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Research Article

Effects of Fluoxetine Administration on Plasma Lipids and Depressive-Like Behavior Induced by Lipid Peroxidation in Cerebral Cortex in Diabetic Rats

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ABSTRACT
Usually, depression is more common in people with diabetes than the general population. The objective of this study was to explore the effects of fluoxetine on lipid peroxidation in the brain, also on plasma lipid levels in diabetic rats after intraperitoneal injection of streptozotocin (60mg/kg). Diabetic rats were treated with fluoxetine for three weeks. The evaluation of depressive-like behavior and locomotor activity was done by using of two behavioral tests, the open field (OFT) and forced swimming (FST). On the last day of the experiment, blood samples were taken to determine blood glucose and plasma lipids, as well as the determination of malondialdehyde (MDA) in the cerebral cortex. It has been noted that treatment of diabetic rats with fluoxetine, significantly increased locomotor activity and reduces the immobility time in (FST). In addition, the same treatment decreases the lipid peroxidation in the cerebral cortex and shows a regulatory effect on glucose and plasma triglycerides.

Keywords: Diabetes, Fluoxetine, Depression, Plasma Lipids, Lipid Peroxidation.

INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder responsible for many degenerative complications and disturbances in glucose, lipid and protein metabolism. The impact of diabetes on the central nervous system has been well documented during the last decade and particularly on neurobehavioral axis[1,2]. Recent studies show that chronic hyperglycemia and overproduction of reactive oxygen species (ROS), resulting in decreased enzymatic antioxidant defense system and high glucose auto-oxidation, are considered the main causes of neurodegenerative diseases[3,4]. It is now known that the onset of depressive-like behavior in people with diabetes is strongly linked to an alteration of the main components of the cell structure of neurons. These cell damage induced by free radicals are more pronounced in neuronal membranes by reason of their high contents of polyunsaturated fatty acids[5]. Many antidepressants are used to treat depressive episodes, but the choice of an adequate antidepressant treatment in depressed subjects suffering from diabetes has become a very important step, given the influence of these drugs on quality of life. In addition, many studies have highlighted the impact of different antidepressants on glucose homeostasis[6,7]. It was noted that selective serotonin reuptake inhibitors (SSRIs) exert regulatory effect on lipid metabolism in diabetic patients[8], thus the fluoxetine treatment in diabetes increases insulin sensitivity and reduces hyperglycemia[9]. The objective of this study was to assess the beneficial effects of fluoxetine administration on dyslipidemia and lipid peroxidation in the cerebral cortex. The latter may be associated with the occurrence of depressive-like behavior in diabetic rats submitted to two behavioral tests, the open field test (OFT) and the forced swim test (FST).

MATERIELS AND METHODS

Animals

Male adult Wistar rats (Pasteur Institute, Algiers, Algeria) were used in this study, (3 months of age, body weight: 200-250g). The animals are subjected to rearing conditions of the animal; temperature 23 °C with 12 hour light / dark cycle. The rats were given free access to water and food, also all the advice for the use and protection of laboratory animals were followed (Council of European Communities, 1987).

Chemicals

Streptozotocin (STZ) and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St-Louis, MO, USA) and fluoxetine was obtained from laboratory (Merinal, Oued S'mar, Algiers, Algeria), while thiobarbituric acid (TBA) was purchased from (Sigma-Aldrich, Germany).

Induction of diabetes and treatment

Diabetes was installed by a single intraperitoneal (ip) injection of 60 mg / kg of STZ, which was previously prepared in citrate buffer (pH= 4.5, 0.1 M). Diabetes confirmation was performed after 72 hours of STZ injection, on a blood sample taken from the caudal vein using a glucometer (Accu Chek Performa). Only rats with

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Table 1: Behavioral parameters of the open field test in experimental groups.

Behavioral parameters	Treatment groups			
	Nondiabetic	Diabetic	Nondiabetic + Fluoxetine	Diabetic + Fluoxetine
Immobility time (sec)	144.90 ± 13.71	207.10 ± 9.74***	109.90 ± 11.08	136.30 ± 7.01$^{\#\#\#}$
Total distance crossed (cm)	961.80 ± 81.46	584.40 ± 87.86***	1014.00 ± 58.31	1008.00 ± 103.20$^{\#\#\#}$
Center square entries	3.40 ± 1.67	0.83 ± 0.75*	3.71 ± 1.60	1.66 ± 1.63
Number of adjustments	7.80 ± 1.92	3.16 ± 2.13**	8.42 ± 3.40	8.33 ± 1.72$^{\#\#}$

$^{*}p <0.05$, $^{**}p <0.01$, $^{***}p <0.001$ vs. Nondiabetic group. $^{\#\#}p <0.01$, $^{\#\#\#}p <0.001$ vs. Diabetic group.

