Egyptian Journal of Basic and Clinical Pharmacology
June 2012, Vol. 2., No. 1: 1-12
http://www.ejbcp.eg.net/

Original Article

Fluoxetine, not Paroxetine, Ameliorates Vascular Dysfunctions in Diabetic/Depressed Rats via Pro-inflammatory Cytokines 'MCP-1 and TNF-α' and Metabolic mechanisms

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A B S T R A C T

Multiple evidences indicate that depression is more prevalent in diabetic subjects than in the general population and increases the risk of vascular complications in diabetes too. Nevertheless, little information is available on vascular effects of antidepressant drugs in diabetes. The current study used diabetic rats exposed to chronic restraint stress (CRS), an animal model of depression, to investigate the vascular effects of selective serotonin reuptake inhibitors 'fluoxetine (FLU) and paroxetine (PAR)'; in diabetic/depressed subjects. Possible role of proinflammatory cytokines, monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) and metabolic changes were investigated as well. For induction of type II diabetes (DM), rats were exposed to high fat diet and streptozotocin (35 mg/Kg, *i.p.*). Diabetic/depressed rats exhibited endothelial dysfunction, confirmed by a significant increase in aortic ring phenylephrine contractile response and Intima/Media ratio as well as a decrease in acetylcholine-dependent relaxation, and these effects were associated with significant elevation of MCP-1 and TNF-α levels in serum and aortic tissue and metabolic dysfunctions, evidenced by alterations in blood glucose, insulin, lipids and insulin sensitivity. Chronic treatment with FLU (10 mg/kg/day, *i.p.*) significantly ameliorated the DM/CRS-induced endothelial and metabolic dysfunctions that were worsened by PAR (10 mg/kg/day, *i.p.*). Moreover, FLU, not PAR reduced the elevated levels of MCP-1 and TNF-α. The present results suggest that chronic treatment with FLU improves vascular dysfunctions in diabetic/depressed rats, partially via its potent anti-inflammatory effect and other via reversing metabolic abnormalities. Conversely, PAR aggravated these diabetic complications. Nevertheless, the antidepressant effect of both drugs appeared to be attenuated in diabetic/depressed rats.

Key Words: Fluoxetine, MCP-1, TNF-α, Paroxetine, Diabetic rats, Endothelial dysfunction

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1. INTRODUCTION

The concurrence of depression and diabetes is a serious problem. Among people with diabetes, whose risk of depression is 50–100% greater than the general population, depression is associated with higher complication and mortality rates *(Rubin et al., 2008)*. Moreover, depression may impair control of glycemia and treatment compliance, as well as increasing the risk of vascular complications in diabetes *(Gomez et al., 2001)*. Therefore, treatment of depression is necessary to improve the quality of life of diabetic patients, to increase treatment compliance, and to decrease the risk of macro-/micro-vascular complications *(Hofmann, 2010)*. However, the response to antidepressants suggested to be altered in diabetics *(Anderson et al., 2010)*.

Moreover, treatment with antidepressants has been suggested to increase risk of diabetes and alter glucose homeostasis in diabetic individuals that could further complicate glycaemic control, which is a limiting factor to prevent or delay microvascular complications *(Andersohn et al., 2009)*. Indeed, several studies indicated that the effect of selective serotonin reuptake inhibitors (SSRIs) on glycemic control is quite controversial *(Kivimäki and Batty, 2012)*. Although, *Paile-Hyvärinen et al. (2007)* reported that paroxetine did not change glucose level in diabetic patients, many

case reports suggested hypoglycemia associated with the use SSRIs, paroxetine, fluoxetine and sertraline in diabetic and nondiabetic patients *(Sawka et al., 2001 ; Zammit, 2012)*. A recent experimental study revealed that paroxetine reduced blood glucose levels in normoglycemic mice *(Kadioglu et al., 2011)*. In contrast, other researchers noted hyperglycemia with paroxetine *(Petty, 1996)*, fluvoxamine and fluoxetine *(Yamada et al., 1999; Carvalho et al., 2004)*.

Vascular inflammation and cardiovascular disease are the leading causes of morbidity and mortality in the diabetic population and remain major public-health issues. TNF-α, IL-6, IL-8, and MCP-1 are proinflammatory cytokines and widely recognized markers of vascular inflammation. The levels of these cytokines are elevated in the blood of many diabetic patients. An increase in circulating levels of TNF-α, IL-6, IL-8, and MCP-1 can lead to increased insulin resistance, vascular inflammation, and the development of vascular disease *(Sushil et al., 2009)*. A recent study demonstrates that fluoxetine suppresses inflammatory responses in lung tissue and inhibits the expression of the inflammatory cytokines IL-1β, TNF-α, MCP-1 and intercellular adhesion molecule-1 (ICAM-1) *(Li XQ et al., 2011)*. Nevertheless, there is no previous study has examined the effect of fluoxetine or paroxetine on the levels of TNF-α, or MCP-1 in diabetic depressed subjects either in clinical or experimental context.

Treatment of depression in diabetic patients is common; however, there is no sufficient data about the effect of antidepressant drugs on the vascular abnormalities associated with diabetes, Consequently, the present work was conducted in diabetic rats exposed to chronic restraint stress, an animal model of depression, to investigate the vascular effects of SSRIs, fluoxetine and paroxetine, in diabetic/depressed subjects; likewise, the possible mechanism through pro-inflammatory cytokines, MCP-1 and TNF-α and metabolic changes was examined.

