



Original Article

Effects of Melatonin on Hepatic and Renal Complication of Diabetes

Safarzadeh Hamed¹, Zargari Felor^{2*}

1. Department of Biology, Marand Branch, Islamic Azad University, Marand, Iran

2. Department of Medical Sciences, Marand Branch, Islamic Azad University, Marand, Iran

Received: 27 Sep 2021

Accepted: 24 Nov 2021

Abstract

Background & Objective: Diabetes is associated with biochemical and pathological changes in liver and kidney tissue. One of the most important mechanisms in causing complications of diabetes is increasing the production of free radicals due to impaired glucose metabolism. Some studies have shown that melatonin is effective in protecting tissues due to its antioxidant role. The aim of this study was to evaluate the effect of melatonin on biochemical factors, histopathological changes of liver and kidney in streptozotocin-induced (STZ) diabetic rats.

Materials & Methods: In this experimental study, 40 adult male rats were randomly divided into 4 groups (n=10): control group, diabetic group, melatonin group (20mg/kg BW) and melatonin + diabetic group. At the end of the 6th week, blood samples were collected. Biomarkers of liver function (alkaline phosphatase: ALP, aspartate aminotransferase: AST, alanine aminotransferase: ALT) and kidney biomarkers (urea, uric acid, BUN, creatinine), serum glucose and histopathological changes of these tissues were evaluated. Data analysis was performed using SPSS and the significance level was $P < 0.05$.

Results: Melatonin treatment significantly ($P < 0.05$) reduced hyperglycemia, hepatic enzymes and renal biochemical factors due to diabetes and improved histopathological changes of liver and kidney tissue.

Conclusions: Melatonin consumption can be effective in improving the renal and kidney complications of diabetes.

Keywords: melatonin, liver, kidney, diabetes

Introduction

Diabetes is a chronic metabolic disorder and one of the most important public health problems in the world, which results from complete or partial defect in insulin secretion or impaired response of body tissues to it. In this disease, various tissues and organs of the body

are affected and their function is impaired (1). The liver plays an important role in maintaining glucose metabolism. B impairing liver function glucose metabolic homeostasis is impaired (2). Hyperglycemia increases the production of free radicals aggravates oxidative stress, alters the metabolism of lipids, proteins, and damages liver and kidney tissues (3). One of the causes of changes in the activity of liver enzymes is the production of free radicals. Studies show that

***Corresponding Author:** Zargari Felor, Department of Medical Sciences, Marand Branch, Islamic Azad University, Marand, Iran

Email: zargarifkb@gmail.com

<https://orcid.org/0000-0002-7827-9713>



levels of liver enzymes (alkaline phosphatase: ALP, aspartate aminotransferase: AST, alanine aminotransferase: ALT) increase in people with diabetes. Increase in these enzymes is a sign of the extent of liver damage (4). Chronic hyperglycemia and consequently, increased oxidative stress are the main causes of diabetic nephropathy and the development of renal dysfunction in diabetics (5). According to studies, prevalence of nephropathy in diabetic patients (Iran: 30.6% (6), China (2.9%) (3), India (34.4% (1) and liver disease (19-20%) (7) is high. Glycosylation of renal glomerular membrane proteins leads to changes in its structure and reduced glomerular filtration (5). In diabetic patients, the balance between antioxidants and free radicals is disturbed and due to the increase in oxidants, the complications of the disease are accelerated. Izuki et al. reported reduction of antioxidants such as vitamin C, E and antioxidant enzymes (8). Melatonin is a lipophilic hormone derived from amino acid tryptophan, secreted by the pineal gland and plays an important role in regulating the light/dark cycle. Studies show the antioxidant properties of melatonin (9), but its effect on diabetes has not been fully investigated (10). Melatonin has 2 types of membrane receptors (MT1, MT2), one type is cytoplasmic receptor (MT3) and another type is nuclear receptor. MT3 receptor is a detoxification enzyme (11). In another word, melatonin has two direct and indirect antioxidant roles. The direct antioxidant function of melatonin is its ability to absorb free radicals. Unlike other antioxidants that require a redox cycle to regeneration, melatonin, through reaction with free radicals, produces stable compounds that are excreted in the urine (12,13). Indirect antioxidant function of melatonin is achieved by increasing the expression of antioxidant enzymes by membrane and nuclear receptors (9). Many studies have shown that melatonin increases superoxide dismutase (SOD) expression (9,14). Some studies also indicate the effect of melatonin on the reduction of lipid profile and blood glucose (10,15,16). Melatonin with antioxidant properties and scavenging free radicals reduces the increase in liver enzymes caused by methotrexate (17).

