MELATONIN AND QUERCETIN IMPROVE DEPRESSIVE-LIKE BEHAVIOR AND DYSLIPIDEMIA IN STREPTOZOTOCIN-INDUCED DIABETES IN RATS

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ABSTRACT

This study was performed to examine and assess the beneficial effects of melatonin and quercetin on depressive disorders and the disturbance of lipid metabolism caused by diabetes mellitus. Diabetes was induced in male rats via intraperitoneal streptozotocin injection (70 mg/kg). Experiments were performed 72 h later. Diabetic rats were treated with melatonin or quercetin during 4 weeks. The anxiolytic and antidepressant-like effects of these two drugs were investigated in the last week of experiment, using the open-field and forced swimming tests. In the last day of the study, plasma insulin and lipid profile were determined in different experimental groups. Treatment with melatonin or quercetin significantly ameliorated anxiety-like and depressive-like behaviors. Furthermore, both melatonin and quercetin reversed the aspects of dyslipidemia observed in rats with diabetes, but the effect of the melatonin was more potent than quercetin, considering the dose used during this study.

Keywords: Diabetes, Melatonin, Quercetin, Mood disorders, Lipid profile.

INTRODUCTION

Diabetes mellitus is a serious metabolic disease, threatening increasingly the public health in the world. Chronic hyperglycemia resulting from defects in either insulin secretion or tissue insulin resistance, which often associated with metabolic abnormalities and severe complications, affecting several organs and systems, such as the heart, kidneys, eyes, blood vessels and the central nervous system (Van Dieren *et al*., 2010). Numerous studies demonstrated the existence of a strong relationship between mood disorders and diabetes and its impact on neuropsychological functioning of diabetic patients (Robinson *et al*., 2013). It was reported that depressive-like behavior and mood disorders, observed during experimental diabetes are due to alterations in monoaminergic systems, serotonergic neurotransmission in particular (Lackovi and Salkovic., 1990; Haider *et al*., 2013).

Melatonin is an indolamine secreted by the pineal gland during the dark phase of the daily light/dark cycle, it is synthesized from the tryptophan. It has been shown that this hormone endowed with antioxidant activity, but also its ability to decrease lipid peroxidation and normalize dyslipidemia in diabetes (Donder *et al*., 1999). Additionally, melatonin could reverse the depressive-like behavior (Brotto et *al*.**,** 2000). This is frequently examined using the forced swimming test (FST) and the open-field test (OFT), which are animal models predictive of anxiolytic and antidepressant-like effects of drugs (Overstreet *et al* ., 2003; Prut and Belzung, 2003).

Quercetin is a flavonoid found in various vegetables and fruits, and known for its anti-diabetic properties that were the subject of several scientific reports in the last years (Ibarra *et al.,* 2014; Eid *et al*., 2015). Recently, several studies have shown the antidepressant activity of quercetin (Holzmann *et al.,* 2015; Demir *et al.,* 2016), and its beneficial effects against disorders of lipid metabolism. (Kobori *et al*,. 2011).

The aim of this study was to assess the ameliorative effects of melatonin and quercetin on depressive-like behavior and dyslipidemia in diabetic rats induced by STZ.

MATERIALS AND METHODS

Animals: Male Wistar rats (Pasteur institute, Algiers, Algeria), weighing (180-210g), were used for the experimental procedures. In order to avoid the animal stress, the animals were subjected to controlled conditions of illumination and temperature room (light cycle of 12 hours day/night, temperature was about 23-25 °C), with free access to food (standard diet) and water. All advice for the handling of laboratory animals were followed (Council of European Communities, 1987).

Chemical products: Melatonin was supplied by Sigma Chemical Co. (St. Louis. MO. USA), Streptozotocin and absolute alcohol were obtained from (Sigma-Aldrich, Germany), while Quercetin was purchased from (Solgar laboratory, France).

Induction of Diabetes and Drug Administration: Before the diabetes induction, the animals were randomly divided into two groups. The first group (Normal control rats, n= 7) was injected with the citrate buffer only.

Diabetes was induced in rats by intraperitoneal injection of a single dose of STZ at the rate of 70 m/kg body weight, STZ was prepared in citrate buffer (pH= 4.5, 0.1 M) (Rushita *et al*., 2013). Hyperglycemia was confirmed 72 hours after STZ injection using a handheld

glucometer (Accu Chek Performa) from a blood sample was taken via the tail vein and confirmed again at ending of the behavioral tests. Only rats with blood glucose levels ≥ 250 mg/dL were considered diabetic.