2. MATERIALS AND METHODS

2.1. Drugs and chemicals:

Fluoxetine hydrochloride and paroxetine hydrochloride hemihydrate powders were dissolved in saline just prior to intraperitoneal injection. Streptozotocin powder was dissolved in 0.05 M citrate buffer, pH= 4.5 *(Srinivasan et al., 2005)*. All drugs and chemicals were purchased from Sigma-Aldrich chemicals Co., USA.

2.2. Animals:

Adult male Wistar rats weighing 200-250 g were obtained from the Egyptian Organisation for Biological Products and Vaccines (VACSERA, Helwan, Egypt). Animals were housed in standard laboratory conditions under a 12 h light/dark cycle, humidity of 55-65% and temperature of 22 ± 3°C, with free access to food and water. The animal experiments were conducted according to the regulations of the Committee of Bioethics of Faculty of Medicine, Ain Shams University, Cairo, Egypt.

2.3. Experimental Models:

Model of type II diabetes "DM" *(Srinivasan et al., 2005)*.

To obtain insulin resistant type II diabetes, rats were fed with high-fat diet (HFD) (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, for 2 weeks followed by low dose streptozotocin (35 mg/kg, *i.p*.).

Model of Chronic Restraint Stress "CRS" *(Bravo et al., 2009)*.

The stress procedure was carried out in a different room. Rats were placed in Plexiglas tubes (25X 8 cm) wide enough to allow comfortable breathing but restricting their movement, for 3 h a day during 5 weeks. Every stress session was carried out between 09.00 Am and 12.0 Pm to avoid any effects because of changes in circadian rhythms.

2.4. Experimental groups:

Fifty-six male Wistar rats were divided into three main groups. Group I; (naive group, n=8); fed with standard rat pellet diet for 10 weeks and not exposed to stress. Group II; (CRS group, n=24) fed standard diet in the first 2 weeks followed by exposure to CRS for 5 weeks and then subdivided into 3 subgroups each of 8 animals; saline-treated CRS, FLU-treated CRS; received fluoxetine 10mg/Kg/day, *i.p. (Ciulla et al., 2007)* and PAR-treated CRS; received paroxetine 10mg/Kg/day, *i.p (Casarotto and Andreatini, 2007)* for 3 weeks after CRS and examined for behavioural tests at the end of 10^{th} week. Group III; (DM-CRS group, n=24); fed with HFD for 2 weeks followed by low dose STZ, *i.p*. for induction of diabetes and then exposed to CRS for 5 weeks. DM-CRS rats were subdivided into 3 subgroups each of 8 animals; saline-treated DM-CRS, FLU-treated DM-CRS and PAR-treated DM-CRS group which received drugs for 3 weeks after CRS then examined at the end of 10^{th} week.

2.5. Behavioural Tests: *(Tõnisaar et al., 2008).*

Forced-swim test: Rats were forced to swim in a vertical glass cylinder (diameter 22.5 cm, height 60 cm) containing water, 35 cm height, maintained at ≈ 25˚C. On the first day, the rats were trained to swim for 15 min. Two days later rats were re-exposed to the forced swimming for 5 minutes. Behaviour was videotaped and immobility time was measured with a stopwatch. Immobility time is the time during which the animal floated on the surface and made only those movements which were necessary to keep it afloat.

Open field examination*:* Rats were placed to the centre of a rectangular arena (60×100 cm), which was divided into 15 equal squares. Parameter registered during 5 min was the number of squares crossed by four feet on one square.

2.6. Vascular tests:

Measuring systolic blood pressure (SBP)

At the end of 10^{th} week, SPB was measured by using indirect tail cuff plethysmography (ADI instrument, Australia). The inflated cuff pressure was computed using power lab/85p (ML 785 software program).

Isolated rat's aortic ring: *(Lorkowska et al., 2006).*

After measuring SBP and taking blood samples from anesthetized rat, the chest was rapidly opened and the descending thoracic aorta was separated and removed to a Petri dish containing Krebs-Henseleit solution. Aortic rings (5-6 mm width) were mounted between 2 parallel stainless steel hooks in a 15 ml organ bath, gassed with 95% O_2 and 5% CO_2 and the temperature was 37ºC. An initial resting tension of 4 g was set and the rings were then allowed to equilibrate for 1-1.5 hours. Isometric responses were measured with a force transducer (K30, Hugo Sacks Electronics, Freiburg, Germany) connected to a bridge coupler type 570 and the trace was displayed on a two-channel recorder (Lineacorder, HSE, WR 3310). Cumulative dose-response curve was constructed by cumulative addition of phenylephrine (30nM- 30 µM) to the bath and EC50 and E max were determined for each curve to assess vascular reactivity. After reaching the plateau of the phenylephrine–induced submaximal contraction (10µM), the rings were relaxed by exposure to a stepwise increase in acetylcholine (ACh) concentration (3µM – 1mM) to assess endothelium-dependent relaxation. Sodium nitroprusside-induced relaxation (Endothelium-independent relaxation) was also assessed using increasing concentrations of sodium nitroporusside (1nM-10µM). The percent relaxation in phenylephrine-induced pre-contraction was determined for all groups and computed.

2.7. Metabolic tests:

Insulin tolerance test *(Zhang et al., 2008).*

At the end of 10^{th} week, insulin (0.75 IU/kg, *i.p.*) was administered and blood glucose concentrations were measured at 0, 30, 60, 90 and 120 minutes by using gluco-check apparatus. The value was presented as a percentage of initial blood glucose level

Body weight

Body weights at the end of 10^{th} week were measured and expressed as mean ±SD g change in comparison to initial body weight.

