

# The Hepatotoxic Effect of Sildenafil Citrate and its Combination with Tramadol on Male Wistar Rats is Ameliorated with Vitamin E Supplementation

## ABSTRACT

**Aims** This study investigated the effect of vitamin E on serum liver enzymes and bilirubin concentrations of male Wistar rats treated with sildenafil citrate (SC) and a combination of SC and tramadol on the background that these drugs cause oxidative damage to the liver.

**Method:** Thirty male Wistar rats (180-200g) were randomly divided into six groups (n=5) thus: control (0.2ml vehicle – olive oil), SC-treated group (Sil) (10mg/kg of SC), SC+tramadol-treated group (Sil+Tra) (10mg/kg of SC and 20mg/kg of tramadol), vitamin E-treated group (Vit E) (100mg/kg of vitamin E), SC+vitamin E-treated group (Sil+Vit E) (received SC and vitamin E) and SC+tramadol+vitamin E-treated group (Sil+Tra+Vit E) (received SC, tramadol and vitamin E). Drugs were administered orally once daily for four weeks after which blood samples were obtained and used to measure serum concentrations of liver enzymes (AST, ALT and ALP) and bilirubin.

**Results:** Serum AST, ALT, ALP, total bilirubin (TB) and unconjugated bilirubin (UCB) concentrations were significantly ( $p<0.001$ ) increased in Sil and Sil+Tra compared with control and Vit E and significantly ( $p<0.01$ ) decreased in Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra. Serum conjugated bilirubin (CB) concentration and percentage conjugation of bilirubin significantly decreased ( $p<0.001$ ) in Sil and Sil+Tra compared with control and Vit E and increased ( $p<0.01$ ) in Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra.

**Conclusion:** SC, administered separately and in combination with tramadol altered liver function. However, vitamin E supplementation was able to ameliorate this alteration in liver function.

**Keywords:** Bilirubin, Liver enzymes, Sildenafil citrate, Tramadol, Vitamin E, Wistar rats

## 1. INTRODUCTION

The phosphodiesterase type 5 inhibitor, sildenafil citrate (SC) and the opioid, tramadol are substances that have been reported to be highly abused in Nigeria (Abdel-Hamid *et al.*, 2016; Nna *et al.*, 2015; Oka *et al.*, 2015). The reasons behind their abuse are prolongation of erection, postponement of ejaculation and enlargement of genital size (Bechara *et al.*, 2010). The male folks have the perception that these medications increase the size of their genitalia. They take the drugs at very high doses even without any erectile or ejaculatory problems (Oka *et al.*, 2015; Nna *et al.*, 2014a). Some combine SC and tramadol because SC prolongs erection and tramadol postpones ejaculation (Oka *et al.*, 2015; Nna *et al.*, 2017). The implication of this is constant bombardment of the liver due to high concentrations of these medications. Both SC and tramadol administered separately and in combination have been reported to damage the liver (Nna *et al.*, 2015; Eweka and Eweka, 2011; Aitici *et al.*, 2005) by causing oxidative stress (Sheweita *et al.*, 2018).

Oxidative stress has been the underlying mechanism behind the aetiology of many human diseases (Baunthiyal *et al.*, 2017). Several antioxidants including vitamin E have been tested for their ability to fight oxidative stress (Pizzino *et al.*,

21 2017). Vitamin E has been reported to exhibit hepatoprotective effect and treat non-alcoholic fatty liver disease (El Hadi *et al.*, 2018; Maheswari *et al.*, 2015; Pacana and Sanyal, 2012). In our previous study, we reported that vitamin E was able to counter the hepatotoxic effect of tramadol (Nwangwa *et al.*, 2019). It is still unknown whether vitamin E can also protect the liver from the reported hepatotoxicity associated with administration of SC and a combination of SC and tramadol. Therefore, in the present study, we investigated the effect of SC and a combination of SC and tramadol on the liver of Wistar rats to validate already established claims. Additionally, we investigated the effect of vitamin E on the liver of these groups of rats to check whether vitamin E would also have the potential to protect the liver from damage that may be caused by SC and its combination with tramadol.

## 29 30 **2. MATERIAL AND METHODS**

### 31 32 **2.1 Experimental animals**

33 This study used thirty male Wistar rats that were of weight, 180-200g. After purchasing the animals from Department of Agriculture, University of Calabar, we housed them in the animal house of Department of Physiology in the same university in properly ventilated wooden cages. The principles of animal handling as laid down in Helsinki's (1964) declaration were adopted. All the animals had free access to rat feed and water *ad libitum* and were exposed to 12/12 hours light/dark cycle at room temperature. The rats were kept for two weeks to explore their new habitat before treatment with drugs started.

### 39 **2.2 Experimental design and drug administration**

40 The thirty rats were randomly assigned into six groups of five rats each:

- 41 1. Control group received 0.2mL of vehicle (olive oil).
- 42 2. Sildenafil-treated group (Sil) received 10mg/kg of SC (Maxheal Laboratories Pvt Ltd., India).
- 43 3. Sildenafil+tramadol-treated group (Sil+Tra) received 10mg/kg of SC and 20mg/kg of tramadol (Glow Pharma pvt Ltd, India).
- 44 4. Vitamin E-treated group (Vit E) received 100mg/kg of vitamin E (Sigma Aldrich, USA).
- 45 5. Sildenafil+Vitamin E-treated group (Sil+Vit E) was given 10mg/kg of SC and 100mg/kg of vitamin E.
- 46 6. Sildenafil+tramadol+vitamin E-treated group (Sil+Tra+Vit E) received SC, tramadol and vitamin E at the
- 47 respective doses used in this study.
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50 All the drugs used were purchased from Anijah Pharmacy, Eta Agbor, Calabar, Nigeria and administered orally and once daily for four weeks.

### 52 **2.3 Collection of blood samples**

53 At the end of the four-week administration period, the rats were sacrificed after being anaesthetized with chloroform. Blood sample were obtained from the heart by cardiac puncture with the use of 5mL syringe attached to 21G needle. The blood samples collected were introduced into pre-labelled plain sample bottles and thereafter allowed for two hours to clot. After clotting, the blood samples were centrifuged at 2500rpm for 10 minutes to get serum. The serum from each sample was used for the measurement of the concentrations of liver enzymes and bilirubin.