Table 2: Various behavioral parameters of forced swimming test in experimental groups

Behavioral parameters	Treatment groups			
	Nondiabetic	Diabetic	Nondiabetic + Fluoxetine	Diabetic + Fluoxetine
Immobility time (sec)	72.20 ± 7.63	127.00 ± 19.40***	49.30 ± 11.80	64.20 ± 19.60$^{\#\#\#}$
Climbing time (sec)	108.00 ± 8.55	81.90 ± 12.00***	103.00 ± 7.74	108.00 ± 8.17$^{\#\#\#}$
Swimming time (sec)	106.10 ± 6.90	59.65 ± 13.42***	149.50 ± 9.55	125.20 ± 12.70$^{\#\#\#}$

$^{***}p <0.001$ vs. Nondiabetic group. $^{\#\#\#}p <0.001$ vs. Diabetic group.

blood glucose levels ≥ 250 mg / dL were considered diabetic. After the diabetes induction, the rats were divided into 4 groups and were treated for 21 days as follows:

The first group (nondiabetic control, n= 5) received intraperitoneal injection of saline (0.9% NaCl). The second group (diabetic control, n = 6) a daily intraperitoneal injection of saline (0.9% NaCl). The third group (Non diabetic + fluoxetine, n = 7) was treated with fluoxetine (20 mg/kg, ip). The fourth group (diabetic + fluoxetine, n = 6) was treated with fluoxetine (20 mg/kg, ip). Fluoxetine was prepared in saline (0.9% NaCl). The volume injected in experimental groups was 0.5 ml/kg body weight.

Behavioral study

Open-field test (OFT)

This test was performed on the 18th day of the experiment. It is designed to measure locomotor activity and the anxiety developed in rats, it consists in placing the animal into a apparatus with a base surrounded by plexiglass parapets whose measures are (70cm × 70cm × 40cm) respectively. This apparatus is divided into 2 equal areas, a peripheral part and a central part which serves as a starting point of the animal for each test. The test takes 5 minutes during which the following variables will be measured: the total distance crossed, the number of entries into the central part, the immobility time and the number of adjustments.

Forced swimming test (FST)

This is one of the most widely used tests for predicting the antidepressant activity and anxiolytic effect of drugs[10]. This is carried out by placing rats in an aquarium of 40 cm high and 30cm × 30cm in size, filled with water at 25 °C, so that the rat does not use his legs to escape by clinging to the edges of the aquarium. The test was conducted in two phases separated by 24 hours. During the first phase (performed on the 20th day of the experiment), the rat was placed for 15 minutes in the aquarium for causing a behavioral despair. The second phase takes 5 minutes during which, swimming time, immobility time and climbing time are recorded.

Determination of biochemical parameters

All rats were decapitated on the last day of the experiment, and a blood collection was carried out through the retro-orbital sinus using a capillary hematocrit washed with EDTA (0, 01%). The collected blood was recovered in heparinized tubes for the determination of different blood parameters. Glucose and plasma lipids were determined by the enzymatic colorimetric method (Kit Spinreact, Girona, Spain). The determination of lipid peroxidation parameter, malondialdehyde (MDA) and the proteins were done in the cerebral cortex. Tissue was homogenized in 5 mL of solution phosphate buffered saline (PBS) (0.1M, pH 7.4) and centrifuged at 10 000 × g for 20 min at 4°C. The obtained supernatant was stored at -20 °C until proteins and MDA determination. The MDA level was measured by the method of Buege and Aust[11]. The assay is based on the reaction of MDA which is the final product resulting from the peroxidation of polyunsaturated fatty acids. In hot acidic medium, the thiobarbituric acid (TBA) reacts with MDA to form a colored complex with peak absorbance at 530 nm. The amount of tissue MDA was expressed in nanomoles per milligrams of protein. The amount of MDA in the tissue was expressed in nanomoles per milligrams of protein. The protein determination in tissues was performed according to the method of Bradford[12].

Statistical analysis

Results were expressed as mean ± standard deviation (S.D), and the analysis of these results was performed by using oneway analysis of variance (ANOVA), followed by Newman-Keuls post hoc comparison. The difference between groups is considered significant when $p <0.05$.