2.8. Biochemical tests:

Fasting rats were anesthetized with urethane (1.2 g/Kg *i.p.*). Blood samples were collected via retro-orbital approach, centrifuged for 15 min to obtain serum that was immediately stored at -80˚C until assayed for:

Total cholesterol, triglyceride and HDL levels (mg/dl) were determined by enzymatic colorimetric method using detection kits (Greiner Diagnostic Gmbh, Germany). **Low density lipoprotein** (LDL) was calculated using the Friedewald formula *(Friedewald et al., 1972)***:** LDL = Total cholesterol – (HDL + Triglyceride/5).

Fasting glucose level was measured using "GLUCOSE Colorimetric PAP Detection Kit" (Greiner Diagnostic Gmbh, Germany).

Fasting insulin was determined by using insulin ELISA kits (MEDGENIX-INS-EASIA, Biosource, Europ S.A). **Insulin resistance:** was derived from fasting serum glucose and insulin using electronic HOMA (homeostasis model for assessment of insulin resistance) calculator program (Version 2.2.2 released 12 December 2007 by Diabetes Trials Unit, University of Oxford, UK, www.dtu.ox.ax.uk/homa).

MCP-1 and TNF-α protein in aortic tissue homogenate and serum

MCP-1 level was determined using ELISA kit for rat MCP-1 (Camarillo, CA, USA). TNF-α protein was determined using commercially available rat TNF-α ELISA kit (Raybiotechmique ®, USA) according to the manufacturer's instructions.

2.9. Histopathological tests:

Abdominal aorta was dissected, part of it was taken for cytokines biochemical study and the other was fixed in 10% formalin. Hematoxylin-Eosin staining was performed on 5 µm sections. Neutrophil sequestration was determined by counting the number of neutrophils using image analyzer (Leica Q 500 MC program, Heidelberg, Germany) in 5 different areas of the aorta, each 200 x 200 µm area , and the numbers obtained from areas were averaged for each animal. Intima/Media Ratio (IMR) = width of intima /width of media at maximal intima thickness**.** IMR was measured as an index of early atherosclerosis *(O'Leary et al., 1999)*.

Statistical analysis:

The results were expressed as means ± S.D. Statistical analysis was performed using computer program "GraphPad Prism", USA, version 4 (2005). Results were analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni's post hoc test to determine differences between treatment groups. Statistical comparisons for nonparametric data in OFT were analyzed using Kruskal-Wallis test followed by post hoc, Dunn's multiple comparisons test. Repeated measures two-way ANOVA was the test utilized for insulin tolerance test to determine the effects of drugs and time factors. Differences were statistically significant at $P< 0.05$.

3. RESULTS

3.1. Effect of fluoxetine (FLU) and paroxetine (PAR) on depressive-like behavior in forced swimming test (FST) and open field test (OFT):

Figure (1) reveals that diabetic and non-diabetic rats exposed to CRS exhibited depressive behavior in the form of significant (P<0.001) elevation in the immobility time when exposed to FST and reduction in the number of crossed squares when exposed to OFT in comparison to control rats. Moreover, this depressive behavior significantly (P<0.001) increased in diabetic rats compared to non-diabetic rats. FLU and PAR significantly (P<0.05) reduced immobility time (FST) in diabetic/stressed rats but this effect was significantly lower than that in non-diabetic rats *(P*<0.001). Both FLU and PAR significantly increased the number of crossed square (OFT) only in non-diabetic rats (P<0.01*&* P<0.001 respectively), while, in diabetic/stressed rats they produced insignificant effect on OFT.

3.2. Effect of FLU and PAR on vascular function in diabetic/depressed rats

DM-CRS rats showed significant (P<0.01) elevation of systolic blood pressure. Both FLU and PAR reduced SBP; however, this reduction was only significant (P<0.05) with FLU (Table 1).

DM-CRS induced significant increase in vascular reactivity to phenylephrine (PhE) which was evidenced by reduction in the EC50 (0.221±0.097 µM vs 0.573±0.125 µM; *P<0.01*) and elevation of Emax (0.79±0.18 vs 0.51±0.09 g tension; *P<0.05*) as compared to control group (Mean ± SD). Endothelial-dependent relaxation was impaired in aorta from rats exposed to DM-CRS evidenced by significant *(P<0.005)* reduction in the maximum % relaxation induced by 1mM acetylcholine (ACh). FLU significantly ameliorated PhE Emax (0.68±0.09 g tension) and EC_{50} (0.340±0.097 µM) and increased ACh-induced relaxation (P<0.01, P<0.05 & P<0.01; respectively) in comparison to DM-CRS vehicle-treated group. Conversely, PAR produced significant (P<0.05) reduction in % ACh–induced relaxation as compared with DM-CRS vehicle-treated group (Figure 2 A & B).

There was no significant difference in sodium nitroprusside-induced relaxation between groups (data not shown).

3.3. Effect of FLU and PAR on metabolic abnormalities in diabetic/depressed rats

It is clear from table (1) that chronic treatment with paroxetine produced significant increase in body weight gain (P<0.001); while, FLU significantly (P<0.05) reduced body weight gain compared to DM-CRS vehicle-treated group. DM+CRS induced significant elevation in serum total cholesterol, triglyceride and LDL; while, insignificantly affected HDL in comparison to control group. Chronic treatment with FLU significantly ameliorated these elevations. On the opposite, PAR induced significant deterioration in lipid profile compared to DM-CRS vehicle-treated group.

Table (2) indicates that DM-CRS induced significant elevation in blood glucose (P<0.001), reduction in blood insulin (P<0.05) and increased insulin resistance by HOMA calculator (P<0.05) compared to control group. Chronic treatment with FLU significantly reduced blood glucose (P<0.05) and conversely, PAR significantly increased (P<0.05) blood glucose compared to DM-CRS group. Both FLU and PAR insignificantly affected insulin level. FLU reduced HOMA index (mean ± SD) from 2.81 ±

0.64 to 2.11 ± 0.56, however this effect was statistically insignificant (P>0.05) compared to DM-CRS group.

