

Hepatotoxicity following separate administration of two phosphodiesterase-5 inhibitors (sildenafil & tadalafil) and opioid (tramadol); evaluation of possible reversal following their withdrawal

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ABSTRACT

The use of phosphodiesterase-5 inhibitors (PDE5i) and tramadol in the absence of erectile and ejaculatory dysfunctions in Nigeria has become a norm. In this study, we comparatively assess the effects of chronic use of these drugs on hepatotoxicity. Fifty male albino wistar rats weighing 180–200g were randomly assigned into 5 groups (n=10) as follows; control, sildenafil (1mg/100g b.w), tadalafil (1mg/100g b.w), tramadol (2mg/100g b.w) and sildenafil+tramadol treated group (1mg/100g and 2mg/100g b.w, respectively). Drugs were orally administered, once, every two days for 8weeks, at the end of which five animals were sacrificed per group (batch 1), while the remaining five animals per group were allowed for another 8weeks without drug administration (batch 2). Serum concentration of liver enzymes (AST, ALT and ALP) and bilirubin were assessed in both batches. Serum concentrations of AST, ALT, ALP, total bilirubin and unconjugated bilirubin were significantly (p<0.001) increased in all treated groups (batch 1), while conjugated bilirubin concentration was significantly (p<0.001) reduced in all treated groups, compared with control. Serum concentrations of AST and ALT were significantly reduced in sildenafil (p<0.01), tadalafil (p<0.05), tramadol (p<0.001) and sildenafil+tramadol (p<0.001) recovery groups, compared with their treated groups. Total and unconjugated bilirubin fractions were significantly (p<0.05 and p<0.01, respectively) reduced in tadalafil recovery group, compared with the treated group. Sildenafil+tramadol recovery group showed significantly (p<0.001) reduced total and unconjugated bilirubin concentrations, compared with the treated group. Chronic administration of PDE5i and tramadol reversibly altered liver functions.

INTRODUCTION

Sildenafil citrate (common name–viagra) and tadalafil (common name–cialis) are widely used for treatment of erectile dysfunction (ED) of various etiologies (Nurnberg *et al.*, 2001; Benchekroun *et al.*, 2003; Gopalakrishnan *et al.*, 2006; Kokkinos *et al.*, 2015). These drugs belong to a class of drugs called phosphodiesterase-5 inhibitors (PDE5i), owing to their potent and selective inhibition of phosphodiesterase type 5 enzyme that breaks down cyclic guanosine monophosphate (cGMP) (Boolell *et al.*, 1996). Inhibiting the degradation of c GMP prolongs

erection, and forms the basis of the efficacy of this class of drugs. Undoubtedly, erectile dysfunction most often occurs together with premature ejaculation (Rosen *et al.*, 2004). Several studies have reported the beneficial effect of the use of tramadol hydrochloride (an opioid drug) to delay ejaculation (Safarinejad & Hosseini, 2006; Salem *et al.*, 2008; Althof *et al.*, 2010; Rowland *et al.*, 2010). This later informed the use of tramadol in patients suffering premature ejaculation. Most recently, there has been an upsurge in the abuse of ED medications. In a population–based study reported by Nna *et al.*, (2014a, b) in Calabar, Nigeria, the young population indulged more in the use of ED medications, even in the absence of erectile or ejaculatory dysfunction.

Their motivation for using these medications revolve round a number of factors, however, the most recurrent factor as reported in that study is; a quest for a bigger genital size.

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Users of sex stimulants have reported their side effects to include stomach pain and headache (Nna *et al.*, 2014a). It is conceivable that there are other side effects which may not be easily discernible like headache and stomach pain, but may be serious enough to affect one's health. It is against this backdrop that the present study seeks to ascertain the effect of chronic use of these drugs (sildenafil, tadalafil and tramadol) on hepatotoxicity, since the liver will be constantly bombarded with large concentrations of these medications. Also, the present study investigates the possibility of reversal of hepatotoxicity following the withdrawal of these drugs.

MATERIALS AND METHODS

Animal preparation and protocol

Fifty (50) male albino wistar rats weighing 180 – 200g were used for this study. The animals were purchased from the Department of Agriculture, in University of Calabar, and housed in well ventilated cages (wooden bottom and wire mesh top) in the animal house of the College of Medical Sciences, University of Calabar. The animals were allowed access to rat feed and water *ad libitum*, and exposed to 12/12 hours light/dark cycle. The animals were allowed 7 days for habituation before commencement of the research. Indeed, the animals were handled in accordance with the laid down principles for animal care as instituted by Helsinki in 1964.

Experimental design and drugs used

The rats were randomly assigned into 5 groups (n = 10) as shown in table 1. The drugs used in this study were; sildenafil citrate (Maxheal Laboratories Pvt Ltd, India), tadalafil (Pfizer, India) and tramadol hydrochloride (Glow Pharma Pvt Ltd, India). The different drugs were administered per oral route, once, every two days, for 8 weeks. This design was selected because it is conceivable that these classes of drugs may not be taken daily. At the end of 8 weeks of treatment, five animals were randomly selected and sacrificed from each group, blood samples were collected for assessment of serum bilirubin concentration and liver enzyme activities. The remaining 5 animals per group were allowed for another 8 weeks, without treatment (recovery phase), but were allowed access to feed and water *ad libitum*.

Table 1: Experimental design.

