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Vitamin E Supplementation Counters the Hepatotoxic Effects of Tramadol on Male Wistar Rats

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Abstract: *This study investigated the effect of vitamin E on serum liver enzymes and bilirubin concentrations of male Wistar rats treated with tramadol on the background that tramadol is widely abused and has been reported to cause oxidative damage to the liver. Twenty male Wistar rats (180-200g) were randomly assigned into four groups of five rats each: Control (0.2mL olive oil as vehicle), tramadol-treated group (Tra) (20mg/kg of tramadol), vitamin E-treated group (Vit E) (100mg/kg of vitamin E) and tramadol+vitamin E-treated group (Tra+Vit E) (received tramadol and vitamin E). Drugs were administered orally, once daily for twenty-eight days after which the rats were sacrificed and blood samples collected and used for measurement of serum concentrations of liver enzymes (AST, ALT and ALP) and bilirubin. Serum concentrations of AST, ALT, ALP, total bilirubin (TB) and unconjugated bilirubin (UCB) were significantly increased ($p<0.05$) in Tra compared with control and decreased ($p<0.05$) in Tra+Vit E compared with Tra. Serum concentration of conjugated bilirubin (CB) and percentage conjugation of bilirubin decreased significantly ($p<0.01$) in Tra compared with control but was not significantly different between Tra and Tra+Vit E. Tramadol therefore altered liver function and vitamin E supplementation was able to counter these alterations but could not counter the negative effect of tramadol on conjugation of bilirubin.*

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

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I. Introduction

The liver is actively involved in the detoxification and metabolism of substances including drugs and this increases the risk of the liver to toxic injuries.

Tramadol is an opioid analgesic acting on the central nervous system to treat moderate to severe pain [1,2]. Its ability to postpone ejaculation and to cause the feeling of a "high" has led to its abuse in Nigeria among the younger population [3-5]. The male folks even in the absence of ejaculatory problems take tramadol at excessively high doses [6] and this poses threat to health. As such, there'll be constant bombardment of the liver with high concentration of tramadol. Experimental studies have shown that tramadol treatment causes hepatotoxicity and alteration in liver function [4,7-9]. These negative effects of tramadol on the liver has been associated with oxidative stress [9].

Vitamin E is a lipid soluble antioxidant which prevents the production of lipid peroxides by scavenging free radicals in biological membranes [10]. Vitamin E has been reported to be effective in the treatment of non-alcoholic fatty liver disease [11,12] and the prevention of oxidative damage to the liver caused by substances such as carbamazepine [13]. Due to the high level of abuse of tramadol and the oxidative damage it causes to the liver, it becomes expedient to investigate whether a power antioxidant like vitamin E will be able to counter the negative effects of tramadol on the liver as no study till date has shown this. This study was therefore conducted to investigate the effect of vitamin E on serum liver enzymes and bilirubin concentrations (as markers for liver damage and functionality) in male Wistar rats treated with tramadol.

II. Materials and Methods

2.1 Experimental Animals

Twenty male Wistar rats weighing 180-200g were bought from the Department of Agriculture, University of Calabar and used for the study. The rats were housed in wooden cages that were properly ventilated and kept in the animal house of the Department of Physiology, University of Calabar. Before the commencement of treatment with the various substances, the rats were allowed for two weeks to acclimatize. Ethical approval was obtained from the Animal Research Ethics Committee of Faculty of Basic Medical Sciences, University of Calabar. The principles for animal care as contained in the declaration of Helsinki [14]

were adopted in the handling of the animals. 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.

2.2 Experimental Design and Drug Administration

The twenty rats were randomly divided into four experimental groups with each group having five rats. The groups were control, tramadol treated-group (Tra), vitamin E-treated group (Vit E) and tramadol+vitamin E-treated group (Tra+Vit E). The control group received 0.2mL of the vehicle (olive oil). The Tra received 20mg/kg of tramadol (Glow Pharma pvt Ltd, India). The Vit E was given 100mg/kg of vitamin E (Sigma Aldrich, USA). The Tra+Vit E received 20mg/kg of tramadol and 100mg/kg of vitamin E. The drugs were obtained from Anijah Pharmacy, Eta Agbor, Calabar, Nigeria. Drug and vehicle administration was done orally and once a day for twenty-eight days.

2.3 Collection of Blood Samples

At the end of the period of administration, all the rats were anaesthetized using chloroform and sacrificed. Blood samples were collected from the animals through cardiac puncture using 5ml syringe attached to 21G needle into pre-labelled plain sample bottles and thereafter allowed for two hours to clot. The clotted blood samples were then centrifuged at 2500rpm for 10 minutes to obtain serum. The sera obtained were then used to measure serum concentrations of liver enzymes and bilirubin.

2.4 Measurement of Serum Concentration of Liver Enzymes

2.4.1 Aspartate Aminotransferase and Alanine Aminotransferase

Reitman and Frankel [15] method was used to measure the concentrations of AST and ALT. The principle behind this is that pyruvate is produced from transamination by ALT and it produces a brown-coloured hydrazine which is measured calorimetrically at 510nM by its reaction with 2,4-dinitrophenylhydrazine. The reaction with AST forms an oxaloacetate which decarboxylates spontaneously to pyruvate which is also measured by hydrazine formation. The calculation is shown below:

$\frac{T-TB}{S-SB}$ X 67/Umol/min/L for AST

$\frac{T-TB}{S-SB}$ X 133/mol/Umin/L for ALT

Where T = Test
TB =Test Blank
S = STD
SB = STD Blank

2.4.2 Alkaline Phosphatase

The modified method of King and Armstrong [16] was used to determine ALP concentration. This is based on the principle that phenol released by enzymatic hydrolysis from phenylphosphate under defined conditions of time, temperature and PH is estimated calorimetrically. A test tube containing a mixture of 1mL of buffer and 1mL of phenylphosphate substrate was placed in a water bath at 37°C for 3 minutes. 0.1ml of serum was added, mixed gently and incubated for 15 minutes and the reaction was halted by adding 0.8mL of 0.5N sodium hydroxide

Control: In a test tube, 1mL substrate was mixed with 0.8mL of 0.5N sodium hydroxide followed by 0.1mL of serum

Standard: 1.1mL of buffer was mixed with 0.1mL of phenol standard (1mg/100mL) and 0.8mL of 0.5N sodium hydroxide.

Blank: 1.1mL of buffer, 1.0mL of water and 0.8mL of 0.5N sodium hydroxide was mixed. To all tubes, 1.2mL of 0.5N $NaHCO_3$ was added with 1mL of $K_3(Fe(CN)_6)$. After each addition, each tubular content was properly mixed and the successive additions adjusted the PH to develop the colour. The 0.0 of reddish-brown colours of 510 nM (nanometer) was read avoiding exposure to strong sunlight.