RESULTS

Effect of fluoxetine on the open field test

The obtained results showed that the increase in immobility time in diabetic rats was highly significant in comparison with non-diabetic rats ($^{***}p <0.001$, table1), also a significant decrease in the total distance crossed, the number of entry in the center part and the number of adjustments in the diabetic group compared to non-diabetic rats ($^{***}p <0.001$, $^{*}p <0.05$, $^{**}p <0.01$, respectively; table1). Treatment with fluoxetine significantly increased ambulatory activity, by increasing the total distance crossed, the number of adjustments and the reduction of immobility time in diabetic rats treated with fluoxetine compared to untreated ($^{\#\#\#}p <0.001$,. $^{\#\#}p <0.01$,. $^{\#\#\#}p <0.001$, respectively; table 1).

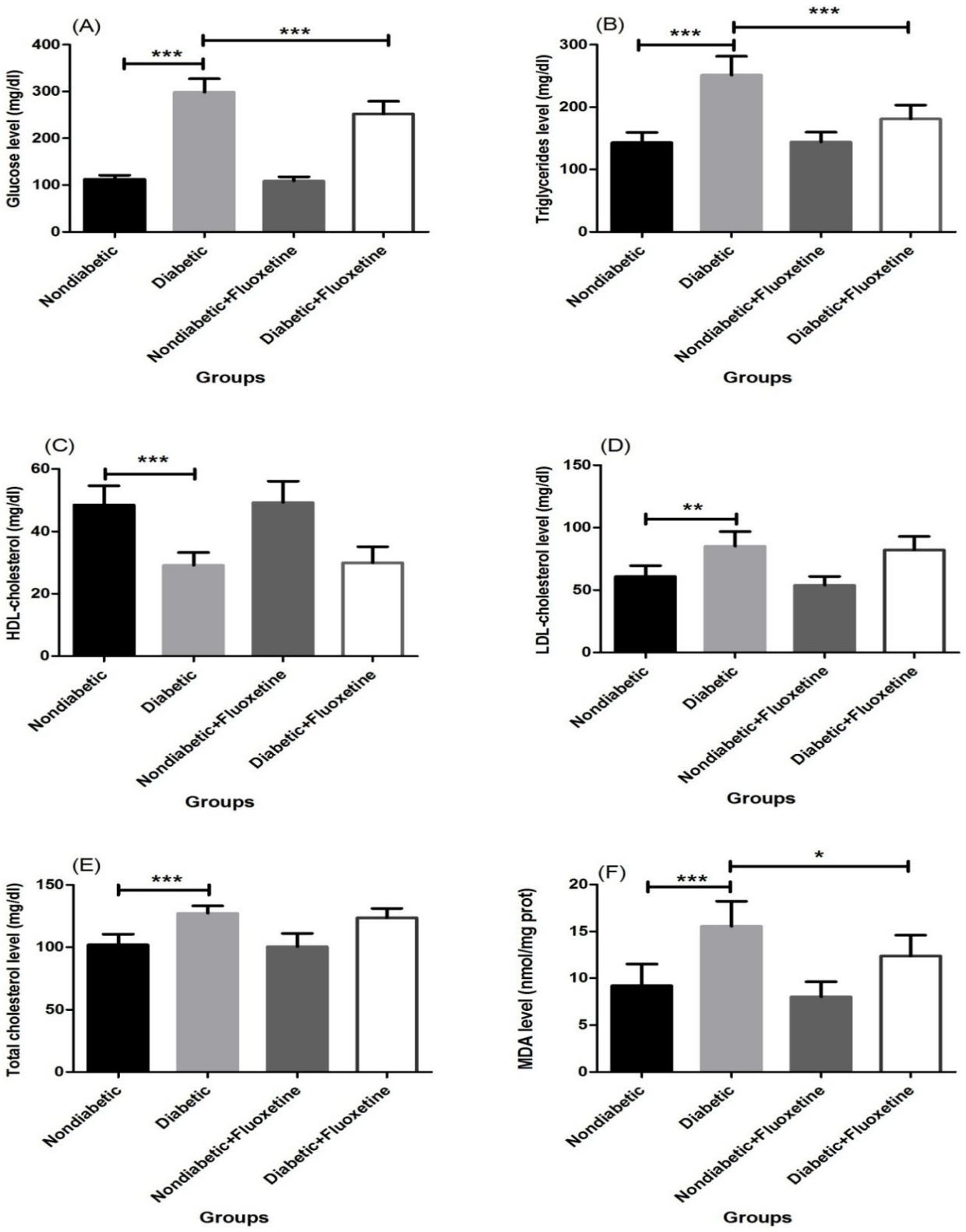

Figure 1: Measurement of biochemical parameters in different groups. (A): Plasma glucose. (B): Triglycerides. (C): HDL-cholesterol. (D): LDL-cholesterol. (E): Total cholesterol. (F): MDA level in cerebral cortex. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Effect of fluoxetine on the forced swimming test(FST)

