Archives of Current Research International

18(1): 1-10, 2019; Article no.ACRI.49801
ISSN: 2454-7077

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

J. N. Nwangwa[1], A. L. Udefa[1*], P. M. Obi[1], C. E. Obeten[1] and S. Okpa[1]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Cross River State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author ALU designed the study and wrote the first draft of the manuscript. Author JNN managed the literature searches, participated in data analysis and edited the first draft. Authors PMO, CEO and SO took part in laboratory analyses. All authors participated in laboratory analyses, read and approved the final manuscript.

Article Information

DOI: 10.9734/ACRI/2019/v18i130123
Editor(s):
(1) Dr. M. A. Elbagermi, Department of Chemistry, Misurata University, Libya.
Reviewers:
(1) K. Ramesh Kumar, S. V. S. Medical College, India.
(2) Mohammed Ismail Khan, ESIC Medical College, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/49801

Original Research Article

Received 15 April 2019
Accepted 19 June 2019
Published 02 July 2019

ABSTRACT

Aims: This study investigated the effect of vitamin E on serum liver enzymes and bilirubin concentrations in 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.
Methods: Thirty male Wistar rats (180-200 g) were randomly divided into six groups (n=5) thus: control (0.2 ml vehicle – olive oil), SC-treated group (Sil) (10 mg/kg of SC), SC+tramadol-treated group (Sil+Tra) (10 mg/kg of SC and 20 mg/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

**Corresponding author: Email: augustineudefa@unical.edu.ng;*

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 [1,2,3]. The reasons behind their abuse are prolongation of erection, postponement of ejaculation and enlargement of genital size [4]. 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 [3,5]. Some combine SC and tramadol because SC prolongs erection and tramadol postpones ejaculation [3,6]. 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 [2,7,8] by causing oxidative stress [7,9].

Oxidative stress has been the underlying mechanism behind the aetiology of many human diseases [10]. Several antioxidants including vitamin E have been tested for their ability to fight oxidative stress [11]. Vitamin E has been reported to exhibit hepatoprotective effect and treat non-alcoholic fatty liver disease [12,13, 14]. In our previous study, we reported that vitamin E was able to counter the hepatotoxic effect of tramadol [15]. 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 male 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.

2. MATERIALS AND METHODS

2.1 Experimental Animals

This study used thirty male Wistar rats that were of weight, 180-200 g. 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 declaration [16] 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.

2.2 Experimental Design and Drug Administration

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

1. Control group received 0.2 mL of vehicle (olive oil).
2. Sildenafil-treated group (Sil) received 10 mg/kg of SC.
3. Sildenafil+tramadol-treated group (Sil+Tra) received 10 mg/kg of SC and 20 mg/kg of tramadol.
4. Vitamin E-treated group (Vit E) received 100 mg/kg of vitamin E.
5. Sildenafil+Vitamin E-treated group (Sil+Vit E) was given 10 mg/kg of SC and 100 mg/kg of vitamin E.
6. Sildenafil+tramadol+vitamin E-treated group (Sil+Tra+Vit E) received SC, tramadol and vitamin E at the respective doses used in this study.

SC (Maxheal Laboratories Pvt Ltd., India), tramadol (Glow Pharma pvt Ltd, India) and vitamin E (Sigma Aldrich, USA) were purchased from Anijah Pharmacy, Eta Agbor, Calabar, Nigeria and administered orally and once daily for four weeks.

2.3 Collection of Blood Samples

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 2500 rpm for 10 minutes to get serum. The serum from each sample was used for the measurement of the concentrations of liver enzymes and bilirubin.

2.4 Measurement of Serum Concentration of Liver Enzymes

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT): Serum concentration of AST and ALT was measured using the method of Reitman and Frankel [17] as described in our previous study [15].

Alkaline Phosphatase: Also, the modified method of King and Armstrong [18] as described in our previous study [15] was used to determine ALP concentration.

2.5 Measurement of Serum Bilirubin Concentration

The method described by Sherlock and Lunec [19] was used to measure serum bilirubin concentration.