The calculation is shown below:

$$\text{Serum ALT (King} - \text{Armstrong Units/100ml} = \frac{\text{Reading of unknown} - \text{Reading of control}}{\text{Reading of standard} - \text{Reading of blank}} X\ 100$$

2.5 Measurement of Serum Bilirubin Concentration

Serum bilirubin concentration was estimated using Powell [17] method. Serum bilirubin is present in two forms namely conjugated (mostly with glucoronic acid) and unconjugated (free bilirubin). Both forms give purple azobilirubin with diazotized sulphanilic acid. Conjugated bilirubin reacts in aqueous solution (direct reaction) whereas unconjugated bilirubin requires an accelerator or solubilizer, such as benzoate urea.

2.5.1 Calculation of Percentage Conjugation of Bilirubin

Percentage conjugation of bilirubin was calculated by multiplying the ratio of serum conjugated bilirubin concentration and 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). Data analysis was performed using statistical package for social sciences (SPSS) (version 20). One-way analysis of variance along with post hoc multiple comparisons test (Least Square Difference) was used to compare mean difference between groups. $P<0.05$ was considered statistically significant.

III. III Results

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

3.1.1 Serum aspartate aminotransferase (AST)

Figure 1 shows serum AST concentration for control, Tra, Vit E and Tra+Vit E which was 34±2.47, 43.4±3.96, 33.6±1.17 and 36.2±2.15 IU/L respectively. Serum AST concentration was significantly ($p<0.05$) increased in Tra compared with control. It was significantly decreased in Vit E compared with Tra.

3.1.2 Serum alanine aminotransferase

Figure 2 shows serum ALT concentration for control, Tra, Vit E and Tra+Vit E which was 47.8±2.56, 66.6±2.54, 47±4.15 and 48.4±0.93 IU/L respectively. Serum ALT concentration was significantly ($p<0.001$) increased in Tra compared with control. It was however significantly ($p<0.001$) decreased in Vit E and Tra+Vit E compared with Tra.

3.1.3 Serum alkaline phosphatase

Figure 3 shows serum ALP concentration for control, Tra, Vit E and Tra+Vit E which was 24.2±0.92, 34.8±1.98, 24.4±0.68 and 28.8±2.31 IU/L respectively. Serum ALP concentration was significantly ($p<0.001$) increased in Tra compared with control. It was however significantly decreased in Vit E ($p<0.001$) and Tra+Vit E ($p<0.05$) compared with Tra.

3.1.4 AST/ALT ratio

Figure 4 shows AST/ALT ratio for control, Tra, Vit E and Tra+Vit E which was 0.71±0.02, 0.66±0.06, 0.74±0.07 and 0.75±0.05 respectively. AST/ALT ratio was not significantly different among the experimental groups.

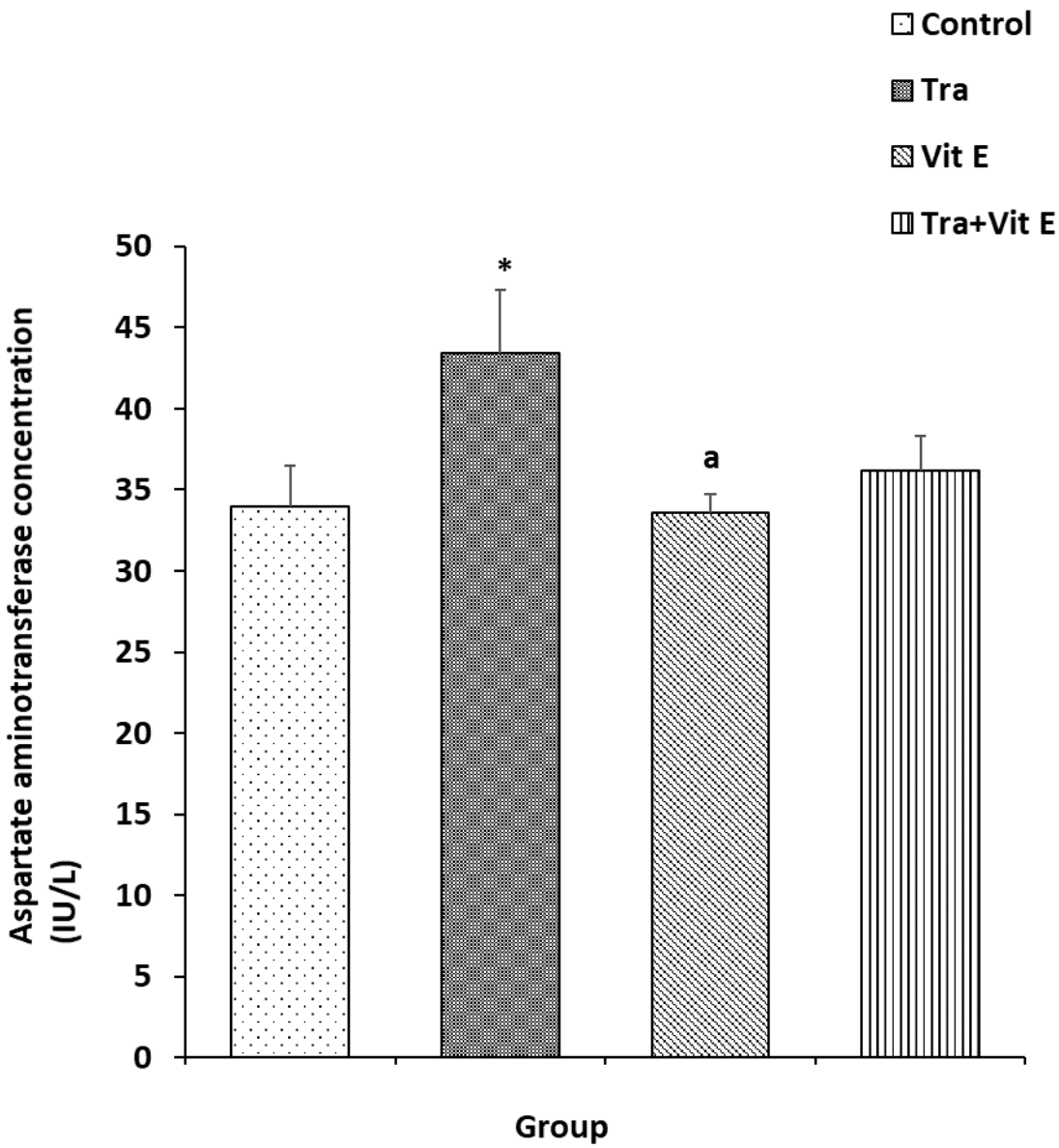

Figure 1: Comparison of aspartate aminotransferase concentration in the different experimental groups. Values are mean±SEM, n = 5.