### 58 **2.4 Measurement of serum concentration of liver enzymes**

#### 59 **2.4.1 Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT)**

60 Serum concentration of AST and ALT was measured using the method of Reitman and Frankel (1957) as described in our previous study (Nwangwa *et al.*, 2019).

#### 63 **2.4.2 Alkaline Phosphatase**

64 Also, the modified method of King and Armstrong (1964) as described in our previous study (Nwangwa *et al.*, 2019) was used to determine ALP concentration.

### 68 **2.5 Measurement of serum bilirubin concentration**

69 The method described by Sherlock and Lunec (1951) was used to measure serum bilirubin concentration.

#### 71 **2.5.1 Calculation of percentage conjugation of bilirubin**

72 Percentage conjugation of bilirubin was calculated by multiplying the ratio of serum conjugated bilirubin concentration to  
73 serum total bilirubin concentration by 100%.

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75 Percentage conjugation of bilirubin =  $\frac{\text{Serum conjugated bilirubin concentration}}{\text{Serum total bilirubin concentration}} \times 100\%$   
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## 77 2.6 Statistical analysis

78 Results are presented as mean  $\pm$  standard error of mean (SEM). The data were analysed using the software, statistical  
79 package for social sciences (SPSS) (version 20). One-way analysis of variance along with post hoc multiple comparisons  
80 (Tukey test) was used to compare mean difference between groups.  $P < 0.05$  was considered statistically significant.

## 81 3. RESULTS AND DISCUSSION

### 82 3.1 Comparison of serum liver enzyme concentration in the different experimental groups

#### 83 3.1.1 Serum aspartate aminotransferase (AST)

84 Figure 1 shows serum AST concentration for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E which was  $30.6 \pm 1.94$ ,  
85  $55.6 \pm 2.16$ ,  $62.8 \pm 1.24$ ,  $31.8 \pm 0.66$ ,  $39 \pm 1.18$  and  $38 \pm 2.19$  IU/L respectively. Serum AST concentration was significantly  
86 increased in Sil ( $p < 0.001$ ), Sil+Tra ( $p < 0.001$ ), Sil+Vit E ( $p < 0.05$ ) and Sil+Tra+Vit E ( $p < 0.001$ ) compared with control. It  
87 was significantly ( $p < 0.001$ ) decreased in Vit E, Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra.

#### 88 3.1.2 Serum alanine aminotransferase

89 Figure 2 shows serum ALT concentration for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E which was  $44.6 \pm 1.69$ ,  
90  $71.6 \pm 1.81$ ,  $78.2 \pm 1.07$ ,  $47 \pm 4.15$ ,  $52.8 \pm 3.34$  and  $54.2 \pm 3.22$  IU/L respectively. Serum ALT concentration was significantly  
91 ( $p < 0.001$ ) increased in Sil and Sil+Tra compared with control. It was significantly decreased in Vit E ( $p < 0.001$ ), Sil+Vit E  
92 ( $p < 0.001$ ) and Sil+Tra+Vit E ( $p < 0.01$ ) compared with Sil. It was also significantly ( $p < 0.001$ ) decreased in Vit E, Sil+Vit E  
93 and Sil+Tra+Vit E compared with Sil+Tra.

#### 94 3.1.3 Serum alkaline phosphatase

95 Serum ALP concentration for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E was  $24.2 \pm 0.92$ ,  $56 \pm 2.14$ ,  $59.4 \pm 1.63$ ,  
96  $24.4 \pm 0.68$ ,  $44.2 \pm 1.39$  and  $41.4 \pm 1.94$  IU/L respectively. Serum ALP concentration was significantly ( $p < 0.001$ ) increased in  
97 Sil, Sil+Tra, Sil+Vit E and Sil+Tra+Vit E compared with control and Vit E. It was significantly ( $p < 0.001$ ) decreased in Vit E,  
98 Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra (Figure 3).

#### 99 3.1.4 AST/ALT ratio

100 AST/ALT ratio is shown in figure 4. AST/ALT ratio for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E was  
101  $0.68 \pm 0.03$ ,  $0.78 \pm 0.03$ ,  $0.80 \pm 0.01$ ,  $0.70 \pm 0.06$ ,  $0.76 \pm 0.07$  and  $0.72 \pm 0.08$  respectively and was not significantly different  
102 among the experimental groups.

103 Sildenafil citrate (SC) and tramadol are used for treating erectile dysfunction and premature ejaculation respectively. Both  
104 SC and tramadol are highly abused with some abusers taking a combination of both drugs (Nna *et al.*, 2015; Oka *et al.*,  
105 2015). Both substances have been reported to cause damage to hepatocytes (Nwangwa *et al.*, 2019; Nna *et al.*, 2015) via  
106 oxidative stress (Sheweita *et al.*, 2018; Eweka and Eweka, 2011). The lipid-soluble vitamin, vitamin E is known to protect  
107 hepatocytes from damage caused by oxidative stress (Maheswari *et al.*, 2015). In the present study, we investigated the  
108 effect of vitamin E on serum liver enzymes and bilirubin concentrations of male Wistar rats treated with SC and a  
109 combination of SC and tramadol.

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112 Serum AST, ALT and ALP concentrations increased significantly in Sil and Sil+Tra compared with control. AST/ALT ratio  
113 was not significantly different among groups. Our findings indicate that SC and its combination with tramadol caused  
114 damage to hepatocytes. Increased concentrations of these enzymes in the Sil and Sil+Tra imply that the administration of  
115 SC and SC+tramadol caused liver damage because these enzymes are released in high amount into the blood when the  
116 liver cells are damaged (Aragon and Younossi, 2010). Also, the increased ALP may be an indication of damage to bone  
117 cells and cholestasis that may cause progressive liver disease - biliary cirrhosis (Ashwood, 1994; Kendall *et al.*, 1970).  
118 Our findings are consistent with the work done by Nna *et al.* (2015) where SC and a combination of SC and tramadol  
119 were shown to increase the concentrations of these enzymes. Aitici *et al.* (2005) and Rukhshanda *et al.* (2014) reported  
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129 that long term tramadol treatment in mice caused necrosis, vacuolization, central vein dilation, haemorrhage, cytolysis and  
130 complete cell membrane degeneration in hepatocytes. However, serum concentrations of AST, ALT and ALP decreased  
131 significantly in Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra (Figures 3). These results indicate that vitamin E  
132 supplementation was able to antagonize the hepatotoxic effects of SC and the combination of SC and tramadol  
133 suggesting its hepatoprotective effect.