Calculation of percentage conjugation of bilirubin: Percentage conjugation of bilirubin was calculated by multiplying the ratio of serum conjugated bilirubin concentration to serum total bilirubin concentration by 100%.

$$\text{Percentage conjugation of bilirubin} = \frac{\text{Serum conjugated bilirubin concentration}}{\text{Serum total bilirubin concentration}} X\ 100\%$$

2.6 Statistical Analysis

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

3. RESULTS AND DISCUSSION

3.1 Comparison of Serum Liver Enzyme Concentration in the Different Experimental Groups

Serum Aspartate Aminotransferase (AST): Fig. 1. shows serum AST concentration for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E which was 30.6±1.94, 55.6±2.16, 62.8±1.24, 31.8±0.66, 39±1.18 and 38±2.19 IU/L respectively. Serum AST concentration was significantly 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 was significantly ($p<0.001$) decreased in Vit E, Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra.

Serum alanine aminotransferase: Fig. 2. shows serum ALT concentration for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E which was 44.6±1.69, 71.6±1.81, 78.2±1.07, 47±4.15. 52.8±3.34 and 54.2±3.22 IU/L respectively. Serum ALT concentration was significantly ($p<0.001$) increased in Sil and Sil+Tra compared with control. It was significantly decreased in Vit E ($p<0.001$), Sil+Vit E ($p<0.001$) 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 and Sil+Tra+Vit E compared with Sil+Tra.

Serum alkaline phosphatase: Serum ALP concentration for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E was 24.2±0.92, 56±2.14, 59.4±1.63, 24.4±0.68, 44.2±1.39 and 41.4±1.94 IU/L respectively. Serum ALP concentration was significantly ($p<0.001$) increased in 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, Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra (Fig. 3.).

AST/ALT ratio: AST/ALT ratio is shown in Fig. 4. AST/ALT ratio for control, Sil, Sil+Tra, Vit E, Sil+Vit E and Sil+Tra+Vit E was 0.68±0.03, 0.78±0.03, 0.80±0.01, 0.70±0.06, 0.76±0.07and 0.72±0.08 respectively and was not significantly different among the experimental groups.

3.2 Comparison of Total Bilirubin (Tb) Concentration in the Different Experimental Groups

Fig. 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 ($p<0001$) increased in Sil+Vit E and Sil+Tra+Vit E compared with Vit E. Serum TB

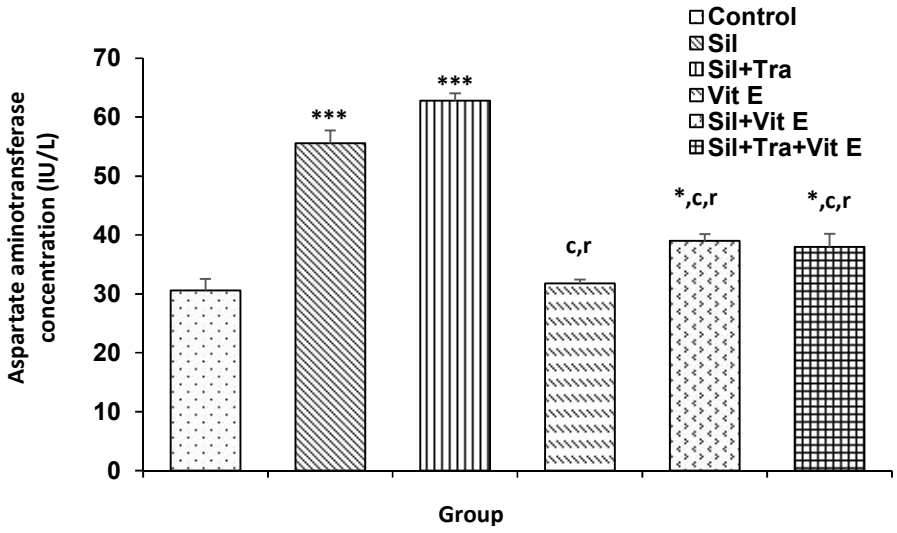

Fig. 1. Comparison of aspartate aminotransferase concentration in the different experimental groups. Values are mean±SEM, n = 5

