Sainz, I. Antolín, F. Herrera, V. Martín, R.J. Reiter, Regulation of antioxidant enzymes: a significant role for melatonin, J. Pineal Res. 36 (1) (2004 Jan) 1–9.
[71] M. Eghbal, A. Eftekhari, E. Ahmadian, Y. Azarmi, A. Parvizpur, A review of biological and pharmacological actions of melatonin: oxidant and prooxidant properties, J Pharmacol Reports 1 (106) (2016) 2.
[72] R.J. Reiter, J.C. Mayo, D.X. Tan, R.M. Sainz, M. Alatorre-Jimenez, L. Qin, Melatonin as an antioxidant: under promises but over delivers, J. Pineal Res. 61 (3) (2016 Oct) 253–278.
[73] A. Galano, D.X. Tan, R.J. Reiter, Melatonin: a versatile protector against oxidative DNA damage, Molecules 23 (3) (2018 Feb 27) 530.
[74] A. Hacışevki, B. Baba, An overview of melatonin as an antioxidant molecule: a biochemical approach, Melatonin molecular biology, clinical and pharmaceutical approaches 5 (2018 Nov 5) 59–85.
[75] M. Kuai, Y. Li, X. Sun, Z. Ma, C. Lin, Y. Jing, Y. Lu, Q. Chen, X. Wu, X. Kong, H. Bian, A novel formula Sang-Tong-Jian improves glycometabolism and ameliorates insulin resistance by activating PI3K/AKT pathway in type 2 diabetic KKAy mice, Biomed. Pharmacother. 84 (2016 Dec 1) 1585–1594.

[76] T.Y. Liu, C.X. Shi, R. Gao, H.J. Sun, X.Q. Xiong, L. Ding, Q. Chen, Y.H. Li, J.J. Wang, Y.M. Kang, G.Q. Zhu, Irisin inhibits hepatic gluconeogenesis and increases glycogen synthesis via the PI3K/Akt pathway in type 2 diabetic mice and hepatocytes, Clin. Sci. 129 (10) (2015 Nov 1) 839–850.
[77] C. Song, D. Liu, S. Yang, L. Cheng, E. Xing, Z. Chen, Sericin enhances the insulin-PI3K/AKT signaling pathway in the liver of a type 2 diabetes rat model, Exp. Ther. Med. 16 (4) (2018 Oct 1) 3345–3352.
[78] F. Wajid, R. Poolacherla, F.K. Mim, A. Bangash, I.H. Rutkofsky, Therapeutic potential of melatonin as a chronobiotic and cytoprotective agent in diabetes mellitus, J. Diabetes Metab. Disord. 19 (2020 Dec) 1797–1825.
[79] M. Tajes Orduna, C. Pelegri Gabalda, J. Vilaplana Hortensi, M. Pallas Lliberia, A. Camins Espuny, An evaluation of the neuroprotective effects of melatonin in an in vitro experimental model of age-induced neuronal apoptosis, J. Pineal Res. 46 (3) (2009 Apr) 262–267.
[80] E. Ha, S.V. Yim, J.H. Chung, K.S. Yoon, I. Kang, Y.H. Cho, H.H. Baik, Melatonin stimulates glucose transport via insulin receptor substrate-1/phosphatidylinositol 3-kinase pathway in C2C12 murine skeletal muscle cells, J. Pineal Res. 41 (1) (2006 Aug), 67-7.
[81] T.C. Nogueira, C. Lellis-Santos, D.S. Jesus, M. Taneda, S.C. Rodrigues, F.G. Amaral, A.M. Lopes, J. Cipolla-Neto, S. Bordin, G.F. Anhê, Absence of melatonin induces night-time hepatic insulin resistance and increased gluconeogenesis due to stimulation of nocturnal unfolded protein response, Endocrinology 152 (4) (2011 Apr 1) 1253–1263.
[82] E. Adeghate, M. Fernandez-Cabezudo, R. Hameed, H. El-Hasasna, M. El Wasila, T. Abbas, B. Al-Ramadi, Orexin-1 receptor co-localizes with pancreatic hormones in islet cells and modulates the outcome of streptozotocin-induced diabetes mellitus, PLoS One 5 (1) (2010 Jan 6) e8587, https://doi.org/10.1371/journal.pone.0008587. PMID: 20062799; PMCID: PMC2799220.
[83] E. Adeghate, A.S. Ponery, D.J. Pallot, J. Singh, Distribution of vasoactive intestinal polypeptide, neuropeptide-Y and substance P and their effects on insulin secretion from the in vitro pancreas of normal and diabetic rats, Peptides 22 (1) (2001 Jan) 99–107, https://doi.org/10.1016/s0196-9781(00)00361-2. PMID: 11179603.
[84] S. Mohsin, H. Elabadlah, M.K. Alotaiba, S. AlAmry, S.J. Almehairbi, M.M.K. Harara, A.M.H. Almuhsin, S. Tariq, F.C. Howarth, E.A. Adeghate, High-density lipoprotein is located alongside insulin in the islets of Langerhans of normal and rodent models of diabetes, Nutrients 16 (2) (2024) 313, https://doi.org/10.3390/nu16020313.
[85] V.P. Singh, A. Bali, N. Singh, A.S. Jaggi, Advanced glycation end products and diabetic complications, KOREAN J. PHYSIOL. PHARMACOL. 18 (1) (2014 Feb) 1–14, https://doi.org/10.4196/kjpp.2014.18.1.1. Epub 2014 Feb 13. PMID: 24634591; PMCID: PMC3951818.
[86] https://lafeber.com/mammals/what-to-know-about-pet-rats-and-sleep/#:~:text=And%20they%20can%20be%20awake,or%2052%25%20of%20the%20day. (accessed on 23 jan2024).
[87] S.M. Simasko, S. Mukherjee, Novel analysis of sleep patterns in rats separates periods of vigilance cycling from long-duration wake events, Behav. Brain Res. 196 (2) (2009 Jan 23) 228.
[88] K.B. Megha, A. Arathi, S. Shikha, R. Alka, P. Ramya, P.V. Mohanan, Significance of melatonin in the regulation of circadian rhythms and disease management, Mol. Neurobiol. (2024 Jan 11), https://doi.org/10.1007/s12035-024-03915-0. Epub ahead of print. PMID: 38206471.
[89] M.J. Honka, A. Latva-Rasku, M. Bucci, K.A. Virtanen, J.C. Hannukainen, K.K. Kalliokoski, P. Nuutila, Insulin-stimulated glucose uptake in skeletal muscle, adipose tissue and liver: a positron emission tomography study, Eur. J. Endocrinol. 178 (5) (2018 May) 523–531, https://doi.org/10.1530/EJE-17-0882. Epub 2018 Mar 13. PMID: 29535167; PMCID: PMC5920018.
[90] E. Cummings, H.S. Hundal, H. Wackerhage, M. Hope, M. Belle, E. Adeghate, J. Singh, Momordica charantia fruit juice stimulates glucose and amino acid uptakes in L6 myotubes, Mol. Cell. Biochem. 261 (1–2) (2004 Jun) 99–104, https://doi.org/10.1023/b:mcbi.0000028743.75669.ab. PMID: 15362491.