Original Article

Effects of melatonin on biochemical factors and food and water consumption in diabetic rats

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Abstract

Background: Diabetic neuropathy is one of the serious problems due to microvessel vasculopathy in diabetes. It has been reported that hyperglycemia and hypertriglyceridemia are the underlying mechanisms in inducing and progression of diabetic neuropathy. The aim of the present study was to investigate the effects of melatonin on serum glucose and lipid levels, as well as food consumption and water intake in streptozotocin-induced diabetic rats.

Materials and Methods: Eighty male Wistar rats were randomly assigned to six groups including; normal control group, diabetic control group and 4 diabetic experimental groups that received melatonin intraperitoneally at doses of 2.5, 5, 10, and 20 mg/kg at the end of sixth week after verification of neuropathy by means of evaluation of sciatic nerve conduction velocity (MNCV), for two weeks. Blood glucose and lipid levels, body weight, the amounts of food consumption, and water intake were determined in all groups at weeks 0 (before diabetes induction), 3, 6, and at the end of eighth week.

Results: Treatment with melatonin reduced significantly the serum glucose ($P < 0.001$) and triglyceride ($P < 0.05$) levels, food consumption ($P < 0.001$), and water intake ($P < 0.001$) in diabetic rats at the end of eighth week. However, melatonin had no significant effect on body weight of diabetic animals.

Conclusions: Treatment with melatonin could improve several signs of diabetes, including hyperglycemia, hypertriglyceridemia, polyphagia, and polydipsia. Therefore, melatonin may be used as an adjunct therapy in the treatment of diabetes.

Key Words: Diabetic neuropathy, food consumption, hyperglycemia, hyperlipidemia, melatonin, water intake

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INTRODUCTION

Diabetic neuropathies consist of complicated and heterogenous disorders including a broad spectrum of abnormalities that affecting peripheral and autonomic nervous system and leading to considerable illness and mortality.[1] Diabetic neuropathy is one of the serious problems due to microvessel vasculopathy in diabetes. About 50% of patients with more than 10 years history

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of diabetes mellitus develop diabetic neuropathy.[2,3] Diabetic neuropathy can be developed due to failures in peripheral sensory and motor nerves and also autonomic nerves. The high glucose concentration around peripheral nerves triggers changes inside the cell that ultimately destroy the nerve cells by means of vascular agents and interstitial changes.[4] Peripheral diabetic neuropathy is the most common form of diabetic neuropathies and is seen at least in 50% of diabetic patients;[5] mostly type 2 diabetics and elder people.[6] It seems that diabetic neuropathy is a multifactorial disease owing to interaction of abnormal metabolite activity and impaired vascular function.[7] The pathogenesis of peripheral diabetic neuropathy is the consequence of complicated reactions among multiple initiative mechanisms of hyperglycemia, including enhanced aldose reductase activity, glycation, non enzymatic glycooxidation, protein kinase C (PKC) activation, impaired insulin signaling, infection,[2] hypertension and disorders of fatty acid and lipid metabolism.[5]

Hyperglycemia induces oxidative stress and acute mitochondrial injury in dorsal root ganglion neurons, but this is not the only underlying mechanism inducing neuropathy in vivo. Fat disorder is also associated with development of neuropathy even in pre-diabetic patients. Fat disorder leads to increased level of oxidized low-density lipoproteins (LDLs) that in turn cause injury to dorsal root ganglion neurons by means of Lectin-like Oxidized LDL Receptor-1 (LOX-1) receptor and results in developing diabetic neuropathy.[8] Recently, it has been reported that the increased level of triglyceride participate in the progression of the disease. It has been found that dorsal root ganglion neurons express oxidized LDL receptor, LOX-1, and activation of this receptor leads to NADPH oxidase activity in vitro, then the oxidative stress causes injury of these neurons. Moreover, the oxidized LDLs significantly reduce the antioxidant potency of these neurons, thus it seems logical to use an antioxidant material to treat diabetic neuropathy, preventing its development or reducing the sign and symptoms of peripheral neuropathy in diabetic patients.[8]