After diabetes installation, diabetic rats were divided into 3 subgroups: The first subgroup (n= 6) received a daily injection of vehicle consisting of 15% ethanol (Diabetic group), the second subgroup (n = 6) was injected by melatonin daily for 4 weeks with a dose of 1 mg/kg (ip) (Diabetic + melatonin) group. The third subgroup (n = 7) received a daily dose of quercetin at the rate of 10 mg/kg (ip) for 4 weeks (Diabetic + quercetin) group.

Melatonin and quercetin treatment was started 3 days after the diabetes induction, melatonin was dissolved in an amount of ethanol, then underwent dilution until the concentration of ethanol became 15%. The required amount of quercetin for injection was also dissolved in ethanol 15%. The administration of the solvent, melatonin and quercetin was performed after 13:30 pm. All the drugs were injected in a constant volume of 1 ml/kg body weight.

Behavioral experiments

Open field test (OFT): Open-field test is often used to study exploratory behavior and locomotor activity in rats. The apparatus used is a square box of plexiglass and consist of square (70×70cm) with high walls (50×50 cm). It is divided into two parts of equal size: a central part and peripheral part. The central part serves as a starting point for the animals. The movement of the animal allows to measure the number of squares crossed and the time spent in each area. Therefore, this test shows the anxious behavior, which is more pronounced when the rat spends more time in the peripheral zone. Variables measured in this test are: the total distance crossed, the immobility time, the number of entries into the central part and the number of adjustments. (This test was performed on the 26th day of melatonin and quercetin administration).

Forced swimming test (FST): Forced swimming test is an animal model frequently used to predict the efficacy of antidepressant treatment (Porsolt *et al*.,1977). The test takes place in rats in two sessions, the pre-test (FST1) and the test (FST2), separated by an interval of 24 hours. The pre-test (on the 27th day of melatonin and quercetin administration) consists of placing the animal for 15 minutes in an aquarium (54cm×30cm×36cm) in diameter, filled with water (26 ° C) to 40 cm height in which the rat cannot use his lower limbs to keep the surface or leak out which submit it to the forced swimming. At the end of this session the rat was immobile. The second session lasts 5 minutes during which the immobility time, the swimming and climbing were measured.

Blood Collection and Determination of biochemical parameters: At the end of the 4 weeks of experiment. All rats were fasted for 16 hours and blood samples were obtained by retro-orbital puncture using micro-hematocrit capillary tube, previously rinsed with EDTA 0.01%. Blood samples were collected in heparinized tubes for determination of blood parameters.

Insulin determination was performed with a chemiluminescence enzyme immunoassay method, using automate analyses (Cobas ®**,** Roche Diagnostics, France). Plasma lipid determination, such as triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol were performed by an enzymatic colorimetric methods (Kit Spinreact, Girona, Spain).

Statistical analysis: Results were expressed as mean ± standard deviation (S.D). The data were analyzed by one-way analysis of variance (ANOVA) and Newman-Keuls test was used for post hoc analyses. Differences were considered statistically significant when $P<0.05$. Graph Pad Prism 5 version 5.04 for windows was used to do analysis.

RESULTS

Effect of melatonin and quercetin on open field test and forced swimming test parameters

Open field test: Diabetic rats showed a significant decrease of total distances crossed and number of adjustments with an increase of immobility time in comparison to normal control group ($P<0.001$, $P<0.001$, $P<0.05$, respectively; Table 1). Melatonin administration increased significantly the locomotor activity by increasing the total distance crossed and the number of inputs at the center and decreasing of immobility time in comparison to diabetic control group ($P<0.001$, $P<0.05$, $P<0.001$, respectively; Table 1). Similar results were obtained for the total distance crossed and immobility time in diabetic rats treated with quercetin ($P<0.05$, Table 1). However, the melatonin effect on the open-field parameters was greater than quercetin, as regards the total distance crossed and immobility time ($P<0.05$, $P<0.01$, respectively; Table 1).

