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Separately Administered Phosphodiesterase-5 Inhibitors (Sildenafil and Tadalafil) and Opioid (Tramadol), Reversibly Alter Serum Lipid Profile in Male Albino Wistar Rats

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ABSTRACT

Several undesired effects following chronic use of phosphodiesterase-5 inhibitors (particularly, sildenafil and tadalafil) and opioid (tramadol) have been reported. This study assessed the effect of chronic administration of sildenafil, tadalafil, tramadol and sildenafil+tramadol (as used in Nigeria today), on serum lipid profile (cardiovascular risk assessment), since alterations may not be easily discernable by their users. Fifty male albino wistar rats weighing 180-200 g were randomly divided into 5 groups (n = 10), thus; control (0.2 mL normal saline), sildenafil treated (10 mg kg^{-1}), tadalafil treated (10 mg kg^{-1}), tramadol treated (20 mg kg^{-1}) and sildenafil+tramadol treated (10 and 20 mg kg^{-1}, respectively) group. The drugs were administered every two days, per oral route, for eight weeks. All animals had access to food and water *ad libitum*. At the end of eight weeks, 5 animals were sacrificed from each group, leaving the remaining 5 per group for another 8 weeks without treatment (recovery phase). Blood was collected from each animal via cardiac puncture and serum lipid profile assessed. Serum total cholesterol was significantly ($p<0.001$) increased in tadalafil treated group, but significantly ($p<0.001$) reduced in tramadol and sildenafil+tramadol treated groups, compared with control. Serum LDL-c concentration was significantly increased in sildenafil ($p<0.01$) and tadalafil ($p<0.001$) treated groups, but significantly reduced in tramadol and sildenafil+tramadol treated groups, compared with control. Cardiac Risk Ratio (CRR) was significantly reduced in tramadol ($p<0.001$) and sildenafil+tramadol ($p<0.01$) treated groups, compared with sildenafil and tadalafil treated groups. The same trend was observed for atherogenic coefficient. Atherogenic index of plasma was significantly ($p<0.05$) reduced in sildenafil+tramadol treated group, compared with control and tadalafil treated groups. Following withdrawal of treatment, serum total cholesterol and LDL-c reduced significantly ($p<0.001$) in tadalafil recovery group, compared with its treated group, while serum lipid profile did not differ significantly between the treated and recovery group of rats administered sildenafil, tramadol and sildenafil+tramadol. Sildenafil and tadalafil exhibited hyperlipidaemic effects, while tramadol and sildenafil+tramadol exhibited hypolipidaemic effects, with poor reversibility.

Key words: Lipid profile, phospodiesterase-5 inhibitors, sildenafil, tadalafil, tramadol

INTRODUCTION

Since the advent of sildenafil (the first orally administered phosphodiesterase-5 [PDE5] inhibitor) for treatment of Erectile Dysfunction (ED), its use has enjoyed wide patronage the world over, following reported effectiveness (Pfizer, 2012). Indeed, the advent of sildenafil and other PDE5 inhibitors saw to the management of ED, thus leaving the male folks with having to battle with the other common male sexual dysfunction-Premature Ejaculation (PE). Like ED, this male sexual dysfunction was conquered following the advent of tramadol hydrochloride in late 1970s. This opioid drug has since been used for treatment of PE (Strebel *et al*., 2004; Safarinejad and Seyyed, 2006; Salem *et al*., 2008).

However, it is regrettable that these drugs are currently widely abused the world over. We previously reported high incidence of abuse of these medications and other sex stimulants in a population-based study conducted in Calabar, Cross River State, Nigeria (Nna *et al*., 2014). Several side effects were reported by respondents who participated in that study and were currently using sex stimulants above their recommended doses.

In a study using male albino wistar rat model, sildenafil, tadalafil and tramadol were shown to be hepatotoxic and reversibility was poor following withdrawal of these drugs (Nna *et al*., 2015). It is likely that some other detrimental effects not easily discernable by recreational users of these drugs may exist. This study therefore seeks to ascertain the impact of chronic administration of these drugs and the new therapy (sildenafil+tramadol combination) currently invoke in Nigeria, on serum lipid profile using albino wistar rat model. This will provide information on the likelihood to suffer cardiovascular disease. Furthermore, possible reversal of alterations in lipid profile following withdrawal of these drugs is also assessed.

MATERIALS AND METHODS

Experimental animals and protocol: A total of 50 male albino wistar rats weighing 180-200 g were obtained from the animal house of the Department of Agriculture, Faculty of Science, University of Calabar, Nigeria. The animals were kept in well ventilated cages, in the animal house of the College of Medical Sciences, University of Calabar, Nigeria. They were allowed access to normal rat chow and water *ad libitum* and exposed to 12/12 h light/dark cycle. The animals were allowed 7 days for acclimatization before commencement of the research. All animals were handled in accordance with the laid down principles for animal care as prescribed in Helsinki's 1964 declaration.

