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Paroxetine Administration Alter some Biochemical Parameters in Male Wistar Rats Over a Systemic Period of Thirty-Five Days

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ABSTRACT

Paroxetine is often used to treat patients with psychotic disorders, one of the side effects of this medication is that it causes erectile dysfunction in such individuals. There is a little or no information on the effect of paroxetine on some biochemical and endothelial markers of experimental models, hence the need for this research. Biochemical and endothelial functional makers in male Wistar rats were evaluated after oral administration of paroxetine for 4, 7, 21, 28 and 35 days. Seventy-two (72) male Wistar rats were grouped into two of thirty-six rats in group A (control) which received normal saline and thirty-six rats in group B (paroxetine-treated) which received 10 mg/Kg body weight of paroxetine hydrochloride for 4, 7, 14, 21, 28, and 35 days respectively. During this period, six animals from the two groups were sacrificed on days 4, 7, 14, 21, 28 and 35 by anaesthesia using diethyl ether, blood was collected into lithium-heparinized bottles and the tissues of interest (penile and heart) of the rats were excised and preserved in ice-cold sucrose-tris buffer. Phosphodiesterase 5, arginase, nitric oxide were evaluated from the isolated tissue homogenates while cGMP, endothelin-1, creatine kinase, lipid profile and testosterone concentrations were evaluated from the plasma. The results revealed that during pre-treatment with paroxetine, there was significant ($p < 0.05$) 85.19 ± 4.64 and 92.58 ± 0.57 respectively PDE 5 inhibition in the penile and cardiac homogenates of rats. Nitric oxide concentration was significantly ($p > 0.05$) 2.96 ± 0.27 and 4.82 ± 0.05 reduced while arginase activities increased significantly ($p > 0.05$) 272.16 ± 5.07 and 201.93 ± 11.82 during paroxetine treatment. Same trend of results were observed with plasma endothelin-1 concentration (12.88 ± 0.78), cGMP concentration (0.14 ± 0.00) and Testosterone concentration (0.46 ± 0.03) was significantly ($p > 0.05$) decreased during paroxetine-treatment. However, plasma creatine kinase 463.6 ± 50.96 , triacyl glycerol 58.61 ± 5.49 , total cholesterol 181.55 ± 9.72 and low-density lipoprotein cholesterol 165.86 ± 9.72 were significantly ($p < 0.05$) increased during paroxetine-treatment. However, no significant ($p > 0.05$) difference was observed in the high-density lipoprotein cholesterol 8.07 ± 0.46 during administration with paroxetine. From this study, it can be concluded that paroxetine administration altered erectile and endothelial markers throughout the period of administration and as such should be prescribed to patients with caution.

Keywords: Paroxetine, cyclic guanosine monophosphate (cGMP), arginase, endothelin-1

Introduction

Selective serotonin reuptake inhibitors (SSRIs) such as paroxetine are effective in treating

psychological disorders and are the most commonly prescribed first-line oral therapy.

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However, their clinical usefulness is often limited by poor observance and discontinuation due to lack of therapeutic response and possibly adverse effects such as causing male sexual disorders (Nantz *et al.*, 2009). Paroxetine is an antidepressant of the selective serotonin reuptake inhibitors (SSRIs) that is used majorly to treat depressive disorders. One of the adverse effects of SSRIs administration to psychotic patients is erectile dysfunction and symptoms of sexual dysfunction have been reported to persist after discontinuation of SSRIs (Simonsen *et al.*, 2016). It also delays orgasm/sex drive and inhibits synthesis of nitric oxide in male rats (Prabhakar and Richard, 2010). Erectile dysfunction is a pathological condition with several predisposing factors such as cardiovascular diseases, atherosclerosis, neuronal diseases and diabetes (Musicki *et al.*, 2015; Gandaglia *et al.*, 2015). The primary mediator of penile erection is nitric oxide (NO) which is produced through the activity of nitric oxide synthase (NOS) expressed in the endothelial and neuronal cells (Forstermann and Sessa, 2011; Kavoussi *et al.*, 2018). There are shreds of evidence that impaired endothelial function lowers nitric oxide (NO) level and significantly reduce the level of other important signaling molecules such as cyclic guanosine monophosphate (cGMP), in the cavernosal smooth muscles (Lugnier, 2006). PDE 5 inhibitors such as sildenafil citrate are prescribed as first line oral therapeutics for the management of erectile dysfunction. However, adverse effects of this synthetic drug which includes blurred vision, persistent headache, heartburn, nasal congestion, delayed flaccidity, diarrhoea and dyspepsia (Moreira *et al.*, 2000; Lichtblau *et al.*, 2015). More so, patients suffering from pulmonary arterial hypertension, incidences of vascular, gastrointestinal and neurologic side effects have been reported and, in some patients, adverse effects may remain intolerable leading to discontinuation of treatment (Lichtblau *et al.*, 2015; Siehr *et al.*, 2015).