**p<0.05, ***p<0.001, vs control*
c = p<0.001 vs Sil

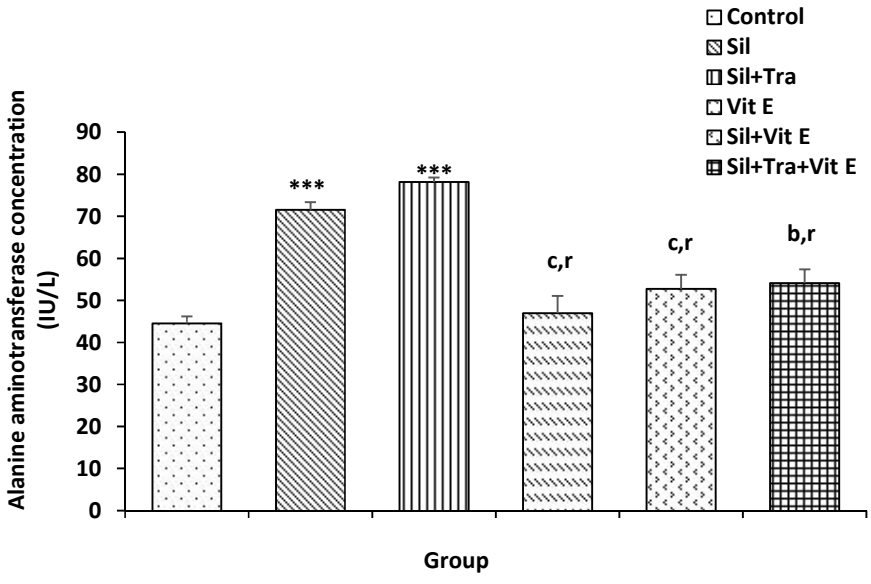

Fig. 2. Comparison of alanine aminotransferase concentration in the different experimental groups. Values are mean±SEM, n = 5

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

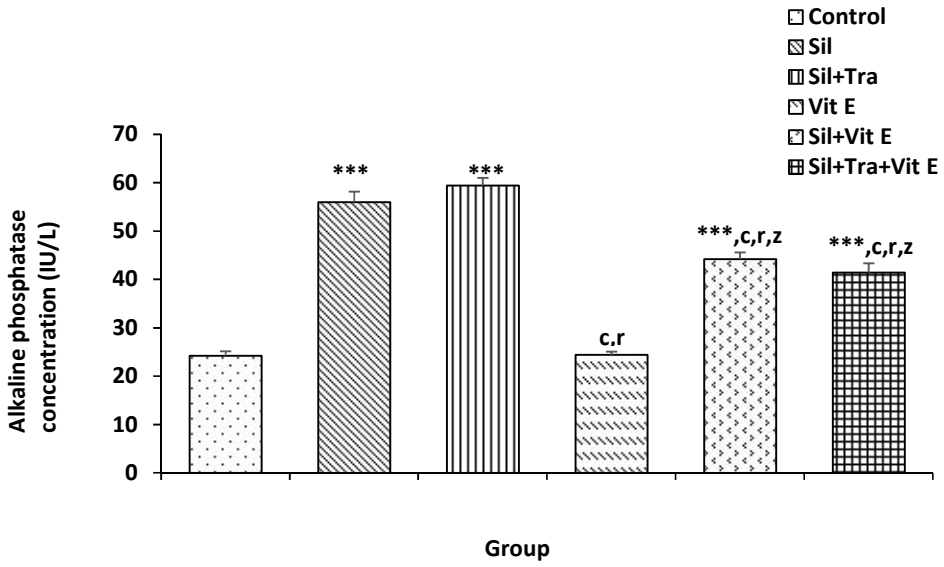

Fig. 3. Comparison of alkalinephosphatase concentration in the different experimental groups. Values are mean±SEM, n = 5