UNDER PEER REVIEW

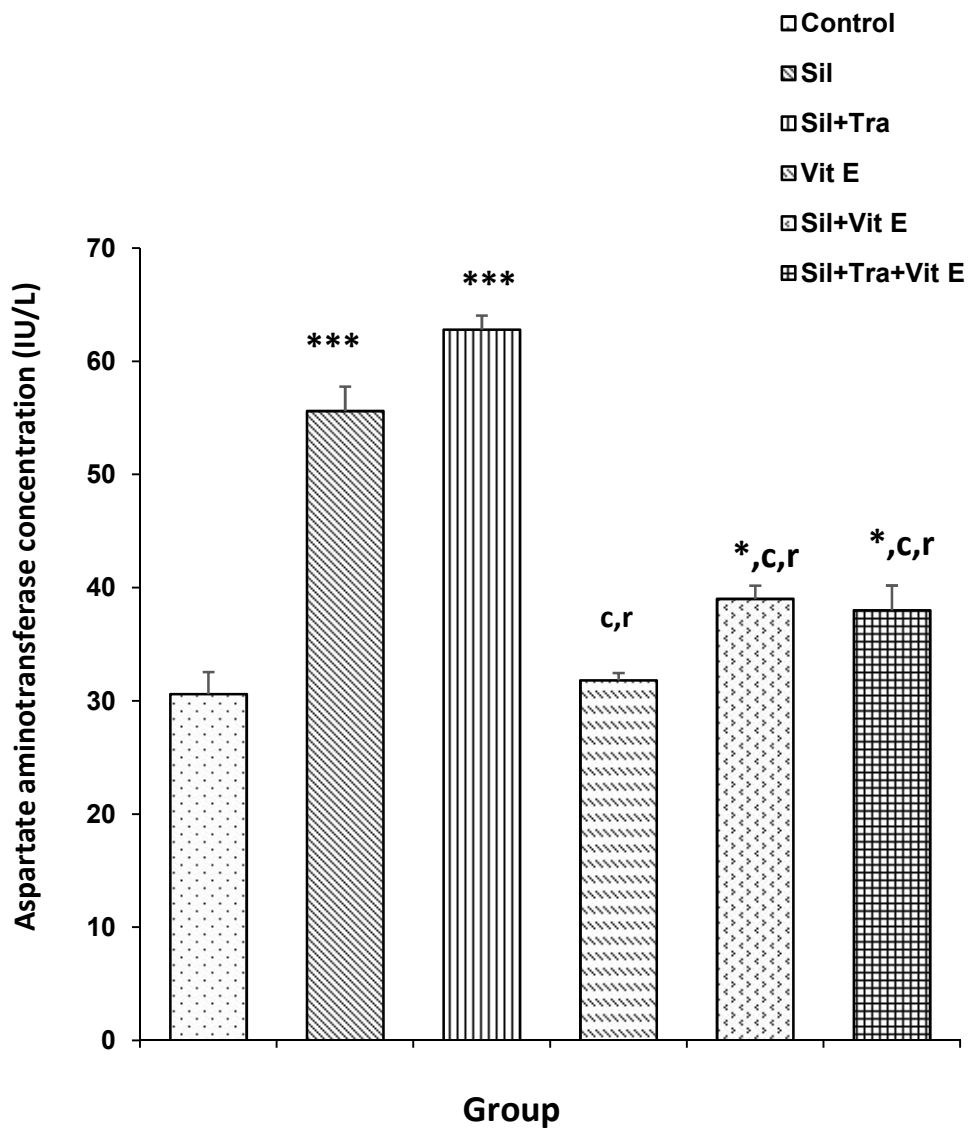


Figure 1: Comparison of aspartate aminotransferase concentration in the different experimental groups. Values are mean±SEM, n = 5.

\*p<0.05, \*\*\*p<0.001, vs control  
 c = p<0.001 vs Sil  
 r = p<0.001 vs Sil+Tra