Melatonin is a neurohormone secreted from pineal gland; it possesses powerful antioxidant properties and is capable of scavenging oxygenous and nitrogenous free radicals. Besides melatonin, its metabolites have also antioxidant properties.[9,10] Melatonin and its metabolites can easily pass the cell membranes and entering the cytosol and cell organelles, so melatonin and its metabolites may exert a very important effect in treating diabetic neuropathy by inducing antioxidant and scavenging free radicals effects. In the study of Montano *et al*., the melatonin effect on feeding behavior has been investigated[11] and it has been shown that drinking time has been reduced and fecal output has been increased. This study aimed to investigate the effects of melatonin on blood sugar and lipid level (as the probable factors of neuropathy), body weight and the amount of water and food consumption (as manifestations of diabetes mellitus) during 24 hours in diabetic rats.

MATERIALS AND METHODS

Eighty male Wistar rats weighing 298 ± 5 g, prepared from animal house of the faculty of Medicine of Mashhad, were randomly assigned to six groups. The rats maintained under standard conditions, temperature 25 ± 2°C, 12 hours light/dark cycle and free access to food and water. Blood samples were taken from retro-orbital cavernous sinus at day zero, and at the end of third, sixth and eighth week of treatment. Then, the blood samples were centrifuged at 4000 × g for 10 minutes and serum isolated and kept at −20°C until measuring the biochemical factors. All biochemical factors were measured by Pars Azmun kits, Iran. Streptozotocin (STZ) (Enzo Life Sciences, USA) was used to induce diabetes mellitus (55 mg/kg, ip).[12] The animals had 12 hrs fasting before STZ injection. Tail blood samples were taken after 72 hrs of STZ injection and blood glucose level examined by glucometer (Easygluco, Infopia Co., Ltd., Korea). The rats with blood glucose level more than 300 mg/dl included in the study, otherwise excluded.

The study groups include: Normal control group (n = 7) received no treatment, diabetic rats were randomly divided into four groups and assigned as: Diabetic control group (n = 7) received normal saline and ethanol 4% (as much as melatonin volume in treatment groups),[13,14] experimental groups 3-6 that received melatonin at doses of 2.5 (n = 4), 5 (n = 7), 10 (n = 7) and 20 mg/kg (n = 4) i.p. All injections were done at 18 o'clock before light off time for 2 weeks. After induction of diabetes, animals were left without treatment for 6 weeks.[15,16]

Different studies have shown that, diabetic neuropathy will develop 4-6 weeks after STZ injection.[15] At the end of sixth week after verification of diabetic neuropathy by means of behavioral (tail-flick for evaluation of warm hyperalgesia, von Frey for evaluation of mechanical allodynia and mechanical hyperalgesia) and electrophysiological tests (MNCV) (for evaluation of sciatic nerve conductive velocity), melatonin (Sigma chemical co., USA) treatment commenced for 2 weeks.[16,17] The blood glucose and lipid levels determination

was done by photometric technique (Convergys 100, Convergent technologies GmbH and Co. KG, Germany). Animals' body weight was measured weekly. The amounts of food and water consumption were also determined. In the first 2 weeks of study, food and water consumption during 24 hrs was measured twice weekly and the mean of them used for that week. Thereafter, food and water intake was assessed weekly.

The animals that had not gained favorable blood glucose level (≥300 mg/kg) or died before the end of study has been excluded from the experiment.

Data were expressed as mean ± SEM. One way ANOVA, post hoc Tukey and LSD were used for exploring the differences between groups. Paired samples *t*-test was used for comparing the results in each group using SPSS 16 software. The level of significance was considered as $P < 0.05$.

RESULTS

Measurement of blood glucose levels in the third week showed a significant increase ($P < 0.001$) in STZ injected groups compared to healthy control and persisted until the end of sixth week. After 2 weeks, at the end of eighth week, there was still significant increase in serum glucose levels in diabetic control group compared to healthy control ($P < 0.001$). However, serum glucose levels reduced significantly in melatonin treatment groups in eighth week (after treatment) compared to sixth week (before treatment) [Table 1].