Phosphodiesterase 5 inhibitors (PDE5i) play a major role in the overall management of erectile dysfunction, but treatment is not limited to PDE5i alone (Ademosun *et al.*, 2019). Literature has stated that co-inhibition of other key enzymes (arginase) involve in regulating biomolecules paramount to penile erection alongside with phosphodiesterase5 could be of pharmacological importance in ED management (Adefegha *et al.*, 2018). There is little or no information on the effects of paroxetine administration on endothelial parameters especially cGMP and endothelin-1 concentrations in experimental animals, hence the justification of this study.

Materials and Methods

Reagents, chemicals and assay kits

Trizma base (Lot no.: BCBJ8442V), Ammonium sulphate (Lot no.: BCBJ0661V), cyclic guanosine monophosphate (cGMP) (Lot no.: BCBJ1515V) and *Crotalus adamanteus* venom (SLBR1148V) were products of Sigma Aldrich Chemical Company, St. Louis, United States of America; Imidazole (Lot no.: B0212) was a product of Santa Cruz Biotechnology, Dallas, Texas; Sildenafil citrate (Lot no.: A471719) was a product of Pfizer, New York; Paroxetine hydrochloride (Lot no.: 615M) was a product of Glaxo Smith Klein (GSK), Brasov, Romania; Tween-80 was a product of BDH Laboratory Chemicals, Poole, England; L-arginine was a product of Burgoyne Burbidges & Co., Mumbai, India; Assay kit for testosterone was a product of Monobind Inc., Lake Forest, USA; Assay kits for cGMP (Lot no.: P104126) and Endothelin-1 (P109997) were products of Biotechne Ltd, Abingdon, United Kingdom; Assay kits for creatine kinase, total cholesterol, triglyceride, low density lipoprotein cholesterol (LDLC), high density lipoprotein cholesterol (HDLC) were products of Randox laboratories Co-Artrim, United Kingdom. All other reagents used were of analytical grade from the Laboratory in Department of Biochemistry.

Experimental animals

Seventy-two adult male Wistar rats (weighing between 210.43 ± 10.04 g) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin.

Induction of erectile and endothelial dysfunction

Thirty-six male rats were induced with erectile dysfunction by oral administration of 10 mg/kg of paroxetine suspension which was prepared daily in Tween-80 (BDH Chemicals, Ltd.; Poole, England), suspended in 9 g/L saline solution for twenty-one days using an oropharyngeal cannula (Chan *et al.*, 2010; Malviya *et al.*, 2011; Yakubu and Nurudeen, 2012). Thirty none - ED rats received only normal saline solution to serve as control. Erectile/ cardiac dysfunctions were confirmed with nitric oxide depletion and elevated levels of phosphodiesterase 5 in the tissues after twenty-one days.

Animal grouping and extract administration

The animals were randomly selected into two groups of thirty-six animals each as follows:

Control: Normal Control (non-induced rats that received 0.75 ml of physiological saline solution)

PXT: Paroxetine (PXT-treated rats that received 0.75ml of 10 mg/Kg body weight of paroxetine hydrochloride solution)

All the animals in the control group received physiological saline solution throughout the period of the study i.e. thirty-five days while animals in the PXT group received 10 mg/Kg body weight of paroxetine hydrochloride for twenty-one days. Six animals were sacrificed from the two groups on days 4, 7, 14, 21, 28 and 35 days. After day 28, the remaining animals in the two groups were given distilled water till day 35. This is referred to as the post-treatment phase of the experiment. All the animals sacrificed were anaesthetized with diethyl ether and blood was collected through jugular puncture into lithium- heparinised tubes in order to obtain clear plasma for analysis. The tissues of

interest were also excised and stored in ice-cold 0.25 M sucrose buffer in order to maintain the tissues integrity until analysis was done.

Determination of phosphodiesterase 5 activity

The method of Butcher and Sutherland (1962) was used for the determination of phosphodiesterase 5 activity.

Calculation

The activity (P) of phosphodiesterase 5 was calculated according to the following formular:

$$P = \frac{V\Delta C}{V_3} \quad (\text{units/ml})$$

Where P is enzyme activity

V is total volume of reaction

ΔC is reaction velocity (Pi/ incubation time of 20 minutes)

V_3 is Volume of enzyme source

Pi is amount of inorganic phosphate released

Determination of concentration of cyclic guanosine monophosphate

This was done according to the manufacturer's guide (R and D Systems) as described by Motterlini *et al.* (2002).

Determination of concentration of Endothelin-1

This was done according to the manufacturer's guide (R and D Systems) as described by Yanagisawa (1994).

Determination of Nitric oxide

The nitric oxide level in tissues were determined using the Griess reagent following the method of Green *et al.* (1982).

Determination of Total cholesterol

The total cholesterol concentration was determined by colorimetric method as described by Fredrickson *et al.* (1967).

Calculation

Total cholesterol =

$$\frac{\text{Absorbance of Sample} \times \text{Concentration of standard}}{\text{Absorbance of Standard}}$$

Concentration of the standard was 208 mg/dl.

Determination of Triglyceride

Triglyceride concentrations in the plasma were determined as described by Searcy (1969).

Calculation:

Triglyceride in sample =

$$\frac{\text{Absorbance of Sample} \times \text{Concentration of Standard}}{\text{Absorbance of Standard}}$$

Concentration of the standard was 192 mg/dl

Determination of High-density lipoprotein cholesterol

HDL-Cholesterol was determined by a colorimetric method described by Burstein and Mortin (1969).