Experimental design and drug administration: The rats were randomly divided into 2 batches (batches A and B), each having 5 groups (n = 5, Table 1). The drugs used in this study [sildenafil (Maxheal Laboratories Pvt Ltd, India), tadalafil (Pfizer, India) and tramadol hydrochloride (Glow Pharma Pvt Ltd, India)] were administered to batches A and B, once, every two days, per oral route, for 8 weeks, as used in our previous study (Nna *et al*., 2015). At the end of 8 weeks of treatment, batch A animals were sacrificed and their blood samples were collected for lipid profile assessment. Batch B animals were allowed for another 8 weeks without drug administration (Table 1-recovery phase), but were allowed access to feed and water *ad libitum*. At the end of 8 weeks recovery period, the animals were sacrificed and their blood samples were also collected for lipid profile assessment.

Serum lipid profile estimation: The animals were sacrificed under chloroform anaesthesia, blood samples were collected from the animals through cardiac puncture into pre-labelled sample

Table 1: Experimental design

Group	No. of animals	Treatment
Batch A-treatment phase		
NC	5	0.2 mL normal saline
Sildenafil-treated	5	Sildenafil (dose = 1 mg/100 g b.wt.)
Tadalafil-treated	5	Tadalafil (dose = 1 mg/100 g b.wt.)
Tramadol-treated	5	Tramadol (dose = 2 mg/100 g b.wt.)
Sild.+Tram.-treated	5	Sildenafil and tramadol (dose = 1 and 2 mg/100 g b.wt., respectively)
Batch B-recovery phase		
NC	5	Treatment withdrawn
Sildenafil-treated	5	Treatment withdrawn
Tadalafil-treated	5	Treatment withdrawn
Tramadol-treated	5	Treatment withdrawn
Sild.+Tram.-treated	5	Treatment withdrawn

bottles and allowed to clot for 30 min. The clotted blood samples were centrifuged to separate the cells from the serum. Sera were then aspirated into labelled vials and stored in the freezer at -15°C pending usage.

Serum Total Cholesterol (TC) and High Density Lipoprotein (HDL-c) concentrations were determined using the enzymatic colorimetric test kit method of Siedel *et al*. (1983), while triglyceride concentration (TG) was determined by method of Negele *et al*. (1992), as used in previous studies (Ofem *et al*., 2014a, b; Nku *et al*., 2014; Essien *et al*., 2014; Ani *et al*., 2015).

Serum concentration of Low Density Lipoprotein (LDL-c) was measured using the Friedewald *et al*. (1972) relationship. Low density lipoprotein-cholesterol was obtained from the difference between serum TC and the sum of HDL-c and VLDL-c as shown below:

$$\text{LDL-c} = \text{TC-(HDL-c+VLDL-c)}$$

The Very Low Density Lipoprotein-cholesterol (VLDL-c) concentration, Cardiac Risk Ratio (CRR), Atherogenic Coefficient (AC) and Atherogenic Index of Plasma (AIP) were obtained mathematically as shown below:

$$\text{VLDL-C (mg dL}^{-1}) = \frac{\text{Triglyceride (TG)}}{2.2}$$

$$\text{Cardiac Risk Ratio (CRR)} = \frac{\text{TC}}{\text{HDL} - \text{c}}$$

$$\text{Atherogenic Coefficient (AC)} = \frac{\text{TC-HDL-c}}{\text{HDL-c}}$$

$$\text{Atherogenic Index of Plasma (AIP)} = \frac{\text{Log TC}}{\text{HDL-c}}$$

Statistical analysis: The data obtained from this study are presented as Mean±Standard Error of Mean (SEM). The one-way analysis of variance (ANOVA) was used for analysis of data within groups, followed by the Least Square Difference (LSD) post hoc multiple comparison, using SPSS software version 17.0 and Microsoft excel (2013 version). Data from batches one and two were compared using student's t-test. The $p<0.05$ was considered significant.

RESULTS

Comparison of the different parameters of serum lipid profile between the different experimental groups after 8 weeks of drug administration: Serum total cholesterol concentration for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group was 3.80±0.20, 3.88±0.16, 6.03±0.13, 1.94±0.43 and 1.59±0.23 mmol L^{-1}, respectively. Serum total cholesterol concentration was significantly increased in tadalafil treated group ($p<0.001$), but significantly reduced in tramadol ($p<0.001$) and sildenafil+tramadol treated group ($p<0.001$), compared with control and sildenafil treated groups. It was also significantly ($p<0.001$) lower in tramadol and sildenafil+tramadol treated groups, compared with tadalafil treated group (Table 2).

