

The Effects of Melatonin On The Oxidative Stress , Protein Glycation , Microalbuminuria and Lipid Profile In Type II Diabetes Mellitus

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ABSTRACT

Previous studies indicated that supplementation with antioxidants has a protective effects against oxidative stress-induced damage in type 2 diabetes. In this study we evaluated the antioxidant effects of melatonin on the oxidative stress parameters and microalbuminuria in type 2 DM patients. 30 patients with type 2 DM were treated with 3mg/day melatonin for 90 days. Erythrocytes and plasma MDA and glutathione, fasting plasma glucose, %HbA_{1C}, microalbuminuria, total plasma protein and lipid profile were measured each 30 days and compared with those obtained from 20 healthy controls. A decrease in MDA levels associated with the elevation in GSH levels were observed, compared with the pre-treatment levels. Fasting plasma glucose, glycated hemoglobin and microalbuminuria were significantly decreased, associated with an improvement in the total cholesterol, HDL-C and LDL-C levels, with respect to the pretreatment values. In conclusion , treatment of type 2 DM patients with melatonin may have protective effects against the oxidative stress-induced damage during the course of type 2 DM.

Key words: Diabetes Mellitus, Oxidative Stress, Melatonin, Microalbuminuria.

الخلاصة

لقد أصبحت ظاهرة ازدياد الاجهاد التأكسدي لدى مرضى النوع الثاني من داء السكري أمراً مسلماً به ، ويلعب دوراً مهماً في ظهور مضاعفات المرض . و عليه فقد تم في هذه الدراسة معالجة (30) مريضاً بالنوع الثاني من داء السكري بجرع يومية (3 ملغم/يوم) من مادة الميلاتونين ولمدة (90) يوماً . وتم قياس المتغيرات التالية :

تركيز (MDA) و (GSH) في البلازما وخلايا الدم ومقدار الكلوكوز في بلازما الدم ودرجة كلوزة خضاب الدم (Hb_{1AC}) والالبومين الدقيق في البول ، واجمالي بروتينات البلازما وتراكيز صور الشحوم المختلفة في البلازما ، قبل البدء بالعلاج وكل 30 يوماً من العلاج ولمدة 90 يوماً .

اتضح من تحليل النتائج بأن استخدام الميلاتونين قد ساهم في تقليل فرط الاجهاد التأكسدي والتقليل من طرح الالبومين في البول وتقليل مقدار الجلوكوز في الدم وكلوزة خضاب الدم ، كما أدى إلى تحسن واضح في مستويات الكولسترول والبروتينات الشحمية عالية واطانة الكثافة مقارنة بمستوياتها قبل البدء بالعلاج . ومن خلال هذه النتائج يمكن الاستنتاج بان للميلاتونين دوراً فاعلاً في حماية الجسم من الضرر الناتج عن فرط الاجهاد التأكسدي لدى مرضى النوع الثاني من داء السكري .

INTRODUCTION

There is currently a great interest in the potential contribution of the increased oxidative stress to diabetes mellitus (DM)^(1, 2). Different abnormal pathways that are strictly related to chronic hyperglycemia of DM, such as non-enzymatic glycation⁽³⁾, polyol pathway⁽⁴⁾ and glucose autooxidation⁽⁵⁾. During the progress of DM, there is a continuous events of metabolic stress, tissue damage and cell death that could lead to both increased free radicals production , and compromised total plasma antioxidant capacity⁽⁶⁾. Chronic complications , which may occur , like retinopathy , nephropathy and dyslipidemia, are among the dangerous consequences that usually accompany diabetes Although the

mechanisms behind their pathogenesis remain poorly understood, recent several mechanisms, including glycation of protein, oxidation of lipoprotein, growth factors alterations, changes in permeability and vascular changes, have been proposed to clarify the link between diabetes and these abnormalities⁽⁷⁾. Melatonin, N-acetyl-5-methoxytryptamine, is a pineal secretory product that affect reproductive functions, modulates immune system activities, inhibits oxidative stress⁽⁸⁾, and regulates circadian rhythms. Recent studies have examined the efficacy of melatonin as an antineoplastic agent^(9, 10, 11) and as an antioxidant^(12, 13).