Our results show that the immobility time was significantly increased in diabetic rats compared with controls ($^{***}p < 0.001$, table 2), whereas climbing and swimming time were decreased significantly in the diabetic group compared to non-diabetic group ($^{***}p < 0.001$, $^{***}p < 0.001$, respectively; table 2). In diabetic rats treated with fluoxetine, we recorded a significant decrease in immobility time compared to untreated diabetic rats ($^{\#\#\#}p < 0.001$, table 2), and we also noted a significant increase

in climbing and swimming time in diabetic group receiving fluoxetine compared to diabetic control group ($^{###}p$ <0.001, $^{###}p$ <0.001, respectively; table 2).

Effect of fluoxetine on biochemical parameters

Glucose and plasma lipids

The results of this study showed a significant elevation of plasma glucose in diabetic rats in comparison with non-diabetic rats ($^{***}p$ <0.001, fig. 1). However, fluoxetine administration in the diabetic group caused a highly significant decrease in glucose compared to untreated diabetic group ($^{***}p$ <0.001, fig. 1). We noted a significant increase in triglycerides, LDL-cholesterol, total cholesterol and a decrease in HDL-cholesterol in diabetic rats compared to non-diabetic rats ($^{***}p$ <0.001, $^{**}p$ <0.01, $^{***}p$ <0.001, $^{***}p$ <0.001, respectively; fig. 1). while, fluoxetine administration in the diabetic group significantly decreased plasma glucose level compared to diabetic control rats ($^{***}p$ <0.001; fig. 1) and induced an improvement in triglycerides ($^{***}p$ <0.001, fig.1). On the other hand, no change observed, regarding the concentrations of LDL-cholesterol, total cholesterol and HDL-cholesterol in diabetic rats treated with fluoxetine.

lipid peroxidation

In this study, we noticed that the MDA level in the cerebral cortex was significantly increased in diabetic rats compared to the non-diabetic group ($^{***}p$ <0.001, fig.1). By cons, treatment with fluoxetine diabetic group significantly reduced the MDA level compared to the untreated diabetic group ($^{*}p$ <0.05, fig.1).

DISCUSSION

Numerous studies have shown that metabolic disorders caused by the persistence of the diabetic state, contributed directly to the onset of depressive-like behavior and reduce locomotor activity in rodents[13]. In this study, we assessed the depressive state and ambulatory activity in diabetic rats by two tests, the open field and forced swimming test. The results show a decrease in locomotor activity in diabetic animals, as well as increased of immobility time in the FST. Treatment with fluoxetine, was able to reverse the depressive and anxious behavior in diabetic rats, this could be explained by the improvement of active behaviors during these two tests. Biochemically, our results showed that chronic hyperglycemia and severe insulin deficiency, observed in diabetes mellitus are associated with lipid peroxidation induced by overproduction of reactive oxygen species (ROS) in the brain. These free radicals are the main cause of the changes observed in brain structures, in view of their high fat content, specifically polyunsaturated fatty acid of neuronal membranes, which leads to cognitive and neurobehavioral deficits[14]. Fluoxetine treatment decreased remarkably the MDA level in the cerebral cortex. In this capacity, several studies have investigated the cytoprotective effects of fluoxetine, like a drug that could restrict excessive production of calcium ions (Ca^{2+}), which directly promotes the generation of (ROS)[15,16]. In addition, other studies have shown the ability of SSRIs such as fluoxetine to inhibit P450 cytochromes, a set of enzymes that may be involved in oxidative stress[17]. At the level of peripheral tissues, diabetes induction caused an impaired glucose metabolism. In our study, the fluoxetine administration in diabetic rats reduced glucose level, this indicates a hypoglycemic effect of this drug[7,18]. Our study, reveals very high levels of triglycerides, LDL-cholesterol, total cholesterol and low levels of HDL-cholesterol induced by diabetes installation. However, we noted that fluoxetine could repair this hypertriglyceridemia, which is in agreement with the results reported by[19], which demonstrated the beneficial effect of fluoxetine on elevated levels of triglycerides that characterizes the metabolic abnormalities in diabetes mellitus. In conclusion, fluoxetine could reduce several complications of diabetes by its protective properties vis-a-vis lipid peroxidation of cell membranes, also by its regulatory effect on glucose homeostasis, which make it a recommended treatment for diabetic patients with depression.

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