Since many studies are needed to show the antioxidant protective effects of melatonin on oxidative stress, the aim of this study was to investigate the role of melatonin in preventing liver and kidney damage in diabetic rats.

Materials & Methods

In this experimental study, 40 male wistar rats (250 ± 10 gr) were purchased from the Pasteur Institute of Tehran, Iran. The animals were kept in standard condition (temperature of $22 \pm 3^\circ\text{C}$, humidity: 40-50%, LD 12:12 cycle). After adaptation, they were randomly divided into 4 groups ($n=10$), which included the control group (group I), diabetic group (group II): ip injection of streptozotocin (STZ: Sigma, USA) 60mg/kg BW, after 72 hr. blood glucose level above 250 mg/dL was considered as diabetic), melatonin group (group III) (melatonin (Sigma, USA) 20mg/kg BW ip injection, every day 9:am 0.5 mL for 6 weeks) and diabetic + melatonin group (group IV). The control and diabetic groups received 1% ethanol-containing saline during treatment. [Melatonin is sparingly soluble in water and soluble in organic solvents such as ethanol. Therefore, it was first dissolved in 1% ethanol and then normal saline was added]. At the end of the sixth week, the animals were anesthetized with ether and blood samples were taken, serum was isolated for biochemical analysis. Hystopathological evaluation was performed using haematoxylin and eosin (H&E) staining and a light microscope. For histological studies, the slices of the liver and kidney were fixed in a 10% formalin solution, 3-5 micrometer-thick sections were examined. Blood glucose, liver enzymes (AST, ALT, ALP), creatinine, urea, uric acid, blood urea nitrogen (BUN), were measured using Pars Azmoon kit (Pars Azmoon Co., Iran) and Auto-analyzer (911Hitachi, Japan). Data were analyzed with SPSS software version 22 using Mann-Whitney U statistical test to examine significant differences between groups. The results were reported as Mean \pm SD and $P < 0.05$ was considered statistically significant. The experimental protocol was approved by the Medical Ethics Committee, and animals received human care in compliance with the guidelines of Tabriz University of

Medical Sciences (National Institutes of Health Publication NO.85-23 Revised 1985).

Results

Results are shown in Table 1. The levels of serum glucose, liver enzymes and renal markers (creatinine, urea, uric acid, BUN) in the diabetic group compared to the control group showed a significant increase ($p<0.05$), melatonin

treatment could significantly reduce glucose, liver and kidney markers. Melatonin alone did not show any significant effects. The results of histopathology of liver and kidney show the presence of necrosis, morphological changes (disorganization and inflammation in diabetic rats, treatment with melatonin restored and reduced cellular changes in kidney (Figure 1.A-C) and liver (Figure 2. A-C) tissue.

Table 1. Effects of melatonin on liver and kidney biomarker values in serum of all groups (Mean \pm SD)

Groups	Glucose (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mg/dL)	BUN (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Group I	103.4 \pm 30	175.2 \pm 4	120.5 \pm 11.9	695 \pm 58.9	0.58 \pm 0.02	29.6 \pm 1.7	63.7 \pm 3.7	2.33 \pm 0.11
Group II	623 \pm 50*	225 \pm 8.3*	205.3 \pm 12.8*	2987 \pm 352.2*	0.73 \pm 0.04*	32.6 \pm 2.5*	70.2 \pm 5.4*	2.31 \pm 0/11
Group III	108.3 \pm 20	169.4 \pm 3.8	112.6 \pm 7.4	686.4 \pm 33.9	0.58 \pm 0.01	29.5 \pm 1	63.3 \pm 2	2.38 \pm 0.07
Group IV	197.22 \pm 87**	61.7 \pm 9**	58.7 \pm 5.8**	852.2 \pm 99.2**	0.20 \pm 0.01**	29.6 \pm 1.5	19.7 \pm 1.7**	0.61 \pm 0.06**

* $p<0.05$ compared to group I

** $p<0.05$ compared to group II

control group (group I), diabetic group (group II), melatonin group (group III), diabetic + melatonin group (group IV).