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

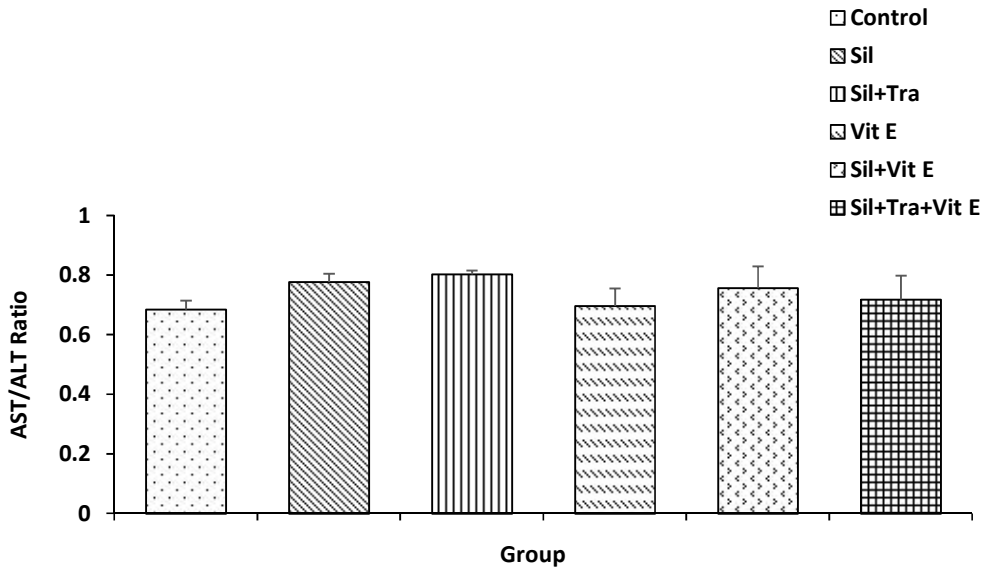

Fig. 4. Comparison of AST/ALT ratio in the different experimental groups. Values are mean±SEM, n = 5

No significant difference among groups

concentration was however significantly (p<0.01) decreased in Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra.

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±1.34, 2.52±0.23, 2.06±0.26, 8.68±0.85, 12.2±1.26 and 11.6±0.83 µmol/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 (Fig. 6).

3.4 Comparison of Serum Unconjugated Bilirubin (Ucb) Concentration in the Different Experimental Groups

Fig. 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±0.46, 28.94±1.26, 34.68±0.95, 1.96±0.27. 10.9±1.13 and 8.42±0.91 µmol/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<0.0010 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 Fig. 8. It was 79.51±1.90, 8.09±0.89, 5.58±0.65, 80.93±3.46, 52.74±2.30 and 58.06±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.

Sildenafil citrate (SC) and tramadol are used for treating erectile dysfunction and premature ejaculation respectively. Both SC and tramadol

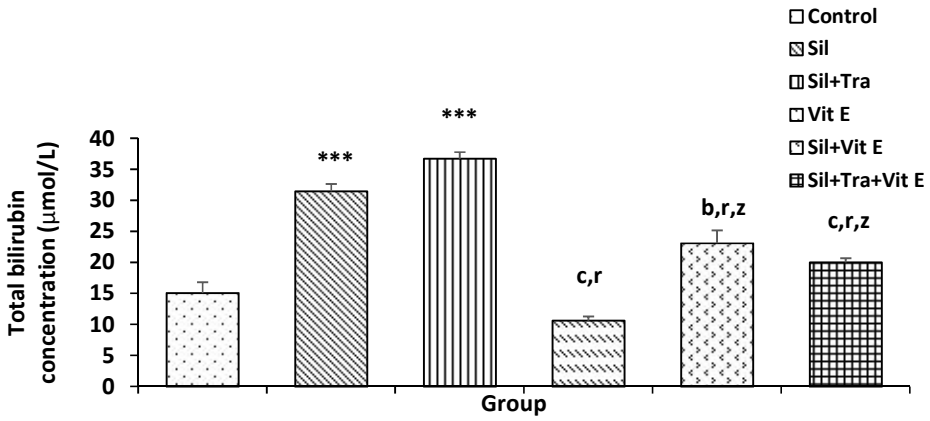

Fig. 5. Comparison of total bilirubin concentration in the different experimental groups. Values are mean±SEM, n = 5

