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Effects of Melatonin and Flaxseeds Oil on Oxidative Stress and Inflammatory Parameters in Type 2 Diabetic Patients

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Abstract

In patients with diabetes, chronic inflammation is characterised by the increase in C-reactive protein (CRP) and tumour necrosis alpha (TNF-α). The consumption of melatonin and flaxseed oil may improve insulin sensitivity and the effectiveness of diabetic medications. The current study investigated the efficacy of melatonin and flaxseed oil in patients with Type 2 diabetes mellitus (T2DM). It involved 43 patients with diabetes who were divided into 3 groups. The first group received a placebo (starch 50mg, n=13), the second group received melatonin (10mg per day, n=14) and the third group received flaxseed oil (1000mg per day, n=16), in addition to prescribed hypoglycaemic medication and a 12-week controlled diet. Fasting blood sugar (FBS), glycated haemoglobin (HbA1c), oxidative stress and inflammatory parameters were measured in each group at 0, 6, and 12 weeks. Melatonin and flaxseed oil administrations resulted in a highly significant increase in glutathione (GSH) levels, a significant decrease in malondialdehyde (MDA), a significant increase in superoxide dismutase (SOD) in the melatonin group and a significant increase in SOD in the flaxseed oil group. The melatonin group and the flaxseed oil group also showed a highly significant decrease in oxidised low-density lipoprotein (ox-LDL).TNF-α was significantly reduced after the respective consumption of melatonin and flaxseed oil. Furthermore, flaxseed oil consumption resulted in a significant decrease in CRP; however, there was no significant difference in CRP due to melatonin consumption.

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1. Introduction

Type 2 diabetes mellitus (T2 DM) is a metabolic disease characterized by hyperglycemia caused by impaired insulin secretion in pancreatic β-cells in response to glucose, or by deficiencies in insulin action on its target tissues. Long-term complications particularly retinopathy, nephropathy, neuropathy and microvascular disease has been linked to chronic hyperglycemia [1].There is increasing clinical and experimental evidence that reactive oxygen species (ROS) and antioxidants (e.g. glutathione (GSH), superoxide dismutase (SOD), etc.) are increased in diabetes, and that the onset of diabetes is strongly related to oxidative stress (OS). In patients with diabetes, hyperglycemia induces free radicals and impairs the defence mechanism of endogenous antioxidant that function is to counteraction toxic OS [2].In addition, chronic inflammation in diabetics is marker by elevated serum level of C-reactive protein (CRP) and proinflammatory cytokine (e.g. interleukin-6 (IL-6), tumor necrosis factor alfa (TNF-α), monocyte chemoattractant protein-1 (MCP-1) ... etc.) [3].TNF-α can cause insulin resistance, diabetic retinopathy, diabetic nephropathy, endothelial dysfunction and atherothrombosis [4]. Melatonin has its own antioxidative effect and also increases the activity of endogenous antioxidative enzymes. It is secreted at night and crosses the blood-brain barrier, where it prevents oxidation disorders of cerebral nerve cells during nocturnal sleep [5]. Melatonin's immunostimulatory effect is dependent not only on its ability to increase cytokine production, but also on its anti-apoptotic and antioxidant properties [6]. Flaxseed has the potential to be a powerful antioxidant. The active constituent of flaxseed (lignan, secoisolaricire-sinoldiglucoside (SDG)) has potent antioxidant properties, lowering ROS, also reducing DNA scissions and lipid peroxidation [7, 8]. Animals consumed flaxseed, flaxseed oil, or flaxseed lignan had lower levels of inflammation, oxidative lung damage, lipid peroxidation, and hyperinsulinemia [8]. In human, flaxseed (oil, lignan, or supplements) was thought to reduce OS by lowering serum inflammation biomarkers (TNF-a, IL-1b, IL-6, CRP), decreasing glucose, glycosylated haemoglobin (HbAlC) concentrations, and lowering insulin resistance [8,9].The aim of the current study was to investigate the effect of melatonin and flaxseed oil on glycaemic control, OS and inflammatory parameters in T2DM patients.