Forced swimming test: In our study, we noted a significant increase of immobility time in diabetic rats compared to normal control group ($P<0.001$, Table 2). Melatonin treatment caused a reduction in immobility time and a significant increase of swimming and climbing time in comparison with the untreated diabetic rats ($P<0.001$, $P<0.001$, $P<0.05$, respectively; Table 2). Quercetin in his turn reduced significantly the immobility time and increased the swimming and climbing time in diabetic rats ($P<0.001$, $P<0.05$, respectively; Table 2). Melatonin reduced the immobility time and increased the swimming time more effectively than did quercetin ($P<0.05$, $P<0.001$; respectively; Table 2).

Insulin and plasma lipid profile: The obtained results show a significant decrease ($P<0.05$, Table 3) of plasma insulin in different diabetic groups treated or not with melatonin and quercetin, compared with normal control group. Triglycerides levels, total cholesterol and LDL-cholesterol levels were increased significantly ($P<0.001$, Table 3) compared with those in the normal control group. As against the HDL-cholesterol levels significant was decreased significantly in this group ($P<0.05$, Table

3). The administration of melatonin changed effectively the high levels of triglycerides, total cholesterol and LDL-cholesterol (P<0.001, Table 3), also the HDL-cholesterol levels was significantly increased (P<0.05, Table 3). Quercetin showed a significant effect on lipid profile in diabetic rats (P<0.001, Table 3), but there was no significant difference in regards to the melatonin and quercetin effects on HDL-cholesterol levels in the diabetic rats.

Table 1. Behavioral parameters on open-field test in the different groups of rats.

Behavioral parameters	Normal control group	Control diabetic group	Diabetic + melatonin group	Diabetic + quercetin group
Total distance crossed (cm)	1027.00±116.80	464.30±123.40^{aa}	1230.00±188.10^{c}	989.90±132.20$^{c, d}$
Immobility time (sec)	162.50±12.79	227.50±17.81^{aa}	181.80±19.26^{c}	203.10±16.88^{b,e}
Center square entries	3.00±1.41	1.16±1.17	4.16±1.94^{b}	2.85±2.03
Number of adjustments	8.28±1.97	3.66±1.96^{a}	5.33±2.94	5.00±3.46

^{a}P<0.05 vs. normal control group, ^{aa}P<0.001 vs. normal control group, ^{b}P<0.05 vs. control diabetic group, ^{c}P<0.001 vs. control diabetic group, ^{d}P<0.05 vs. Diabetic+melatonin group, ^{e}P<0.01 vs. Diabetic+melatonin group.

Table 2. Behavioral parameters in the forced swimming test in the different groups of rats.

Behavioral parameters	Normal control Group	Control diabetic group	Diabetic + melatonin group	Diabetic +quercetin group
Immobility time (sec)	69.80±10.11	127.00±23.00^{aa}	39.02±11.10^{c}	62.92±16.10^{c,d}
Swimming time (sec)	118.80±12.86	58.74±12.41^{aa}	160.00±9.44^{c}	117.40±19.89^{c,ee}
Climbing time (sec)	111.80±12.17	86.61±13.45^{a}	105.30±10.46^{b}	115.70±15.71^{b}

^{a}P<0.05 vs. normal control group, ^{aa}P<0.001 vs. normal control group, ^{b}P<0.05 vs. control diabetic group, ^{c}P<0.001 vs. control diabetic group, ^{d}P<0.05 vs. Diabetic+melatonin group, ^{e}P<0.01 vs. Diabetic+melatonin group, ^{ee}P<0.001 vs. Diabetic+melatonin group.

Table 3. Plasma insulin and lipid profile in the different groups of rats.

Biochemical parameters group	Normal control group	Control diabetic group	Diabetic + melatonin	Diabetic +quercetin
Insulin (ng /ml)	6.14±1.46	4.538±8.61^{a}	4.570±0.80^{a}	4.03±1.09^{a}
Triglycerides (mg /dL)	195.60±8.61	281.4±10.16^{aa}	227.20±14.53^{c}	253.10±12.31^{c,e}
Total cholesterol (mg /dL)	102.71±5.08	138.60±2.14^{aa}	106.19±3.10^{c}	113.50±4.01^{c,e}
LDL cholesterol (mg /dL)	54.39±5.18	96.65±5.10^{aa}	69.66±3.59^{c}	82.06±4.77^{c,ee}
HDL cholesterol (mg /dL)	35.47±4.70	27.32±3.92^{a}	35.28±5.27^{b}	36.37±5.07^{b}