Serum triglyceride concentration for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group was 2.51±0.20, 0.95±0.22, 3.55±0.21, 1.54±0.39 and 0.96±0.18 mmol L^{-1}, respectively. Serum TG concentration was significantly reduced in sildenafil ($p<0.001$), tramadol ($p<0.05$) and sildenafil+tramadol ($p<0.001$) treated groups, but significantly increased in tadalafil treated group ($p<0.01$), compared with control. Serum TG concentration was significantly ($p<0.01$) increased in tadalafil treated group, compared with sildenafil treated group, but significantly ($p<0.001$) reduced in tramadol and sildenafil+tramadol treated groups, compared with tadalafil treated group (Table 2).

Serum concentration of HDL-c was 0.27±0.02, 0.18±0.01, 0.32±0.05, 0.25±0.05 and 0.25±0.06 mmol L^{-1} for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group, respectively. There was no significant difference in serum HDL-c concentration between the treated groups and control. However, serum HDL-c concentration was significantly ($p<0.05$) higher in tadalafil treated group compared with sildenafil treated group (Table 2).

Serum VLDL-c concentration was 1.14±0.09, 0.43±0.10, 1.61±0.10, 0.70±0.18 and 0.44±0.08 mmol L^{-1} for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group, respectively. Serum concentration of VLDL-c was significantly increased in tadalafil treated group ($p<0.01$), but significantly reduced in sildenafil ($p<0.001$), tramadol ($p<0.05$) and sildenafil+tramadol ($p<0.001$) treated groups, compared with control. It was also significantly ($p<0.001$) increased in tadalafil treated group, compared with sildenafil treated group and significantly ($p<0.001$) reduced in tramadol and sildenafil+tramadol treated groups, compared with tadalafil treated group (Table 2).

Serum LDL-c concentration for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group was 2.39±0.21, 3.27±0.15, 4.09±0.18, 0.99±0.24 and 0.90±0.20 mmol L^{-1}, respectively. Serum LDL-c concentration was significantly increased in sildenafil ($p<0.01$) and tadalafil ($p<0.001$) treated groups, but significantly ($p<0.001$) reduced in tramadol and sildenafil+tramadol treated groups, compared with control. It was also significantly ($p<0.01$) increased in tadalafil treated group, but significantly ($p<0.001$) reduced in tramadol and sildenafil+tramadol treated

Table 2: Comparison of serum lipid profile in the different experimental groups after 8 weeks of treatment with the various drugs

Parameters	Control	Sildenafil	Tadalafil	Tramadol	Sild.+Tram.
Total cholesterol (mmol L^{-1})	3.80±0.20	3.88±0.16	6.03±0.13^{a,d}	1.94±0.43^{a,d,q}	1.59±0.23^{a,d,q}
TG (mmol L^{-1})	2.51±0.20	0.95±0.22^{a}	3.55±0.21^{b,d}	1.54±0.39^{c,q}	0.96±0.18^{a,q}
HDL-c (mmol L^{-1})	0.27±0.02	0.18±0.01	0.32±0.05^{f}	0.25±0.05	0.25±0.06
VLDL-c (mmol L^{-1})	1.14±0.09	0.43±0.10^{a}	1.61±0.10^{b,d}	0.70±0.18^{c,q}	0.44±0.08^{a,q}
LDL-c (mmol L^{-1})	2.39±0.21	3.27±0.15^{b}	4.09±0.18^{a,e}	0.99±0.24^{a,d,q}	0.90±0.20^{a,d,q}
CRR	14.05±0.35	22.34±2.08^{e}	20.81±4.14	8.15±1.22^{d,r}	9.63±3.38^{e,r}
AC	13.05±0.35	21.34±2.08^{e}	19.81±4.14	7.15±1.22^{d,r}	8.63±3.38^{e,r}
AIP	0.97±0.05	0.70±0.08	1.06±0.05^{f}	0.77±0.07	0.62±0.20^{c,r}

Values are Mean±SEM, n = 5. a: $p<0.001$, b: $p<0.01$, c: $p<0.05$ vs control, d: $p<0.001$, e: $p<0.01$, f: $p<0.05$ vs sildenafil, q: $p<0.001$, r: $p<0.01$ vs tadalafil, CRR: Cardiac risk ratio, AC: Atherogenic coefficient, AIP: Atherogenic index of plasma

groups, compared with sildenafil treated group. Serum LDL-c concentration was significantly ($p<0.001$) reduced in tramadol and sildenafil+tramadol treated groups, compared with tadalafil treated group (Table 2).

Comparison of CRR, AC and AIP between the different experimental groups after 8 weeks of drug administration: Cardiac risk ratio for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group was 14.05±0.35, 22.34±2.08, 20.81±4.14, 8.15±1.22 and 9.63±3.38, respectively. Cardiac risk ratio was significantly ($p<0.05$) increased in sildenafil treated group, compared with control. The CRR was significantly reduced in tramadol ($p<0.001$) and sildenafil+tramadol ($p<0.01$) treated groups, compared with sildenafil treated group. It was also significantly ($p<0.01$) reduced in sildenafil and sildenafil+tramadol treated groups, compared with tadalafil treated group (Table 2).