Group	No. of animals	Treatment
1 (NC)	10	Distil water
2 (Sildenafil)	10	Sildenafil (dose = 1mg/100g b.w.)
3 (Tadalafil)	10	Tadalafil (dose = 1mg/100g b.w.)
4 (Tramadol)	10	Tramadol (dose = 2mg/100g b.w.)
5 (Sild.+Tram.)	10	Sildenafil & Tramadol (dose = 1mg/100g b.w. & 2mg/100g b.w., respectively)

Collection of blood samples

Under chloroform anaesthesia, blood samples were collected from the animals through cardiac puncture into pre-labelled sample bottles, and allowed to clot for 30 minutes. 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.

Measurement of serum concentration of liver enzymes

Serum concentrations of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were determined using the method described by Reitman and Frankle (1957), modified by Bhutia *et al.*, (2006).

Measurement of serum bilirubin concentration

Serum bilirubin concentration was measured using the method described by Sherlock and Lunec (1951).

Percentage conjugation of bilirubin

The percentage of serum total bilirubin that was conjugated (SCB) was obtained mathematically using the formula below, as previously used by Nna *et al.*, (2014c).

Percentage SCB =

$$\frac{\text{Serum concentrated bilirubin concentration}}{\text{Serum total bilirubin concentration}} \times 100$$

Statistical analysis

The data obtained from this study are presented as mean \pm SEM. The one way analysis of variance (ANOVA) was used to analyse the data, following by post hoc multiple comparison (least square difference procedure). Also, student's t – test was used to compare the difference between treated and recovery groups. *P* values <0.05 were considered significant. Computer software, SPSS (version 17.0) and Microsoft excel (2010 version) analyser were used for the analysis of data.

RESULTS

Comparison of serum concentration of liver enzymes and bilirubin in the different experimental groups following 8 weeks of treatment with the various drugs

Serum AST concentration

After 8 weeks of drug administration, serum AST concentration for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups were 16.40 \pm 1.54, 39.60 \pm 3.57, 84.20 \pm 3.28, 104.40 \pm 3.11 and 57.20 \pm 4.57 IU/L, respectively. Serum AST concentration was significantly (*p*<0.001) increased in all treated groups, compared with control group. Serum AST concentration was significantly (*p*<0.001) higher in tadalafil, tramadol and sildenafil + tramadol treated groups, compared with sildenafil treated group. It was also significantly (*p*<0.001) higher in tramadol treated group, compared with tadalafil treated group. Serum AST concentration was significantly (*p*<0.001) lower in sildenafil + tramadol treated group, compared with tadalafil and tramadol treated groups (table 2).

Table 2: Comparison of serum concentration of liver enzymes and bilirubin in the different experimental groups after 8 weeks of treatment with the various drugs.

Parameter	Control	Sildenafil	Tadalafil	Tramadol	Sild. + Tram.
AST (IU/L)	16.40 ± 1.54	39.60 ± 3.57 ^a	84.20 ± 3.28 ^{a,d}	104.40 ± 3.11 ^{a,d,p}	57.20 ± 4.57 ^{a,d,p,x}
ALT (IU/L)	34.20 ± 0.37	57.60 ± 3.54 ^a	78.60 ± 3.78 ^{a,d}	64.80 ± 3.62 ^{a,q}	46.80 ± 2.15 ^{b,f,p,x}
ALP (IU/L)	31.00 ± 2.30	56.74 ± 4.65 ^a	54.95 ± 3.92 ^a	59.79 ± 4.90 ^a	54.18 ± 1.58 ^a
AST:ALT	0.48 ± 0.04	0.70 ± 0.07	1.09 ± 0.09 ^{a,e}	1.64 ± 0.12 ^{a,d,p}	1.24 ± 0.13 ^{a,d,y}
Total Bilirubin (µmol/L)	11.92 ± 0.40	20.20 ± 0.56 ^c	24.99 ± 1.68 ^b	52.40 ± 4.92 ^{a,d,p}	30.96 ± 3.10 ^{a,f,x}
Conjugated Bilirubin (µmol/L)	8.90 ± 1.21	3.62 ± 0.30 ^a	4.04 ± 0.42 ^a	5.58 ± 0.15 ^{a,f}	1.48 ± 0.18 ^{a,f,q,x}
Unconjugated Bilirubin (µmol/L)	3.51 ± 0.31	16.58 ± 0.80 ^b	20.95 ± 1.42 ^a	46.82 ± 5.00 ^{a,d,p}	29.48 ± 3.25 ^{a,r,z}
Percentage Conjugation of Bilirubin (%)	74.56 ± 9.86	18.09 ± 1.88 ^a	16.16 ± 1.25 ^a	11.23 ± 1.55 ^a	5.14 ± 0.99 ^a

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;

p = p<0.001, q = p<0.01, r = p<0.05 vs tadalafil; x = p<0.001, y = p<0.01, z = p<0.05 vs tramadol

Serum ALT concentration

Serum ALT concentration for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups were 34.20 ± 0.37, 57.60 ± 3.54, 78.60 ± 3.78, 64.80 ± 3.62 and 46.80 ± 2.15 IU/L, respectively. Serum ALT concentration was significantly increased in sildenafil (p<0.001), tadalafil (p<0.001), tramadol (p<0.001) and sildenafil + tramadol treated group (p<0.01), compared with control. Tadalafil treated group had significantly (p<0.001) higher serum ALT concentration, while sildenafil + tramadol treated group had a significantly lower (p<0.05) ALT concentration, compared with sildenafil treated group (Table 2.0). Serum ALT concentration was significantly lower in tramadol treated group (p<0.01) and sildenafil + tramadol treated group (p<0.001), compared with tadalafil treated group. Also, serum ALT concentration was significantly (p<0.001) lower in sildenafil + tramadol treated group, compared with tramadol treated group (table 2.0).

