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EFFECTS OF MELATONIN AND FLUOXETINE ON OXIDATIVE STRESS PARAMETERS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Résumé

L'hyperglycémie chronique s'accompagne d'une production excessive des radicaux libres par divers mécanismes. Ceci est un signe indicateur du stress oxydatif témoin des complications chroniques liées au diabète sucré. L'objectif de cette étude est d'évaluer les effets bénéfiques de la mélatonine et de la fluoxétine sur les désordres du métabolisme lipidique induits par le diabète, ainsi que sur les paramètres du stress oxydatif au niveau des érythrocytes chez les rats rendus diabétiques suite à une injection intrapéritonéale de streptozotocine (60 mg/kg). Il a été noté que les deux traitements exercent un effet régulateur sur la glycémie et les paramètres du profil lipidique, cependant seule la mélatonine pourrait diminuer la lipoperoxydation et améliorer le statut antioxydant des érythrocytes.

Mots clés: *Diabète sucré, Mélatonine, Fluoxétine, Profile lipidique, Stress oxydatif.*

Abstract

Chronic hyperglycemia is accompanied by excessive production of free radicals by various mechanisms. This is an indicator of the oxidative stress that is involved in the worsening of chronic complications related to diabetes mellitus. The objective of this study was to evaluate the beneficial effects of melatonin and fluoxetine on diabetes-induced lipid metabolism disorders, as well as on oxidative stress parameters in erythrocytes of diabetic rats following an intraperitoneal injection of streptozotocin (60 mg/kg). It was noted that both treatments exert a regulatory effect on blood glucose and lipid profile parameters, however only melatonin could decrease the lipid peroxidation and improve the antioxidant status in erythrocytes.

Key words: *Diabetes mellitus, Melatonin, Fluoxetine, Lipid profile, Oxidative stress*

ملخص

يرتبط ارتفاع السكر في الدم المزمن مع الإفراط في إنتاج الجذور الحرة من خلال آليات مختلفة . يعد هذا علامة منبهة لأكسدة التي تشارك في تفاقم المضاعفات المزمنة لمرض السكري . الهدف من هذه الدراسة هو تقييم التأثيرات المفيدة لميلاتونين والفلوكستين على اضطرابات استقلاب الدهون الناجمة عن مرض السكري وكذا على تأشيرات الأكسدة في خلايا الدم الحمراء عند الجرذان المصابة بداء السكري بعد الحقن داخل الصفاق بالستربتوزوتوسين (60 ملغ / كلغ). لوحظ أن لكلا العلاجان تأثير تنظيمي على مستوى السكر في الدم وعلى تأشيرات الدهون، لكن وحدها الميلاتونين قادرة على تقليل من بيروكسيد الدهون وتحسين وضع مضادات الأكسدة في الكريات الحمراء.

الكلمات المفتاحية: *داء السكري، الميلاتونين، الفلوكستين، مستوى الدهون ، الإجهاد التأكسدي*

Chronic hyperglycemia caused by diabetes mellitus is seem to be frequently associated with disorders of the general metabolism and characterized by an overproduction of free radicals by various mechanisms (Matough *et al*., 2012; Tangvarasittichai, 2015). These free radicals are the main cause of metabolic abnormalities and degenerative complications of diabetes mellitus that could affect several organs and functions.

The use of animal models in experimental diabetes, particularly STZ-induced diabetes, allows a better understanding of the pathophysiology of type 1 diabetes which is characterized by its classic symptoms, like weight loss, polydipsia, polyphagia and polyuria (Cooke & Plotnick, 2008). In addition, diabetes induced by injection of STZ, is characterized by an accumulation of free radicals which cause degeneration and necrosis of pancreatic β cells.

Other studies (Chawla *et al*., 2014) have shown that diabetes mellitus is accompanied by alterations in the different pathways of carbohydrate, lipid and protein metabolism, facilitating the implementation of different processes which might be involved in the aggravation of vascular complications. In this context, it has been demonstrated that the accumulation of advanced glycation end products (AGE) induced by the elevation of non-enzymatic protein glycation and auto-oxidation of glucose, lead to an increase in oxidative stress (Rahimi *et al*., 2005; Guillet, 2010).

Melatonin an indolamine responsible for controlling the circadian biological cycles, which also intervenes at other levels to act as an antioxidant involved in the cellular protection systems of organisms, because of its ability to neutralize many free radicals and improve the activity of antioxidant enzymes (Tan *et al*., 2002; Rodriguez *et al*., 2004).