In insulin tolerance test, glucose concentration declined rapidly after insulin administration in control group; while, in diabetic/stressed rats showed significant (P<0.001) reduction in insulin response denoting the development of a diabetic model which is an analogue to type 2 diabetes mellitus with insulin resistance and hyperglycemia. At the 120th minute sample, chronic treatment with FLU significantly improved the insulin response by reducing glucose concentrations (P<0.001) versus DM-CRS vehicle-treated group. In opposition, PAR induced more insulin resistance evidenced by reduction in insulin response; though, it did not reached to a significant level (Figure 3).

3.4. Effect of SSRIs on serum and aortic proinflammatory cytokines, MCP-1 and TNF-α level in diabetic/depressed rats.

DM-CRS rats exhibited significant (P<0.001) increase in serum MPC-1 and TNF-**α** level as compared with control group. FLU, but not PAR significantly reduced MPC-1 and TNF-**α** levels (P<0.01, P<0.001 respectively) in comparison to DM-CRS vehicle-treated group (Figure 4A &B). The same was noticed in aortic tissue homogenate, exposure to DM-CRS induced significant increase in MPC-1 and TNF- **α** levels (P<0.001, P<0.01 respectively) compared to control group and FLU significantly ameliorated MPC-1 and TNF- **α** levels (P<0.001, P<0.0 respectively) compared to diabetic vehicle-treated group. Although, PAR produced insignificant effect on serum pro-inflammatory cytokines it could produce significant (P<0.05) elevation in MPC-1 in aortic tissue (Figure 5 A & B).

3.5. Effect of tested drugs on aortic intima/ media ratio (IMR) and neutrophil infiltration in diabetic/depressed rats:

Exposure to DM-CRS model induced significant (P<0.001) increase in aortic IMR compared to control group. Chronic treatment with FLU significantly (P<0.01) ameliorated IMR in comparison to DM-CRS vehicle-treated group. On the opposite, PAR showed significant (P<0.05) increase in IMR compared to DM-CRS vehicle-treated group (figure 6). DM-CRS model produced marked infiltration of aortic subendothelium and adventitia with neutrophil cells, indicating vascular wall inflammation and FLU, but not PAR, could produce significant (P<0.01) reduction in average neutrophil count compared to DM-CRS, denoting a potent anti-inflammatory effect (Figure 7 A & B).

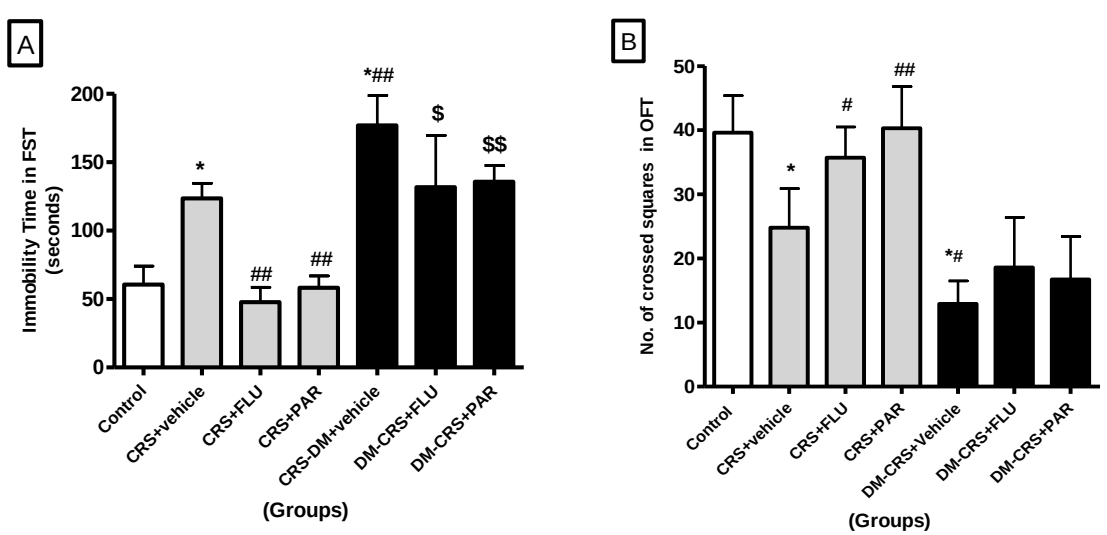

Figure 1: Effect of chronic treatment with fluoxetine (FLU), 10 mg/kg/day i.p, and paroxetine (PAR), 10 mg/kg/day i.p, on: (A) Immobility time (seconds) in forced swimming test (FST), and (B) The number of crossed squares in open field test (OFT) of diabetic and non-diabetic rats exposed to chronic restraint stress (CRS). Data are means ± SD; (n = 6-8/ group). *P<0.001 *vs* control group; $^{\#}P$<0.01, $^{\#\#}P$<0.001 *vs* CRS + vehicle treated group; $^{\$}P$<0.05 *vs* DM-CRS + vehicle treated group.