*p<0.05 vs Control
a = p<0.05 vs Tra

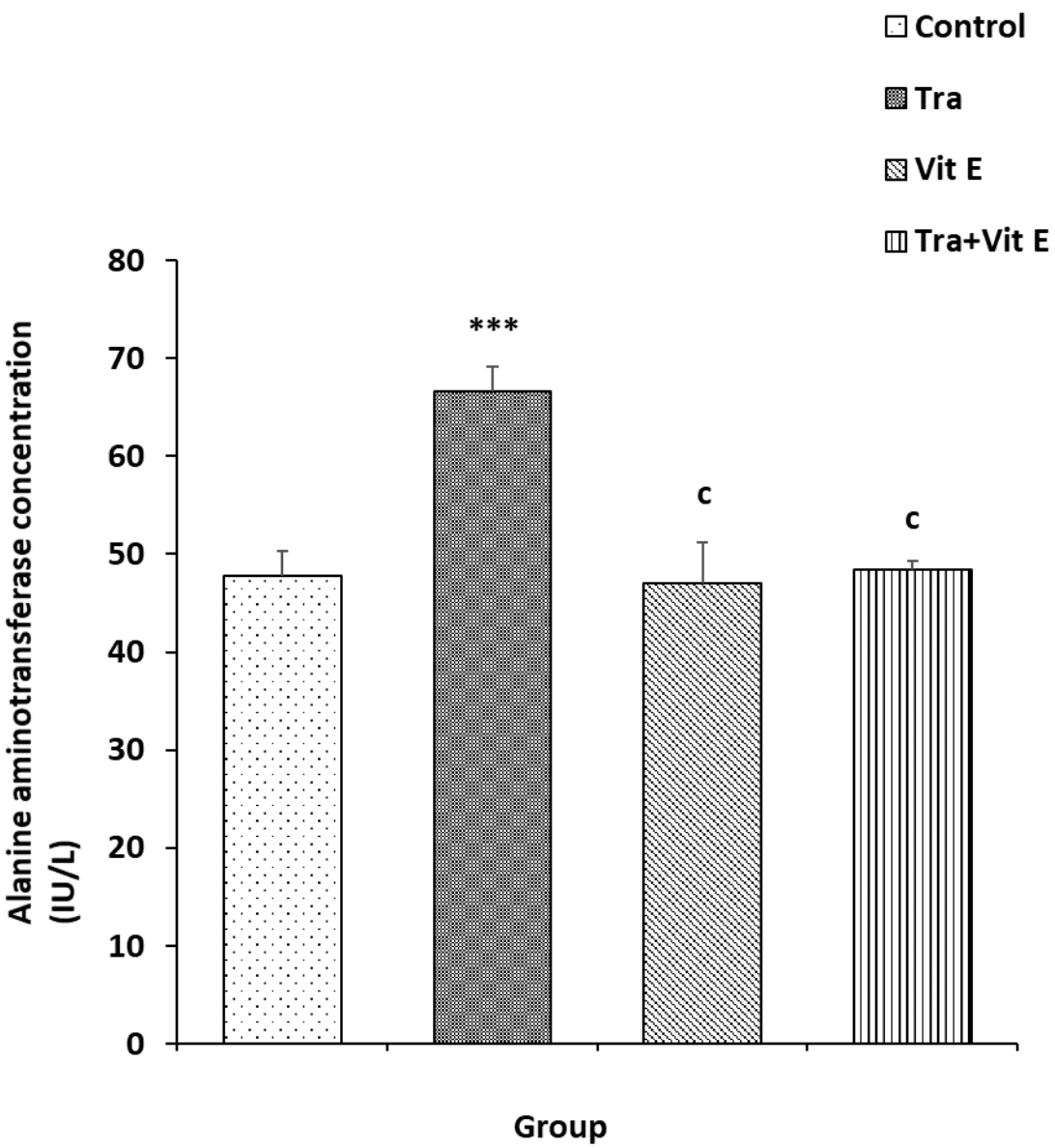

Figure 2: Comparison of alanine aminotransferase concentration in the different experimental groups. Values are mean±SEM, n = 5.