Currently, fluoxetine which is a selective serotonin reuptake inhibitor, widely used in clinical practice for treatment of depressive disorders and anxiety for its effects less troublesome than other antidepressants. Numerous studies show that the fluoxetine plays a dominant role in the restoration of oxidative damage by improving cellular antioxidant status following a decline caused by oxidative stress (Behr *et al*., 2012; Erman *et al*., 2015). Besides these effects, this drug could influence carbohydrate and lipid homeostasis during diabetes mellitus (Gülseren *et al*., 2005; Biagetti & Corcoy, 2013).

The objective of this study was to evaluate the beneficial effects of melatonin and fluoxetine on the parameters of oxidative stress in erythrocytes and on the disturbances of lipid and glucose metabolism following the installation of diabetes.

MATERIAL AND METHODS

Animals

Adult male *Wistar* rats (Pasteur institute, Algiers, Algeria) were used in this study, aged ten weeks and weighing 200 to 260 g. They are reared in polyethylene cages, lined with a litter of wood chips and acclimatized for 3 weeks under laboratory conditions (a normal temperature, a regular day/night light system and free access to food and water).

Chemicals

All chemicals including melatonin and fluoxetine were all obtained from Sigma (St. Louis, MO).

Induction of diabetes and drug administration

Diabetes was induced by a single intraperitoneal injection of STZ of 60 mg / kg dissolved in citrate buffer (0.1 M, pH 4.5). The rats were initially divided into two groups, the control group (non diabetic, n=7) which was injected (ip) with saline, the rats of the other group received a single dose of STZ. Three days after the injection of STZ, the confirmation of the diabetes installation was performed from the lateral tail vein by using a glucometer. Only rats with glycemia > (250 mg/dl) were considered diabetic (n=19) and selected for the experiments. Diabetic rats were randomly divided into three groups: the first diabetic group (Diabetic, n = 6) who received injections of physiologic saline. The second group (Diabetic + melatonin, n = 6), treated daily with melatonin (10 mg / kg, ip) which was dissolved in 1% ethanol. The third group (Diabetic + Fluoxetine, n = 7) which received fluoxetine (15 mg / kg, ip) diluted in physiological saline. For each group the treatment was given for 28 days.

Determination of biochemical parameters

- Glucose level and lipid profile

On the last day of this experiment, blood samples were taken from the retro-orbital venous plexus using a heparinized microcapillary tubes to measure biochemical parameters such as serum glucose, profile lipid parameters. The blood collected in dry tubes undergoes centrifugation at 3000 rpm/min for 15 minutes; the obtained serum is stored at -20°C for subsequent use.

Glucose level was measured by enzymatic colorimetric method using commercial Kit (Spinreact, Spain). Triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol were determined by commercial kit (Biolabo, France).

- Oxidative stress parametres

The RBCs were hemolyzed by the addition of two volumes of distilled water and then incubated for 15 min in the refrigerator, then the mixture was centrifuged at 4000 rpm/min for 10 minutes to remove cell debris. The supernatant constitutes the erythrocyte lysates which will serve for the determination of oxidative stress parameters.

Malondialdehyde (MDA) determination in erythrocytes was carried out according to the method of Buege & Aust (1984), MDA is the final product of lipid peroxidation and reacts with thiobarbituric acid (TBA) to form an absorbent complex at 530 nm.

Reduced glutathione (GSH) assay is based on the reaction of the reducing group (SH) contained in the sample and 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB). This reaction releases an aromatic derivative, thionitrobenzoic acid (TNB) measured at 412 nm (Ellman, 1959).
Catalase (CAT) activity was determined by the method of Aebi. (1984), The reaction is based on the degradation of hydrogen peroxide (H_2O_2). This activity is measured at 240 nm.

The concentration of total proteins in erythrocytes was determined by the assay of Bradford (1976), using bovine serum albumin (BSA) as standard.

Statistical analysis

Results were expressed as means ± standard error of mean (SEM). 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 $P < 0.05$.

RESULTS

Effect of treatment on body weight, water intake and food intake

During our daily monitoring of water consumption for 28 days, we found that the volume of water consumed increased significantly ($P<0.001$; table 1) in diabetic group compared to non-diabetic. On the other hand, our results showed no significant effect after treatment with melatonin and fluoxetine in diabetic rats.

The results illustrated in Table 1 show that daily food consumption has increased considerably in diabetic rats compared to non-diabetics ($P<0.001$; table 1). Nevertheless, there was no change in food consumption in the diabetic group treated with melatonin and fluoxetine.