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However, despite the widespread use of melatonin, there is minimal information on its use as a pharmacological approach in the treatment of DM and its complications. There is also minimal information on the toxicology of high pharmacological doses⁽¹⁴⁾. Based on LD₅₀ values in animals, it appears that melatonin has an extremely low acute toxicity⁽¹⁵⁾.

This study determined whether treatment with melatonin altered the clinical and biochemical abnormalities associated with DM in patients with type II diabetes mellitus.

PATIENTS and METHODS

This study was carried out on 30 patients who have type II DM for at least 5 years, with age range (40–80 years) at the National Diabetes Center, Al–Mustansiria University. All the selected patients were previously maintained on oral hypoglycemic drugs and diet restriction, but with poor glycemic control. They are treated with 3 mg/day of melatonin in a capsule dosage form, prepared specially for this purpose, given orally at bed time for three months.

Twenty healthy subjects, with the same age ranges as that of patients were selected and served as controls for comparison.

Fasting blood samples were collected from all subjects by vein puncture, before starting treatment with melatonin (as base line samples), and then after each 30 days of treatment to follow the changes in the studied parameters.

All blood samples were collected in heparinized tubes. Erythrocytes were separated by centrifugation at 3000 r.p.m. for 10 min. at 4°C, after the removal of the puffy coat, the erythrocytes were washed twice with ice-cooled saline containing 2.5 mM sodium azide to inhibit catalase activity⁽¹⁶⁾. Urine samples were collected from all subjects at the early morning before starting treatment and every 30 days for the evaluation of microalbuminuria⁽¹⁷⁾.

Erythrocytes and plasma malondialdehyde (MDA) levels were analyzed according to the method of Stocks and Dormandy (1971)⁽¹⁷⁾. Erythrocytes and plasma glutathione levels were measured according to the method of Godin *et al.* (1988)⁽¹⁸⁾. Plasma glucose levels were evaluated using a ready made kit (LABKIT, Spain) according to the method of Barhan and Trindoe (1972)⁽¹⁹⁾, and glycated hemoglobin (Hb_{A1C}) level was determined according to the method of Sushil (2000)⁽²⁰⁾.

Total plasma protein concentrations were determined according to the Reinhold (1953) method⁽²¹⁾, while hemoglobin (Hb) levels

were estimated according to the method of Drapkin and Austin (1935)⁽²²⁾.

Plasma lipid profile was evaluated through the measurement of total plasma cholesterol according to the method of Richmond (1974)⁽²³⁾, and triglycerides levels according to the method of Fossati and Principe (1982)⁽²⁴⁾, while Burstein *et al.* (1970)⁽²⁵⁾ method was utilized for the measurement of high density lipoprotein–cholesterol (HDL–c) levels from which the plasma concentrations of the low density lipoprotein–cholesterol (LDL–c) levels were calculated indirectly using Burtis and Ashwood formula (1999)⁽²⁶⁾. Statistical analysis of data was done by two–comparison of mean values, utilizing Student's t–test, P–values less than 0.05 was considered significant.

RESULTS

Combination of the routinely used measures for glycemic control with 3 mg/day melatonin, produced significant reduction in MDA levels in plasma (after 60 days), and erythrocytes (after 30 days) Table (1). The level of reduction in MDA reached values which are comparable, or even lower than that found in corresponding controls after 90 days of treatment.

Table (1): Effects of treatment with 3mg/day melatonin on plasma and erythrocytes MDA levels in type 2 diabetic patients.

Treatment period	n	Plasma MDA μmol/L	Erythrocytes MDA nmol/g Hb
Control	20	1.36 ± 0.1	16.98 ± 0.86
Zero time	30	1.98 ± 0.2*	19.89 ± 0.98*
30 days	30	1.54 ± 0.11	15.63 ± 1.19**
60 days	30	1.21 ± 0.15**	12.89 ± 0.8**
90 days	30	0.97 ± 0.09**	12.55 ± 0.62**

Results represent mean ± standard error.

n = number of subjects.

*** Significantly different with respect to control (P < 0.05).**

**** Significantly different with respect to zero time (P < 0.05).**

Table (2) showed that the oxidant stress–induced depletion of GSH in the plasma was improved after 30 days of treatment with 3 mg/day of melatonin (58%), and continuous

treatment led to further elevation in plasma GSH levels, which nearly matched their values in normal controls after 90 days of treatment.