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

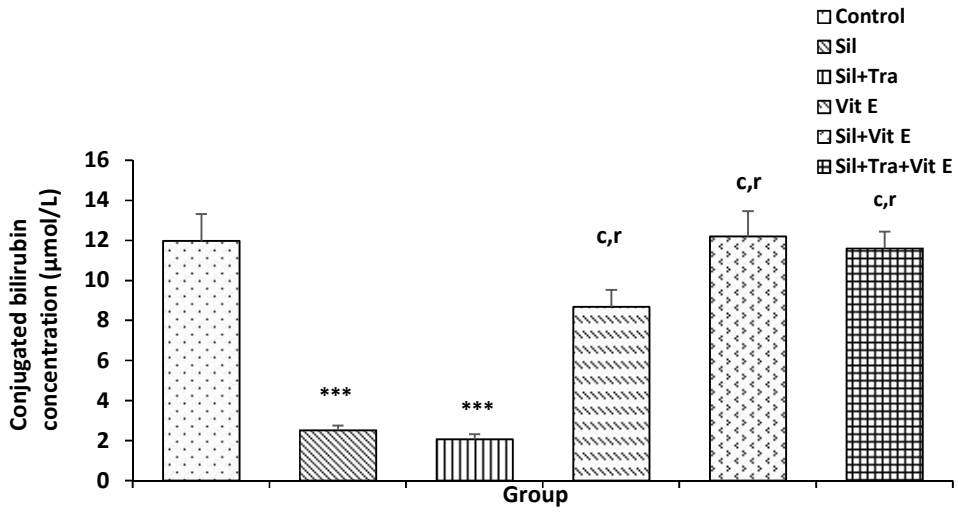

Fig. 6. Comparison of conjugated bilirubin concentration in the different experimental groups. Values are mean±SEM, n = 5

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

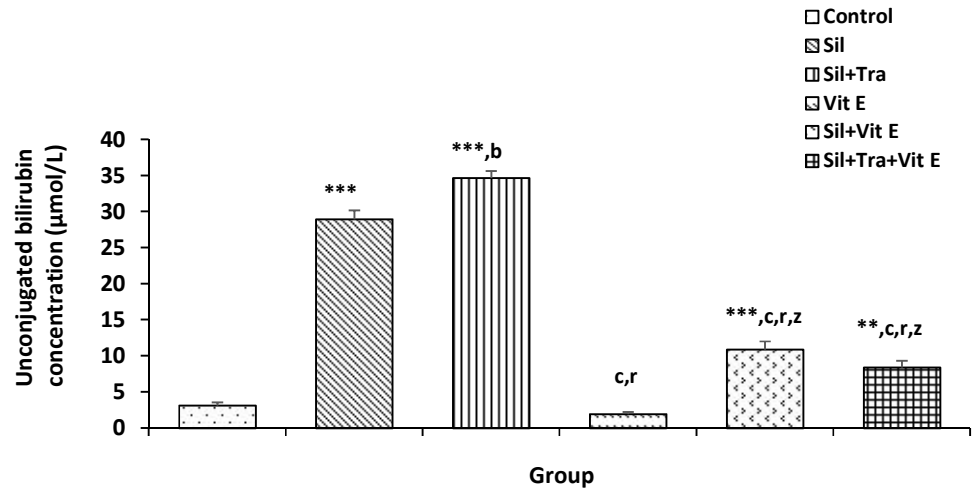

Fig. 7. Comparison of unconjugated bilirubin concentration in the different experimental groups. Values are mean±SEM, n = 5

***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

are highly abused with some abusers taking a combination of both drugs [2,3]. Both substances have been reported to cause damage to hepatocytes [2,15] via oxidative stress [7, 9]. The lipid-soluble vitamin, vitamin E is known to protect hepatocytes from damage caused by oxidative stress [13]. In the present study, we investigated the effect of vitamin E on serum liver enzymes and bilirubin concentrations in male Wistar rats treated with SC and a combination of SC and tramadol.

Serum AST, ALT and ALP concentrations increased significantly in Sil and Sil+Tra compared with control. AST/ALT ratio was not significantly different among groups. Our findings indicate that SC and its combination with tramadol caused damage to hepatocytes. Increased concentrations of these enzymes in the Sil and Sil+Tra imply that the administration of SC and SC+tramadol caused liver damage because these enzymes are released in high amount into the blood when the liver cells are damaged [20]. Also, the increased ALP may be an indication of damage to bone cells and cholestasis that may cause progressive liver disease - biliary cirrhosis [21, 22]. Our findings are consistent with the work done by Nna *et al.* [2] where SC and a combination of SC and tramadol were shown to increase the concentrations of these enzymes. Aitici *et al.* [8] and Rukhshanda *et al.* [23] reported that long term tramadol treatment in mice caused necrosis, vacuolization, central vein dilation, haemorrhage, cytolysis and complete cell membrane degeneration in hepatocytes. However, serum concentrations of AST, ALT and ALP decreased significantly in Sil+Vit E and Sil+Tra+Vit E compared with Sil and Sil+Tra (Fig. 3). These results indicate that vitamin E supplementation was able to antagonize the hepatotoxic effects of SC and the combination of SC and tramadol suggesting its hepatoprotective effect.