This study showed a reduction in body weight in the untreated diabetic group when compared to non-diabetic control at the 4th week ($P<0.001$; table 1) of treatment. However, we did not report any recovery of body weight in the diabetic animals treated with melatonin and fluoxetine.

Effect of treatment on serum glucose and lipid profile

The results obtained show an increase in serum glucose level of diabetic rats compared to those without diabetes ($P<0.001$; table 1). On the other hand, daily administration of melatonin and fluoxetine for 28 days reduces glycemia in diabetic animals ($P<0.05$, $P<0.001$, respectively; table 1).

In this study, we noted a highly significant increase in total and LDL-cholesterol in the diabetic group when compared to non-diabetic ($P<0.01$, $P<0.001$, respectively; table 1), But a significant reduction of these two parameters was noted only in the diabetic animals treated with melatonin ($P<0.05$; table 1). With respect to HDL-cholesterol, we noted a decrease in this parameter in diabetic rats compared to normal rats and no treatment had influence on HDL-cholesterol levels in diabetic rats. We also found that triglyceride levels of diabetic animals increased significantly in comparison with control rats ($P<0.001$; table 1), by contrast for diabetics treated with melatonin and fluoxetine, we noted a decrease in triglyceridemia ($P<0.01$, $P<0.01$, respectively; table 1).

Effect of melatonin and fluoxetine treatment on oxidative stress parameters of erythrocytes

We have noticed an increase in lipid peroxidation in erythrocytes of diabetic rats when compared to controls ($P<0,01$; fig. 1), and a significant decrease in GSH levels was observed in the same group of rats ($P<0,01$; fig. 1), In contrast, melatonin treatment has been able to prevent the increase of lipid peroxidation and depletion of GSH in erythrocytes of diabetic animals ($P<0.05$, $P<0.01$, respectively; fig. 1).

In addition, CAT activity decreased significantly in the diabetic group compared to control group ($P<0.05$; fig. 1). But, no change in activity of this enzyme was observed in diabetic rats treated with melatonin or fluoxetine.

Table 1. Effect of melatonin and fluoxetine treatment on water intake, food intake and body weight in control and diabetic rats

Group	water intake (ml/24)	Food intake	Body weight (g)	
(g/24h)				
			Initial	Final
Non diabetic	52.71±5.00	16.51±1.82	231.50±5.15	263.60±2.55
Diabetic	93.83±5.11***	31.92±2.35***	230.00±4.31	178.10±4.40***
Diabetic + melatonin	97.33±3.67	27.73±1.83	234.30±3.10	179.10±3.98
Diabetic +fluoxetine	100.70±2.52	28.04±1.09	231.60±3.10	169.20±4.28

*** P< 0.001 vs. Non diabetic group.

Table 2. Effect of melatonin and fluoxetine treatment on blood glucose and lipid profile parameters

Biochemical parameters	Non diabetic	Diabetic	Diabetic + melatonin	Diabetic +fluoxetine
Glucose (mg /dL)	91.78±6.91	296.90±14.06***	240.10±22.03#	179.40±16.99###
Triglycerides (mg /dL)	124.60±9.09	256.00±15.79***	184.50±13.84##	193.81±12.16##
Total cholesterol (mg /dL)	101.60±5.23	140.30±6.69**	112.80±7.41#	126.90±5.60
LDL-cholesterol (mg /dL)	47.39±4.62	93.32±7.48***	66.32±5.81#	83.49±5.34
HDL-cholesterol (mg /dL)	35.87±3.61	26.49±2.34	35.44±4.17	29.94±4.41