Serum ALP concentration

Serum ALP concentration after 8 weeks of drug treatment was 31.00 ± 2.30, 56.74 ± 4.65, 54.95 ± 3.92, 59.79 ± 4.90 and 54.18 ± 1.58 IU/L, for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups. Serum ALP concentration was significantly (p<0.001) increased in sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups, compared with control (table 2.0).

AST/ALT ratio

The AST/ALT ratio after 8 weeks of drug treatment was 0.48 ± 0.04, 0.70 ± 0.07, 1.09 ± 0.09, 1.64 ± 0.12 and 1.24 ± 0.13, for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups, respectively. The ratio of AST to ALT was significantly (p<0.001) higher in tadalafil, tramadol and sildenafil + tramadol treated groups, compared with control. It was also significantly higher in tadalafil (p<0.01), tramadol (p<0.001) and sildenafil + tramadol treated groups (p<0.001), compared with sildenafil treated group.

The ratio of AST to ALT was significantly (p<0.001) higher in tramadol treated group, compared with tadalafil treated group, and significantly (p<0.01) lower in sildenafil + tramadol treated group, compared with tramadol treated group (table 2.0).

Serum total bilirubin concentration

Serum total bilirubin concentration for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups were 11.92 ± 0.40, 20.20 ± 0.56, 24.99 ± 1.68, 52.40 ± 4.92 and 30.96 ± 3.10 µmol/L, respectively. Serum total bilirubin concentration was significantly higher in sildenafil (p<0.05), tadalafil (p<0.01), tramadol (p<0.001) and sildenafil + tramadol treated group (p<0.001), compared with control. Total bilirubin concentration was significantly higher in tramadol (p<0.001) and sildenafil + tramadol treated group (p<0.05), compared with sildenafil treated group, and significantly (p<0.001) higher in tramadol treated group, compared with tadalafil and sildenafil + tramadol treated groups (table 2.0).

Serum conjugated bilirubin concentration

Following 8 weeks of drug administration, serum conjugated bilirubin concentration for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups were 8.90 ± 1.21, 3.62 ± 0.30, 4.04 ± 0.42, 5.58 ± 0.15 and 1.48 ± 0.18 µmol/L, respectively. Serum conjugated bilirubin concentration was significantly (p<0.001) lower in sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups, compared with control. Serum conjugated bilirubin concentration was significantly (p<0.05) higher in tramadol treated group, but significantly (p<0.05) lower in sildenafil + tramadol treated group, compared with sildenafil treated group. Serum concentration of conjugated bilirubin was significantly lower in sildenafil + tramadol treated group, compared with tadalafil (p<0.01) and tramadol (p<0.001) treated groups (table 2.0).

Serum unconjugated bilirubin concentration

After 8 weeks of drug administration, serum unconjugated bilirubin concentration for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups were 3.51 ± 0.31, 16.58 ± 0.80, 20.95 ± 1.42, 46.82 ± 5.00 and 29.48 ± 3.25 µmol/L, respectively. Serum unconjugated bilirubin concentration was significantly higher in sildenafil (p<0.01), tadalafil (p<0.001), tramadol (p<0.001) and sildenafil + tramadol (p<0.001) treated groups, compared with control. Serum unconjugated bilirubin concentration was significantly (p<0.001) higher in tramadol treated group, compared with sildenafil treated

group. Serum concentration of unconjugated bilirubin was significantly higher in tramadol ($p < 0.001$) and sildenafil + tramadol ($p < 0.05$) treated groups, compared with tadalafil treated group. Serum concentration of unconjugated bilirubin was significantly ($p < 0.05$) lower in sildenafil + tramadol treated group, compared with tramadol treated group (table 2.0).

Percentage conjugation of serum total bilirubin

After 8 weeks of drug administration, the percentage conjugation of serum total bilirubin for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups was 74.56 ± 9.86 , 18.09 ± 1.88 , 16.16 ± 1.25 , 11.23 ± 1.55 and 5.14 ± 0.99 %, respectively. Percentage conjugation of serum total bilirubin was significantly ($p < 0.001$) lower in sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups, compared with control (table 2).

Comparison of serum concentration of liver enzymes and bilirubin in the different experimental groups after 8 weeks of withdrawal of treatment

Serum AST concentration

After 8 weeks of withdrawal of treatment, serum AST concentration for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups was 15.20 ± 1.02 , 23.00 ± 1.92 , 66.80 ± 4.47 , 38.00 ± 2.26 and 15.00 ± 1.14 IU/L, respectively. Serum AST concentration was significantly higher in sildenafil ($p < 0.05$), tadalafil ($p < 0.001$) and tramadol ($p < 0.001$) treated groups, compared with control. Serum AST concentration was significantly higher in tadalafil ($p < 0.001$) and tramadol ($p < 0.001$) treated groups, but significantly lower in sildenafil + tramadol treated group ($p < 0.05$), compared with sildenafil treated group. It was also significantly ($p < 0.001$) lower in tramadol and sildenafil + tramadol treated groups, compared with tadalafil treated group. Serum AST concentration was significantly ($p < 0.001$) lower in sildenafil + tramadol treated group, compared with tramadol treated group (table 3).