Calculation:

HDL-Cholesterol conc. (mg/dl) =

$$\frac{\text{Absorbance of Sample} \times \text{Conc. of Standard}}{\text{Absorbance of Standard}}$$

Concentration of standard was 0.443 mg/dl.

Determination of Low-density lipoprotein Cholesterol

The serum low-density lipoprotein cholesterol was assayed using the polyvinyl sulphate (PVS) reaction as described by Demacker *et al.* (1984).

Calculation:

$$\text{LDL-C (mg/dl)} = \text{Total cholesterol (mg/dl)} - 1.5 \times \text{Supernatant cholesterol (mg/dl)}$$

Determination of plasma creatine kinase activities

The method described by Witt and Trendelenburg (1982) was used for the quantitative determination of creatine kinase in the plasma.

Calculation:

$$\text{Creatine kinase (U/l)} = 4127 \times (\Delta \text{Absorbance/minute} \times \text{dilution factor})$$

4127 is the standard factor used in preparation of the enzyme kit.

Determination of Testosterone

The serum testosterone concentration was quantitatively determined using the direct human testosterone enzyme immunoassay (EIA) kit described by Tietz (1995).

Calculation of testosterone concentration:

The serum testosterone concentration of the sample was extrapolated from the calibration curve for testosterone.

Statistical analysis

All data were presented as the mean of six determinations \pm standard error of mean (S.E.M.). Statistical evaluation was done by one-way analysis of variance (ANOVA), followed by Duncan's posthoc test for multiple comparisons using Graphpad prism 5. Values were considered statistically significant at $p < 0.05$.

Results

The penile PDE 5 activity in paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days is as shown in Figure 1. PDE 5 activities significantly ($p < 0.05$) 85.19 ± 4.64 increased in penile of rats treated with paroxetine (10 mg/Kg b. wt.) when compared to those administered normal saline (control). The enzyme activity in paroxetine-treated rats increased as the period of administration increased; activity of the enzyme after 21 days of administration was significantly ($p < 0.05$) higher than on days 4, 7 or 14. The

increased activity of PDE 5 decreased significantly ($p < 0.05$) one week (day 28) after paroxetine was discontinued but was not significantly increased ($p < 0.05$) during the post-treatment week (day 35) after the administration of paroxetine was discontinued and the values determined were significantly ($p < 0.05$) different from those of day 21.

Activity of cardiac PDE 5 in paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days is shown on Figure 2. PDE 5 activities significantly ($p < 0.05$) increased in cardiac tissues of rats treated with paroxetine when compared with the control. The enzyme activity in paroxetine-treated rats at 21 days of administration was significantly ($p < 0.05$) higher than on day 4 (first plot on the curve). The elevated activity of cardiac PDE 5 persisted for two weeks after the administration of paroxetine was discontinued and the values determined on day 28 or 35 were not significantly ($p < 0.05$) different from those of day 21.

Figure 3 shows the activity of penile arginase in paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days. Arginase activities significantly ($p < 0.05$) increased in penile tissues of rats treated with paroxetine when compared with the control. The enzyme activity in paroxetine-treated rats was not significantly ($p < 0.05$) different on the various days monitored; although activity of the enzyme on day 7 was significantly ($p < 0.05$) higher than on day 4, penile arginase activities on days 14 and 21 was not significantly ($p < 0.05$) different from those of day 4. The increased activity of penile arginase persisted after the discontinuation of paroxetine.

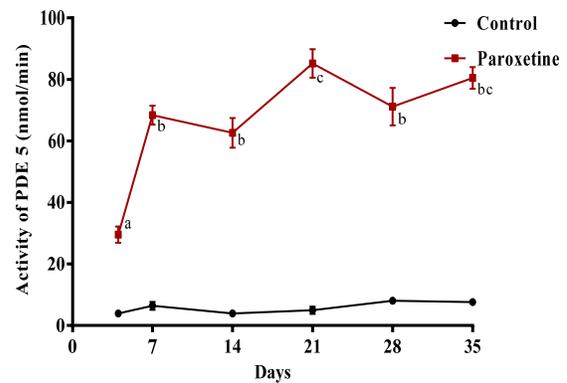


Figure 1: Time course of penile phosphodiesterase 5 activities in paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Values are mean of six determinations \pm SEM; plotted values with different superscript are significantly ($p < 0.05$) different from each other.

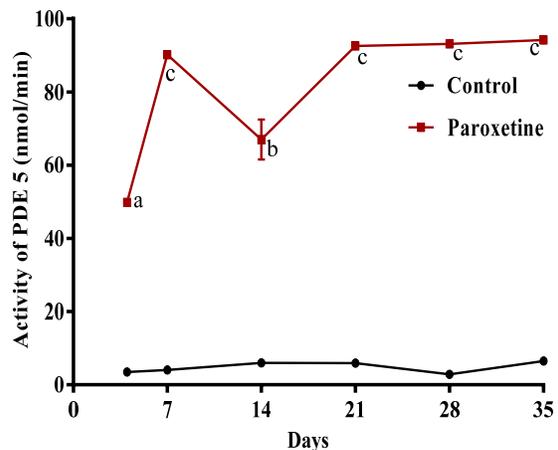


Figure 2: Time course of cardiac phosphodiesterase 5 activities in paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Values are mean of six determinations \pm SEM; plotted values with different superscript are significantly ($p < 0.05$) different from each other.