2. Experimental Procedure

2.1. Patients Selection

This study included 43 patients (26 females and 17 males) with type 2 diabetes on sulfonylurea (glibenclamide), aged 40-60 years (48.39±7.89 SD), and with a disease duration of five to ten years who attended the Endocrinology and Diabetes Center, Baghdad, Iraq, for the period from October 2012 to April 2014.

2.2. Exclusion Criteria

This study excluded patients with liver or kidney disease, as well as those with cardiovascular disease. Also, patients taking insulin therapy or other anti-diabetic medications, antioxidants (aspirin), hypolipidemic agents, anti-inflammatory or nonsteroidal anti-inflammatory drugs were excluded. In addition, any medications should be considered. Also, pregnant women and breast feeding are excluded.

2.3. Inclusion Criteria

This study included patients who had already taken the maximum dose of sulfonylurea (glibenclamide) (15 mg per day) for two weeks with a regulated diet but had poor glycemic control as indicated by abnormal fasting blood sugar (FBS) and HbAlC.

2.4. Study Design

Three groups of patients were randomly established as following: 1) Group A (n=13) patients were administered a placebo starch powder in a capsule (50 mg per day) (Ingredion, India). 2) Group B (n=14) patients were administered melatonin powder 10mg as hard gelatin capsule (Amuun, Egypt) once day (10mg per day) at night. 3) Group C (n=16) patients were administered flaxseed oil 500 mg soft gelatin capsule twice daily (1000 mg per day) (Emad factory for herbal oils, Iraq) after meals. All patients in these groups were also received glibenclamide and a restricted diet for 12 weeks.

2.5. Specimen Collection and Evaluation

To track changes in the examined parameters, ten (10 ml) blood samples after 12 hours fasting were taken through venipuncture and serum was separated from all patients at 0 (baseline), 6 and 12 week(s) of treatment. FBS was measured using a ready-to-use kit, as described in [10]. HbAlC was measured using the VARIANT

hemoglobin AlC program, which based on ion exchange high performance liquid chromatography [11].Serum malondialdehyde (MDA) levels were estimated using the method of [12], which was modified by [13]. Serum glutathione (GSH) levels were determined using the method of [14]. Serum superoxide dismutase (SOD) levels were determined using the method described in [15], and serum oxidized LDL (ox-LDL) levels were determined using an enzyme immunoassay (ELISA) kit described in [16]. TNF-α levels in serum were determined according to [17]. CRP levels were determined using the method described in [18].

2.6. Statistical Analysis

Except for HbAlC, all results were expressed in mmol/L. The paired t-test and analysis of variance (ANOVA) were used to determine the level of significance, with $P<0.05$ considered significant.

3. Results and Discussion

Diabetes is commonly treated with oral hypoglycemic medication, glycosidase enzyme inhibitors, or injected insulin [19], these synthetic agents, however, can have serious side effects [20]. Furthermore, for thousands of years, herbal treatments for diabetes have been used in traditional medicine, they used by approximately 60% of the world's population, and this figure is growing by the day [21]. There are over 800 plant species exhibit hypoglycemic activities [22]. Because of the low cost, ease of availability, and lack of side effects, there has been an increase in the use of herbal products as anti-diabetic [23, 19].

3.1. Effects of Melatonin and Flaxseeds Oil on Glycemic Control

After 12 weeks of treatment in comparison with base line (0week), results showed that there was no significant difference ($P>0.05$) in FBS for the both placebo and flaxseed groups, while a high significant decrease ($P<0.01$) in FBS for the melatonin group. Also, in comparison with placebo group, there was no significant difference in FBS of the flaxseed group ($P>0.05$), while a significant decrease in the melatonin group at the same duration. Regarding HbA1c, in comparison to the baseline value after 12 weeks of treatment, there was no significant difference in the placebo group's HbA1c ($P>0.05$), a significant decrease in the flaxseed group's HbA1c ($P<0.05$), and a high significant decrease in the melatonin group's HbA1c ($P<0.001$). And in comparison, with placebo group, there was no significant difference in HbA1c of the flaxseed group, whereas a significant decrease in HbA1c for melatonin group (Table 1).