Serum ALT concentration

Serum ALT concentration for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups was

35.2 ± 0.80 , 44.4 ± 1.40 , 55.00 ± 3.39 , 45.60 ± 4.79 and 41.40 ± 1.03 IU/L, respectively. Serum ALT concentration was significantly higher in sildenafil ($p < 0.05$), tadalafil ($p < 0.001$) and tramadol ($p < 0.05$) treated groups, compared with control. Tadalafil and sildenafil + tramadol treated groups had significantly ($p < 0.05$) lower serum ALT concentration, compared with sildenafil treated group.

Serum ALT concentration was significantly ($p < 0.05$) lower in tramadol and sildenafil + tramadol treated groups, compared with tadalafil treated group (table 3.0).

Serum ALP concentration

Serum ALP concentration after 8 weeks of withdrawal of treatment was 30.40 ± 2.87 , 61.60 ± 3.78 , 50.20 ± 4.15 , 87.80 ± 4.47 and 50.40 ± 1.89 IU/L, for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups. Serum ALP concentration was significantly ($p < 0.001$) increased in sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups, compared with control. Serum ALP concentration was significantly ($p < 0.001$) higher in tramadol treated group, but significantly ($p < 0.05$) lower in tadalafil and sildenafil + tramadol treated groups, compared with sildenafil treated group. Serum ALP concentration was significantly ($p < 0.001$) higher in tramadol treated group, compared with tadalafil and sildenafil + tramadol treated groups (table 3.0).

AST/ALT ratio

The AST/ALT ratio after 8 weeks of withdrawal of treatment was 0.43 ± 0.03 , 0.52 ± 0.04 , 1.23 ± 0.09 , 0.87 ± 0.09 and 0.36 ± 0.02 , for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups, respectively. The ratio of AST to ALT was significantly ($p < 0.001$) higher in tadalafil and tramadol treated groups, compared with control. It was also significantly ($p < 0.001$) higher in tadalafil and tramadol treated groups, compared with sildenafil treated group.

The ratio of AST to ALT was significantly ($p < 0.001$) lower in tramadol treated group, compared with tadalafil treated group, and significantly ($p < 0.001$) lower in sildenafil + tramadol treated group, compared with tramadol treated group (table 3).

Table 3: Comparison of serum concentration of liver enzymes and bilirubin in the different experimental groups following withdrawal of the various drugs for 8 weeks.

Parameter	Control	Sildenafil	Tadalafil	Tramadol	Sild.+Tram.
AST (IU/L)	15.20 ± 1.02	23.00 ± 1.92^c	$66.80 \pm 4.47^{a,d}$	$38.00 \pm 2.26^{a,d,p}$	$15.00 \pm 1.14^{b,p,x}$
ALT (IU/L)	35.2 ± 0.80	44.4 ± 1.40^c	$55.00 \pm 3.39^{a,f}$	$45.60 \pm 4.79^{c,r}$	$41.40 \pm 1.03^{f,r}$
ALP (IU/L)	30.40 ± 2.87	61.60 ± 3.78^a	$50.20 \pm 4.15^{a,f}$	$87.80 \pm 4.47^{a,d,p}$	$50.40 \pm 1.89^{a,f,x}$
AST:ALT	0.43 ± 0.03	0.52 ± 0.04	$1.23 \pm 0.09^{a,d}$	$0.87 \pm 0.09^{a,d,p}$	$0.36 \pm 0.02^{b,x}$
Total Bilirubin ($\mu\text{mol/L}$)	11.90 ± 0.79	18.17 ± 2.24^b	19.94 ± 0.88^a	$44.86 \pm 1.73^{a,d,p}$	$10.79 \pm 0.55^{d,p,x}$
Conjugated Bilirubin ($\mu\text{mol/L}$)	8.78 ± 1.14	5.70 ± 0.99^b	5.47 ± 0.48^b	$9.24 \pm 0.33^{c,d}$	$1.78 \pm 0.07^{a,e,q,x}$
Unconjugated Bilirubin ($\mu\text{mol/L}$)	3.12 ± 0.47	12.47 ± 2.23^a	14.47 ± 0.84^a	$35.62 \pm 1.79^{a,d,p}$	$9.53 \pm 0.55^{b,r,x}$
Percentage Conjugation of Bilirubin (%)	72.73 ± 5.30	32.68 ± 7.05^a	27.52 ± 2.15^a	20.73 ± 1.15^a	$16.69 \pm 1.15^{a,f}$

Values are mean \pm 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; p = $p < 0.001$, q = $p < 0.01$, r = $p < 0.05$ vs tadalafil;., x = $p < 0.001$, y = $p < 0.01$ vs tramadol

Serum total bilirubin concentration

Serum total bilirubin concentration for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups was 11.90 ± 0.79 , 18.17 ± 2.24 , 19.94 ± 0.88 , 44.86 ± 1.73 and 10.79 ± 0.55 $\mu\text{mol/L}$, respectively. Serum total bilirubin concentration was significantly higher in sildenafil ($p < 0.01$), tadalafil ($p < 0.001$) and tramadol ($p < 0.001$) treated groups, compared with control. Total bilirubin concentration was significantly ($p < 0.001$) higher in tramadol treated group, but significantly ($p < 0.001$) lower in sildenafil + tramadol treated group, compared with sildenafil treated group.

It was also significantly ($p < 0.001$) higher in tramadol treated group, but significantly ($p < 0.001$) lower in sildenafil + tramadol treated group, compared with tadalafil treated group. Serum total bilirubin concentration was significantly ($p < 0.001$) lower in sildenafil + tramadol treated group, compared with tramadol treated group (table 3.0).