Table 1: Blood glucose concentration (mg/dl) in experimental groups

Group	Before treatment (at the end of 6th week)	After treatment (at the end of 8th week)
Normal control	148.29±11.61	143.91±8.19
Diabetic control	356.20±11.3	347.17±13.17
Melatonin 2.5 mg/kg BW	361.4±7.43	280.59±2.97***
Melatonin 5 mg/kg BW	357.87±5.93	281.3±39.66*
Melatonin 10 mg/kg BW	366.35±7.43	276.84±28.52*
Melatonin 20 mg/kg BW	364.98±7.96	289.59±28.86*

Data are presented as Mean ± SEM. *$P < 0.05$, ***$P < 0.001$ vs diabetic group

The serum triglyceride level increased in all diabetic groups compared to normal control in the third week ($P < 0.01$) and also more increment was seen in all diabetic groups in the sixth week ($P < 0.001$). Melatonin treatment reduced serum triglyceride level at the end of eighth week ($P < 0.05$) compared to diabetic control group but no significant reduction observed between different treatment groups and healthy control [Figure 1].

Table 2 illustrates the changes of body weight in the experimental groups. Body weight reduced considerably after induction of diabetes. Melatonin treatment had no significant effect on body weight [Table 2].

Figure 2 illustrates the amount of food consumption during 24 hrs. Polyphagia occurred after inducing the diabetes in the third week compared to control group ($P < 0.001$), overeating persisted by the end of sixth week. Treatment with melatonin at doses of 2.5, 10, and 20 mg/kg significantly reduced food consumption in diabetic groups compared to untreated diabetic group ($P < 0.001$). However, treatment with melatonin at a dose of 10 mg/kg had no effect on food consumption [Figure 2].

Figure 3 illustrates the amount of water intake during 24 hrs. Polydipsia occurred after induction of diabetes in the third week compared to control group ($P < 0.001$). Melatonin treatment at doses of 2.5, 5, and 20 mg/kg significantly reduced water intake ($P < 0.001$), but melatonin at a dose of 10 mg/kg had no effect on water intake.

The Sciatic nerve conduction velocity reduced after sixth week by 47.2% compared to healthy control group. Different doses of melatonin increased motor nerve conduction velocity at the end of eighth week, it was only significant at doses of 5 mg/kg ($P < 0.01$) and 10 mg/kg ($P < 0.001$) compared to diabetic control group.

DISCUSSION

Neuropathy is a serious common complication of diabetes mellitus both in autonomic and

Table 2: Effects of melatonin on body weight (g) in experimental groups

Group/Time	Week 0	3rd week	6th week	8th week
Normal control	261.48±5.73	280.9±6.52	287.83±5.53	295.25±5.72
Diabetic control	312.54±6.03	256.21±4.59	239.12±8.73	238.57±15.88
Melatonin 2.5 mg/kg	258.56±5.25	256.67±7.39	244±11.17	229±25.39
Melatonin 5 mg/kg	303.95±6.92	241.13±4.37	229.27±10.26	242.43±7.26
Melatonin 10 mg/kg	313±6.51	273.56±8.03	253.38±9.67	241.38±12.22
Melatonin 20 mg/kg	303.25±4.33	249.25±6.91	234.86±9.98	242.25±5.51