Results for the activity of penile arginase seven days after withdrawal of paroxetine treatment (day 28) was significantly ($p < 0.05$) different from other days of treatment (days 4, 7, 14, 21 and 35) while that of 14 days after paroxetine discontinuation (day 35) were significantly ($p < 0.05$) lower than the penile arginase activity observed on days 7 and 28 but not significantly ($p \geq 0.05$) different from enzyme activity of days 4, 14, and 21 (Figure 3). Generally, there was a significant ($p < 0.05$) difference between the paroxetine-treated group and the control.

Arginase activity of heart of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days is shown in Figure 4. Arginase activities significantly ($p < 0.05$) increased in heart of rats treated with paroxetine when compared to the control. In contrast to arginase activities in the penile (Figure 3), the enzyme activity in heart of paroxetine-treated rats significantly ($p < 0.05$) increased as days increased. The elevated activity of cardiac arginase persisted for weeks after the administration of paroxetine was stopped, although post-treatment plotted values determined after 14 days were significantly ($p < 0.05$) lower than plotted values noted during treatment period on day 21.

Concentration of nitric oxide in the penile and heart of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days are shown on Figures 5 and 6 respectively. Concentration of nitric oxide significantly ($p < 0.05$) reduced in both tissues following administration of paroxetine to rats for more than 7 days, when compared to control. Concentration of nitric oxide estimated in the penile decreased as days of treatment increased; nitric oxide level observed on day 21 was significantly ($p < 0.05$) lower than those determined on day 4, 7 and 14 (Figure 5). Similarly, cardiac nitric oxide concentration following administration of paroxetine to rats was also time dependent; concentration on days 14 and 21 was significantly ($p < 0.05$) lower than that of days 4 and 7 (Figure

6). The reduced concentration was partly restored in both tissues after the administration of paroxetine was discontinued for a week (day 28) and penile nitric oxide concentration on day 35 was observed to be significantly ($p < 0.05$) different from every other day except day 28.

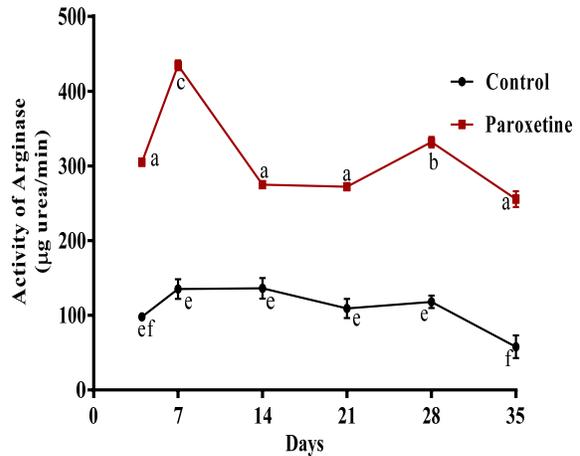


Figure 3: Time course of penile arginase activities in paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Values are mean of six determinations \pm SEM; plotted values with different superscript are significantly ($p < 0.05$) different from each other.

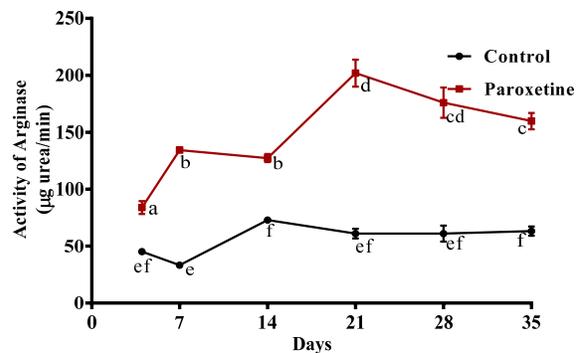


Figure 4: Time course of cardiac arginase activities in paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days.

Values are mean of six determinations \pm SEM; plotted values on the graph with different superscript are significantly ($p < 0.05$) different from each other.

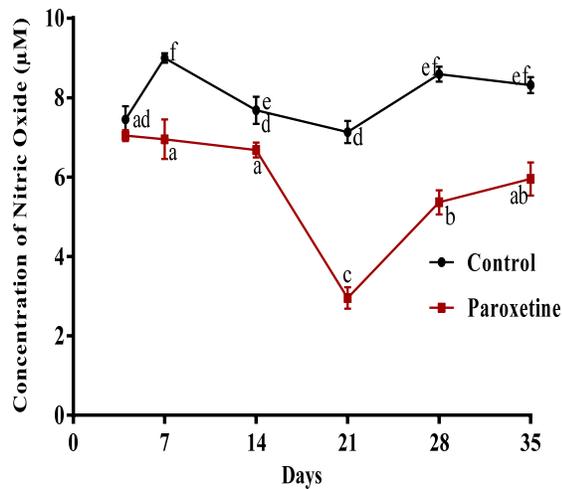


Figure 5: Time course of concentration of penile nitric oxide of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Values are mean of six determinations \pm SEM; plotted values with different superscript are significantly ($p < 0.05$) different from each other.