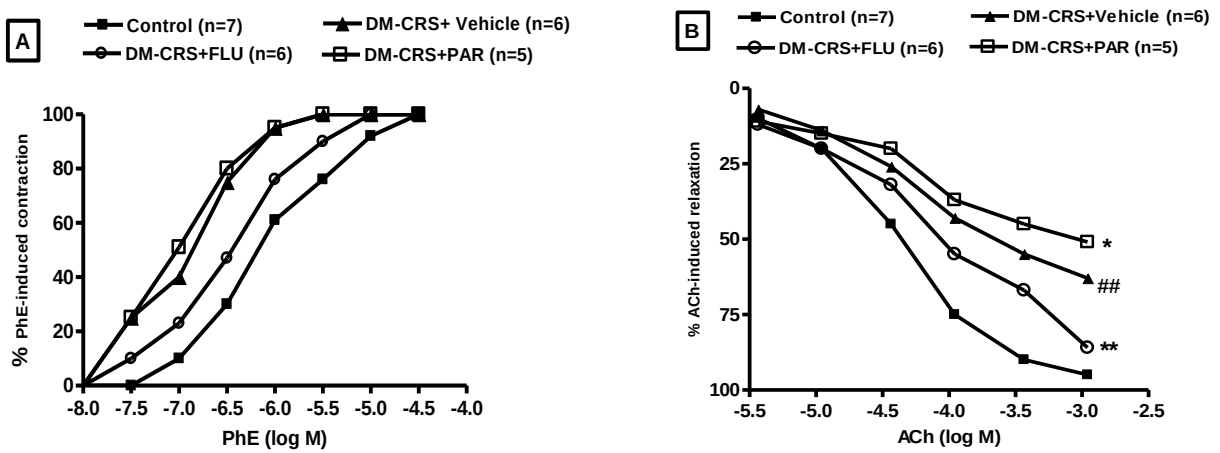

Figure 2: Effect of chronic treatment with fluoxetine (FLU), 10 mg/kg/day, *i.p*, and paroxetine (PAR), 10 mg/kg/day, *i.p*, on phenylephrine (PhE)-induced contraction (A) and % acetylcholine (ACh)-induced relaxation (B) in isolated aortic ring of diabetic Wistar rats exposed to chronic restraint stress (CRS). Data are means; $^{\#\#}P$<0.005 in *vs* control group; $^{*}P$<0.05, $^{**}P$<0.01 *vs* DM-CRS + vehicle treated group.

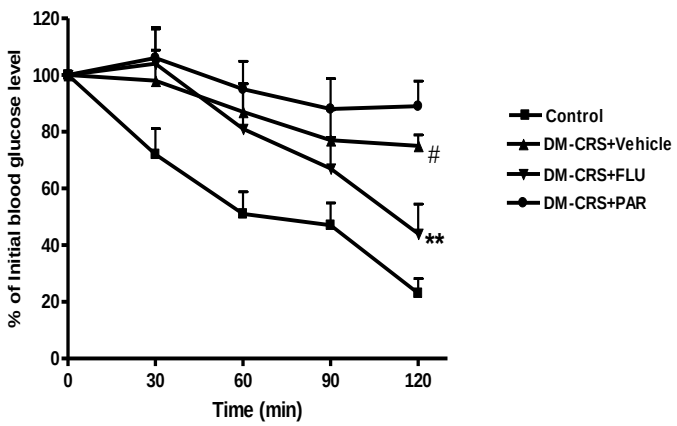

Figure 3: Effect of chronic treatment with fluoxetine (FLU), 10 mg/kg/day i.p, and paroxetine (PAR), 10 mg/kg/day i.p, on percentage of initial blood glucose level during insulin tolerance test (ITT) in diabetic Wistar rats exposed to chronic restraint stress (CRS) at 0, 30, 60, 90 and 120 minutes. Data are means ± SD; (n = 5/ group). $^{\#}P$<0.001 *vs* control group; $^{**}P$<0.001 *vs* DM-CRS + vehicle-treated group.

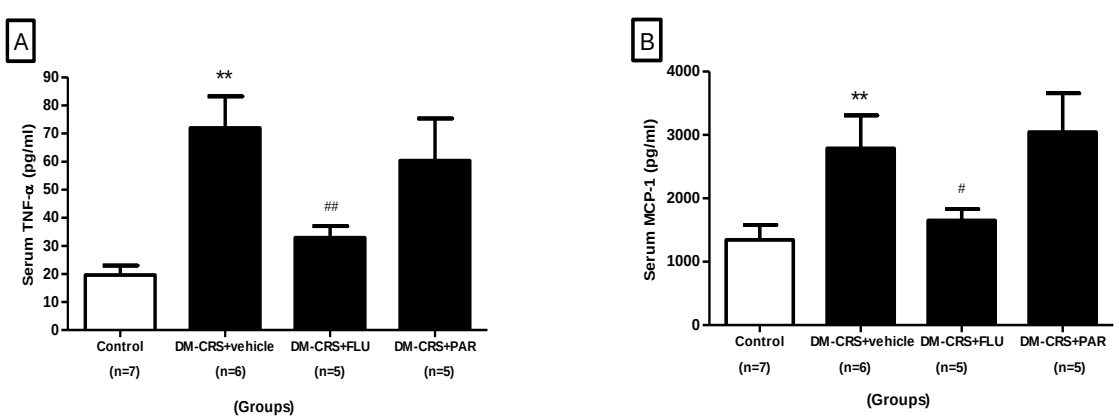

Figure 4: Effect of chronic treatment with fluoxetine (FLU), 10 mg/kg/day *i.p*, and paroxetine (PAR), 10 mg/kg/day *i.p*, on ELISA assay of serum TNF- α (A) and MCP-1 (B) levels in diabetic (DM) Wistar rats exposed to chronic restraint stress (CRS). Data are means ± SD. $^{**}p$ <0.001 *vs* control group; $^{\#}p$<0.01, $^{\#\#}p$<0.001 *vs* DM-CRS + vehicle-treated group.