Data are presented as Mean ± SEM

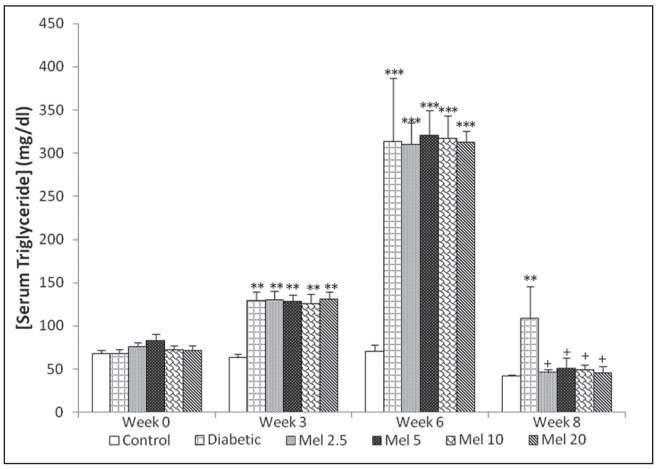

Figure 1: Effect of melatonin on triglyceride level. Data are presented as Mean ± SEM. Data analysis was performed by one-way ANOVA, followed by post hoc LSD, ** $P < 0.01$, *** $P < 0.001$ *vs* control group, + $P < 0.05$ *vs* diabetic group

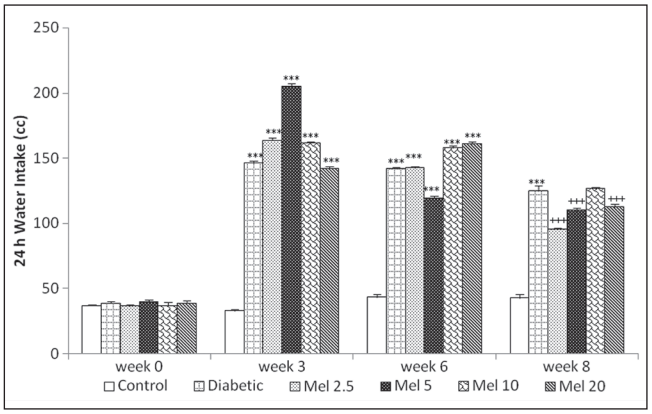

Figure 3: The level of daily water intake before (at 0, 3rd, and 6th weeks) and after (at 8th week) melatonin treatment. Data are presented as Mean ± SEM. Data analysis was performed by one-way ANOVA and post hoc Tukey, ***$P < 0.001$ *vs* control group, +++ $P < 0.001$ *vs* diabetic group

somatic nerves with an array of serious signs and symptoms in several organs. Hyperglycemia damages peripheral nerves by developing systemic and neuronal oxidative stress. The new idea states that disorder of fats involves in neuropathy development. High fat diet *per se* can cause neuropathy independent of hyperglycemia.[8] Several large scale studies undertaken on type 2 diabetic patients revealed that premature disorder of fats is the major independent risk factor in developing diabetic neuropathy.[18,19] Enhancement of lipid peroxidation in diabetic rats is correlated to increased level of blood triglyceride.[20] These sets of information show that why control of blood glucose level *per se*, is not sufficient for prevention of diabetic complications and usually combined therapy is required.[8]

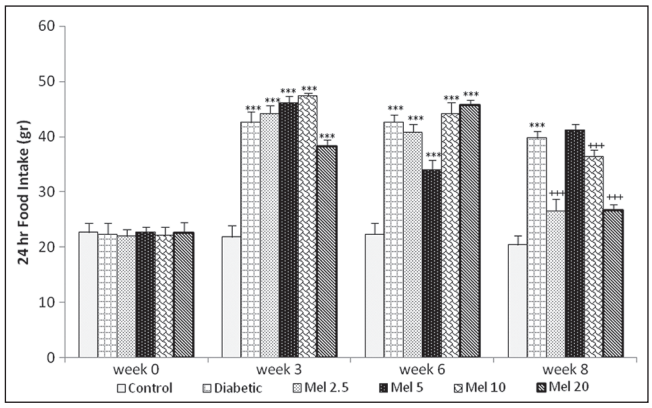

Figure 2: The amount of 24 hrs food consumption before (at week 0, 3rd, and 6th weeks) and after (at 8th week) melatonin treatment. Data are presented as Mean ± SEM. Data analysis was performed by one-way ANOVA and post hoc Tukey, ***$P < 0.001$ *vs* control group, +++ $P < 0.001$ *vs* diabetic group