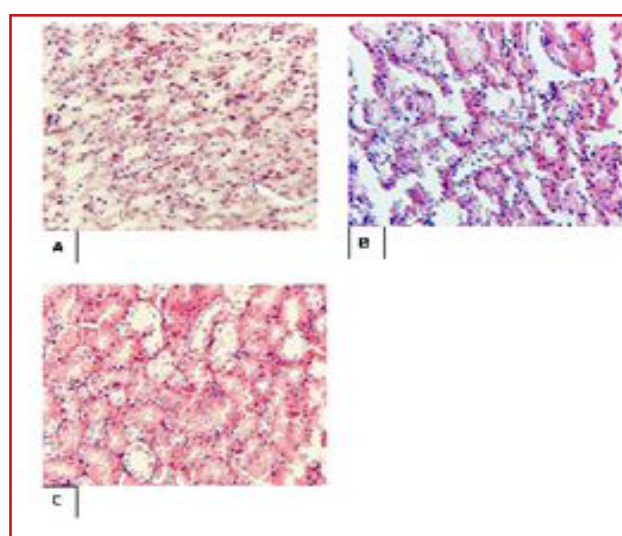


Figure1. Histological structure of kidney in control group (A), diabetic group (B) and diabetic+ melatonin group (C) (H&E X100)

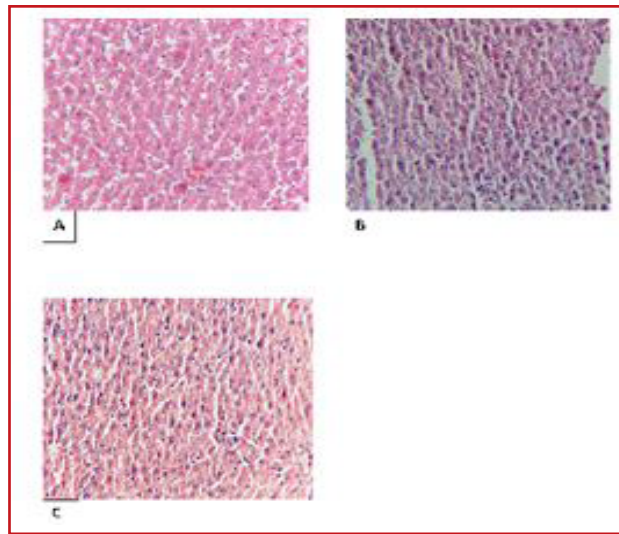


Figure 2. Histological structure of liver in control group (A), diabetic group (B) and diabetic+ melatonin group (C) (H&E X100)

Discussion

In the present study, melatonin treatment was able to improve liver and kidney damage by reducing their markers and serum levels of glucose. Streptozotocin increases blood glucose by necrosis and destruction of pancreatic beta cells (18). Streptozotocin injection increases the activity of NADH dehydrogenase and cytochrome c oxidase so with the leakage of electrons from the inner mitochondrial membrane, the production of ROS increase in pancreas, liver and kidney (19). STZ reduces the activity of antioxidant enzymes by production of ROS and induces the process of apoptosis (20). According to recent studies, melatonin plays a strong role in controlling blood sugar by increasing insulin sensitivity (21)(patients taking antihyperglycemic drugs should be careful). Melatonin protects B cells (with low antioxidant content) by neutralizing ROS (accumulation of ROS damages macromolecules such as protein, DNA, lipids (22). Sener et al. showed that treatment with melatonin reduced BUN, creatinine, ALT, AST in acetaminophen-exposed rats, which was consistent with our study (23). Increased serum levels of liver enzymes indicate liver damage. The researchers attributed the effect of melatonin to its role in scavenging free radicals and stimulating antioxidant enzymes.

Maarman et al. reported that melatonin has amelioration effects on increasing uric acid (by induction of antioxidant enzyme expression) (24). The protective role of melatonin in renal impairment during arsenic poisoning (25), cisplatin –induced nephrotoxicity (26), and pathological renal changes in diabetic rats have been demonstrated (27). Hyperglycemia increases apoptosis (20) and accelerates the glycation of circulating proteins and may initiate a series of auto-oxidation reaction that finally leads to the accumulation of glycation end products (AGEs) (5). These products have oxidizing power and accelerate tissue damage by free radicals. Increased oxidative stress damage may also play an important role in the pathology of functional and morphological changes of liver due to diabetes. Some studies show a protective role of melatonin in hepatotoxicity with methotrexate (28), non-alcoholic fatty liver (29), oxidative stress (30) which is consistent with our study. In these studies, due to the reduction of liver enzymes, improvement in histopathological changes (31), emphasis has been placed on the antioxidant role of melatonin. Based on our unpublished data, significant changes in levels of antioxidant enzymes in serum and pancreas tissue were observed in the rats treated with



melatonin that indicate its antioxidant properties.