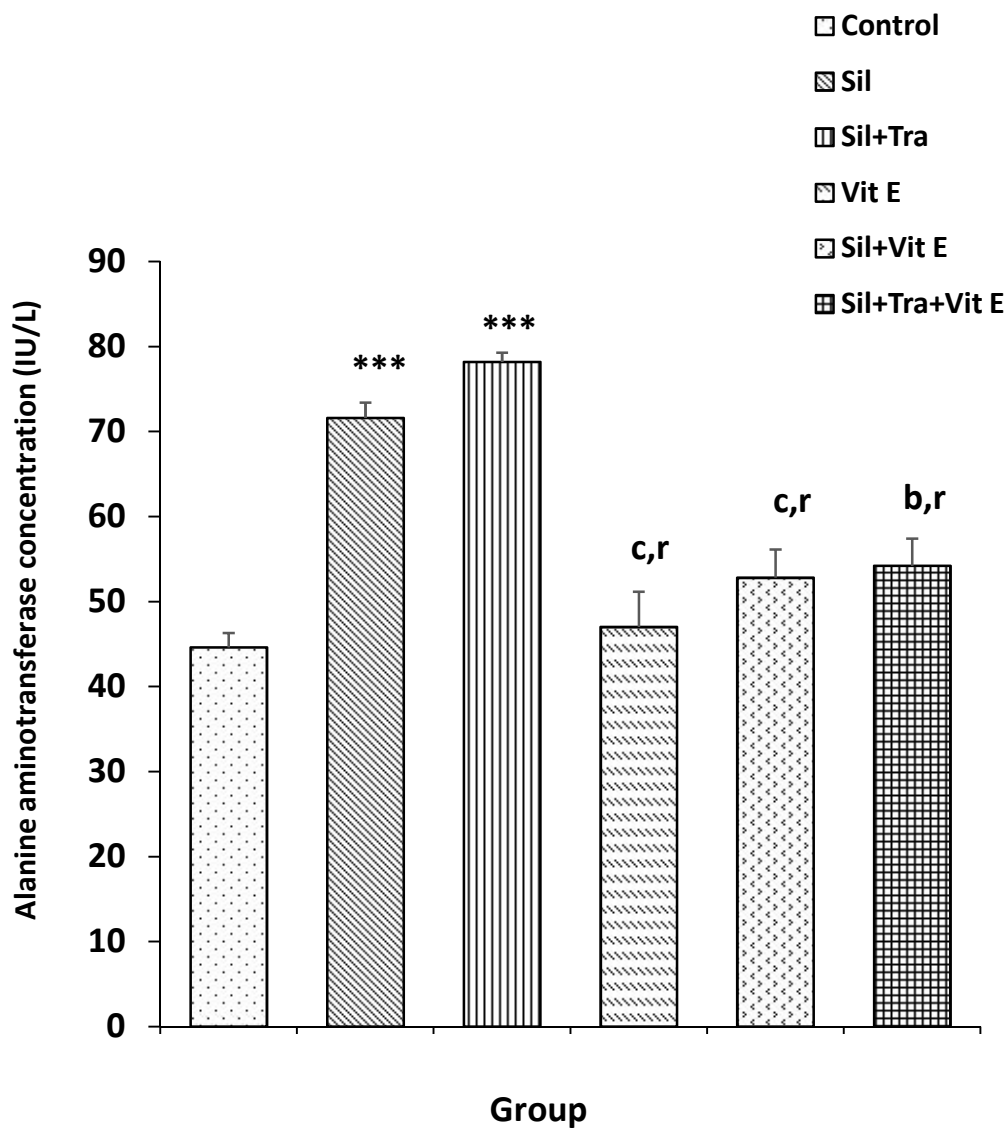


Figure 2: Comparison of alanine aminotransferase concentration in the different experimental groups. Values are mean±SEM, n = 5.

\*\*\*p<0.001, vs control  
 b = p<0.01, c = p<0.001 vs Sil  
 r = p<0.001 vs Sil+Tra

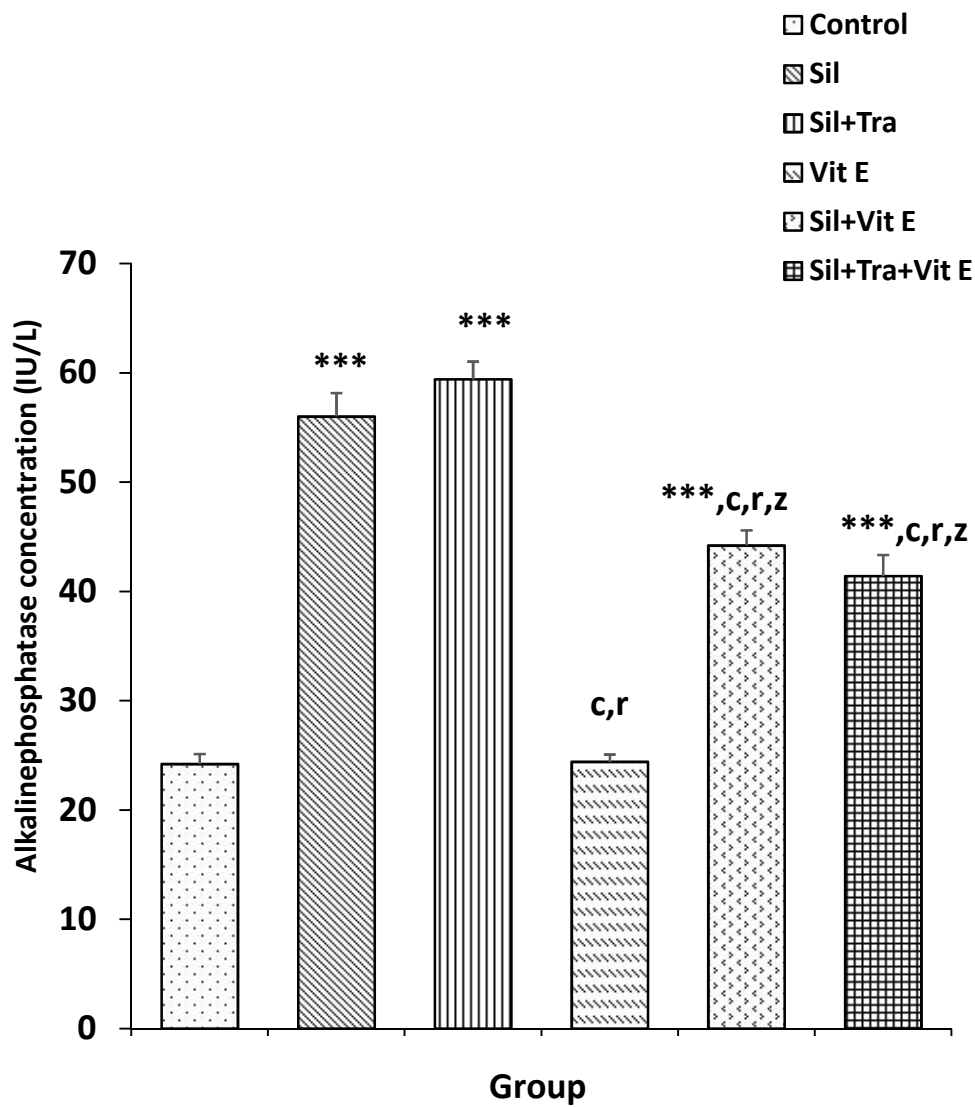


Figure 3: Comparison of alkaline phosphatase concentration in the different experimental groups. Values are mean $\pm$ SEM, n = 5.