Serum conjugated bilirubin concentration

Following 8 weeks of withdrawal of drug administration, serum conjugated bilirubin concentration for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups was 8.78 ± 1.14 , 5.70 ± 0.99 , 5.47 ± 0.48 , 9.24 ± 0.33 and 1.78 ± 0.07 $\mu\text{mol/L}$, respectively.

Serum conjugated bilirubin concentration was significantly lower in sildenafil ($p < 0.01$), tadalafil ($p < 0.01$) and sildenafil + tramadol ($p < 0.001$) treated groups, compared with control. Serum conjugated bilirubin concentration was significantly ($p < 0.01$) higher in tramadol treated group, but significantly ($p < 0.01$) lower in sildenafil + tramadol treated group, compared with sildenafil treated group. It was also significantly ($p < 0.01$) higher in tramadol treated group, but significantly ($p < 0.01$) lower in sildenafil + tramadol treated group, compared with tadalafil treated group. Serum concentration of conjugated bilirubin was significantly ($p < 0.001$) lower in sildenafil + tramadol treated group, compared with tramadol treated group (table 3.0).

Serum unconjugated bilirubin concentration

After 8 weeks of withdrawal of drug administration, serum unconjugated bilirubin concentration for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups was 3.12 ± 0.47 , 12.47 ± 2.23 , 14.47 ± 0.84 , 35.62 ± 1.79 and 9.53 ± 0.55 $\mu\text{mol/L}$, respectively. Serum unconjugated bilirubin concentration was significantly higher in sildenafil ($p < 0.001$), tadalafil ($p < 0.001$), tramadol ($p < 0.001$) and sildenafil + tramadol ($p < 0.01$) treated groups, compared with control. Serum unconjugated bilirubin concentration was significantly ($p < 0.001$) higher in tramadol treated group, compared with sildenafil treated group.

Serum concentration of unconjugated bilirubin was significantly higher in tramadol ($p < 0.001$) treated group, but significantly ($p < 0.01$) lower in sildenafil + tramadol treated group, compared with tadalafil treated group. Serum concentration of

unconjugated bilirubin was significantly ($p < 0.001$) lower in sildenafil + tramadol treated group, compared with tramadol treated group (table 3.0).

Percentage conjugation of serum total bilirubin

After 8 weeks of withdrawal of drug administration, the percentage conjugation of serum total bilirubin for control, sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups was 72.73 ± 5.30 , 32.68 ± 7.05 , 27.52 ± 2.15 , 20.73 ± 1.15 and 16.69 ± 1.15 %, respectively. Percentage conjugation of serum total bilirubin was significantly ($p < 0.001$) lower in sildenafil, tadalafil, tramadol and sildenafil + tramadol treated groups, compared with control. Percentage conjugation of serum total bilirubin was significantly ($p < 0.05$) lower in sildenafil + tramadol treated group, compared with sildenafil treated group (table 3.0).

Comparison of serum concentration of liver enzymes and bilirubin between treated and recovery groups

Serum AST concentration

There was no significant difference in serum AST concentration between the treated and recovery control groups. Serum AST concentration was significantly reduced in sildenafil ($p < 0.01$), tadalafil ($p < 0.05$), tramadol ($p < 0.001$) and sildenafil + tramadol ($p < 0.001$) recovery groups, compared with their respective treated groups (figure 1).

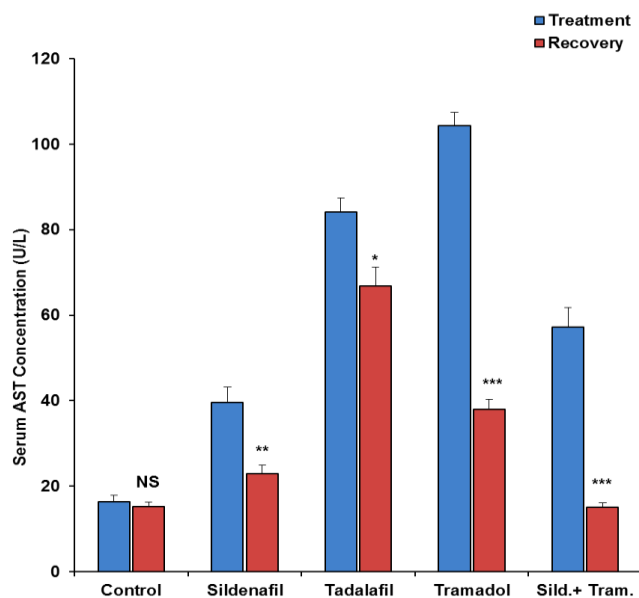


Fig. 1: Comparison of serum AST concentration between the treated and recovery groups. Values are mean \pm SEM, $n = 5$.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs treatment; NS = not significant.

Serum ALT concentration

There was no significant difference in serum ALT concentration between the treated and recovery control groups. Serum ALT concentration was significantly reduced in sildenafil ($p < 0.01$), tadalafil ($p < 0.01$) and tramadol ($p < 0.05$) recovery groups, compared with their respective treated groups. There was

no significant difference in serum ALT concentration between the treated and recovery groups of animals administered sildenafil + tramadol (figure 2).