Conclusion

It seems that due to the oxidative effects of diabetes on the structure of liver and kidney and change of biochemical markers, the use of antioxidant compounds such as melatonin by reducing the number of biochemical parameters and improving structural changes can improve the hepatic and renal complication of diabetes.

Acknowledgment

We wish to thank the Office of Research Islamic Azad University, Marand Branch for supporting this study (thesis code :12530520962001). (National Institutes of Health Publication NO.85-23 Revised 1985).

Conflict of interest

None declared.

References

- 1.Hussain S, Jamali MC, Habib A, Hussain MS, Akhtar M, Najmi AK. Diabetic kidney disease: An overview of prevalence, risk factors, and biomarkers. *Clinical Epidemiology and Global Health*. 2021; 9:2-6.
- 2.Guerra S, Mamede AC, Carvalho MJ, Laranjo M, Tralhão JG, Abrantes AM, et al. Liver diseases: what is known so far about the therapy with human amniotic membrane? *Cell and tissue banking*. 2016;17(4):653-63.
- 3.Zhang XX, Kong J, Yun K. Prevalence of diabetic nephropathy among patients with type 2 diabetes mellitus in China: a meta-analysis of observational studies. *Journal of diabetes research*. 2020;1-11 //doi.org/10.1155/2020/2315607
- 4.Salahshoor MR, Mohammadi MM, Roshankhah S, Najari N, Jalili C. Effect of *Falcaria vulgaris* on oxidative damage of liver in diabetic rats. *Journal of Diabetes & Metabolic Disorders*. 2019;18(1):15-23.
- 5.Suryavanshi SV, Garud MS, Barve K, Addepalli V, Utpat SV, Kulkarni YA. Triphala Ameliorates Nephropathy via Inhibition of TGF- β 1 and Oxidative Stress in Diabetic Rats. *Pharmacology*. 2020;105(11-12):681-91.
- 6.Rabieenia E, Jalali R, Mohammadi M. Prevalence of nephropathy in patients with type 2 diabetes in Iran: A systematic review and meta-analysis based on geographic information system (GIS). *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2020; 14:1543-1550.
- 7.Ciardullo S, Monti T, Perseghin G. High prevalence of advanced liver fibrosis assessed by transient elastography among US adults with type 2 diabetes. *Diabetes Care*. 2021;44(2):519-25.
- 8.Ibuki FK, Bergamaschi CT, da Silva Pedrosa M, Nogueira FN. Effect of vitamin C and E on oxidative stress and antioxidant system in the salivary glands of STZ-induced diabetic rats. *Archives of Oral Biology*. 2020; 116:104765.
- 9.Morvaridzadeh M, Sadeghi E, Agah S, Nachvak SM, Fazelian S, Moradi F, et al. Effect of melatonin supplementation on oxidative stress parameters: a systematic review and meta-analysis. *Pharmacological Research*. 2020; 161:105210.
- 10.Garaulet M, Qian J, Florez JC, Arendt J, Saxena R, Scheer FA. Melatonin effects on glucose metabolism: time to unlock the controversy. *Trends in Endocrinology & Metabolism*. 2020;31(3):192-204.
- 11.Imenshahidi M, Karimi G, Hosseinzadeh H. Effects of melatonin on cardiovascular risk factors and metabolic syndrome: a comprehensive review. *Naunyn-Schmiedeberg's archives of pharmacology*. 2020;393(4):521-36.
12. Reiter RJ, Tan DX, Terron MP, Flores LJ, Czarnecki Z. Melatonin and its metabolites: new findings regarding their production and their radical scavenging actions. *Acta Biochimica Polonica*. 2007;54(1):1-9.
- 13.Nuszkiewicz J, Woźniak A, Szewczyk-Golec K. Ionizing radiation as a source of oxidative stress—the protective role of melatonin and vitamin D. *International Journal of Molecular Sciences*. 2020;21(16):5804.
- 14.Feng TY, Li Q, Ren F, Xi HM, Lv DL, Li Y, et al. Melatonin Protects Goat Spermatogonial Stem Cells against Oxidative Damage during Cryopreservation by Improving Antioxidant Capacity and Inhibiting Mitochondrial Apoptosis Pathway. *Oxidative Medicine and Cellular Longevity*. 2020; 2020:1-16
- 15.Farokhian A, Niroomand M, Khalili D, Darafshi A, Pirsalehi A, et al. Safety and Efficacy of Melatonin in the Lipid Profile of Patients with Type 2 Diabetes Mellitus: A Randomized Clinical Trial. *Iranian Journal of Endocrinology and Metabolism*. 2020;22(3):221-9.
- 16.Rezayat AA, Nour MG, Bondarsahebi Y, Hozhabrossadati SA, Amirkhanlou F, Rezayat SA, et al. Effect of melatonin therapy on the non-alcoholic steatohepatitis patients: a systematic review and meta-analysis on clinical trial studies. *European Journal of Pharmacology*. 2021; 905:174154.
- 17.Montasser AO, Saleh H, Ahmed-Farid OA, Saad A, Marie MA. Protective effects of *Balanites aegyptiaca* extract, Melatonin and Ursodeoxycholic acid against hepatotoxicity induced by Methotrexate in male rats. *Asian Pacific journal of tropical medicine*. 2017;10(6):557-65.
- 18.Arora S, Ojha SK, Vohora D. Characterisation of streptozotocin induced diabetes mellitus in swiss albino mice. *Global Journal of Pharmacology*. 2009;3(2):81-4.
- 19.Prakash A, Kalra JK, Kumar A. Neuroprotective effect of N-acetyl cysteine against streptozotocin-induced memory dysfunction and oxidative damage in rats. *Journal*