In our study, blood glucose and triglyceride levels increased after STZ injection. Melatonin treatment for 2 weeks reduced blood glucose and triglyceride level in neuropathic diabetic groups. As reported by Anwar *et al*., melatonin treatment of 200 µg/kg i.p. for 15 days reduced significantly serum glucose levels.[21] Melatonin treatment for 8 weeks (100 and 200 µg/kg i.p.) has reduced blood glucose to normal level.[22,23] Effect of melatonin on glucose homeostasis in healthy rats is controversial. According to Lima study, glucose tolerance test was impaired and adipose tissue glucose transporter was reduced in healthy rats after pinealectomy[24] but in another study by Bizot Espiard, pinealectomy and melatonin treatment had no effect on glucose homeostasis and insulin responsiveness.[25] It was reported by Prunet- Marcassus *et al*. that melatonin had no effect on plasma insulin level in rats with high fat diet but reduced plasma glucose by 13%. Furthermore, the blood glucose and insulin level tend to increase in pinealectomized rats with high fat diet, and melatonin treatment could prevent this effect to some extent.[26] The glucose reducing effects of melatonin may be due to increased insulin sensitivity in peripheral tissues and by acting on receptors located on hepatocytes and pancreatic ß cells.[27] Melatonin can affect metabolism of glucose and secretion of insulin from pancreas.[28]

In the study of Zhang *et al*., melatonin treatment had no effect on triglyceride level.[29] Hoyos *et al*. have also reported that melatonin had no effect on cholesterol and triglyceride level in rats with normal diet.[30] Sener *et al*. have reported the reduction of hepatic triglyceride level in mice with high cholesterol diet.[31] Melatonin treatment has restored blood triglyceride level to normal before and after STZ injection.[22] In our study, melatonin showed significant reduction

compared to diabetic control group but regarding healthy control no significant change noted. Plasma melatonin can control fatty acid transport in rat inguinal fat pad through receptor mediated Gi- protein coupled signal transduction pathway that leads to reduce cAMP and inhibit fatty acid transport.[32]

In the rat osteosarcoma ROS 17/2.8 cell line, melatonin clearly inhibits the accumulation of triglycerides from fatty acids.[33]

Different studies have shown that lipid reduction effect of melatonin is more powerful than vitamin E, probably due to its antioxidant effect.[34]

In our study, melatonin treatment also reduced diabetic signs and symptoms such as polyphagia, polydipsia, and polyuria. Furthermore, the amount of food and water consumption decreased without changing of body weight. It has been reported by de Oliveira *et al*. that 1 mg/kg melatonin in drinking water has reduced hyperglycemia, polyphagia and polydipsia in rats[35] that congruent to our study results.

In Negi *et al*. study (2010), melatonin administration (10 and 3 mg/kg orally) showed no effect on body weight in STZ-injected rats.[16] As reported by Srinivasan *et al*. (2012), obesity, body weight, and metabolic changes due to obesity was decreased by melatonin in experimental animals.[36] It has been shown that melatonin treatment in normal adult rats and high cholesterol-fed rats reduced body weight and abdominal fat without any effect on food intake or total body fat.[26,37] The possible mechanisms of reducing body weight and fat mass may include the direct effect of melatonin on brown fat[38] and indirect effect by sympathetic nervous system.[39] The brown fat cells have binding sites for melatonin.[38] The antioxidant effects of melatonin may also reduce body weight and fats. These melatonin effects may be exerted by MT1 and MT2 receptors that exist in different tissues.[36] Melatonin had no effect on body weight in our study; this can be due to shorter time span (2 weeks vs. 10 weeks in some studies).

CONCLUSIONS

Treatment with melatonin could improve several signs of diabetes, including hyperglycemia, triglyceridemia, polyphagia, and polydipsia. Therefore, melatonin may be used as adjunct therapy in the treatment of diabetes.

Limitations of study

The diabetes *per se* leads to a high rate of mortality among experimental animals during long-lasting studies, so statistical analysis is not meaningful in some cases. In this experiment the number of animals in each group dropped from 12 to 7 or 4 except in control group.

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