Table (2): Effects of treatment with 3mg/day melatonin on plasma and erythrocytes glutathione (GSH) levels in type 2 diabetic patients

Treatment period	n	Plasma GSH $\mu\text{mol/L}$	Erythrocytes GSH $\mu\text{mol/g Hb}$
Control	20	0.36 \pm 0.07	8.29 \pm 0.1
Zero time	30	0.13 \pm 0.01*	6.26 \pm 0.43*
30 days	30	0.21 \pm 0.01**	6.79 \pm 0.57
60 days	30	0.27 \pm 0.02**	6.8 \pm 0.42
90 days	30	0.29 \pm 0.02**	7.0 \pm 0.51

Results represent mean \pm standard error.

n = number of subjects.

* Significantly different with respect to control (P < 0.05).

** Significantly different with respect to zero time (P < 0.05).

However, erythrocytes GSH levels which are severely depleted due to DM–induced oxidant stress, showed a non–significant increase (P > 0.05), even after 90 days of treatment with melatonin.

As a result of treatment with 3 mg/day melatonin for 90 days, fasting plasma glucose (FPG) and glycated Hemoglobin (Hb_{A1C}) levels were significantly reduced (26% and 11% respectively) compared with pretreatment levels (Table 3).

Table (3): Effects of treatment with 3mg/day melatonin on fasting blood glucose (FPG) and Glycated hemoglobin (Hb_{A1C}) levels in type 2 diabetic patients.

Treatment period	n	FPG mg/dl	Hb _{A1C} %
Control	20	94 \pm 4.6	5.4 \pm 0.28
Zero time	30	190 \pm 22*	9.46 \pm 0.6*
30 days	30	153 \pm 15	-
60 days	30	173 \pm 17	8.7 \pm 0.59
90 days	30	135 \pm 11**	7.2 \pm 0.4**

Results represent mean \pm standard error.

n = number of subjects.

* Significantly different with respect to control (P < 0.05).

** Significantly different with respect to zero time (P < 0.05).

As indicated in Table (4) , microalbuminuria and total plasma protein levels were severely affected by DM–induced oxidant stress, where 52% increase and 16%

decrease in both parameters were observed (Table 4) respectively, and were significantly different compared to controls .

Table (4): Effects of treatment with 3mg/day melatonin on the microalbuminuria and total plasma protein levels in type 2 diabetic patients.

Treatment period	n	Microalbuminuria mg/L	Total plasma protein g/dl
Control	20	169 \pm 4.1	8.55 \pm 0.34
Zero time	30	258 \pm 38*	7.23 \pm 0.12*
30 days	30	169 \pm 43	7.47 \pm 0.09
60 days	30	157 \pm 41**	7.35 \pm 0.08
90 days	30	140 \pm 30**	8.9 \pm 0.1**

Results represent mean \pm standard error.

n = number of subjects.

* Significantly different with respect to control (P < 0.05).

** Significantly different with respect to zero time (P < 0.05).

Melatonin treatment resulted in significant reduction in microalbuminuria (39%) after 60 days, with further decrease after 90 days (42%). However, total plasma protein levels started to show significant elevation ($P < 0.05$) after 90 days of treatment only (Table 4).

Table (5) clearly demonstrated that, the abnormal lipid profile in DM state, respond very well to melatonin treatment, where the

elevated total cholesterol values started to decrease significantly (20%, $P < 0.05$) after 60 days, reaching a level which was lower than that observed in controls after 90 days. Triglyceride levels showed (22%) decrease after 90 days of treatment, but this was still non-significant compared with pre-treatment values.

Table (5): Effects of treatment with 3 mg/day melatonin on the lipid profile in the plasma of type 2 diabetic patients

Treatment period	n	TC mg/dl	TG mg/dl	HDL-c mg/dl	LDL-c mg/dl
Control	20	184 ± 5.8	114 ± 18.1	37 ± 0.95	125 ± 5.8
Zero time	30	211 ± 16.7	211 ± 33.7*	33 ± 0.83*	132 ± 14.2
30 days	30	170 ± 15.1	201 ± 29.9	38 ± 1.9**	100 ± 11.1
60 days	30	168 ± 9.4**	183 ± 22.8	42 ± 3.5**	84 ± 8.2**
90 days	30	147 ± 11**	164 ± 18.9	43 ± 3.3**	71 ± 9.2**

Results represent mean ± standard error.

n = number of subjects.