\*\*\*p<0.001, vs Control  
 c = p<0.001 vs Sil  
 r = p<0.001 vs Sil+Tra  
 z = p<0.001 vs Vit E

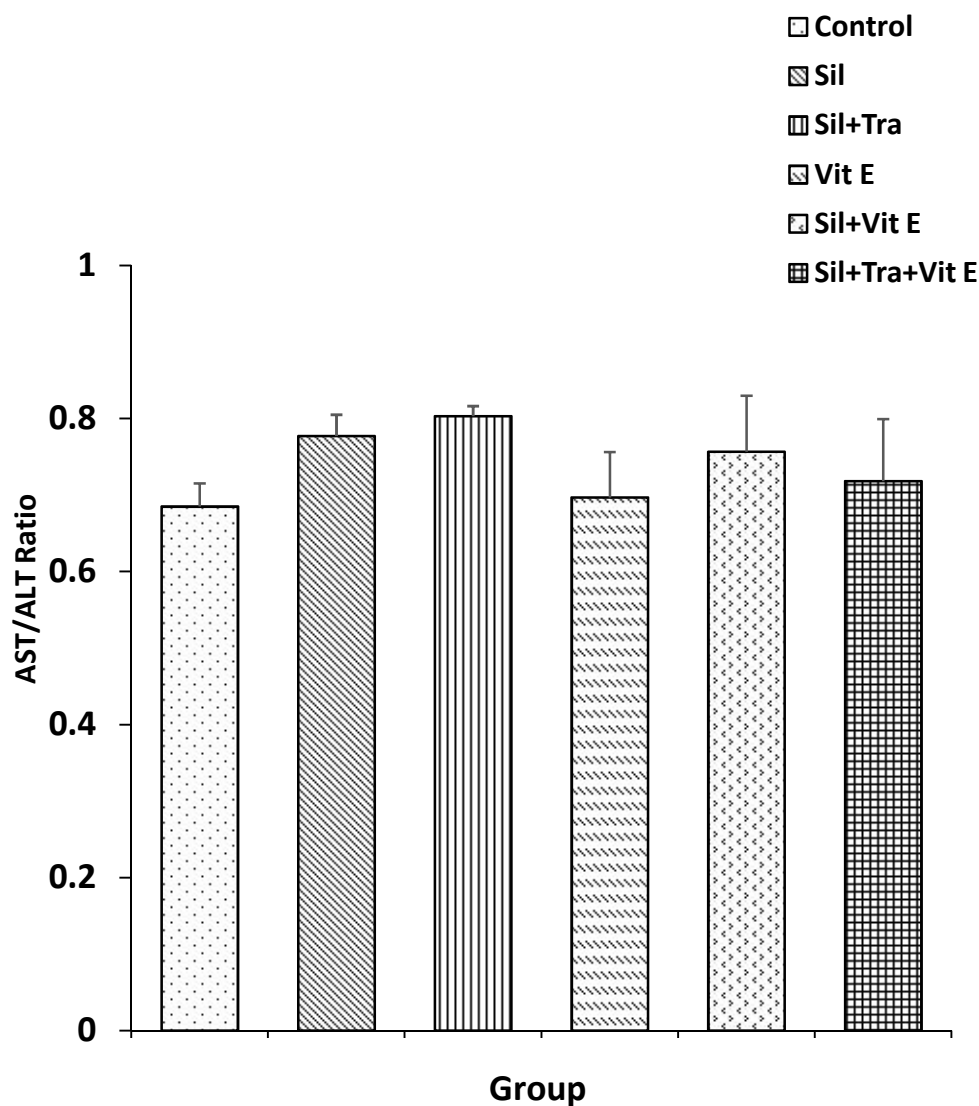


Figure 4: Comparison of AST/ALT ratio in the different experimental groups. Values are mean±SEM, n = 5.

No significant difference among groups.

### 3.2 Comparison of total bilirubin (TB) concentration in the different experimental groups

Figure 5 shows serum TB concentration for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E which was 15.1±1.71, 31.46±1.17, 36.74±1.06, 10.64±0.68, 23.1±2.08 and 20.02±0.68 µmol/L respectively. Serum TB concentration was significantly (p<0.001) increased in Sil and Sil+Tra compared with control and Vit E. It was significantly (p<0.001) increased in Sil+Vit E and Sil+Tra+Vit E compared with Vit E. Serum TB concentration was however significantly (p<0.01) decreased in Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra.

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### 3.3 Comparison of serum conjugated bilirubin (CB) concentration in the different experimental groups

Serum CB concentration for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E was  $11.98 \pm 1.34$ ,  $2.52 \pm 0.23$ ,  $2.06 \pm 0.26$ ,  $8.68 \pm 0.85$ ,  $12.2 \pm 1.26$  and  $11.6 \pm 0.83$   $\mu\text{mol/L}$  respectively. Serum CB concentration was significantly ( $p < 0.001$ ) decreased in Sil and Sil+Tra compared with control. It was however significantly ( $p < 0.001$ ) increased in Vit E, Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra (Figure 6).

### 3.4 Comparison of serum unconjugated bilirubin (UCB) concentration in the different experimental groups

Figure 7 shows serum UCB concentration in the different experimental groups. Serum UCB concentration for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E was  $3.12 \pm 0.46$ ,  $28.94 \pm 1.26$ ,  $34.68 \pm 0.95$ ,  $1.96 \pm 0.27$ ,  $10.9 \pm 1.13$  and  $8.42 \pm 0.91$   $\mu\text{mol/L}$  respectively. Serum UCB concentration was significantly increased in Sil ( $p < 0.001$ ), Sil+Tra ( $p < 0.001$ ), Sil+Vit E ( $p < 0.001$ ) and Sil+Tra+Vit E ( $p < 0.01$ ) compared with control and Vit E. It was significantly ( $p < 0.01$ ) increased in Sil+Tra compared with Sil. However, serum UCB concentration was significantly ( $p < 0.001$ ) decreased in Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra.