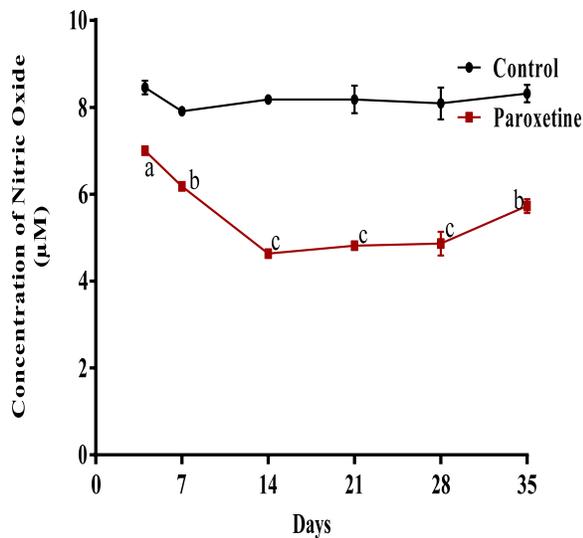


Figure 6: Time course of concentration of cardiac nitric oxide of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Values are mean of six determinations \pm SEM; point values with different superscript are significantly ($p < 0.05$) different from each other.

Figure 7 shows concentration of plasma cGMP of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days. Concentration of cGMP significantly ($p < 0.05$) reduced following administration of paroxetine to rats when compared to the control. cGMP concentration in plasma was not significantly ($p \geq 0.05$) different as days of treatment with paroxetine increased. The reduced concentration of plasma cGMP persisted even after paroxetine had been discontinued for two weeks (day 21 - 35).

Concentration of endothelin-1 in the plasma of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days is shown on Figure 8. In contrast to concentration of plasma cGMP, concentration of plasma endothelin-1 significantly ($p < 0.05$) increased in rats treated with paroxetine when compared to the control. However, as days of treatment with paroxetine increased, plasma endothelin-1 significantly ($p < 0.05$) decreased. Conversely, endothelin-1 concentration was significantly ($p < 0.05$) increased for two weeks after the administration of paroxetine was discontinued (days 28 and 35).

Figure 9 shows concentration of plasma testosterone of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days. Concentration of testosterone significantly ($p < 0.05$) reduced following administration of paroxetine to rats, when compared to control. Plasma concentration of testosterone decreased as the days increased, plotted values determined after 21 days of administration of paroxetine to rats was significantly ($p < 0.05$) lower than on day 4 (first plot on the curve). The reduction in concentration of plasma testosterone persisted for two weeks (days 21 - 35) after the administration of paroxetine was discontinued.

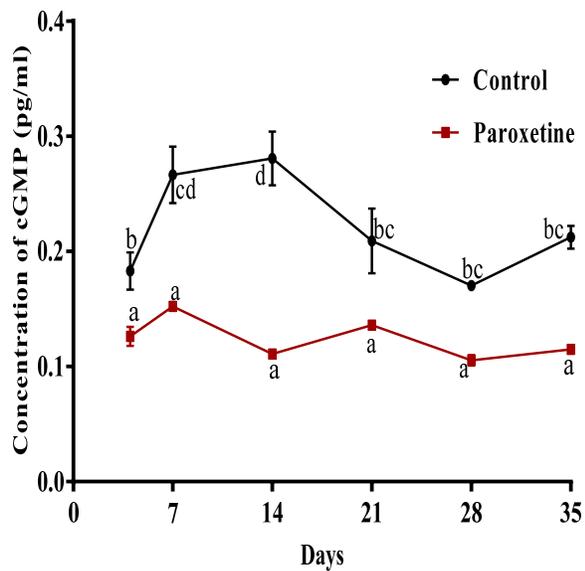


Figure 7: Time course of concentration of cyclic guanosine monophosphate (cGMP) in plasma of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Values are mean of six determinations \pm SEM; plotted values with different superscript are significantly ($p < 0.05$) different from each other.

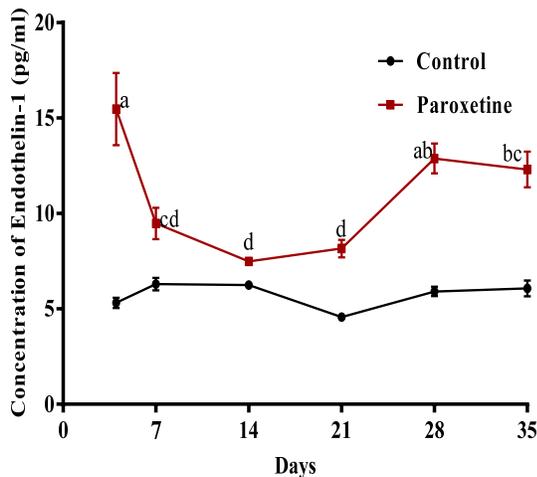


Figure 8: Time course of concentration of endothelin-1 in the plasma of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Values are mean of six determinations \pm SEM; plotted values with different superscript are significantly ($p < 0.05$) different from each other.