Serum total bilirubin (TB) and unconjugated bilirubin (UCB) concentrations increased significantly in Sil and Sil+Tra compared with control (Figs. 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 (Figs. 6 and 8). Our findings are in line with the report of Nna et al. [2]

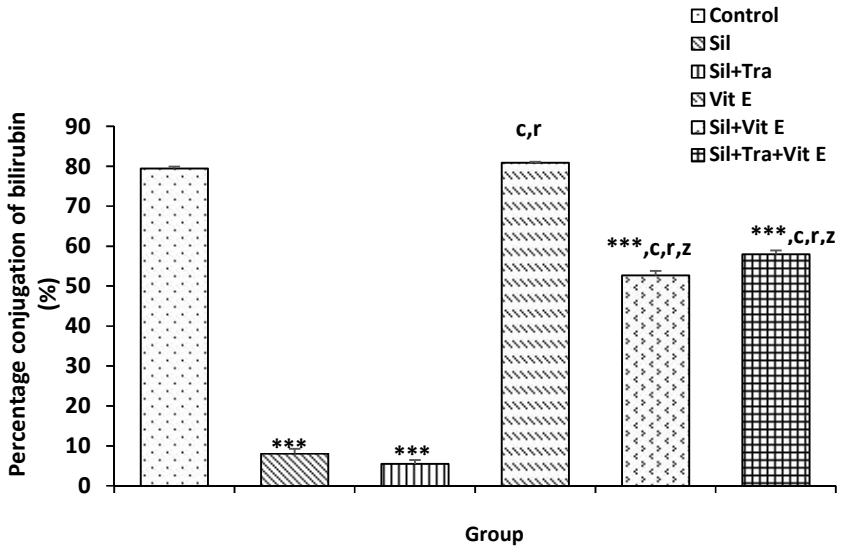

Fig. 8. Comparison of percentage conjugation of bilirubin in the different experimental groups. Values are mean±SEM, n = 5

****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

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 [24]. SC, administered separately and in combination with tramadol has been reported to damage the liver cells [2,7]. Tramadol administration alone has also been reported to damage the liver cells [9,25]. UCB is formed form 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 [26]. 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.

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

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abdel-Hamid IA, Andersson KE, Waldinger MD, Anis TH. Tramadol abuse and sexual function. Sexual Medicine Reviews. 2016;4(3):235-246.
2. Nna VU, Akpan UP, Okon VE, Atangwho IE. Hepatotoxicity following separate administration of two phosphodiesterase-5 inhibitors (sildenafil & tadalafil) and opioid (tramadol); evaluation of possible reversal following their withdrawal. J App Pharm Sci. 2015;5(8):105-113.
3. Oka VO, Udefa AL, Nna VU, Owu DU. Sildenafil citrate and tramadol administered separately and in combination affects basal metabolic rate, triiodothyronine (T_3) and cortisol levels in albino wistar rats. Trends in Medical Research. 2015;10(3):51-62.
4. Bechara A, Casabé A, De Bonis W, Helien A, Bertolino MV. Recreational use of phosphodiesterase type 5 inhibitors by healthy young men. The Journal of Sexual Medicine. 2010;7:3736-3742.
5. Nna VU, Ani EJ, Ofutet EO, Ofem OE, Iroh CE, Osim EE. Recurrent side effects following chronic recreational use of sexual stimulants among male subjects in Calabar, Cross River State, Nigeria. Der Pharmacia Lettre. 2014;6(6):56-61.
6. Nna VU, Udefa AL, Ofutet EO, Osim EE. Testicular and epididymal histology of rats chronically administered high doses of PDE5 inhibitors and tramadol. Nigerian Journal of Physiological Sciences. 2017;32:55-61.
7. Eweka AO, Eweka A. The effects of sildenafil citrate on the liver and kidneys of adult Wistar rats (Rattus norvegicus) – A histological study. Sexual Dysfunctions - Special Issues; 2011.
   Available:http://www.intechopen.com/books/sexual-dysfunctions-special-issues/the-effects-of-sildenafil-citrate-on-theliver-and-kidneys-of-adult-wistar-rats-rattus-norvegicus-a
8. Atici S, Cinel I, Cinel L, Doruk N, Eskandari G, Oral V. Liver and kidney toxicity in chronic use of opioids: an experimental long term treatment model. J Biosci. 2005;2:245-252.
9. Sheweita SA, Almasmari AA, El-Banna SG. Tramadol-induced hepato- and nephrotoxicity in rats: Role of curcumin and gallic acid as antioxidants. PLoS ONE. 2018;13(8):e0202110.