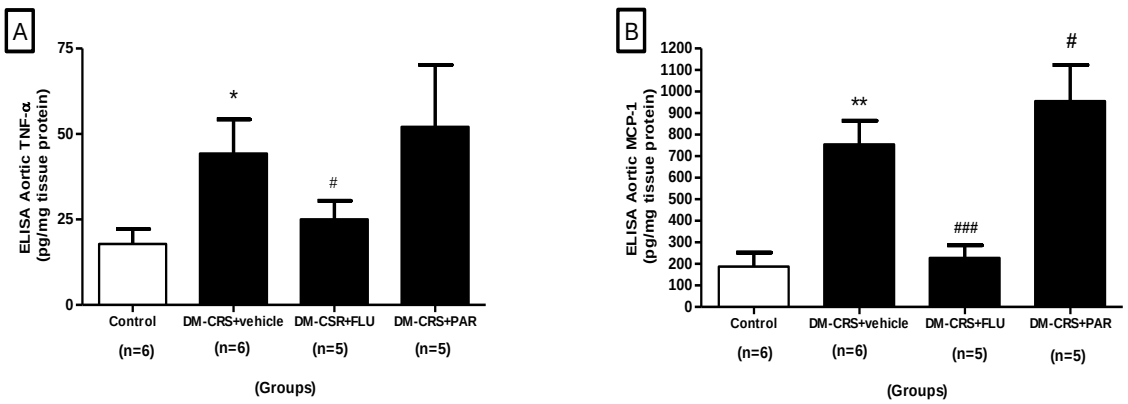

Figure 5: Effect of chronic treatment with fluoxetine (FLU), 10 mg/kg/day i.p, and paroxetine (PAR), 10 mg/kg/day i.p, on aortic tissue ELISA assay of tumor necrosis factor-α "TNF-α" (A) and monocyte chemotactic protein-1 "MCP-1" (B) levels in diabetic (DM) Wistar rats exposed to chronic restraint stress (CRS). Data are means ± SD. $^{*}p$ <0.01, $^{**}p$ <0.001 *vs* control group; $^{\#}p$<0.05, $^{\#\#\#}p$<0.001 *vs* DM-CRS + vehicle-treated group.

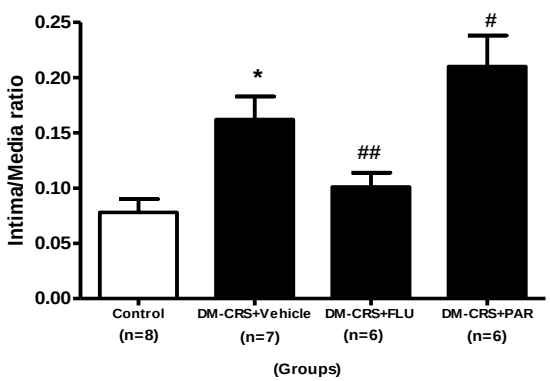

Figure 6: Effect of chronic treatment with fluoxetine (FLU), 10 mg/kg/day, *i.p*, and paroxetine (PAR), 10 mg/kg/day, *i.p*, on aortic intima/ media (IMR) ratio in diabetic Wistar rats exposed to chronic restraint stress (CRS). Data are means ± SD; $^{*}P<0.001$ *vs* control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$ *vs* DM-CRS + vehicle-treated group.

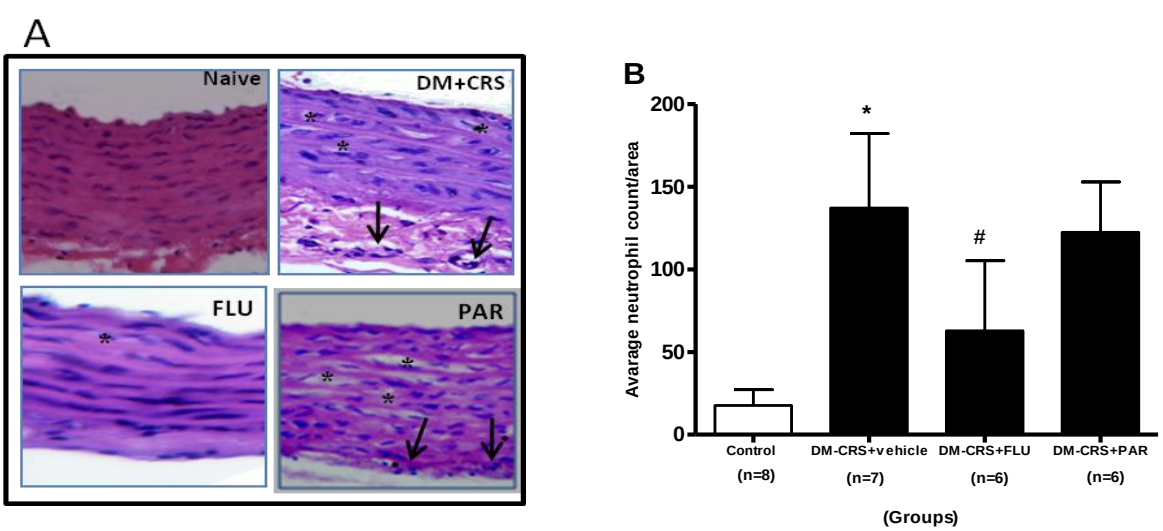

Figure 7: Effect of chronic treatment with fluoxetine (FLU), 10 mg/kg/day, *i.p*, and paroxetine (PAR), 10 mg/kg/day, *i.p*, on (A): aortic histology "H&E X400" showing multiple neurophil infiltrations (arrows) and foam cells (stars) in DM-CRS untreated and PAR-treated groups; and on (B): average neutrophil cell count/200 x 200 µm aortic area in diabetic Wistar rats exposed to chronic restraint stress (CRS). Data are means ± SD. $^{*}P<0.001$ *vs* control group; $^{\#}P<0.01$ *vs* DM-CRS + vehicle-treated group.