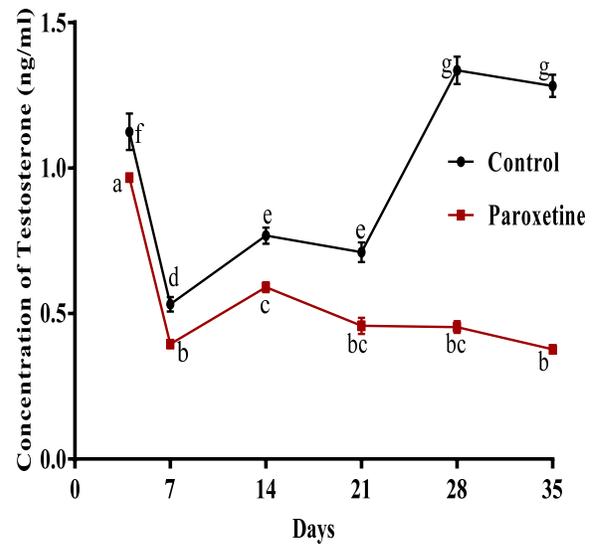


Figure 9: Time course of concentration of testosterone in the plasma of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Values are mean of six determinations \pm SEM; plotted values with different superscript are significantly ($p < 0.05$) different from each other.

Activity of creatine kinase in the plasma of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days is shown in Figure 10. Plasma creatine kinase activity significantly ($p < 0.05$) increased in rats treated with paroxetine when compared to the control. Plasma creatine kinase activity was significantly ($p < 0.05$) different over paroxetine treatment period of 21 days. The increased activity of creatine kinase persisted for two weeks after the administration of paroxetine was discontinued, although post-treatment values (on days 28 and 35) were significantly ($p < 0.05$) lower than paroxetine-treatment values on day 14.

Concentrations of cholesterol and triglycerides in plasma of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days is shown in Table 1. Concentration of cholesterol and triglycerides in rats administered normal saline (control) were not significantly ($p \geq 0.05$) different from each other over the experimental period of 35

days. Administration of paroxetine to rats for ≥ 7 days resulted in significant ($p < 0.05$) increased in concentration of plasma cholesterol and triglycerides, when compared to the control. Concentration of the lipids observed in the plasma increased as days of treatment increased; levels determined on day 21 were significantly ($p < 0.05$) higher than those determined on days 4 and 7 (Table 1). The elevated concentration of cholesterol and triglycerides persisted after the administration of paroxetine was discontinued for two weeks (days 28 and 35), although values obtained for cholesterol were significantly ($p < 0.05$) lower than those of treatment values on day 21 but higher than values on day 4, 7 and 14 (Table 1).

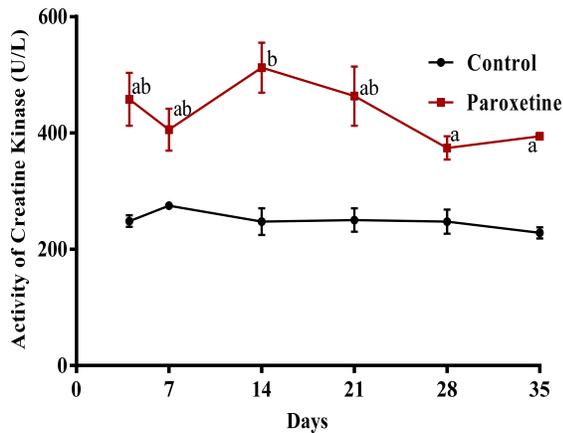


Figure 10: Time course of creatine kinase activities in plasma of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Values are mean of six determinations \pm SEM; plotted values with different superscript are significantly ($p < 0.05$) different from each other.

Table 2 shows the concentration of lipoprotein-cholesterol concentrations in plasma of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days. Although calculated values were between 7 - 10 mg/dl, fluctuations were recorded in the level of HDL-cholesterol over the experimental period in rats administered normal saline (control). Similarly, administration of paroxetine over a period of 21 days did not significantly ($p > 0.05$) alter the concentration of HDL-cholesterol. However, concentration of LDL-cholesterol was significantly ($p < 0.05$) higher in rats treated with paroxetine, when compared with the control. The LDL-cholesterol concentration progressively and significantly ($p < 0.05$) increased as days of treatment with paroxetine increased. Withdrawal of the paroxetine treatment for two weeks partly reduced the increased concentration of LDL-cholesterol, values obtained were significantly ($p < 0.05$) lower than those of paroxetine - treatment values on day 21 but higher than values obtained on day 4, 7 and 14 (Table 2).

Time course of cardiac and arterogenic indices of paroxetine-treated rats are shown in Table 3. Similar trend in relation to LDL-cholesterol were observed. There was progressive and significant ($p < 0.05$) increase in the level of paroxetine-treated rats over a period of 21 days and discontinuation of drugs partly reduced the predisposition of the rats to arterogenic and cardiac risks (Table 3).