Atherogenic coefficient for animals in the control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group was 13.05±0.35, 21.34±2.08, 19.81±4.14, 7.15±1.22 and 8.63±3.38, respectively. Atherogenic coefficient was significantly ($p<0.05$) higher in sildenafil treated group, compared with control. It was significantly lower in tramadol ($p<0.001$) and sildenafil+tramadol ($p<0.01$) treated groups, compared with sildenafil treated group. It was also significantly ($p<0.01$) lower in tramadol and sildenafil+tramadol treated groups, compared with tadalafil treated group (Table 2).

Results of AIP was 0.97±0.05, 0.70±0.08, 1.06±0.05, 0.77±0.07 and 0.62±0.20 for animals in the control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group, respectively. Atherogenic index of plasma was significantly ($p<0.05$) lower in sildenafil+tramadol treated group, compared with control ($p<0.05$) and tadalafil treated group ($p<0.01$). It was significantly ($p<0.05$) higher in tadalafil treated group, compared with sildenafil treated group (Table 2).

Comparison of the different parameters of serum lipid profile between the different experimental groups after 8 weeks recovery period: After 8 weeks of withdrawal of drug administration, serum total cholesterol concentration for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group was 3.72±0.19, 4.27±0.16, 3.75±0.29, 2.51±0.59 and 1.93±0.02 mmol L^{-1}, respectively. Serum total cholesterol concentration was significantly lower in tramadol ($p<0.05$) and sildenafil+tramadol ($p<0.001$) treated groups, compared with control. It was also significantly ($p<0.001$) lower in tramadol and sildenafil+tramadol treated groups, compared with sildenafil treated group. Serum total cholesterol concentration was significantly lower in tramadol ($p<0.05$) and sildenafil+tramadol ($p<0.001$) treated groups, compared with tadalafil treated group (Table 3).

Table 3: Comparison of serum lipid concentration in the different experimental groups following withdrawal of the various drugs for 8 weeks

Parameters	Control	Sildenafil	Tadalafil	Tramadol	Sild.+Tram.
Total cholesterol (mmol L^{-1})	3.72±0.19	4.27±0.16	3.75±0.29	2.51±0.59^{c,d,s}	1.93±0.02^{a,d,q}
TG (mmol L^{-1})	2.42±0.22	1.84±0.56	3.31±0.18^{e}	1.57±0.36^{q}	1.35±0.09^{c,q}
HDL-c (mmol L^{-1})	0.26±0.04	0.28±0.07	0.27±0.05	0.25±0.03	0.26±0.02
VLDL-c (mmol L^{-1})	1.10±0.10	0.84±0.25	1.50±0.08^{e}	0.71±0.17^{q}	0.61±0.04^{c,q}
LDL-c (mmol L^{-1})	2.36±0.26	3.15±0.31	1.98±0.24^{e}	1.54±0.44^{d}	1.06±0.04^{b,d,s}
Cardiac risk ratio	15.11±1.82	18.43±3.97	15.64±3.03	9.69±1.09^{f}	7.52±0.58^{c,e,s}
Atherogenic coefficient	14.11±1.82	17.43±3.97	14.64±3.03	8.69±1.09^{f}	6.52±0.58^{c,e,s}
Atherogenic index of plasma	0.97±0.07	0.79±0.13	1.11±0.08	0.71±0.18^{s}	0.71±0.05^{s}

Values are Mean±SEM, n = 5. a: $p<0.001$, b: $p<0.01$, c: $p<0.05$ vs control, d: $p<0.001$, e: $p<0.01$, f: $p<0.05$ vs sildenafil, q: $p<0.001$, r: $p<0.01$, s: $p<0.05$ vs tadalafil

Serum triglyceride concentration for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group was 2.42±0.22, 1.84±0.56, 3.31±0.18, 1.57±0.36 and 1.35±0.09 mmol L^{-1}, respectively. Serum TG concentration was significantly lower in sildenafil+tramadol treated group ($p<0.05$), compared with control. Serum TG concentration was significantly ($p<0.01$) higher in tadalafil treated group, compared with sildenafil treated group and significantly ($p<0.001$) lower in tramadol and sildenafil+tramadol treated group, compared with tadalafil treated group (Table 3).

Serum concentration of HDL-c following 8 weeks recovery period was 0.26±0.04, 0.28±0.07, 0.27±0.05, 0.25±0.03 and 0.26±0.02 mmol L^{-1} for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group, respectively. There was no significant difference in serum HDL-c concentration between the control and treated groups after 8 weeks recovery period (Table 3).