This results are consistent with other [24] that noticed that dietary milled flaxseed and flaxseed oil consumption for 12 weeks had no effect on HbA1c or fasting blood glucose concentrations. Similarly, flaxseed oil supplementation had no effect on glycemic control in people with T2DM [25]. Furthermore, studies looking into the potential benefits of lowering the dietary ratio have failed to show a positive effect on insulin sensitivity and glycemic control [26]. Another research reveals that taking high doses of flaxseed oil (60 mg/kg) had no effect on glycemic control in T2DM [27]. In a research conducted by [8], flaxseed supplementation was proven to lower insulin resistance. Despite the fact that insulin concentration was not changed significantly, the HOMA-IR index (homeostasis model assessment of insulin resistance) was significantly lower following flaxseed supplementation, reflecting a decrease in insulin resistance. In contrast, [28, 29] reported that decrease in HOMA-IR after supplementing with flaxseed showed no significant changes in insulin concentration. This could explain the current study's findings that administrations of flaxseed oil have no significant effect on FBS levels; however, it significantly lowers HbA1C levels.

Recently, pineal hormone has been shown to influence insulin secretion, carbohydrate metabolism, and blood glucose levels. The pineal gland and a few other organs locally synthesize melatonin (N-acetyl-5-methoxytryptamine) [30]. Melatonin appears to have a diabetes-preventive effect, whereas pinealectomy increases the risk of diabetes [31]. Melatonin's main function is to regulate seasonal and circadian rhythms, with high levels at night and low levels throughout the day. Insulin levels are also adapted to day and night variations due to melatonin-dependent synchronization [32]. In this regard, the findings of this study are consistent with previous findings that melatonin at a dose of 10 mg per day for 12 weeks was found to be effectively reduces FBS and HbA1c levels. Melatonin has the potential to impact diabetes and metabolic disorders not only through altering insulin release, but also by protecting pancreatic β-cells from oxidative damage due to their low antioxidative capacity, in the pancreas, melatonin receptors have been identified [32,33]. Melatonin can also act on target cells through attaching to membrane-bound receptors [34]. In both animal and human investigations,

melatonin has been demonstrated to help regulate blood glucose levels. Over a 5-month period, continuous therapy with prolonged-release melatonin (2 mg) lowers HbA1c levels and improves glycemic control. Furthermore, patients with T2DM who were given melatonin (6 mg) for three months had better glycemic control [30].

Table 1: FBS and HBA1c levels in each group at varied treatment periods.

Group	FBS (mmol/l)			HbA1c (%)		
	0 week	6 weeks	12 weeks	0 week	6 weeks	12 weeks
Placebo (n=13)	11.68+3.64	10.62+2.94 *	11.62+2.79	8.35+1.93	8.6+1.94	8.15+1.61
Melatonin (n=14)	10.39±2.42	9.15±2.78 *	7.64±1.83 **††	8.36±1.13	7.76±1.03 **	6.91±1.15 **†
Flaxseed (n=16)	11.69±5.13	10.97±3.38	10.49±3.21	8.37±1.71	7.41±1.82 **	7.74±1.3 *

Comparison with baseline: *= significant (P<0.05),**=highly significant (P<0.001).
Comparison with placebo over the corresponding duration:†= significant (P<0.05), ††= highly significant (P<0.001).

3.2. Effects of Melatonin and Flaxseeds Oil on Oxidative Stress

Diabetes causes an increase in oxidative stress due to a variety of factors. Hyperglycemia produces reactive oxygen species (ROS), which cause lipid peroxidation and membrane damage via glucose auto-oxidation [35]. In the current study, there was no significant difference in GSH, MDA, and ox.LDL of the placebo group after 12 weeks of treatment in comparing with baseline (0 week), however results showed that both flaxseed and melatonin groups had a high significant increase in GSH and SOD, but a high significant decrease in MDA and in ox.LDL. In addition, after 12 weeks of treatment, there was a high significant increase in GSH and a high significant decrease in MDA of the flaxseed and melatonin treated groups when compared to a placebo-treated group at the same duration. A significant increase in SOD in the melatonin groups, a high significant increase in SOD in the flaxseed treated group, and a high significant decrease in ox.LDL in the flaxseed and melatonin treated groups, as shown in Table 2.