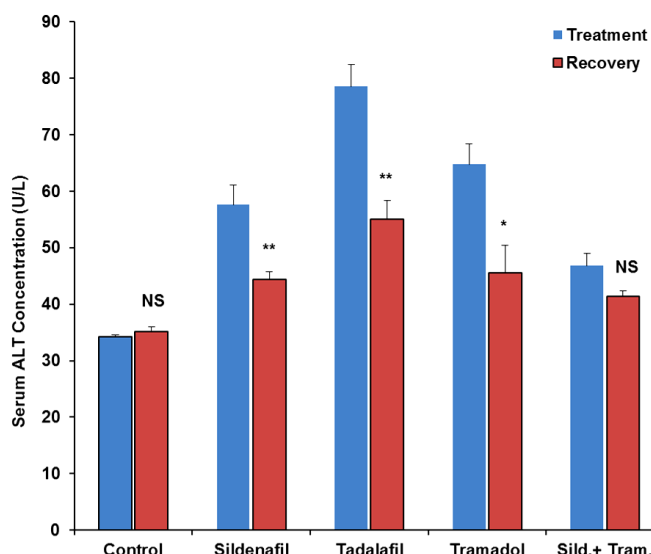


Fig. 2: Comparison of serum ALT concentration between the treated and recovery groups. Values are mean \pm SEM, n = 5.
*p<0.05, **p<0.01 vs treatment; NS = not significant.

Serum ALP concentration

There was no significant difference in serum ALP concentration between the treated and recovery control groups. Also, serum ALP concentration for sildenafil, tadalafil and sildenafil + tramadol recovery groups were not significantly different from their respective treated groups. Furthermore, serum ALP concentration was significantly ($p<0.01$) increased in tramadol recovery group, compared with its treated group (figure 3).

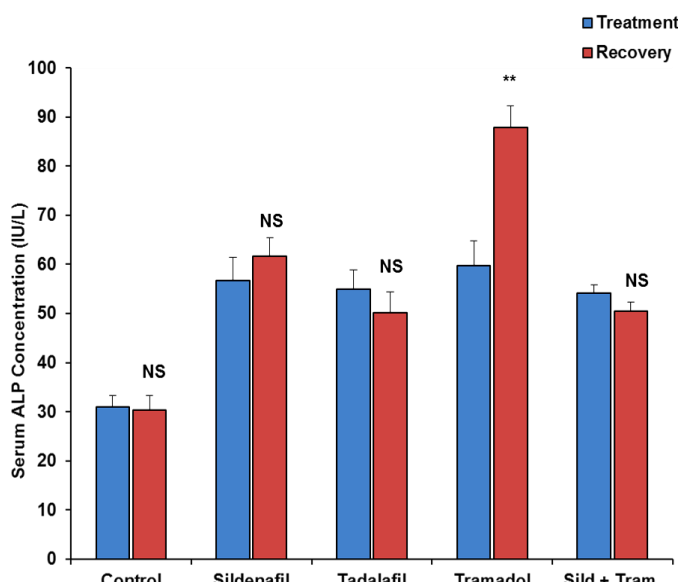


Fig. 3: Comparison of serum ALP concentration between the treated and recovery groups. Values are mean \pm SEM, n = 5.
**p<0.01 vs treatment; NS = not significant.

AST/ALT ratio

There was no significant difference in AST:ALT ratio between the treated and recovery control groups. The ratio of AST to ALT in sildenafil and tadalafil recovery groups did not significantly differ from the treated groups. Furthermore, AST:ALT ratio was significantly reduced in tramadol ($p<0.001$) and sildenafil + tramadol ($p<0.001$) recovery groups, compared with their respective treated group (figure 4).

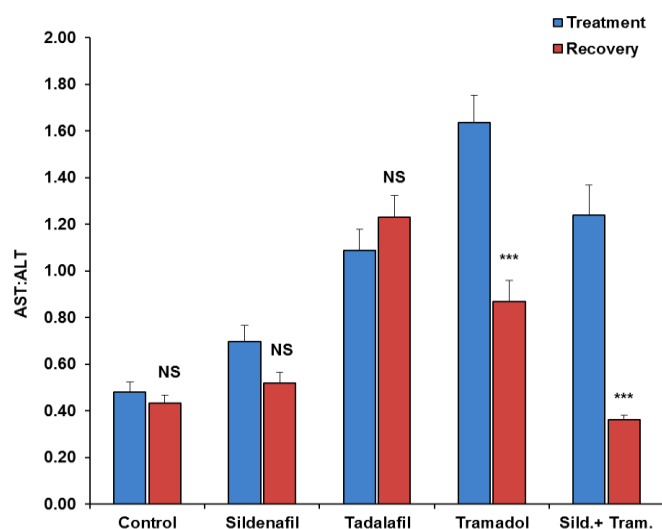


Fig. 4: Comparison of AST/ALT ratio between the treated and recovery groups. Values are mean \pm SEM, n = 5.
***p<0.001 vs treatment; NS = not significant.

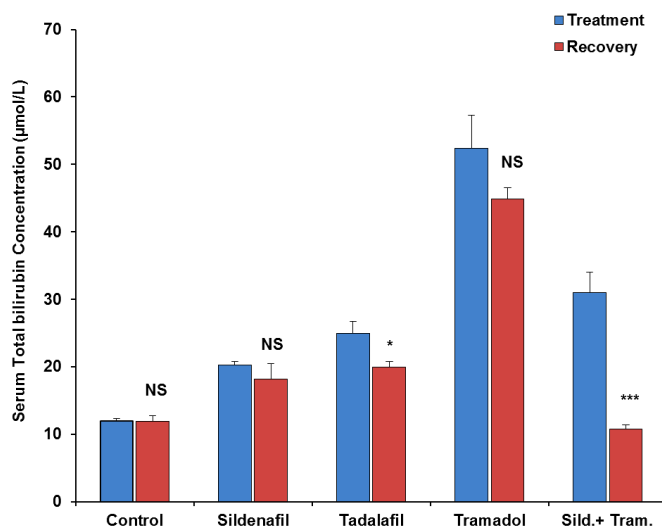


Fig. 5: Comparison of serum total bilirubin concentration between the treated and recovery groups. Values are mean \pm SEM, n = 5.
*p<0.05, ***p<0.001 vs treatment; NS = not significant

Serum total bilirubin concentration

There was no significant difference in serum total bilirubin concentration between the treated and recovery control groups. Serum total bilirubin concentration was significantly reduced in tadalafil ($p<0.05$) and sildenafil + tramadol ($p<0.001$)

recovery groups, compared with their respective treated groups. There was no significant difference in serum total bilirubin concentration between the treated and recovery groups of animals administered sildenafil and tramadol, respectively (figure 5).