### 3.5 Comparison of percentage conjugation of bilirubin in the different experimental groups

Percentage conjugation of bilirubin in the different experimental groups is shown in figure 8. It was  $79.51 \pm 1.90$ ,  $8.09 \pm 0.89$ ,  $5.58 \pm 0.65$ ,  $80.93 \pm 3.46$ ,  $52.74 \pm 2.30$  and  $58.06 \pm 3.9$  % for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E respectively. Percentage conjugation of bilirubin was significantly ( $p < 0.001$ ) decreased in Sil, Sil+Tra, Sil+Vit E and Sil+Tra+Vit E compared with control and Vit E. It was however significantly ( $p < 0.001$ ) increased in Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra.

Serum total bilirubin (TB) and unconjugated bilirubin (UCB) concentrations increased significantly in Sil and Sil+Tra compared with control (Figures 5 and 7). A rise in bilirubin concentration in blood can result from increased production, decreased conjugation, decreased secretion by the liver or blockage of the bile ducts. In our study, serum conjugated bilirubin concentration and percentage conjugation of bilirubin decreased significantly in Sil and Sil+Tra compared with control (Figures 6 and 8). Our findings are in line with the report of Nna *et al.* (2015) and suggest that SC and the combination of SC and tramadol caused functional hepatotoxicity which reduces the liver's ability to conjugate bilirubin thereby causing a rise in total and UCB concentrations. Liver damage, presence of immature red blood cells or Gilbert syndrome causes a rise in serum bilirubin concentration (Volpe, 2003). SC, administered separately and in combination with tramadol has been reported to damage the liver cells (Nna *et al.*, 2015; Eweka and Eweka, 2011). Tramadol administration alone has also been reported to damage the liver cells (Elmanama *et al.*, 2015; Sheweita *et al.*, 2018). UCB is formed from phagocytosis of haemoglobin (Hb) released from red blood cells that have been destroyed or haemolysed. The increased concentrations of TB and UCB observed in our study imply that separate administration of SC and a combination of SC and tramadol may have caused red blood cell haemolysis that led to the hyperbilirubinaemia since bilirubin is produced from catabolism of haemoglobin (Sgro *et al.*, 2006). However, in the groups administered vitamin E, a decrease in serum TB and UCB concentrations and an increase in serum CB concentration were observed compared to the groups treated with SC and a combination of SC and tramadol indicating the protective role of vitamin E against functional hepatotoxicity associated with administration of SC and a combination of SC and tramadol.

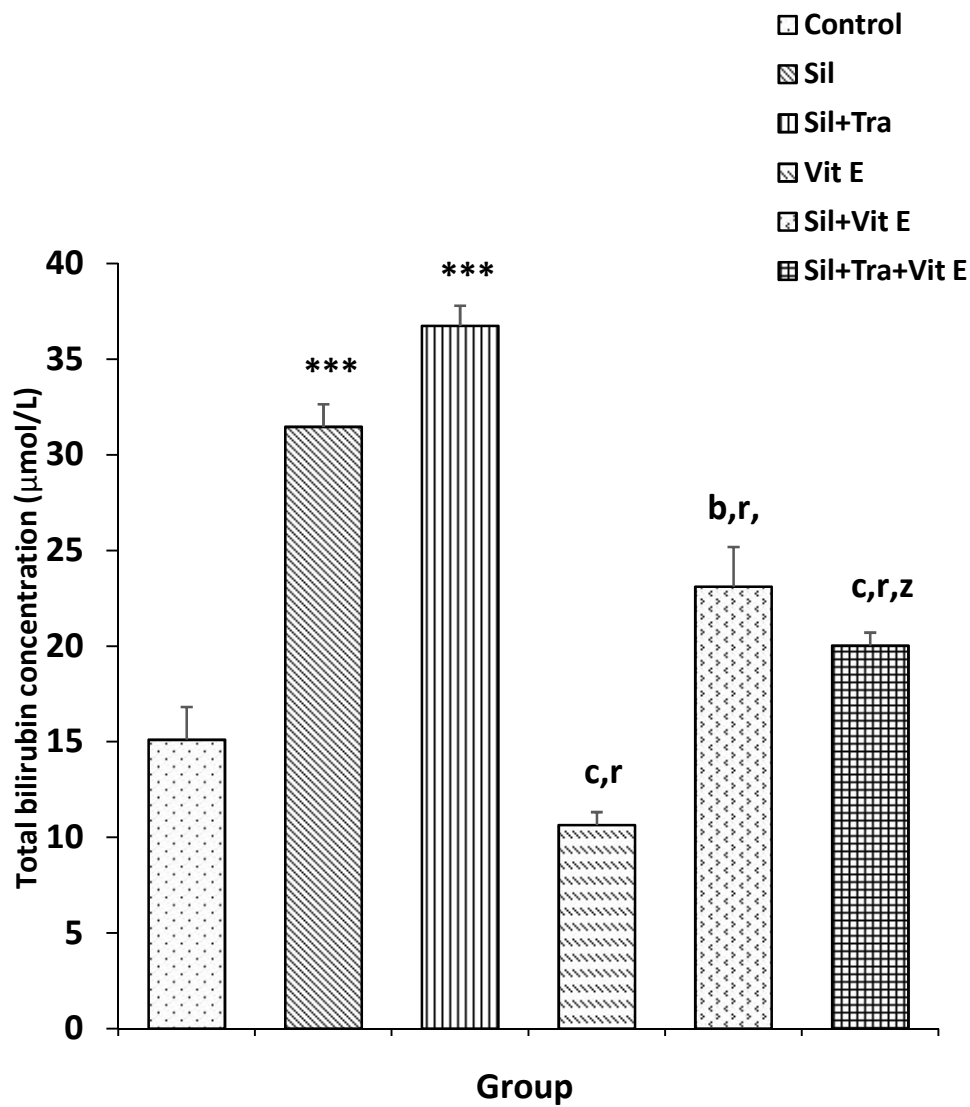


Figure 5: Comparison of total bilirubin concentration in the different experimental groups. Values are mean±SEM, n = 5.