Table 1: Time course of concentrations of cholesterol and triglycerides in the plasma of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Days	Cholesterol (mg/dl)		Triglycerides (mg/dl)	
	Control	Paroxetine	Control	Paroxetine
4	23.80 ± 0.74 ^a	36.67 ± 1.74 ^a	24.37 ± 0.23 ^a	30.74 ± 1.20 ^{ab}
7	24.80 ± 0.26 ^a	48.39 ± 3.30 ^b	22.14 ± 1.34 ^a	36.94 ± 3.26 ^{bc}
14	23.60 ± 1.82 ^a	59.11 ± 5.90 ^b	26.43 ± 1.61 ^a	51.65 ± 1.30 ^{de}
21	25.25 ± 1.39 ^a	181.55 ± 9.72 ^d	23.58 ± 0.04 ^a	58.61 ± 5.49 ^e
28*	22.94 ± 1.53 ^a	83.13 ± 7.15 ^c	18.78 ± 0.29 ^a	42.78 ± 3.50 ^{cd}
35**	23.46 ± 0.35 ^a	85.06 ± 0.17 ^c	25.52 ± 0.05 ^a	49.68 ± 2.63 ^{de}

Values are mean of six determinations ± SEM; values along a column with different superscript are significantly ($p < 0.05$) different from each other; * = 7 and ** = 14 days after paroxetine was discontinued.

Table 2: Time course of lipoprotein-cholesterol profiles in the plasma of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Days	HDL-cholesterol (mg/dl)		LDL-cholesterol (mg/dl)	
	Control	Paroxetine	Control	Paroxetine
4	9.58 ± 0.16 ^b	8.51 ± 0.23 ^{ab}	4.75 ± 0.74 ^a	19.53 ± 1.74 ^{bc}
7	8.59 ± 0.42 ^b	9.42 ± 0.41 ^{ab}	7.21 ± 0.26 ^a	29.90 ± 3.30 ^{cd}
14	7.51 ± 0.27 ^a	7.69 ± 0.15 ^a	8.41 ± 1.82 ^a	43.83 ± 5.90 ^d
21	8.45 ± 0.54 ^{ab}	8.10 ± 0.69 ^a	8.03 ± 1.34 ^a	165.84 ± 9.71 ^f
28*	7.44 ± 0.37 ^a	8.07 ± 0.46 ^a	8.22 ± 1.53 ^a	66.84 ± 7.15 ^e
35**	8.69 ± 0.41 ^{ab}	10.10 ± 0.68 ^b	15.32 ± 0.34 ^b	65.26 ± 0.17 ^e

Values are mean of six determinations ± SEM; values along a column with different superscript are significantly ($p < 0.05$) different from each other; * = 7 and ** = 14 days after paroxetine was discontinued.

Table 3: Time course of cardiac and arterogenic indices of paroxetine-treated rats over a period of 21 days and post-treatment period of 14 days

Days	Cardiac index		Artherogenic index	
	Control	Paroxetine	Control	Paroxetine
4	2.49 ± 0.04 ^a	4.31 ± 0.08 ^a	0.48 ± 0.06 ^a	2.29 ± 0.11 ^a
7	2.91 ± 0.12 ^{ab}	5.13 ± 0.25 ^a	0.91 ± 0.07 ^{ab}	3.13 ± 0.26 ^a
14	3.13 ± 0.13 ^b	7.68 ± 0.57 ^b	1.10 ± 0.17 ^b	5.65 ± 0.60 ^b
21	2.99 ± 0.03 ^b	22.61 ± 0.78 ^c	0.98 ± 0.04 ^b	20.60 ± 0.70 ^c
28*	3.08 ± 0.09 ^b	9.85 ± 0.34 ^b	1.09 ± 0.12 ^b	7.88 ± 0.09 ^b
35**	3.76 ± 0.14 ^c	8.54 ± 0.56 ^b	1.75 ± 0.09 ^c	6.53 ± 0.49 ^b

Values are mean of six determinations ± SEM; values along a column with different superscript are significantly different from each other; * = 7 and ** = 14 days after paroxetine was discontinued.

Discussion

PDE 5 is a cGMP specific enzyme that is most prominently found in the corpus cavernosum of the penis and also in appreciable amount in the heart tissue (Bobin *et al.*, 2016). Elevation in the activity of PDE 5 has been reported in cases of erectile dysfunction (Yuan *et al.*, 2013). The use of anti-psychotics such as paroxetine has also been linked to erectile dysfunction (Garcia *et al.*, 2017). In this study, sub-chronic administration of paroxetine for 21 days caused progressive increase in the activity of PDE 5 by ~ 16 folds, (Figure 1) establishing erectile dysfunction in rats. Persistence in elevated levels of PDE 5 was observed following discontinuation of paroxetine for two weeks, indicating post-paroxetine induced sexual dysfunction in rats.