Serum conjugated bilirubin concentration

There was no significant difference in serum conjugated bilirubin concentration between the treated and recovery control groups. Serum conjugated bilirubin concentration was significantly ($p < 0.001$) increased in tramadol recovery group, compared with its treated group. Although serum conjugated bilirubin concentration was increased in sildenafil, tadalafil and sildenafil + tadalafil recovery groups, there were not significantly different from their respective treated groups (figure 6).

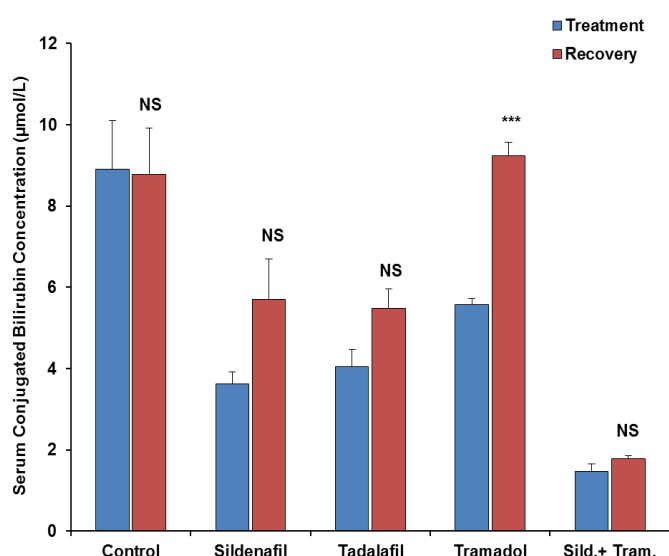


Fig. 6: Comparison of serum conjugated bilirubin concentration between the treated and recovery groups. Values are mean \pm SEM, $n = 5$. *** $p < 0.001$ vs treatment; NS = not significant

Serum unconjugated bilirubin concentration

There was no significant difference in serum unconjugated bilirubin concentration between the treated and recovery control groups. Also, there was no significant difference in serum unconjugated bilirubin concentration in sildenafil and tramadol recovery groups, compared with their respective treated groups. Serum unconjugated bilirubin concentration was significantly reduced in tadalafil ($p < 0.01$) and sildenafil + tramadol ($p < 0.001$) recovery groups, compared with their respective treated groups (figure 7).

Percentage conjugation of serum total bilirubin

There was no significant difference in percentage conjugation of serum total bilirubin between the treated and recovery control groups. Percentage conjugation of serum total bilirubin was significantly higher in tadalafil ($p < 0.01$), tramadol ($p < 0.01$) and sildenafil + tramadol recovery groups, compared with their respective treated groups. There was no significant difference in percentage conjugation of serum total bilirubin

between sildenafil recovery and treated groups, though the recovery group showed higher percentage conjugation of serum total bilirubin (figure 8).

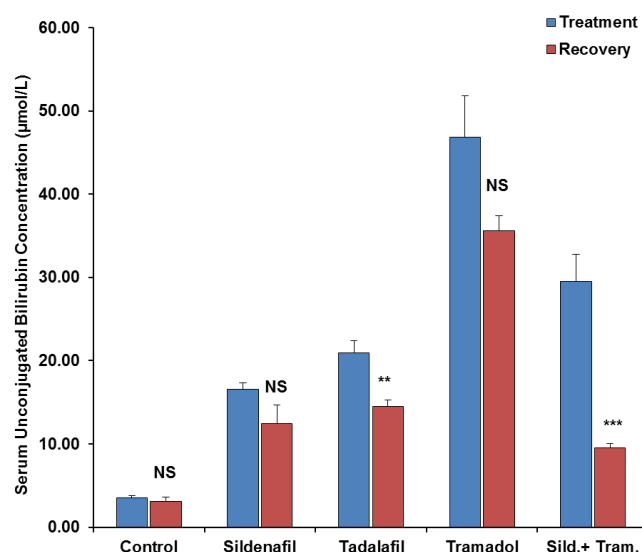


Fig. 7: Comparison of serum unconjugated bilirubin concentration between the treated and recovery groups. Values are mean \pm SEM, $n = 5$. ** $p < 0.01$, *** $p < 0.001$ vs treatment; NS = not significant.