** P<0.01; *** P<0.001 vs. Non diabetic group. # P<0.05; ## P<0.01; ### P<0.001 vs. Diabetic group.

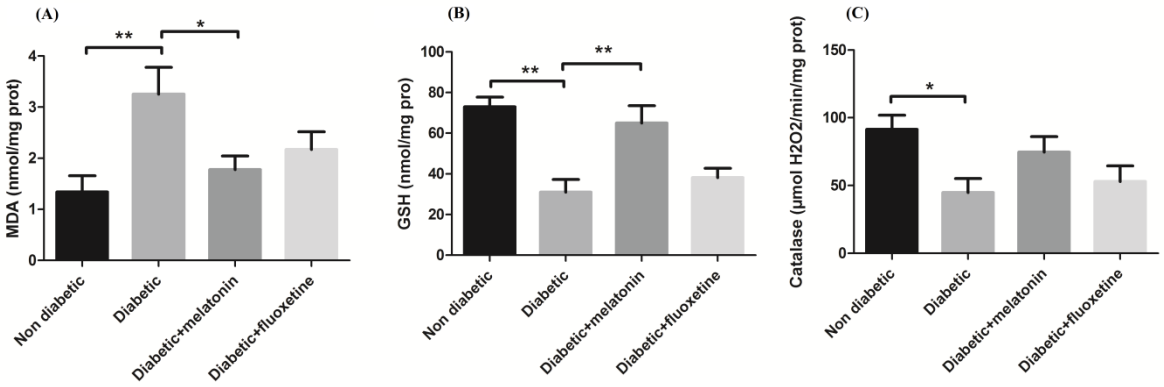

Fig. 1: Measurement of oxidative stress parameters in erythrocytes after 28 days of melatonin and fluoxetine treatment in the experimental groups. (A): MDA (Malondialdehyde). (B): GSH (Reduced glutathion). (C): Catalase activity. (* p<0.05; ** p<0.01).

DISCUSSION

In the present study, induction of diabetes by STZ resulted in a loss of body weight in rats. This reduction in body weight can be attributed to the acceleration of lipid and protein catabolism caused by peripheral non-use of glucose by insulin-sensitive tissues, which leads to muscle atrophy and loss of tissue proteins (Widmaier *et al*., 1995). Under our experimental conditions, prolonged treatment with melatonin and fluoxetine does not cause any change in body weight in diabetic animals. These results are in agreement with the findings of Ha *et al*., 1999: Jiang *et al*., 2016, who reported that treatment of diabetic rats with melatonin had no effect on body weight.

Polyphagia is one of the major characteristics of diabetes mellitus, the feeling of hunger, implies that glucose in the blood cannot be used by the cells due to lack of insulin. Indeed, during our experimental period, we observed a significant increase in dietary intake in diabetic rats compared to normal rats. Similar results have been reported by Akbarzadeh *et al*. (2007). In addition, our results showed polydipsia in diabetic rats, from the first days of diabetes. These results are in line with those of literature (Akbarzadeh *et al*., 2007; Haider *et al*., 2013).

In our study, we found that STZ caused a very high levels of cholesterol and triglycerides in the diabetic group, which is consistent with the results of Elberry *et al*. (2015); Adam *et al*. (2016); Achi *et al*. (2017). However, an improvement in lipid profile was observed in melatonin treated diabetic rats, which could be explained by decreased triglyceride, total cholesterol and LDL-C levels. These results support those described by (Baydas *et al*., 2002; Anwar *et al*., 2003; Agil *et al*., 2011). On the other hand, the effect of fluoxetine on dyslipidemia seems lower than that of melatonin, we noted an improvement only in triglyceridemia in diabetic rats treated with fluoxetine. This result confirms the one previously recorded by our laboratory, namely a reduction in triglyceridemia in diabetic rats receiving fluoxetine (Rebai & Boudah, 2016). In this context, Daubresse *et al*. (1996) and Ye *et al*. (2011) report that fluoxetine decreases hypertriglyceridemia in patients with type 2 diabetes.

Because of their high oxygen consumption, high content of polyunsaturated fatty acids (PUFAs) and low enzymatic antioxidant defense, erythrocytes show increased susceptibility to oxidative stress induced by diabetes. The results of the present study showed an imbalance of redox state in erythrocytes. This can be explained by MDA rate which has increased significantly in diabetic animals, and the decrease in GSH levels following their action of neutralization of free radicals. The same effects have been demonstrated by other authors in experimental diabetes induces a disturbance of the antioxidant system, an increase in lipid peroxidation and depletion of GSH in erythrocytes (Vural *et al*., 2001; Ozkol *et al*., 2013). Treatment of diabetic animals with melatonin prevented depletion of GSH and reduced lipoperoxidation. It should be noted that fluoxetine does not appear to have any beneficial effect on erythrocyte redox status. These results demonstrate the antioxidant properties of melatonin already reported by several studies (Montilla *et al*.,1998; Vural *et al*., 2001; Anwar and Meki, 2003).

CONCLUSION

This study showed that melatonin and fluoxetine have a protective effect against metabolic disorders caused by diabetes mellitus, specifically on blood parameters, they improve lipid profile and reduce hyperglycemia, but melatonin is endowed with strong antioxidant activity than fluoxetine.

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