Serum VLDL-c concentration was 1.10±0.10, 0.84±0.25, 1.50±0.08, 0.71±0.17 and 0.61±0.04 mmol L^{-1} for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group, respectively. Serum VLDL-c concentration was significantly lower in sildenafil+tramadol treated group, compared with control. It was significantly ($p<0.01$) higher in tadalafil treated group, compared with sildenafil treated group, but significantly ($p<0.001$) lower in tramadol and sildenafil+tramadol treated groups, compared with tadalafil treated group (Table 3).

Serum LDL-c concentration after 8 weeks recovery period for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group was 2.36±0.26, 3.15±0.31, 1.98±0.24, 1.54±0.44 and 1.06±0.04 mmol L^{-1}, respectively. Serum LDL-c concentration was significantly ($p<0.01$) lower in sildenafil+tramadol treated group, compared with control. Serum LDL-c concentration was significantly lower in tadalafil ($p<0.01$), tramadol ($p<0.001$) and sildenafil+tramadol ($p<0.001$) treated groups, compared with sildenafil treated group. Serum LDL-c concentration was significantly ($p<0.05$) reduced in sildenafil+tramadol treated group, compared with tadalafil treated group (Table 3).

Comparison of CRR, AC and AIP between the different experimental groups after 8 weeks recovery period: Cardiac risk ratio for control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group was 15.11±1.82, 18.43±3.97, 15.64±3.03, 9.69±1.09 and 7.52±0.58 respectively. Cardiac risk ratio was significantly ($p<0.05$) lower in sildenafil+tramadol treated group, compared with control. The CRR was significantly reduced in tramadol ($p<0.05$) and sildenafil+tramadol ($p<0.01$) treated groups, compared with sildenafil treated group. It was also significantly ($p<0.05$) reduced in sildenafil+tramadol treated group, compared with tadalafil treated group (Table 3).

After 8 weeks recovery period, atherogenic coefficient for animals in the control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group was 14.11±1.82, 17.43±3.97, 14.64±3.03, 8.69±1.09 and 6.52±0.58, respectively. Atherogenic coefficient was significantly ($p<0.05$) lower in sildenafil+tramadol treated group, compared with control. It was significantly lower in tramadol ($p<0.05$) and sildenafil+tramadol ($p<0.01$) treated groups, compared with sildenafil treated group. It was also significantly ($p<0.01$) lower in sildenafil+tramadol treated group, compared with tadalafil treated group (Table 3).

Result of AIP after recovery was 0.97±0.07, 0.79±0.13, 1.11±0.08, 0.71±0.18 and 0.71±0.05 for animals in the control, sildenafil, tadalafil, tramadol and sildenafil+tramadol treated group, respectively. The values of AIP for the treated groups were not significantly different

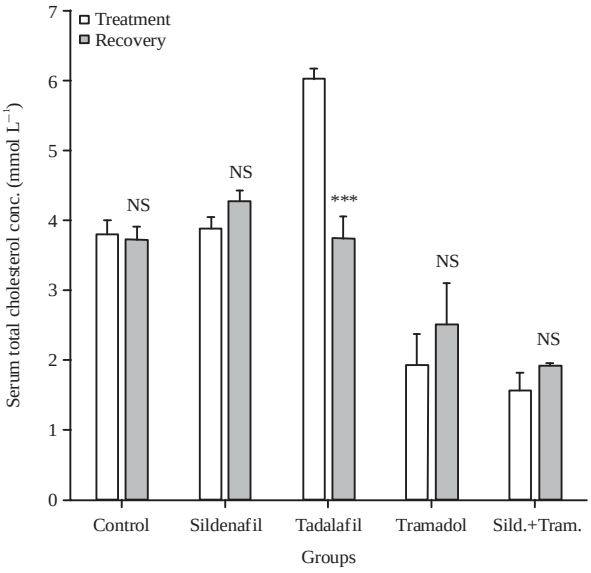

Fig. 1: Comparison of serum total cholesterol concentration between the treated and recovery groups. Values are Mean±SEM, n = 5. ***p<0.001 vs treated group, NS: Not significantly different from treated group

different from that of control. However, AIP was significantly (p<0.05) lower in tramadol and sildenafil+tramadol treated groups, compared with tadalafil treated group (Table 3).

Comparison of parameters of lipid profile between the treated and recovery batches of animals: The was no significant difference in the serum concentrations of total cholesterol between the treated and recovery group of animals for control, sildenafil, tramadol and sildenafil+tramadol groups. The tadalafil recovery group had significantly (p<0.001) lower serum total cholesterol, compared with the treated group (Fig. 1). The values of serum triglyceride (Fig. 2), high density lipoprotein concentration (Fig. 3), very low density lipoprotein cholesterol (Fig. 4), cardiac risk ratio (Fig. 5), atherogenic coefficient (Fig. 6) and atherogenic index of plasma (Fig. 7) for the recovery groups were not significantly different from the treated groups. Serum concentration of LDL-c was significantly (p<0.001) reduced in the tadalafil treated group, compared with its corresponding recovery group (Fig. 8). There was no significant difference in serum LDL-c concentration between the treated and recovery batches of animals for control, sildenafil, tramadol and sildenafil+ tramadol groups.