***p<0.001 vs control
c = p<0.001 vs Tra

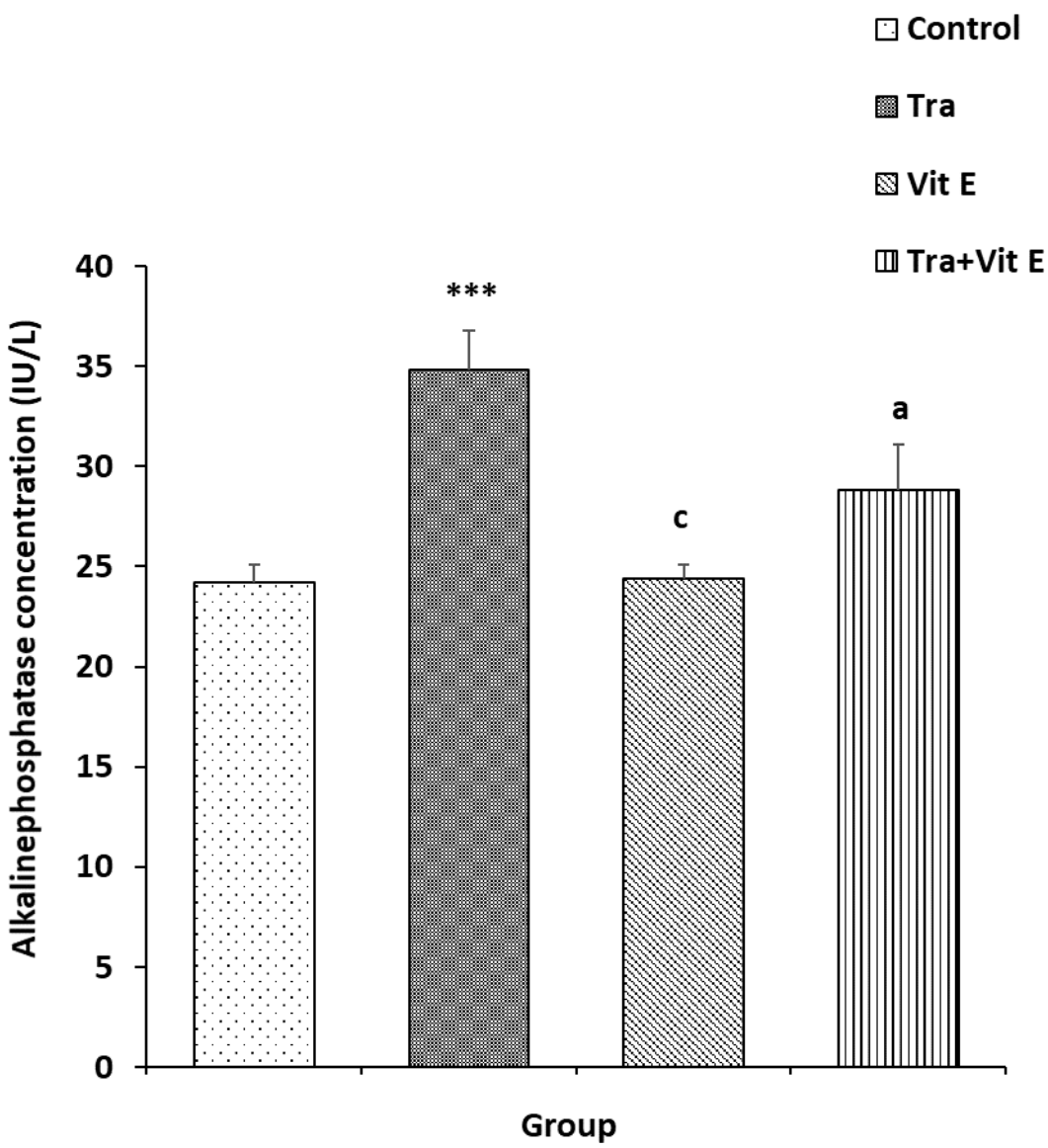

Figure 3: Comparison of alkalinephosphatase concentration in the different experimental groups. Values are mean±SEM, n = 5.

***p<0.001, vs control
a = p<0.05, c = p<0.001 vs Tra

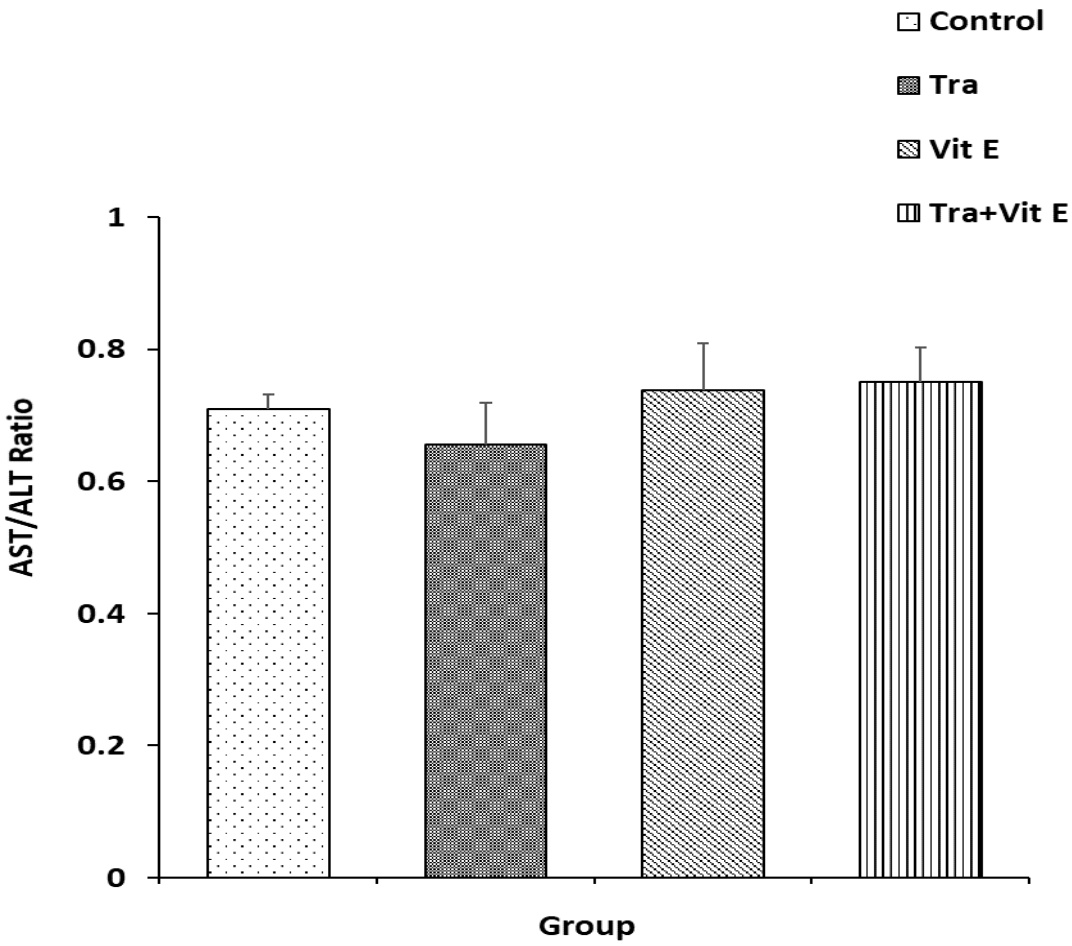

Figure 4: Comparison of AST/ALT ratio in the different experimental groups. Values are mean±SEM, n = 5.

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, Tra, Vit E and Tra+Vit E which was 13.7±1.52, 20.96±0.81, 10.64±0.68 and 15±2.36 µmol/L respectively. Serum TB concentration was significantly ($p<0.01$) increased in Tra compared with control. It was significantly decreased in Vit E ($p<0.001$) and Tra+Vit E ($p<0.01$) compared with Tra.

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

Serum CB concentration for control, Tra, Vit E and Tra+Vit E was 6.58±1.32, 2.82±0.18, 5.34±0.63 and 3.18±0.34 µmol/L respectively. Serum CB was significantly ($p<0.001$) decreased in Tra compared with control. It was significantly ($p<0.01$) increased in Vit E compared with Tra. Serum CB concentration was significantly decreased in Tra+Vit E compared with control ($p<0.001$) and Vit E ($p<0.05$) (Figure 6).

3.4 Comparison of unconjugated bilirubin concentration in the different experimental groups

Serum UCB concentration for control, Tra, Vit E and Tra+Vit E was 7.12±2.31, 18.14±0.78, 5.3±0.86 and 11.82±2.18 µmol/L respectively. Serum UCB was significantly increased in Tra ($p<0.001$) and Tra+Vit E ($p<0.05$) compared with control. It was significantly decreased in Vit E ($p<0.001$) Tra+Vit E ($p<0.01$) compared with Tra. Serum UCB was significantly ($p<0.01$) increased in Tra+Vit E compared with Vit E (Figure 7).