Biochemical parameters	Normal control group	Control diabetic group	Diabetic + melatonin group	Diabetic +quercetin group
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Total cholesterol (mg /dL)	102.71±5.08	138.60±2.14^{aa}	106.19±3.10^{c}	113.50±4.01^{c,e}
LDL cholesterol (mg /dL)	54.39±5.18	96.65±5.10^{aa}	69.66±3.59^{c}	82.06±4.77^{c,ee}
HDL cholesterol (mg /dL)	35.47±4.70	27.32±3.92^{a}	35.28±5.27^{b}	36.37±5.07^{b}

^{a}P<0.05 vs. normal control group, ^{aa}P<0.001 vs. normal control group, ^{b}P<0.05 vs. control diabetic group, ^{c}P<0.001 vs. control diabetic group, ^{d}P<0.05 vs. Diabetic+melatonin group, ^{e}P<0.01 vs. Diabetic+melatonin group, ^{ee}P<0.001 vs. Diabetic+melatonin group.

DISCUSSION

Findings of this study support the hypothesis that diabetes mellitus plays a role in the development of depression-like behavior and anxiety in diabetic patients. Induction of diabetes with STZ altered locomotor activity in the open-field test. Melatonin administration attenuated the anxiogenic behavior, this is proved by the increase of total distance crossed and the decrease of the immobility time. Similar results were reported by Golus and King, (1981). In this context, it has been postulated that the anxiolytic effect of melatonin is related to melatoninergic receptors (MT2) stimulation (Ochoa-Sanchez *et al.,* 2012).

In addition, diabetic rats showed a high immobility time compared to non diabetics in the forced swimming test, reflecting despair-like behavior (Wayhs *et al.,* 2010). This is may be linked to the dysregulation of serotonergic neurotransmission which is involved in the pathophysiology of depression (Haider *et al.,* 2013). On the other hand, melatonin could reverse behavioral alterations, indicated by a lower immobility time, which explains its antidepressant-like effect (Ergun *et al.,* 2006; Detanico *et al.,* 2009).

It is well known that antidepressant-like activity of melatonin, comes from its interaction with the serotonergic system (Micale *et al*., 2006) and more precisely with 5-HT2A receptors, where it could act as an antagonist (Eison *et al.,* 1995).

In the current study, we have noted that quercetin administration in diabetic rats improved locomotor activity in the open-field test, by the increasing the total distance crossed and the decreasing the immobility time. Our study demonstrated clearly the antidepressant-like effect of quercetin in diabetic rats submitted to forced swimming test, when quercetin reduced the immobility time. Available reports suggest that quercetin inhibits the monoamine oxidase, an enzyme involved in the metabolism of monoamines in the brain (Lee *et al*., 2001). The role of quercitin in the modulation of several neurotransmitter systems, such as serotonin, GABA and nitric oxide has also been studied (Bonilla-Jaime *et al.,* 2015). In this regard, Lee *et al.,* 2005 reported that quercetin may regulate the serotonergic neurotransmission by inhibiting 5-HT3 receptors and it is well known that 5HT3 receptor antagonists exert anxiolytic action (Harmer *et al.,* 2006). There are also reports that quercetin might have beneficial effects on corticotrophin releasing factor (CRF) induced anxiety and depression-like behaviors (Bhutada *et al.,* 2010).

On plasma parameters, ours results show no effect of melatonin and quercetin on insulin levels in diabetic rats, this is probably due to the administration of these two drugs which was done after development of the diabetes. In the current, it was also found that melatonin significantly reduced the triglycerides, total cholesterol and LDL-cholesterol levels, while it increases HDL-cholesterol levels. This result is in agreement with those provided by Montilla *et al*., (1998). Quercetin administration to diabetic rats drastically improved lipid profile by decreasing the triglycerides, total cholesterol and LDL-cholesterol levels and increasing HDL-cholesterol levels. These findings correlate with the results reported by Jeong *et al*., (2012).

In conclusion, melatonin and quercetin could prevent the complications of diabetes mellitus, either by their beneficial effects in diabetic patients suffering depressive disorders or, even by its ameliorative effect on dyslipidemia. Melatonin seems more effective than quercetin, as regards the improvements in depressive behavior and lipid profile.

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