DISCUSSION

Some undesired effects (predominantly, headache and stomach pain) have been associated with chronic use of sex stimulants like; PDE5 inhibitors, tramadol hydrochloride (an opioid drug used for treatment of PE), herbal therapies, among others (Nna *et al*., 2014). This present study assessed serum lipid profile following chronic administration of ED and PE medications, since alteration in parameters of lipid profile may not be easily discernable, until it becomes life threatening.

Atherogenic indices are powerful indicators of the risk of heart disease; the higher the values, the higher the risk of developing cardiovascular disease and vice versa. In this present study,

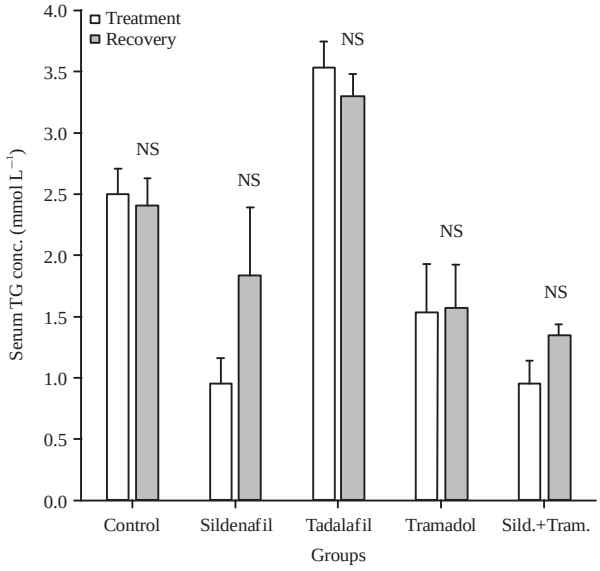

Fig. 2: Comparison of serum TG concentration between the treated and recovery groups. Values are Mean±SEM, n = 5. NS: Not significantly different from treated group

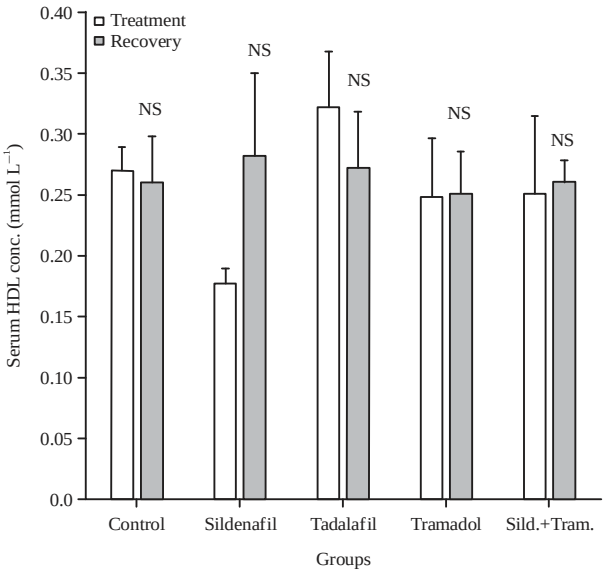

Fig. 3: Comparison of serum HDL cholesterol concentration between the treated and recovery groups. Values are Mean±SEM, n = 5. NS: Not significantly different from treated group

serum total cholesterol was significantly increased following administration of tadalafil, but significantly lowered by tramadol and sildenafil+tramadol, while sildenafil administered alone had no significant effect on serum total cholesterol. Tadalafil-treated group had the highest concentrations of total cholesterol, TG and LDL-c.

Steroid hormones depend largely on serum lipids for their synthesis, although acetyl Co-enzyme A also contributes to the pool of substrates (Guyton and Hall, 2010). The concentration of serum total cholesterol determines in part, the concentration of steroid hormones in serum.

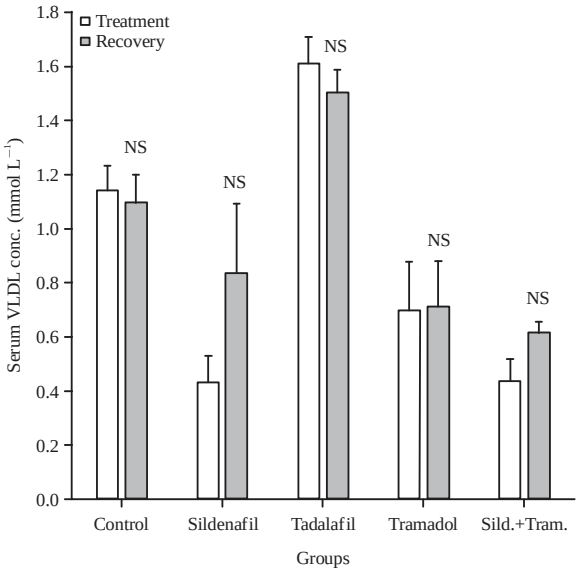

Fig. 4: Comparison of serum VLDL cholesterol concentration between the treated and recovery groups. Values are Mean±SEM, n = 5