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

Figure 8 shows percentage conjugation of bilirubin for control, Tra, Vit E and Tra+Vit E which was 51.45±12.58, 13.5±0.82, 50.74±6.45 and 22.80±3.00 % respectively. Percentage conjugation of bilirubin was significantly ($p<0.01$) decreased in Tra compared with control. It was significantly ($p<0.01$) increased in Vit E compared with Tra. Percentage conjugation of bilirubin was significantly ($p<0.05$) decreased in Tra+Vit E compared with control and Vit E.`

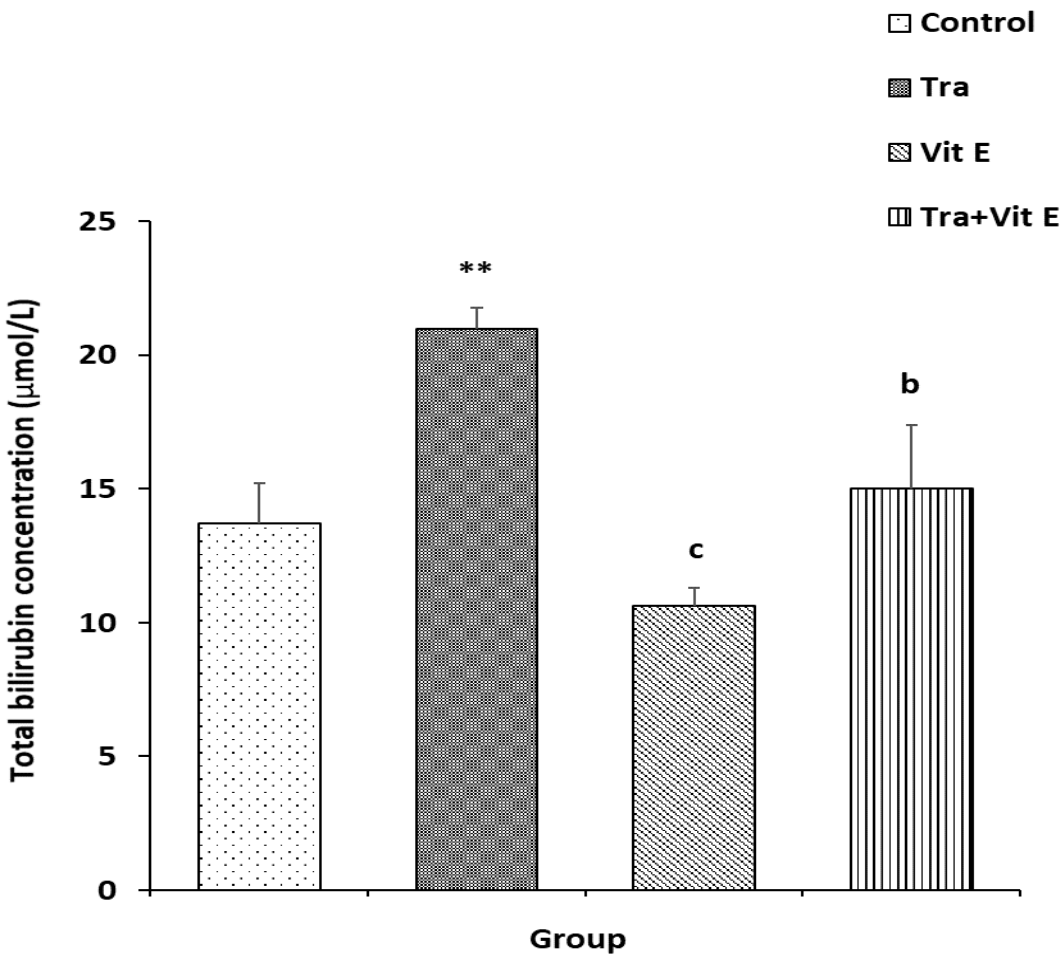

Figure 5: Comparison of total bilirubin concentration in the different experimental groups. Values are mean±SEM, n = 5.