\*\*\*p<0.001, vs control  
 b = p<0.01, c = p<0.001 vs Sil  
 r = p<0.001 vs Sil+Tra  
 z = p<0.001 vs Vit E

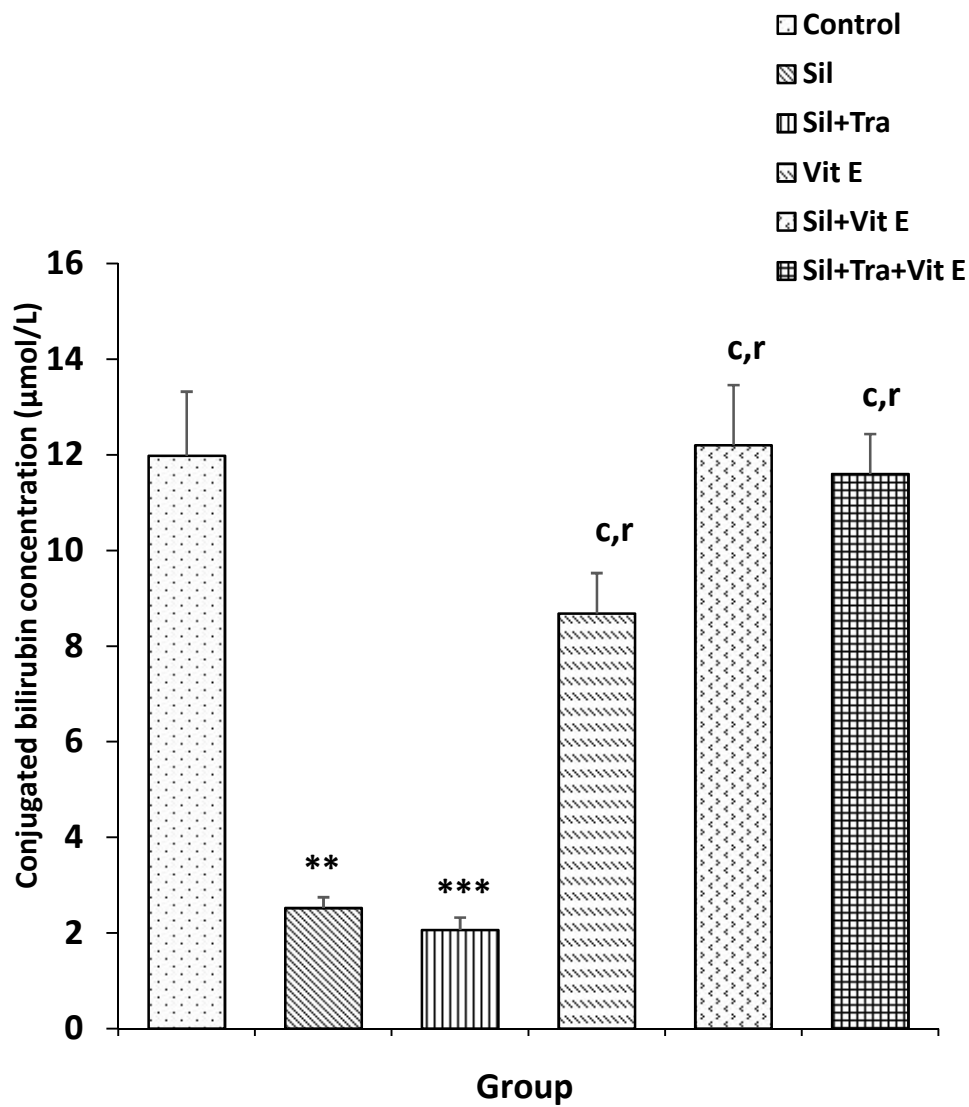


Figure 6: Comparison of conjugated bilirubin concentration in the different experimental groups. Values are mean±SEM, n = 5.