Flaxseed oil lowered oxidized glutathione levels and maintained low GSH levels in erythrocytes, showing that it may boost antioxidant activity in human erythrocytes, according to [36]. A prior study [37] found similar results in erythrocytes from diabetic rats. This event revealed that flaxseed oil was abundant in PUFA (n-3 polyunsaturated fatty acid) and that the PUFA's numerous double bonds protected the membrane from oxidative stress [38]. Furthermore, flaxseed oil has been shown to transfer phenolic hydrogen to a peroxyl free radical of a peroxidized PUFA in a previous study. By suppressing the radical chain reaction, this method can prevent PUFA peroxidation in cellular or subcellular membrane phospholipids [39]. Several investigations in diabetic models have shown that glucose can enter the cell and activate the polyol pathway, resulting in nicotinamide adenine dinucleotide phosphate (NADPH) depletion [40].

Melatonin has antioxidant properties in both homogenized tissues and living organisms [41]. One study [42] assessed the effect of melatonin supplementation on oxidative stress parameters in elderly primary essential hypertensive patients. MDA levels were significantly reduced after taking 5 mg of melatonin every day for 30 days. Although there have been no clinical studies on the effect of melatonin on LDL oxidation. In one study, the antioxidative effect of melatonin on ox-LDL-induced disruption of nitric oxide synthesis in the human umbilical artery, which could be the fundamental source of endothelial dysfunction in preeclampsia. Melatonin appears to protect against ox-LDL-induced inhibition of NO production in the endothelium of human umbilical arteries, most likely by scavenging hydroxyl radicals [43]. Another study investigated melatonin and its physiological metabolites' antiatherogenic characteristics by assessing their effects on the radical-initiated production of ox-LDL. Melatonin appears to have little antiatherogenic properties in terms of LDL oxidation, although its main breakdown product 6-hydroxy melatonin inhibits LDL oxidation in a way that vitamin E does [44].Melatonin and other treatments that reduce the inflammatory and oxidative effects of diabetes mellitus may have a significant influence on people by preventing or decreasing retinal complication. Melatonin reduces

oxidative stress in diabetic rats, reducing retinal histological alterations [30].

Table 2: Oxidative stress parameters in each group at varied treatment periods.

Group	GSH(µmol/l)			MDA(µmol/l)			SOD(µmol/l)			ox. LDL(U/ml)		
	0 week	6 weeks	12 weeks	0 week	6 weeks	12 weeks	0 week	6 weeks	12 weeks	0 week	6 weeks	12 weeks
Placebo (n=13)	0.49± 0.05	0.56± 0.12*	0.51± 0.07	1.72± 0.29	1.95± 0.34	1.79± 0.27	32.0± 6.48	32.0± 6.7	34.0± 5.42*	45.6± 7.95	42.6± 6.33	45.8± 9.43
Flaxseed (n=16)	0.54± 0.05†	0.65± 0.06 **†	0.86± 0.14 **††	1.81± 0.19	1.24± 0.1 **††	0.96± 0.1 **††	27.3± 9.45	37.1± 12.54 **	50.3± 14.43 **b	45.1± 3.99	34.5± 4.75 **††	28.4± 4.97 **††
Melatonin (n=14)	0.49± 0.06	0.64± 0.08 **	0.77± 0.07 **††	1.65± 0.3	1.27± 0.28 **††	1.01± 0.23 **††	29.5± 4.42	33.5± 5.21 **	41.5± 5.9 **†	49.5± 6.69	40.0± 1.66 **	33.4± 4.31 **††

Comparison with baseline: *= significant (P<0.05),**=highly significant (P<0.001).
Comparison with placebo over the corresponding duration: †= significant (P<0.05), ††= highly significant (P<0.001).

3.3. Effects of Melatonin and Flaxseeds Oil on Inflammatory Markers

C-reactive protein is an acute phase protein whose presence in the blood is regarded to be a sign of chronic inflammation [45]. The level of C-reactive protein has been shown to predict cardiovascular disease and to induce the expression of adhesion molecules [46]. Table (3) showed that after treatment for 12 weeks in terms of TNF-α and CRP in comparison with placebo, there was a significant (P<0.05) and high significant (P<0.001) reduction in TNF-α in both flaxseed and melatonin groups, respectively. Also, there was a significant reduction in CRP in flaxseed group (P<0.05), but there was no significant difference in CRP in melatonin group (P>0.05). Flaxseed has anti-inflammatory properties [47]. In humans, supplementation with flaxseed lowered serum concentrations of TNF, CRP, glucose, HbAlC and improved insulin sensitivity [48]. Animals fed flaxseed, flaxseed oil, or flaxseed lignan had lower levels of inflammation, oxidative lung damage, lipid peroxidation, and hyperinsulinemia [49], these findings are consistent with the findings of the current study (Table 3). Flaxseed supplementation was hypothesized to reduce oxidative stress, thereby decreasing inflammation biomarkers and insulin resistance. Human serum TNF-α has been shown to be significantly reduced by flaxseed oil [50].