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

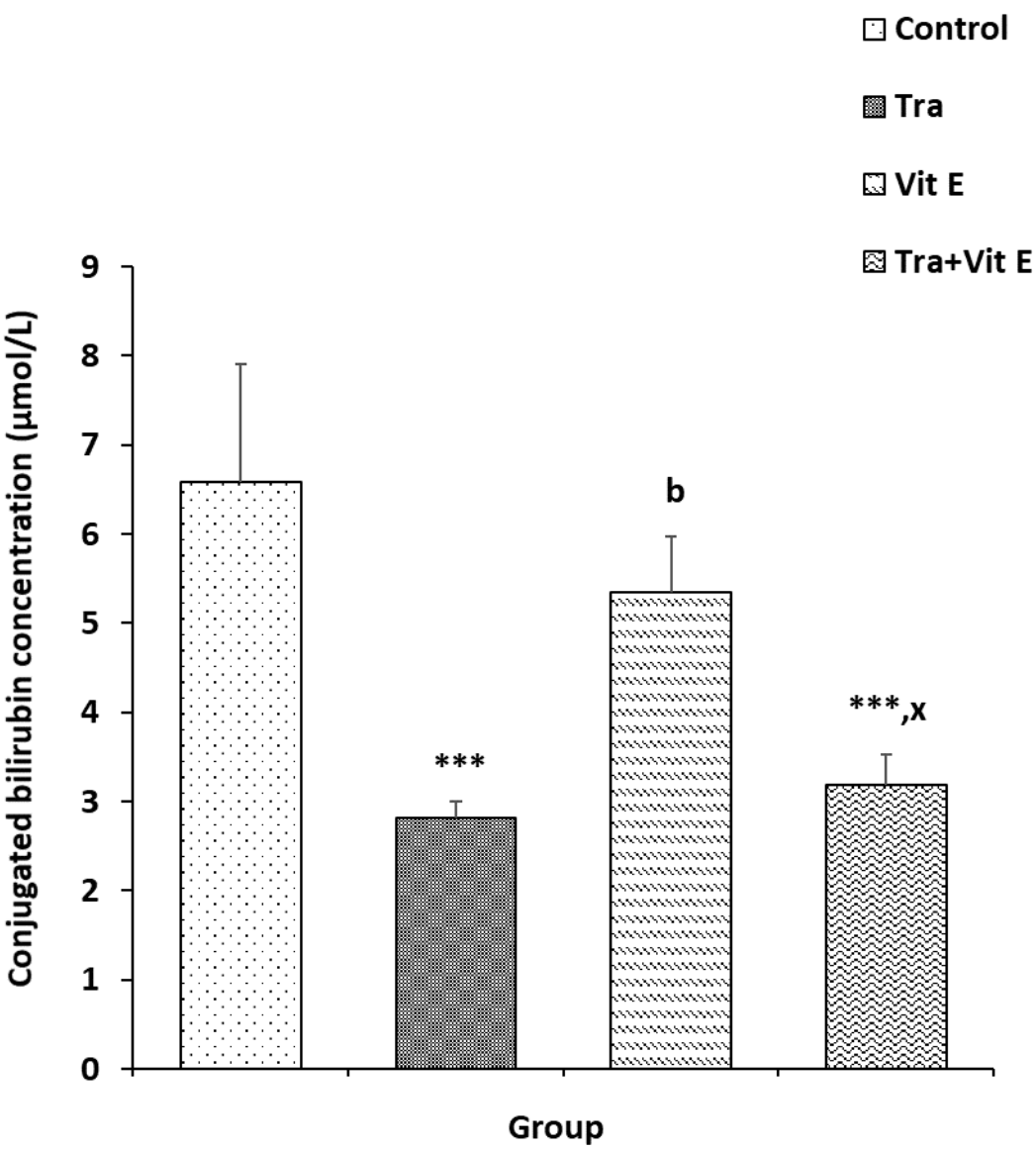

Figure 6: Comparison of conjugated bilirubin concentration in the different experimental groups. Values are mean±SEM, n = 5.

***p<0.001, vs control
b = p<0.01 vs Tra
x = p<0.05 vs Vit E.

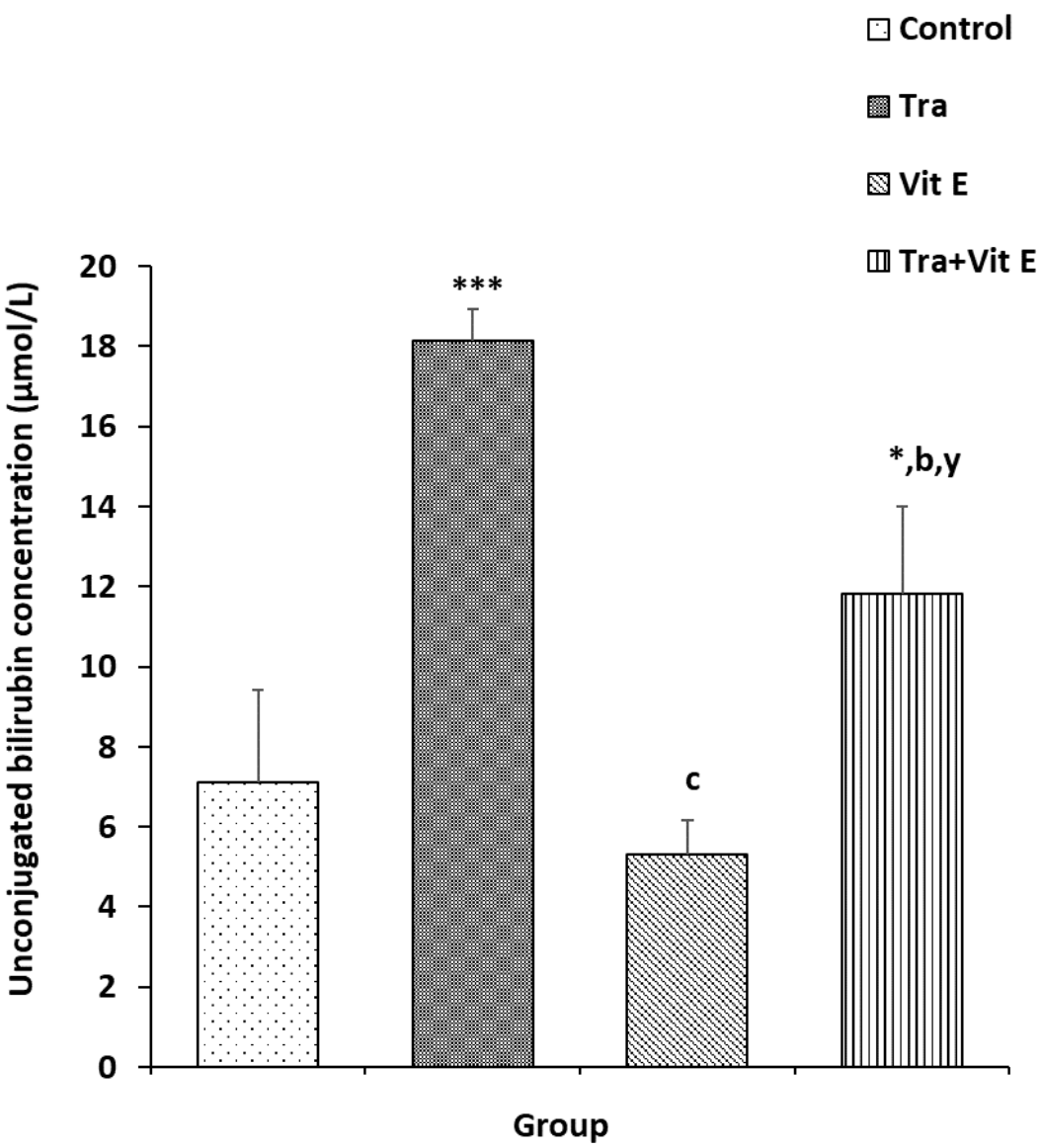

Figure 7: Comparison of unconjugated bilirubin concentration in the different experimental groups. Values are mean±SEM, n = 5.

*p<0.05, ***p<0.001, vs control
b = p<0.01, c = p<0.001 vs Tra
y = p<0.01 vs Vit E.