Table 1: Effect of chronic treatment with fluoxetine (FLU) 10 mg/kg/day, *i.p,* and paroxetine (PAR), 10 mg/kg/day, *i.p,* on body weight change (g), systolic blood pressure (SBP) and lipid profile (mg/dl) in diabetic (DM) Wistar rats exposed to chronic restraint stress (CRS).

Parameter	Control	DM-CRS +Vehicle	DM-CRS + FLU	DM-CRS + PAR
BW change (g)	98.7±25.7	$31.1^{\#\#}$±17.9	8.9^{**}±4.8	89.9^{***}±22.7
SBP (mmHg)	95±10.5	$176.2^{\#}$±23.5	140.3^{*}±18.7	165.3±12.8
TC (mg/dl)	92.2±15.7	$210.2^{\#\#\#}$±25.3	145.8^{**}±17.4	275.7^{*}±24.6
TG (mg/dl)	84.1±17.2	$290.4^{\#\#\#}$±39.3	220.4^{*}±28.8	282.3±42.1
HDL (mg/dl)	39.3±7.8	30.5±5.1	33.9±8.6	22.4^{*}±3.4
LDL (mg/dl)	41.3±6.9	$112.0^{\#\#}$±12.5	77.9^{**}±13.6	201.4^{***}±37.3

Data are means ± SD; (n = 6/ group). $^{\#}P<0.01$, $^{\#\#}P<0.005$, $^{\#\#\#}P<0.001$ *vs* control group; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs* DM-CRS vehicle-treated group.
TC: Total Cholesterol, TG: Triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein

Table 2: Effect of chronic treatment with fluoxetine (FLU) 10 mg/kg/day i.p, and paroxetine (PAR) 10 mg/kg/day i.p, on fasting blood glucose (FBG), fasting blood insulin (FBI) levels and HOMA index in diabetic Wistar rats exposed to chronic restraint stress (CRS).

Parameter	Control	DM-CRS +Vehicle	DM-CRS + FLU	DM-CRS + PAR
FBG (mmol/l)	4.69±0.96	$16.8^{\#\#}$±3.1	13.1*±1.8	20.90*±3.05
FBI (µU/ml)	20.71±3.54	$11.09^{\#}$±3.13	12.87±4.12	12.66±3.08
HOMA-index	2.36±0.33	$2.81^{\$}$±0.64	2.11±0.56	2.60±0.73

Data are means ± SD; n = 6/group. $^{\$}P<0.05$, $^{\#}P<0.005$, $^{\#\#}P<0.001$ *vs* control group; $^{*}P<0.05$ *vs* DM-CRS vehicle-treated group
HOMA = homeostasis model for assessment of insulin resistance

4. DISCUSSION

The present work demonstrated that diabetic/depressed rats exhibited vascular dysfunction as evidenced by a significant increase in systolic blood pressure, aortic PhE- contractile response (decreased EC50 & increased Emax) and IMR with significant decrease in endothelial-dependent relaxation. These vascular abnormalities were associated with metabolic changes as increase in blood glucose, total cholesterol (TC), triglyceride (TG) and LDL and reduced insulin level and insulin sensitivity (measured by HOMA index and ITT). Chronic treatment with fluoxetine significantly ameliorated these vascular and metabolic dysfunctions. On the other hand, paroxetine exerted the opposite effect, since it produced more deterioration in most of metabolic and endothelial dysfunctions induced by DM-CRS. Moreover, fluoxetine significantly showed reduction while, paroxetine showed elevation in blood glucose level and body weight.

Multiple recent studies have reported that HFD-fed and low-dose STZ-treated rats serve as an animal model for type 2 diabetes *(Srinivasan et al., 2005; Zhang et al., 2008)*. In all these studies, exposure to HFD-STZ induced hyperglycemia, dyslipidemia, hypertension and insulin resistance. The significant increase in PhE-contractile response and the decrease in ACh-dependent relaxation noted in aortic rings isolated from diabetic/depressed rats might be related to the systemic metabolic abnormalities. Moreover, *Bartuoe et al. (2005)* mentioned that hypertriglyceridemia and insulin resistance induce endothelial dysfunction and impairment of nitric oxide (NO)-dependent vasodilator responses to ACh. Deficiency of endothelial-derived NO is believed to be the primary defect that links insulin resistance and endothelial dysfunction *(Bourgoin et al., 2008)*.

Indeed, fluoxetine and paroxetine had controversial effects on blood glucose levels. Several case reports suggested hypoglycemia associated with the chronic use of SSRIs, paroxetine, fluoxetine and sertraline in diabetic and nondiabetic patients *(Sawka et al., 2001; Zammit, 2012)*. Moreover, experimental studies revealed a reduction in blood glucose level with paroxetine chronic treatment in normoglycemic mice *(Kadioglu et al., 2011)* and with fluoxetine acute treatment in diabetic rats *(Gomez et al., 2001)*. In contrast, other researchers declared a case report of hyperglycemia with chronic use of paroxetine *(Petty, 1996)*. Carvalho and his colleagues (2004) suggested that acute central injection of fluoxetine in the third ventricle induced a hyperglycaemia via increasing brain serotonergic activity. Additionally, *Yamada et al. (1999)* reported that acute treatment with fluoxetine and fluvoxamine induced hyperglycemia in mice.

The discrepancies regarding the effects of fluoxetine and paroxetine on glycemic control that are stated above might be explained by observing their effects in diverse circumstances, comorbidities, doses, routes, scheduling or species. Furthermore, none of these studies investigated the effect of chronic treatment with fluoxetine or paroxetine on blood glucose level in diabetic/depressed Wistar rats as in the present work.