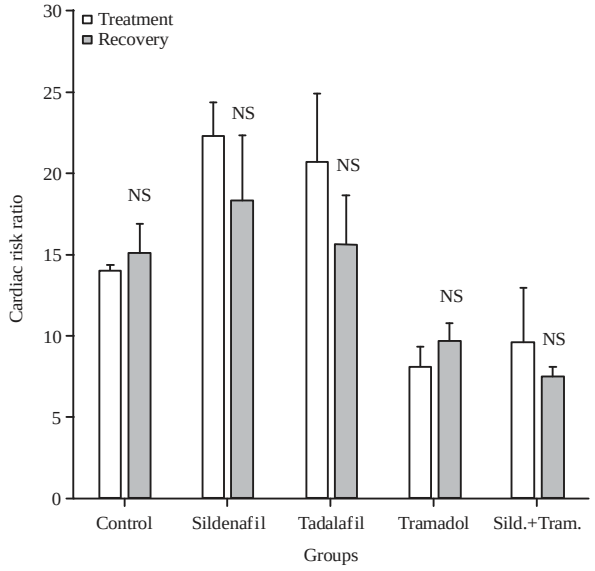

Fig. 5: Comparison of cardiac risk ratio between the treated and recovery groups. Values are Mean±SEM, n = 5, NS: Not significantly different from treated group

Consistent with this present study, previous reports had demonstrated that tramadol reduced serum concentration of total cholesterol (Abdellatief *et al*., 2014; Ahmed and Kurkar, 2014). The consequence of this is that steroid hormones concentration will likely be raised in tadalafil treated group and lowered in tramadol and sildenafil+tramadol treated groups, respectively. The significant reduction in serum total cholesterol observed in sildenafil+tramadol treated group may be attributed to the effect of tramadol hydrochloride since sildenafil administered in isolation did not significantly affect total cholesterol.

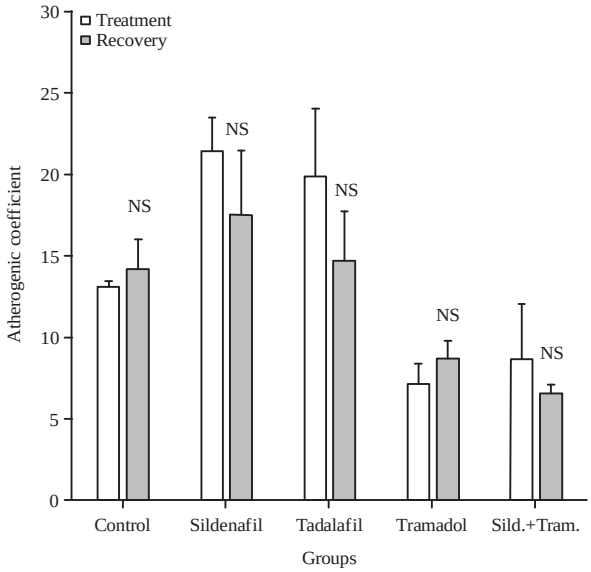

Fig. 6: Comparison of atherogenic coefficient between the treated and recovery groups. Values are Mean±SEM, n = 5, NS: Not significantly different from treated group

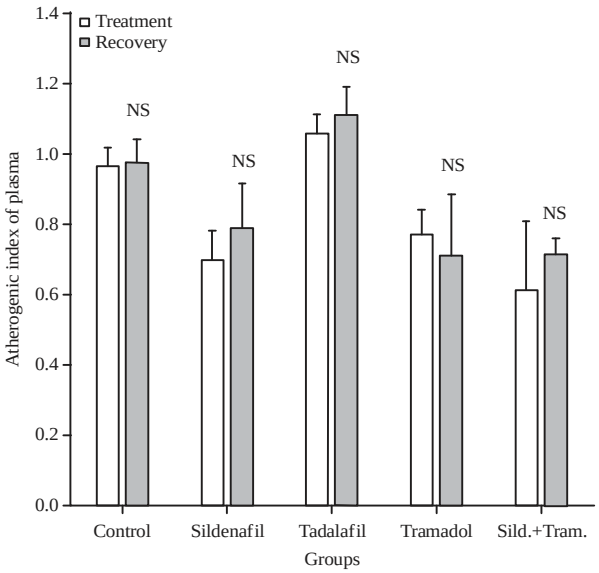

Fig. 7: Comparison of atherogenic index of plasma between the treated and recovery groups. Values are Mean±SEM, n = 5, NS: Not significantly different from treated group