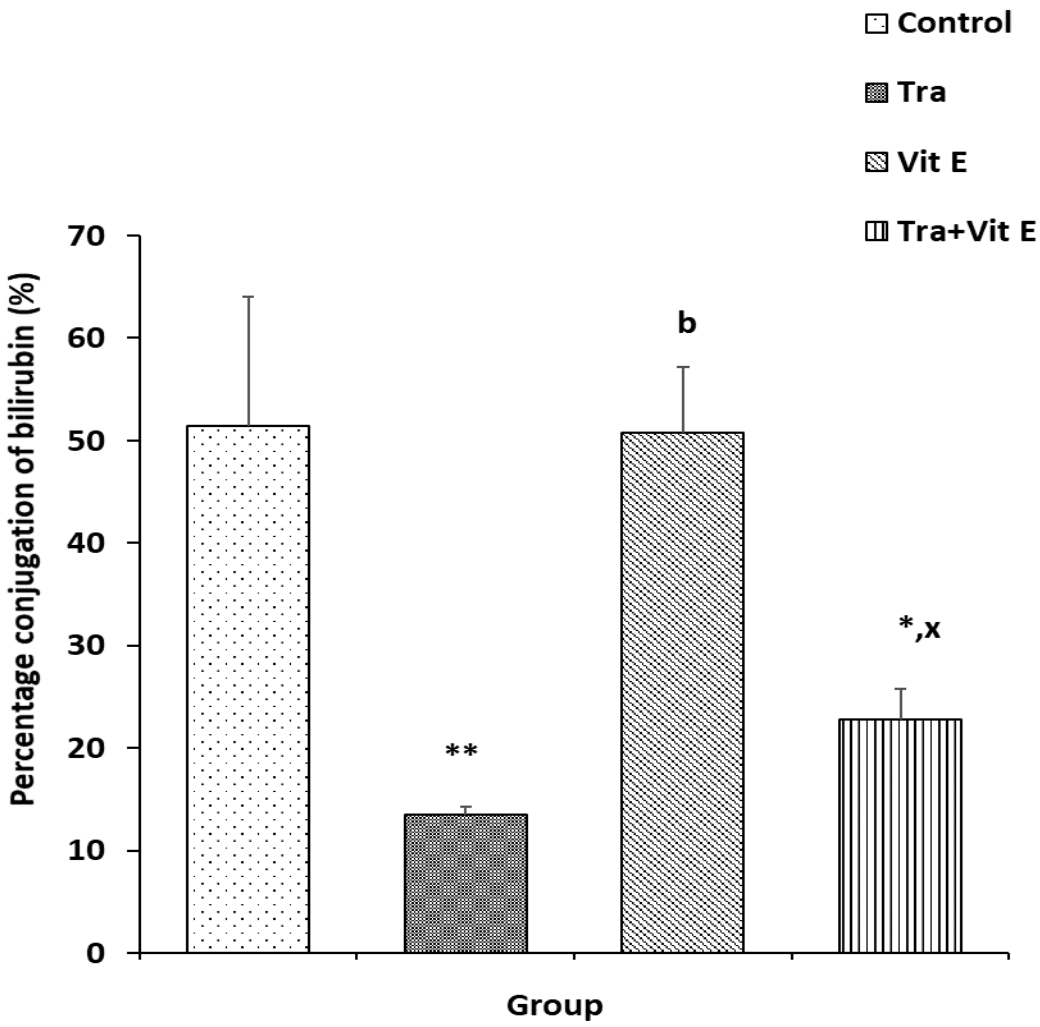

Figure 8: Comparison of percentage conjugation of bilirubin in the different experimental groups. Values are mean±SEM, n = 5.

*p<0.05, **p<0.01 vs control
b = p<0.01 vs Tra
x = p<0.05 vs Vit E.

IV. Discussion

The liver is a metabolic organ of the body responsible for glycogen storage, detoxification of substances, synthesis of plasma proteins and production of bile [18]. Tramadol is a centrally acting analgesic and has been reported to impact negatively on hepatocytes [4] via oxidative stress [9]. Vitamin E is a lipid-soluble antioxidant that has been reported to protect hepatocytes from oxidative damage caused by several substances [13]. This study was carried out to investigate the effect of vitamin E on serum liver enzymes and bilirubin concentrations in male Wistar rats treated with tramadol.

Serum AST, ALT and ALP concentrations increased significantly in the Tra group compared with control. Except AST, all other liver enzymes decreased significantly in the Tra+Vit E compared with Tra

(Figures 1, 2 and 3). AST/ALT ratio was not significantly different among groups. These results are in tandem with the work done by Nna *et al.* [4] and indicate that tramadol impacted negatively on the liver cells (hapatocytes). In their Study, Nna *et al.* [4] reported that tramadol administered to male Wistar rats for a period of 8 weeks resulted in elevated levels of serum AST, ALT and ALP. Increased levels of AST, ALT and ALP are indicative of liver damage. These enzymes are released in greater amounts into the blood of humans and animals when hepatocytes are damaged [19]. Death of hepatocytes either by necrosis or apoptosis leads to increase in AST and ALT concentrations. ALP takes part in bone mineralization and its activity rises in bone disease and hepatobiliary disease. Thus increased ALP concentration is also an indication of damage to bone cells and cholestasis which probable results in progressive liver disease – biliary cirrhosis [20, 21]. Long term tramadol treatment in mice was shown to cause necrosis, vacuolization, central vein dilation, haemorrhage, cytolysis and complete cell membrane degeneration in hepatocytes [7, 8]. The results of our study show that vitamin E supplementation exhibited hepatoprotective effect as serum concentration of the liver enzymes decreased significantly in the Tra+Vit E compared with Tra. This implies that vitamin E supplementation protected the hepatocytes from oxidative damage that was probably associated with tramadol treatment.

Total bilirubin (TB) concentration was significantly increased in Tra compared with control and decreased in Vit E and Tra+Vit E compared with Tra. Conjugated bilirubin (CB) concentration and percentage conjugation of bilirubin were significantly decreased in Tra and Tra+Vit E compared with control. They were also significantly decreased in Tra+Vit E compared with Vit E. Unconjugated bilirubin (UCB) concentration was significantly increased in Tra and Tra+Vit E compared with control. However, UCB concentration was significantly decreased in Tra+Vit E compared with Tra. Our result is consistent with a previous study [4] which showed that tramadol treatment in male Wistar rats caused an increase in serum TB and UCB concentrations and a decrease in serum CB concentration and percentage of conjugation of bilirubin. Elmanama *et al.* [22] measured the levels of liver enzymes and bilirubin in the blood of males who were addicted to taking tramadol for over five years and observed an increase in their serum levels of AST, ALT, ALP, total bilirubin and direct bilirubin. Increased bilirubin concentration in blood is due to increased synthesis, reduced conjugation, reduced secretion by the liver or blockage of the bile ducts. UCB is formed form phagocytosis of haemoglobin (Hb) released from red blood cells that have been destroyed or haemolysed. In the liver, about 80% of the UCB is conjugated with glucuronic acid and the reaction is catalysed by uridinediphosphateglucuronyltransferase. The liver then excretes the CB in bile into the gut where some is lost in faeces and the rest reabsorbed through the intestinal mucosa back into the blood. Increased level of CB causes obstructive jaundice while increased level of UCB causes haemolytic jaundice [23]. Elevated serum bilirubin concentration may arise from damage to liver, presence of immature red blood cells or Gilbert syndrome [24]. Tramadol has been reported to cause damage to hepatocytes via oxidative stress [9]. The present results indicate that treatment with tramadol may have caused haemolysis of red blood cells (RBCs) which led to hyperbilirubinaemia as TB and UCB concentrations were increased significantly in the Tra compared with control. Increased level of UCB in blood is due to increased haemolysis of RBCs [25], absence of glucuronyl transferase or hepatocellular disease [23]. It may also mean that tramadol treatment reduced the rate of conjugation of UCB in the liver which probably explains why serum UCB concentration was increased in the Tra and CB and percentage of conjugation of bilirubin reduced in this group. Vitamin E supplementation however exhibited an opposing effect by reducing the destruction of RBCs that is probably associated with tramadol treatment. This is seen in figures 4 and 6 where TB and UCB concentrations were significantly decreased in Tra+Vit E compared with Tra. Vitamin E supplementation was unable to counter the effect on CB caused by tramadol treatment as no significant difference was observed in serum CB concentration between Tra and Tra+Vit E (Figure 5).