\*\*\*p<0.001 vs control

c = p<0.001 vs Sil

r = p<0.001 vs Sil+Tra

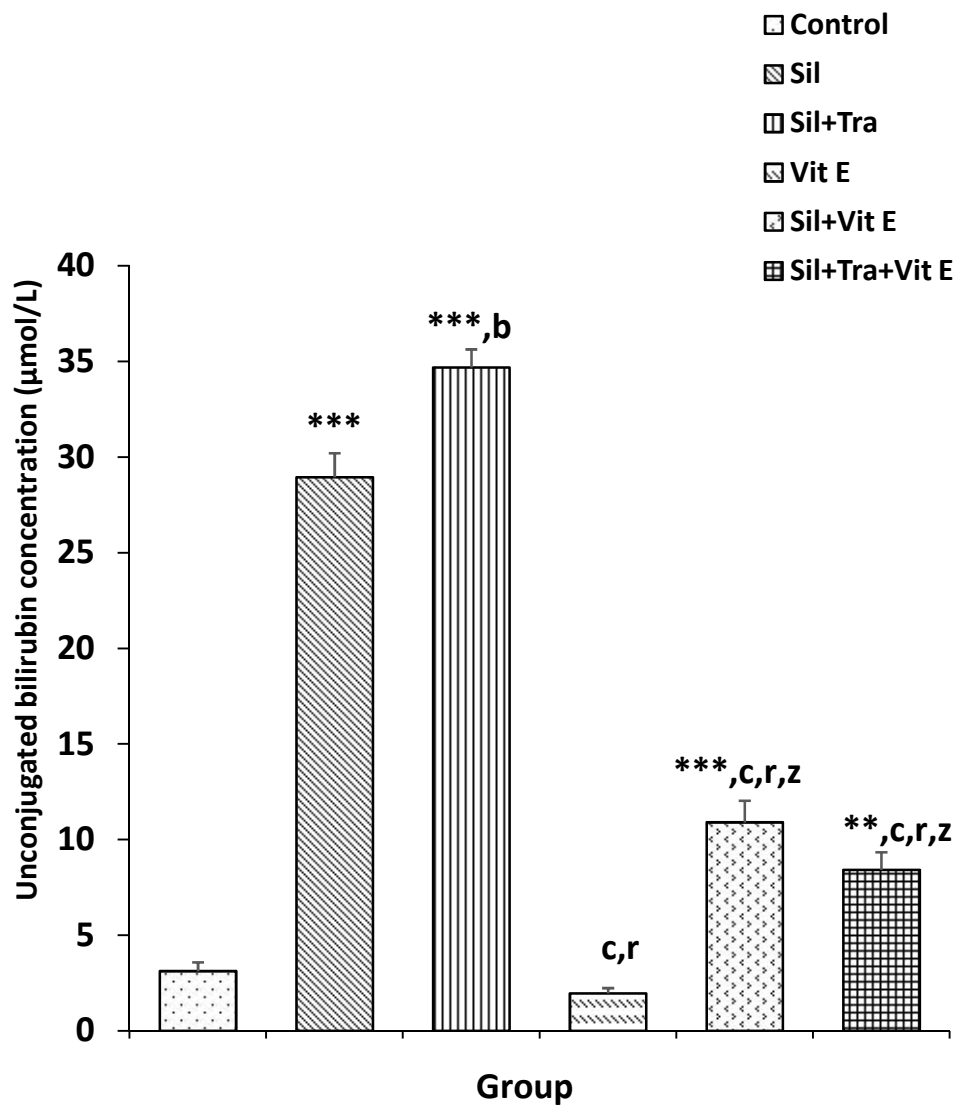


Figure 7: Comparison of unconjugated bilirubin concentration in the different experimental groups. Values are mean±SEM, n = 5.

\*\*p<0.001, \*\*\*p<0.001 vs control  
 b = p<0.01, c = p<0.001 vs Sil  
 r = p<0.001 vs Sil+Tra  
 z = p<0.001 vs Vit E

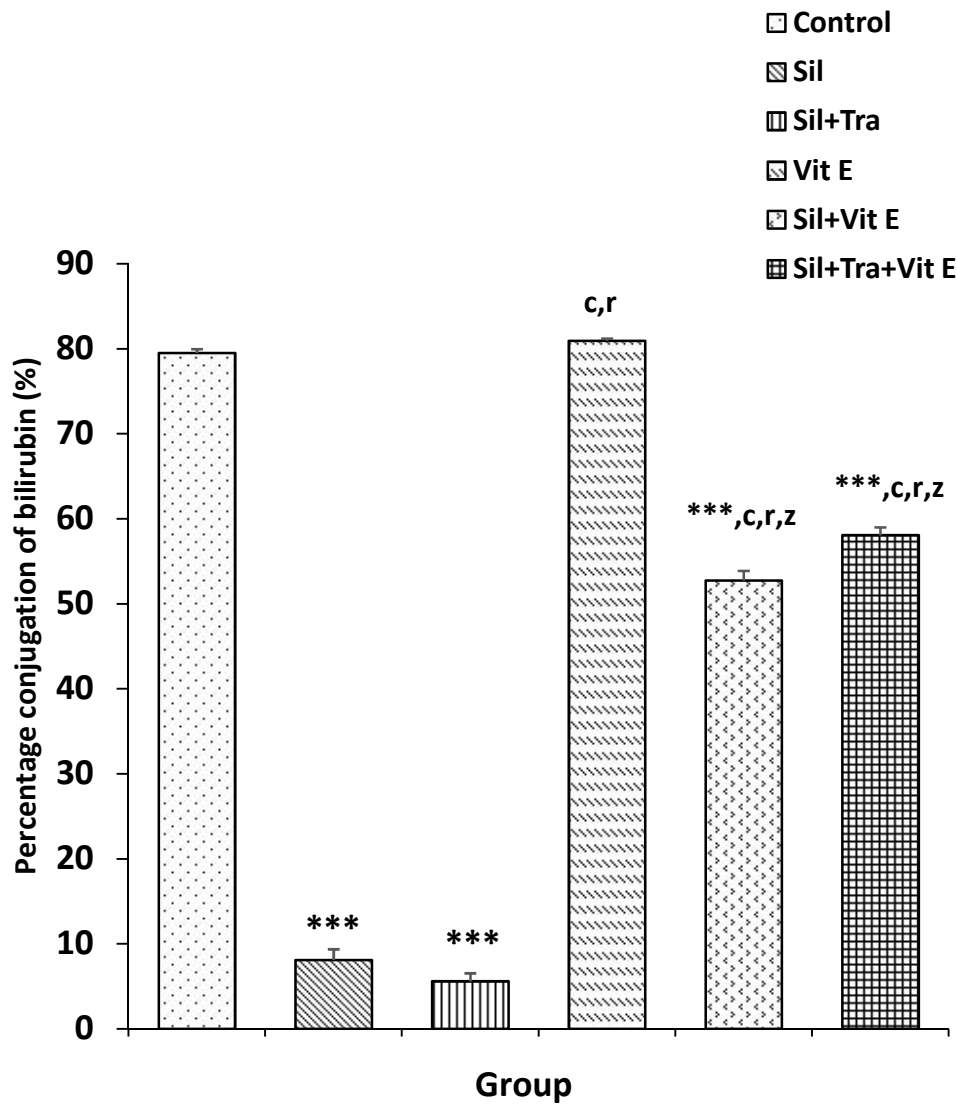


Figure 8: Comparison of percentage conjugation of bilirubin in the different experimental groups. Values are mean±SEM, n = 5.

\*\*\*p<0.001 vs control  
 c = p<0.001 vs Sil  
 r = p<0.001 vs Sil+Tra  
 z = p<0.001 vs Vit E

#### 4. CONCLUSION

Administration of sildenafil citrate, separately and in combination with tramadol increased serum concentrations of liver enzymes and bilirubin and decreased the liver's ability to conjugate bilirubin indicating alteration in liver function.

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197 Supplementation with vitamin E was effective in countering this alteration in liver function. This may be attributed to its  
198 antioxidant effect.

## 199 200 201 **CONSENT**

202  
203 Not applicable

## 204 205 **ETHICAL APPROVAL**

206 This research was approved by the Animal Ethics Committee of the Faculty of Basic Medical Sciences, University of  
207 Calabar, Nigeria. The Helsinki's 1964 laid down principles for the care and use of animals were strictly adhered to.

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