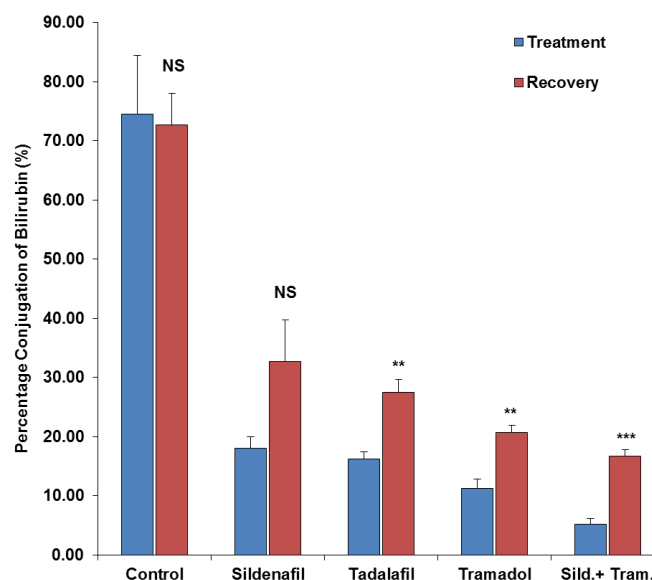


Fig. 8: Comparison of percentage of bilirubin conjugated by the liver between the treated and recovery groups. Values are mean \pm SEM, $n = 5$. ** $p < 0.01$, *** $p < 0.001$ vs treatment; NS = not significant.

DISCUSSION

The liver plays a major role in metabolism, and has a number of functions in the body, including glycogen storage, plasma protein synthesis, detoxification of most substances, and production of bile; an alkaline compound which aids in digestion (Gartner and Hiatt, 2000). Furthermore, the liver is known to form some metabolites in the process of metabolizing various substances. These metabolites (if harmful) are then packaged for

excretion. This appears to form the basis of the liver damage observed in this present study since a high concentration of the metabolites of these drugs will be formed by the liver during their metabolism.

Serum aminotransferase assays are the most common laboratory tests for the detection of liver diseases. Commonly available tests include alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase, serum bilirubin, prothrombin time and serum albumin concentration, among others. They reflect different functions of the liver which include; excreting anions, hepatocellular integrity, formation and the subsequent free flow of bile, and protein synthesis. The present study showed significant increase in serum concentrations of AST, ALT and ALP in the groups administered sildenafil, tadalafil, tramadol and sildenafil + tramadol. Serum concentrations of AST and ALP were highest in tramadol treated group, while ALT concentration was highest in tadalafil treated group. The raised liver enzymes activity after 8 weeks of drug administration is indicative of liver damage. Eweka & Eweka, (2011) had previously reported that sildenafil administered for 6 weeks resulted in severe hepatotoxicity as evident in their study which showed atrophic and degenerative changes in the liver.

Bilirubin concentration is elevated in the blood either by increased synthesis, reduced conjugation, reduced secretion by the liver, or blockage of the bile ducts. In cases of increased production, or decreased conjugation, the unconjugated or indirect form of bilirubin will be elevated. Again, the group administered tramadol had the highest serum concentration of total and unconjugated bilirubin. This shows that hepatotoxicity may have been greatest in this group. Serum conjugated bilirubin concentration was lowest in the group administered sildenafil + tramadol. A similar trend was observed for percentage conjugation of serum total bilirubin. In this study, the elevated bilirubin concentration may be as a result of decreased conjugation by the liver since the conjugated bilirubin concentration was relatively low in the treated groups. Again, this points to possible functional hepatotoxicity which reduces the liver's ability to conjugate bilirubin. Worthy of note also, is the fact that, the medications may be negatively affecting red blood cells and causing hemolysis. This may account for the increased total bilirubin concentration observed in the treated groups, since bilirubin is a product of hemoglobin catabolism. Unconjugated hyper bilirubinemia is caused by accelerated erythrocyte hemolysis (Sgro *et al.*, 2006) such as that seen in the new born (erythroblastosis fetalis), absence of glucuronyl transferase, or hepatocellular disease (Guyton & Hall, 2010). Conjugated hyper bilirubinemia on the other hand, is caused by obstruction of the biliary ducts, as with gallstones or hepatocellular diseases, such as, cirrhosis or hepatitis. Elevated serum bilirubin may also be caused by the effects of many different drugs like; antibiotics, barbiturates, steroids or oral contraceptives. In chronic acquired liver diseases, the serum bilirubin concentration is usually normal until a significant amount of liver damage has occurred, and cirrhosis is present. Our study portrays a chronic liver damage. A decrease in the percentage

conjugation of serum total bilirubin in the treated groups (18.09 ± 1.88 , 16.16 ± 1.25 , 11.23 ± 1.55 and 5.14 ± 0.99 %, for sildenafil, tadalafil, tramadol and sildenafil + tramadol group, respectively) compared with that of control (74.56 ± 9.86 %) further supports the fact that chronic liver damage may be implicated in the treated groups.

Following withdrawal of the various medications, evidence of reversal of hepatotoxicity was observed. From the results shown in table 3, where the reversal values for serum concentration of liver enzymes and bilirubin were compared with control, it appears that reversal of hepatotoxicity was poor. Serum concentration of liver enzymes and bilirubin still remained significantly different from control values (table 3). Furthermore, comparing the recovery groups with their respective treated groups (Figures 1–8), we observed that hepatotoxicity was poorly reversed.

CONCLUSION

Chronic administration of either sildenafil, tadalafil, tramadol or a combination of sildenafil and tramadol (as used in Nigeria today), caused significant alteration in liver functions as evident in the increased serum concentration of liver enzymes and bilirubin, and decrease in the percentage conjugation of serum bilirubin. The alteration was highest in the tramadol treated group and lowest in sildenafil treated group. None of the treated groups recorded complete reversal of hepatotoxicity following withdrawal of treatment. Hence, we strongly advocate for a campaign against abuse of ED medications.

Conflict of interest

We hereby declare that we have no conflict of interest.

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