V. Conclusion

Tramadol treatment increased serum liver enzymes and bilirubin concentrations and decreased the ability of the liver to conjugate bilirubin indicating that it is hepatotoxic and alters liver function. Except for the reduced conjugation of bilirubin, vitamin E supplementation countered the negative effects of tramadol. This may be due to its antioxidant effect.

References

[1]. A.O. Abdel-Zaher, M.S. Abdel-Rahman, F.M Elwasei, Protective effect of *Nigella sativa* oil against tramadol-induced tolerance and dependence in mice: role of nitric oxide and oxidative stress, *Neurotoxicology 32(6), 2011,* 725-733.

[2]. A. Elkhateeb, I. El Khishin, O. Megahed, F. Mazen, Effect of *Nigella sativa* Linn oil on tramadol-induced hepato-and nephrotoxicity in adult male albino rats, *Toxicol Rep, 2, 2015,* 512-519.

[3]. E.A. Salem, S.K. Wilson, N.K. Bissada, J.R. Delk, W.J. Hellstrom, M.A. Cleves, Tramadol HCl has promise in on-demand use to treat premature ejaculation, *Journal of Sexual Medicine, 5, 2008,* 188-193.

[4]. V.U. Nna, U.P. Akpan, V.E. Okon, I.J. Atangwho, 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, 5(8), 2015,* 105-113.

[5]. H.B. Osadolor, J.A. Omo-Erhabor, Effects of tramadol on fertility hormones (follicle stimulating hormone, luteinizing hormone, prolactin, testosterone, estrogen and β-HCG) in laboratory rabbits. *British Journal of Medicine & Medical Research, 14(8), 2016,* 1-11.

[6]. V.U. Nna, E.J. Ani, E.O. Ofutet, O.E. Ofem, C.E. Iroh, E.E. Osim, Recurrent side effects following chronic recreational use of sexual stimulants among male subjects in Calabar, Cross River State, Nigeria. *Der Pharmacia Lettre, 6(6), 2014a,* 56-61.

[7]. S. Atici, I. Cinel, L. Cinel, N. Doruk, G. Eskandari, V. Oral, Liver and kidney toxicity in chronic use of opioids: An experimental long term treatment model. *J Biosci, 2, 2005,* 245-252.

[8]. S. Rukhshanda, I. Razia, N.A. Muhammad, Z. Anum, I. Javed, S.A. Muhammad, Effects of tramadol on histopathological and biochemical parameters in mice (*Mus musculus*) model. *Global J Pharmacol. 1, 2014,* 14-19.

[9]. S.A. Sheweita, A.A. Almasmari, S.G. El-Banna, Tramadol-induced hepato- and nephrotoxicity in rats: Role of Curcumin and Gallic acid as antioxidants, *PLoS ONE, 13(8), 2018,* e0202110.

[10]. C.G. Fraga, R.F. Arias, S.F. Llesuy, O.R. Koch, A. Boveris, Effect of vitamin E and selenium deficiency on rat liver chemiluminescence, *Biochem J, 242, 1987,* 383-386.

[11]. H. El Hadi, Vettor, M. Rossato, Vitamin E as a treatment for nonalcoholic fatty liver disease: Reality or myth? *Antioxidants (Basel), 7(1), 2018,* 12. doi: 10.3390/antiox7010012

[12]. T. Pacana, A.J. Sanyal, Vitamin E and non-alcoholic fatty liver disease. *Curr Opin Clin Nutr Metab Care, 15(6), 2012,* 641-648.

[13]. E. Maheswari, G.R. Saraswathy, T. Santhranii. Influence of Vitamin E on hepatotoxicity and oxidative stress. *International Journal of Research in Pharmacy and Biosciences, 2(3), 2015,* 30-38.

[14]. Helsinki. World Medical Association Declaration of Helsinki. Adopted by the 18th WMA General Assembly, Helsinki, Finland, 1964.

[15]. S, Reitman, S. Frankel, Determination of aminotransaminases in serum. *American Journal of Clinical Pathology, 28, 1957,* 50-56.

[16]. E.J. King, A.R. Armstrong, Canadian Medical Association, *Journal, 31, 1964,* 376.

[17]. A.B. Powell, Total bilirubin measurement by photometry on a blood gas analyzer: potential for use in neonatal testing at the point of care. *Clin Chem, 47(10), 1957,* 1845-1847.

[18]. L.P. Gartner, J.L. Hiatt, Color Atlas of Histology. Lippincott Williams & Wilkins, 2000.

[19]. G. Aragon, Z.M. Younossi, When and how to evaluate mildly elevated liver enzymes in apparently healthy patients, *Cleveland Clinic Journal of Medicine, 77(3), 2010,* 195-204.

[20]. E.R. Ashwood, Tietz textbook of clinical chemistry, 2nd ed., WB Saunders Co., Philedelphia, U.S.A, 1994.

[21]. M.J. Kendall, R. Cockel, J. Becker, C.F. Hawkins, Raised serum alkaline phosphatase in rheumatoid Disease: An index of liver dysfunction? *Ann. Rheum, 29, 1970,* 537-540.

[22]. A.A. Elmanama, N.E.S. Abu Tayyem, H.N. Essawaf, M. Ikram, I.M. Hmaid, Tramadol-Induced liver and kidney toxicity among abusers in Gaza Strip, Palestine. *Jordan Journal of Biological Sciences, 8(2), 2015,* 133-137.

[23]. A.C. Guyton, J.E. Hall, Textbook of Medical Physiology. 10th Edn. W.B. Saunders, Philadelphia, PA., USA, 2011.

[24]. J.J. Volpe, Bilirubin and Brain Injury, in Neurology of the Newborn, 2003.

[25]. M. Sgro, D. Campbell, V. Shah, Incidence and causes of severe neonatal hyperbilirubinemia in Canada. *Canadian Medical Association Journal, 175(6), 2006,* 587-590.

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