* Significantly different with respect to control ($P < 0.05$).

** Significantly different with respect to zero time ($P < 0.05$).

HDL-c levels started to increase significantly after 30 days of treatment with melatonin (15%, $P < 0.05$). Maximum increase in HDL-c value was observed after 90 days of treatment (30%), which is higher than that observed for controls (Table 5). However, LDL-c levels started to decrease as a response for melatonin treatment only after 60 days of treatment (36%, $P < 0.05$). After 90 days, 46% decreases in LDL-c values were observed, which is highly significant compared with pre-treatment values, and even lowered than those observed in controls.

DISCUSSION

Melatonin has been suggested to have potent antioxidant properties that may prevent the development of cancer, atherosclerosis, and other consequences of aging, however, these hypothetical effects are unproven⁽²⁷⁾. Thus conclusive studies regarding the relevance of antioxidant properties of melatonin in the prevention of diseases, like diabetes mellitus, and their complications are not widely available. In this study, we evaluated the possible antioxidant activity of melatonin in ameliorating the oxidant stress state during DM, and the results shown in table (1) very well indicated that this low dose of melatonin resulted in significant reduction in MDA production in plasma and erythrocytes of DM patients. This result clearly indicated the promising

antioxidant activity, especially when correlated with the observed elevation of the soluble antioxidant, the glutathione (table 2). Higher doses may be required to achieve more improvement in the antioxidant profile, since animal studies showed that, for melatonin to show a potent antioxidant activity *in vivo*, very high daily doses may be required (10 mg/kg/day)⁽²⁸⁾. Researchers have discovered melatonin to have the most powerful antioxidant properties, it scavenges the most damaging free radical, the hydroxyl radical, five times better than GSH, and is twice as effective in deactivating the peroxy radical as vitamin E⁽⁸⁾.

Montilla *et al.* (1998) demonstrated that melatonin show a marked protective effect against oxidant stress resulted from hyperglycemia and protein glycosylation in experimental animals, two pathogenic cornerstones indicative of diabetic complications⁽²⁹⁾.

The results presented in table (3), indicated a significant reduction in FPG and Hb_{A1C} levels after 90 days of treatment with melatonin. The presented data and the preliminary findings of others⁽³⁰⁾ suggest that melatonin is protective against DM-induced damage, even at physiologic levels. Even if only pharmacologically relevant, the findings have important implications in that melatonin has no known toxicity and readily absorbed when administered by oral rout.

Antioxidants now a days are found in many clinical trials, to play a role in the amelioration of the microvascular changes during diabetic nephropathy, and this was clearly demonstrated by Nakamura *et al.* (1999)⁽³¹⁾, who observed the beneficial effects of Vit. E on microalbuminuria in DM nephropathy. Table (4) clearly demonstrated the effect of a 3 mg/day melatonin in the reduction of microalbuminuria after 60 days, and this consequently reflected on the improvement of total plasma protein after 90 days, and these results seems to be compatible with those observed by Ha *et al.* (1999), where treatment with melatonin decrease the incidence of early glomerulopathy in diabetic rats⁽³²⁾.

The ability of melatonin to inhibit the impairment of lipid profile in DM patients was studied, and the results presented in table (5) showed that treatment with 3 mg/day melatonin improves the lipid profile through the reduction of TC and LDL-c levels, associated with significant elevation in HDL-c levels. It is postulated that inhibition of LDL-c oxidation by antioxidants might protect against the development of atherosclerosis⁽³³⁾, and it is noted that, in both human and animal studies, resistance of LDL-c to oxidation has been associated with decreased vascular complications⁽³⁴⁾, and protection by antioxidants decreases susceptibility to vascular disturbances due to other diseases like DM⁽³⁵⁾.

Recent studies suggested that HDL-c has an antioxidant effect on LDL-c, thus Klimov *et al.* (1993) reported that HDL-c was protective against LDL-c oxidation, and this effect was concentration dependent⁽³⁶⁾. Therefore, the use of melatonin alone or with other antioxidants, may contributed to the protection of LDL-c oxidation and restore the endogenously present HDL-c molecules to play the protective role effectively.

In conclusion, melatonin through reducing remarkably the degree of lipid peroxidation, hyperglycemia, protein glycation, might give a hope to a promising perspective of this product in the treatment of diabetic complications.

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