Similarly, PDE 5 activity in the cardiac tissue of rats treated with paroxetine was also elevated (Figure 2). Recent studies have highlighted relationship between erectile dysfunction and cardiovascular diseases (Gandaglia *et al.*, 2014). The role of nitric oxide and endothelial factors in modulating cardiac and erectile functions have also been elucidated (Vlachopoulos *et al.*, 2008). Levels of nitric oxide in both (penile and cardiac) tissues of paroxetine-treated rats were reduced, signifying attenuated activities of nitric oxide synthase. Paroxetine is a known inhibitor of nitric oxide synthase (Finkel *et al.*, 1996). Nitric oxide synthase catalyses conversion of L-arginine to nitric oxide; leading to vasodilation of the smooth muscles and penile erection.

Soluble guanylyl cyclase is activated in the presence of nitric oxide and the enzyme converts guanine triphosphate (GTP) to cyclic guanine monophosphate (cGMP). In paroxetine-induced erectile dysfunction, reduction in concentration of plasma cGMP was observed in rats following administration of paroxetine for 4 days and the reduced concentration persisted during the 21 days of treatment as well as following discontinuation of paroxetine for 2 weeks.

Concentration of cGMP in corpus cavernosum smooth muscle cells is dependent on the balance between its production and utilization by soluble guanylyl cyclase and PDE 5 respectively (Lau *et al.*, 2006). Decreased production of endothelium-derived nitric oxide has been implicated in the pathogenesis of not only erectile dysfunction but also cardiovascular diseases (Lara *et al.*, 2003).

Treatment of rats with paroxetine may also influence the activities of other enzymes involved in erectile, endothelial and vascular functions such as arginase. Thakur and Nivsarkar (2017) reported that rats exposed to paroxetine for 5 days showed alterations in the expression of arginase activity; similarly, in this study, 4 days exposure of rats to paroxetine resulted in increased activity of arginase to ~ 1.5 and 2.3 folds in penile and cardiac tissues respectively. Arginase is a manganese-containing enzyme that converts arginine to urea and ornithine. In penile tissue, arginase competes with nitric oxide synthase for L-arginine (Ruth *et al.*, 2014; Lacchini *et al.*, 2015); thus, when there is reduction in nitric oxide synthase activity as evident by low nitric oxide activity in the penile tissue, L-arginine was readily utilized by arginase and this phenomenon may be responsible for higher activity of arginase in the tissues. Arginase reciprocally regulates nitric oxide synthesis as well as endothelial and cardiac functions (Berkowitz *et al.*, 2003; Durante *et al.*, 2007).

In correlation with activity of arginase especially in the cardiac tissue, level of plasma endothelin-1 was increased in paroxetine-treated rats. In contrast to vasodilating nitric oxide, endothelins are vasoconstricting peptides that are produced primarily in the endothelium with a crucial role in vascular homeostasis and are implicated in a number of vascular diseases (Agapitov and Haynes, 2002). Endothelin-1 encoded by *EDN1* gene is a pleiotropic molecule and one of three isoforms found in humans. Endothelin-1 is a putative modulator of erectile dysfunction; Christ

et al. (1995) suggested that the physiological relevance of endothelin-1 to corporal physiology may be related to its ability to augment the contractile responses of other vasomodulators present in the human corpora, perhaps modulating the contractile responses to sympathetic activity. Similarly, Kaiser *et al.* (2004) concluded that endothelin-1 is the best independent predictor of erectile dysfunction suggesting that biochemical measures of endothelial damage are detectable independent of other risk factors. Inhibition of this peptide may prevent pulmonary vasoconstriction and decreases pulmonary vascular resistance (Angus *et al.*, 2017).

Prolonged administration of paroxetine resulted not only in elevation of plasma endothelin-1 (Figure 8) but also concomitant reduction in concentration of plasma testosterone (Figure 9). A component increased risk conferred by erectile dysfunction could be testosterone deficiency (Giltay *et al.*, 2010). Testosterone is an anabolic steroid and the primary sex hormone that plays a crucial role in the development of the male reproductive tissues. Yakubu and Jimoh (2015) showed that paroxetine administration to rats significantly reduced concentration of testosterone, concluding that sexual function was impaired in paroxetine-treated rats. Similar findings have also been reported by other researchers (Ajiboye *et al.*, 2016; El-Sheikh *et al.*, 2017). Low testosterone may increase levels of total and LDL cholesterol and thus, predisposed rats to cardiovascular risks (Carson and Rosano, 2012).

Administration of paroxetine increased concentration of creatine kinase, indicating myocardial infarction may be associated with paroxetine-treatment in rats (Figure 10). Evidence from study involving large number of patients showed clinical relationship between the triad of erectile dysfunction, endothelial dysfunction and cardiovascular diseases (Vlachopoulos *et al.*, 2008). Endothelial dysfunction is the initial step

of the atherosclerotic process involving many vascular districts, including penile and coronary circulation. The onset of sexual dysfunction is sometimes considered as a marker of subclinical systemic vascular disease, thus there is need for treatment of risk factors and conditions associated with erectile dysfunction.

Conclusions

From this study, administration of paroxetine to animals significantly affects the activities of phosphodiesterase5, and arginase; endothelial parameters such as cyclic guanosine monophosphate, endothelin-1 and nitric oxide levels. These activities suggest some possible mechanisms underlying the use of paroxetine to induce erectile dysfunction in experimental models. It also predisposes individuals to atherosclerosis considering the results of the lipid profile.

Competing interests

Authors have none to declare.

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