The risk of suffering cardiovascular disease correlates positively with serum concentration of LDL-c and negatively with serum concentration of HDL-c. Tramadol administered alone and in combination with sildenafil, appears to be beneficial and cardioprotective. This is because the concentration of LDL-c was significantly reduced in both tramadol and sildenafil+tramadol treated groups, after eight weeks of drug administration. On the other hand, sildenafil administered alone and tadalafil both appear to be atherogenic, since they significantly increased serum LDL-c concentration. Although sildenafil did not significantly raise serum total cholesterol in the treated

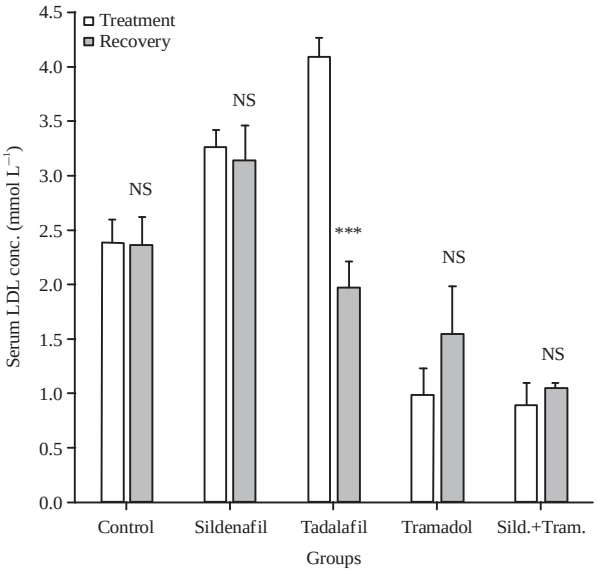

Fig. 8: Comparison of serum LDL-c cholesterol concentration between the treated and recovery groups. Values are Mean±SEM, n = 5. ***p<0.001 vs treated group, NS: Not significantly different from treated group

group, it significantly raised serum LDL-c concentration, decreased TG concentration, increased cardiac risk ratio and atherogenic coefficient. This trend further supports the fact that sildenafil may be atherogenic following prolong use. Furthermore, sildenafil treated group which had a significantly higher serum total cholesterol compared with tramadol and sildenafil+tramadol treated groups, had lower TG and HDL-c concentrations compared with tramadol and sildenafil+tramadol treated groups, though the difference was not significant. Therefore, tramadol attenuated the atherogenic effects of sildenafil in the group treated with sildenafil+tramadol combination. Atherogenic markers were lowest in the groups treated with tramadol and sildenafil+tramadol, thus, showing the beneficial effects of tramadol and sildenafil+tramadol in protecting against cardiovascular diseases.

Although acetyl co-enzyme A and serum cholesterol both contribute to the pool of substrates for steroid hormone (example; testosterone) synthesis, serum lipids (cholesterol) appears to be the dominant substrate. Apart from atherogenic benefits offered by recreational use of tramadol and sildenafil+tramadol, results of this present study further suggest that, serum levels of testosterone in tramadol and sildenafil+tramadol treated groups may be negatively affected, considering the decreased serum total cholesterol in these groups. Reduced serum concentration of testosterone (a steroid hormone) following tramadol administration, has been previously reported (Abdellatief *et al*., 2014; Ahmed and Kurkar, 2014). This effect may be attributed to the depletion of serum total cholesterol (substrate for testosterone synthesis) by tramadol.

Following withdrawal of treatment for eight weeks, serum total cholesterol increased in tramadol and sildenafil+tramadol recovery groups (Table 3). Despite the increase, serum total cholesterol was still significantly lower than control values. When the treated groups were compared with the recovery groups, serum total cholesterol was higher in tramadol and sildenafil+tramadol recovery groups, compared with their respective treated groups (Fig. 1). Apart

from serum total cholesterol and LDL-c which significantly ($p<0.001$) reduced in tadalafil recovery group, compared with its treated group (Fig. 1 and 5), all other parameters assessed showed insignificant reversibility when values from the recovery groups were compared with their respective treated groups (Fig. 1-8).

CONCLUSION

The recreational use of PDE5 inhibitors (notably, sildenafil and tadalafil) alter serum lipid profile, with poor reversibility following their withdrawal. Considering the serum cholesterol depleting effects of tramadol and sildenafil+tramadol, it is important to tame their abuse, as this could affect processes that depend largely on serum lipids for their proper functioning (example; synthesis of steroid hormones). On the other hand, in view of the serum lipid lowering effects of tramadol and sildenafil+tramadol, it will be worthwhile to assess the possibility of treating hyperlipidaemia with these drugs using hyperlipidaemic rat models.

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