of basic and clinical physiology and pharmacology. 2015;26(1):13-23.

20. Francés DE, Ronco MT, Monti JA, Ingaramo PI, Pisani GB, Parody JP, et al. Hyperglycemia induces apoptosis in rat liver through the increase of hydroxyl radical: new insights into the insulin effect. *Journal of Endocrinology*. 2010;205(2):187.

21. Doosti-Irani A, Ostadmohammadi V, Mirhosseini N, Mansournia MA, Reiter RJ, Kashanian M, et al. The effects of melatonin supplementation on glycemic control: a systematic review and meta-analysis of randomized controlled trials. *Hormone and metabolic research*. 2018;50(11):783-90.

22. Pourhanifeh MH, Hosseinzadeh A, Dehdashtian E, Hemati K, Mehrzadi S. Melatonin: new insights on its therapeutic properties in diabetic complications. *Diabetology & metabolic syndrome*. 2020;12(1):1-20.

23. Şener G, Şehirli AÖ, Ayanoğlu-Dülger G. Protective effects of melatonin, vitamin E and N-acetylcysteine against acetaminophen toxicity in mice: a comparative study. *Journal of pineal research*. 2003;35(1):61-8.

24. Maarman GJ, Andrew BM, Blackhurst DM, Ojuka EO. Melatonin protects against uric acid-induced mitochondrial dysfunction, oxidative stress, and triglyceride accumulation in C2C12 myotubes. *Journal of Applied Physiology*. 2017;122(4):1003-10.

25. Dutta S, Saha S, Mahalanobish S, Sadhukhan P, Sil PC. Melatonin attenuates arsenic induced nephropathy via the regulation of oxidative stress and inflammatory signaling

cascades in mice. *Food and Chemical Toxicology*. 2018; 118:303-16.

26. Ali BH, Abdelrahman A, Al Suleimani Y, Manoj P, Ali H, Nemmar A, et al. M. Effect of concomitant treatment of curcumin and melatonin on cisplatin-induced nephrotoxicity in rats. *Biomedicine & Pharmacotherapy*. 2020; 131:110761.

27. Kurçer Z, Parlakpınar HA, Vardi Nİ, Tasdemir SE, Iraz MU, Fadillioglu ER, et al. Protective effects of chronic melatonin treatment against renal ischemia/reperfusion injury in streptozotocin-induced diabetic rats. *Experimental and clinical endocrinology & diabetes*. 2007;115(06):365-71.

28. Khokhar A, Qayyum A, Khan MW. Protective effect of melatonin against methotrexate induced hepatotoxicity in mice. *PAFMJ*. 2017;67(1):126-30.

29. Gonciarz M, Gonciarz Z, Bielanski W, Mularczyk A, Konturek PC, Brzozowski T, et al. The effects of long-term melatonin treatment on plasma liver enzymes levels and plasma concentrations of lipids and melatonin in patients with nonalcoholic steatohepatitis: a pilot study. *Journal of Physiology and Pharmacology*. 2012;63(1):35.

30. Carrasco C, Beatriz A, José R. Effects of melatonin on the oxidative damage and pancreatic antioxidant defenses in cerulein-induced acute pancreatitis in rats. *Hepatobiliary & Pancreatic Diseases International* 2014;13(4): 442-446.

31. Moradpour R, Shokri M, Abedian S, Amiri FT. The protective effect of melatonin on liver damage induced by mobile phone radiation in mice model. *International Journal of Radiation Research*. 2020;18(1):133-41.