Available:https://doi.org/10.1371/journal.pone.0202110
10. Baunthiyal M, Singh V, Dwivedi S. Insights of antioxidants as molecules for drug discovery. International Journal of Pharmacology. 2017;13(7):874-889.
11. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: Harms and benefits for human health. Oxidative Medicine and Cellular Longevity; 2017.
DOI: org/10.1155/2017/8416763
12. El Hadi H, Vettor, Rossato M. Vitamin E as a treatment for nonalcoholic fatty liver disease: Reality or myth? Antioxidants (Basel). 2018;7(1):12.
DOI: 10.3390/antiox7010012
13. Maheswari E, Saraswathy GR, Santhranii T. Influence of Vitamin E on hepatotoxicity and oxidative stress. International Journal of Research in Pharmacy and Biosciences. 2015;2(3):30-38.
14. Pacana T, Sanyal AJ. Vitamin E and non-alcoholic fatty liver disease. Curr Opin Clin Nutr Metab Care. 2012;15(6):641-648.
DOI: 10.1097/MCO.0b013e328357f747
15. Nwangwa JN, Udefa AL, Obeten CE, Obi PM, Adie PU, Kanu UJ. Vitamin E supplementation counters the hepatotoxic effects of tramadol on male Wistar rats. IOSR Journal of Dental and Medical Sciences. 2019;18(6).
16. Helsinki. World Medical Association Declaration of Helsinki. Adopted by the 18th WMA General Assembly, Helsinki, Finland; 1964.
17. Reitman S, Frankel S. Determination of aminotransaminases in serum. American Journal of Clinical Pathology. 1957;28:50-56.
18. King EJ, Armstrong AR. Canadian Medical Association. Journal. 1964;31:376.
19. Sherlock S, Lunec J. Free radical and antioxidant system in health and disease. British Journal of Hospital Medicine. 1951;43:334-344
20. Aragon G, Younossi ZM. When and how to evaluate mildly elevated liver enzymes in apparently healthy patients. Cleveland Clinic Journal of Medicine. 2010;77(3):195-204.
21. Ashwood ER. Tietz textbook of clinical chemistry. 2nd ed. Philadelphia USA: WB Saunders Co.; 1994.
22. Kendall MJ, Cockel R, Becker J, Hawkins CF. Raised serum alkaline phosphatase in rheumatoid Disease: An index of liver dysfunction? Ann. Rheum. 1970;29:537-540.
23. Rukhshanda S, Razia I, Muhammad NA, Anum Z, Javed I, Muhammad SA. Effects of tramadol on histopathological and biochemical parameters in mice (*Mus musculus*) model. Global J Pharmacol. 2014;114-19.
24. Volpe JJ. Bilirubin and Brain Injury, in Neurology of the Newborn. 2003;521-546.
25. Elmanama AA, Abu Tayyem NES, Essawaf HN, Hmaid IM. Tramadol-Induced liver and kidney toxicity among abusers in Gaza Strip, Palestine. Jordan Journal of Biological Sciences. 2015;8(2):133-137.
26. Sgro M, Campbell D, Shah V. Incidence and causes of severe neonatal hyperbilirubinemia in Canada. Canadian Medical Association Journal. 2006;175(6):587-590.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/49801