Original Article

Effects of Melatonin on Hepatic and Renal Complication of Diabetes

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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 aminoteransferase: 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

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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±3ºc, 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 an 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 ±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±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±30	175.2±4	120.5±11.9	695±58.9	0.58±0.02	29.6±1.7	63.7±3.7	2.33±0.11
Group II	623±50*	225±8.3*	205.3±12.8*	2987±352.2*	0.73±0.04*	32.6±2.5*	70.2±5.4*	2.31±0/11
Group III	108.3±20	169.4±3.8	112.6±7.4	686.4±33.9	0.58±0.01	29.5±1	63.3±2	2.38±0.07
Group IV	197.22±87**	61.7±9**	58.7±5.8**	852.2±99.2**	0.20±0.01**	29.6±1.5	19.7±1.7**	0.61±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).

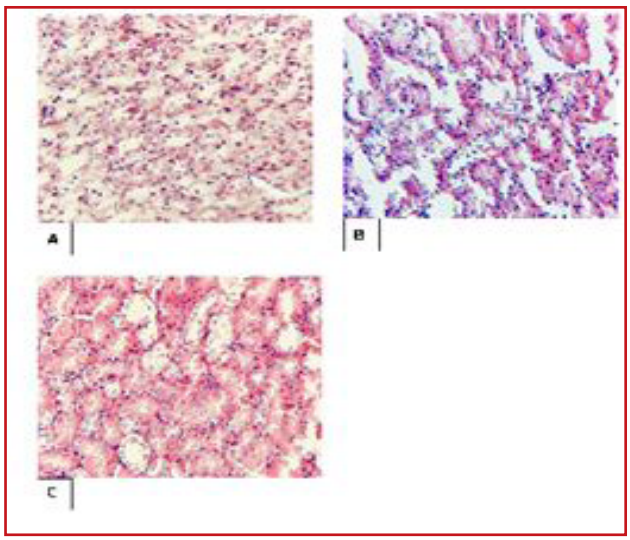

Figure1. Histological structure of kidney in control group (A), diabetic group (B) and diabetic+ melatonin grou-p (C) (H&E X100)

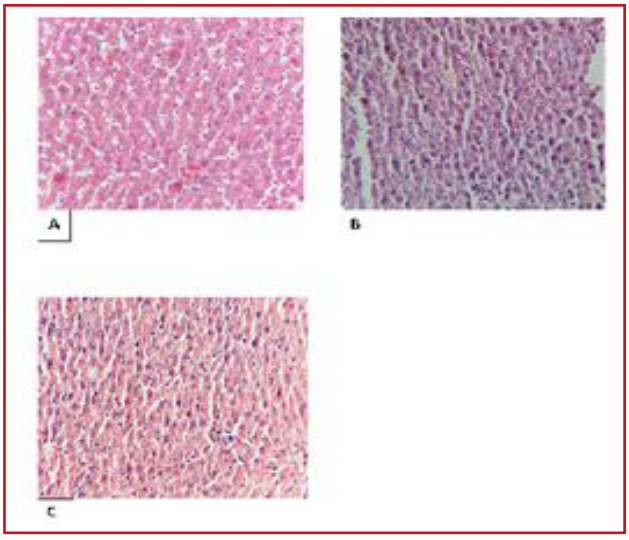

Figure 2. Histological structure of liver in control group (A), diabetic group (B) and diabetic+ melatonin group (C) (H&E X100)

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 pancrease, 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

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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

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Conflict of interest

None declared.

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