Fluoxetine was also shown to decrease insulin resistance in non-depressed patients with type II diabetes mellitus independently on weight loss ***(****Maheux et al., 1997)* and in non-diabetic obese patients *(Araya et al., 1995)*. The reduction in TG and TC levels induced by fluoxetine in the current work is consistent with *Ghaeli et al. (2006)* and who reported that fluoxetine was shown to decrease TG and TC levels in patients with diabetes and major depressive disorder. On the other side, chronic administration of paroxetine was reported to induce a significant increase in TC and LDL-C as well as inducing insulin resistance *(Kim and Yu, 2005; Le Melledo et al., 2009)***.** Recently, paroxetine was proved to inhibit insulin signaling via activation of insulin receptor substrate-1 (IRS-1) kinases in rat hepatoma Fao cells and considered as potential inducer of cellular insulin resistance *(Levkovitz et al., 2007)*. Conversely, *Weber-Hamann et al., (2006)* reported that paroxetine increased the sensitivity to insulin in non-diabetic depressed patients.

Even within a class, individual antidepressants differ in their binding affinity at different receptors. Different receptors are known to mediate insulin resistance and insulin secretion; binding at the H_1 and 5-HT_{2C} receptors mediates weight gain and insulin resistance, whereas M_3 muscarinic receptors in the pancreatic β-cells play a critical role in regulating insulin secretion ***(****Jindal and Keshavan, 2008)***.** Among the SSRIs, paroxetine has the greatest affinity at M_3 muscarinic receptors *(Stanton et al., 1993)*, which may explain its increased diabetogenic risk even in absence of weight gain.

The reduction in blood pressure by usage of antidepressant drugs in the current study was also reported by other investigators *(Golding et al., 2005)***.** A significant blood pressure lowering effect of fluoxetine was reported in DOCA-hypertensive rats *(Fuller et al., 1979)***.** Furthermore, *Pacher et al. (1999)* reported that fluoxetine elicited substantial dilations of isolated skeletal muscle arterioles, a response which is mediated by inhibition of Ca^{2+} channel(s) or interference with the signal transduction by Ca^{2+} in the vascular smooth muscle cells.

No previous experimental study investigated the effect of fluoxetine or paroxetine on endothelial dysfunctions induced by diabetes. However, recent experimental study revealed that fluoxetine increased the NO-dependent relaxation in the unpredictable chronic mild stress model of depression in mice *(Isingrini et al., 2012)*. On the other hand, paroxetine was mentioned to have in-vitro and in-vivo inhibitory effects on NO synthase, and this may translate into its possible deleterious effect on endothelial function *(Finkel et al., 1996)***.** Shimbo and his colleagues (2000) also reported that paroxetine inhibited endothelial-dependent vasodilatation. Indeed, the ability of fluoxetine to reduce the elevated blood lipids, insulin resistance and body weight in the present work might explain its favorable effect on endothelial function and the opposite was noted with paroxetine. From the above mentioned data, fluoxetine seems to protect against cardiovascular complications in diabetes. Conversely, paroxetine might exacerbate diabetic complications.

Additionally, it can be noticed from the present results that the vascular abnormalities in DM-CRS rats were associated with significant increase in serum and aortic level of pro-inflammatory cytokines, MCP-1 and TNF-α, and with marked aortic infiltration with neutrophils. Chronic administration of fluoxetine, but not paroxetine, could reduce levels of MCP-1 and TNF-α and ameliorate neutrophil infiltrations indicating a potent anti-inflammatory action that might provide a possible mechanism for its favorable vascular effect in diabetic/depressed rats. *Sushil et al. (2009)* noticed the elevation of TNF-α and MCP-1 blood level in many diabetic patients that may lead to increased insulin resistance, vascular inflammation, and the development of vascular disease. Furthermore, fluoxetine inhibits the expression of the inflammatory cytokines TNF-α, MCP-1 and intercellular adhesion molecule-1 (ICAM-1) in lung tissue *(Li XQ et al., 2011)* and rheumatoid arthritis *(Sacre et al., 2010)***.**

Finally, the present results also revealed that DM-CRS rats exhibited depressive-like behavioural syndrome, measured by increased duration of immobility in forced-swimming test and decreased number of squares crossed by the animals in the open field test. Chronic treatment with fluoxetine and paroxetine could ameliorate depressive-like behaviours induced by DM-CRS model but to a lesser degree than in non-diabetic rats. Similarly, *Miyata et al. (2004)* reported that the efficacy of SSRIs as antidepressant drugs, such as fluoxetine, fluvoxamine, and desipramine, was decreased by diabetes in the mouse tail suspension test that may be mediated by reduced response of 5-HT_{1A} receptors.

5. CONCLUSION

The present results suggest that chronic treatment with fluoxetine, but not paroxetine, ameliorates vascular dysfunctions in diabetic/depressed rats, partially through its potent anti-inflammatory effect as evidenced by reduction of pro-inflammatory cytokines 'MCP-1 and TNF-α' and neutrophil infiltration, and via reversing metabolic abnormalities as well. Nevertheless, the antidepressant effect of both SSRIs appeared to be attenuated in diabetic/depressed rats; hence, further research on other members of SSRIs or another class of antidepressants e.g tricyclic antidepressants is advised. Likewise, future research on possible drug interaction between fluoxetine and oral antidiabetic drugs in type II diabetes is warranted.

Acknowledgment

The author thanks Dr. Safaa M Shaker, Associate Professor of histology, (Department of histology, Faculty of medicine, Ain Shams University, Cairo, Egypt) for her skilled technical assistance with histological processing.

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