The current study's results on melatonin's effects on CRP and TNF-α were consistent with [51] who found that melatonin treatment reduces pro-inflammatory and oxidative stress, two factors that contribute to insulin resistance, metabolic syndrome, diabetes, and cardiovascular disease. Melatonin has the capacity to influence the inflammatory response by acting directly on the cells and tissues involved. Melatonin can suppress the expression and activity of key enzymes involved in the inflammatory process (phospholipase A2, 5-lipooxygenase, and cyclooxygenase 2) by acting on its high-affinity specific receptors i.e. pertussis-toxin-sensitive membrane receptors (MT1 and MT2), as well as nuclear binding sites found in macrophages, lymphocytes, and other cells [52]. Raygan *et al.* studied 60 patients with diabetes and coronary artery disease, patients were randomly assigned to one of two groups: (placebo or 10 mg melatonin). For a period of 12 weeks, melatonin had the expected effects on glycemic control, HDL-cholesterol, CRP, MDA, NO, and GSH [53]. The etiology of diabetic retinopathy is complicated by inflammation and oxidative stress. As a result, medications like melatonin that reduce the inflammatory and oxidative effects of diabetes mellitus may have a significant influence on individuals by avoiding or reducing retinal complications [30].Melatonin, through a nuclear factor-kappa B-dependent mechanism, lowered the expression of inducible nitric oxide synthase and prevented the overexpression of inflammatory cytokines (TNF-α) and CRP [54].

It was found that an experimental thermal shock causes OS and a strong inflammatory response, as well as an elevation in CRP levels, which can be reversed by melatonin treatment [55].Melatonin's anti-inflammatory properties can be related to its ability to inhibit pro-inflammatory cytokines as well as its ability to reduce the production of various adhesion molecules such intercellular adhesion molecules, vascular cell adhesion molecules, and endothelial cell selection [56, 57]. Further studies are required to investigate the role of other agents for safe, efficient, and cost effective surrogate treatment for T2DM. Metals are important in a variety of metabolic pathways, including glucose metabolism. Zinc oxide, silver, gold, and core-shell silver-gold

nanoparticles have all been shown to have anti-diabetic properties [58-60]. Furthermore, [61] discovered that in human primary adipocytes, superparamagnetic iron oxide nanoparticles suppress the majority of high-risk genes involved in the development of T2DM.

Table (3): Inflammatory markers in each group at varied treatment periods.

Group	TNF-α (pg/ml)			CRP (µg/ml)		
	0 week	6 weeks	12 weeks	0 week	6 weeks	12 weeks
Placebo (n=13)	65.92±9.25	69.0±8.18	69.46±9.42 *	5.22±1.88	6.05±2.01	5.25±1.79
Flaxseed (n=16)	60.44±6.78	61.69±9.27 †	61.31±8.14 †	5.04±2.26	4.13±1.58 **†	3.09±1.45 **†
Melatonin (n=14)	63.36±6.82	59.43±8.23 *†	51.36±6.33 **††	5.68±1.09	4.85±1.01 **	4.06±0.92 **

Comparison with baseline: *= significant (P<0.05),**=highly significant (P<0.001).
Comparison with placebo over the corresponding duration: †= significant (P<0.05), ††= highly significant (P<0.001).

4. Conclusions

Melatonin outperformed Flaxseed oil in terms of glycemic control and TNF-α, while Flaxseed oil outperformed Melatonin in terms of CRP. According to the findings, using flaxseed oil or melatonin as an adjuvant medication could help with diabetes management in T2DM patients via mechanisms of upregulating peripheral tissue responses to available insulin at receptor levels in conjunction with potent antioxidant effects.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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