Outcome of acute and chronic effect of tramadol consumption on liver enzymes, renal function, and electrolyte levels in wistar rats

OSSAI, NDUKA RICHARD[1] OJIEH, ANTHONY EMEKA[1] and NWOGUEZE, CHUKWUEBUKA BARTHOLOMEW[2]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria.
[2]Department of Human Physiology, College of Health Sciences, Evangel University, Akaeze, Ebonyi State Nigeria.
*Corresponding author. E-mail:waddlepaddero@gmail.com. Tel:+2340806239379

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Tramadol overdose had been known to cause acute liver failure and its products are excreted by kidney via urine; this makes the kidney a primary target of toxicity, especially in cases of misuse and overdose. This research aims to evaluate the outcome of acute and chronic consumption of tramadol on liver enzymes, renal functions and electrolytes level in Wistar Rats. Sixty (60) adult Wistar rats for this study was grouped into six groups of 5 male, 5 female and was treated with tramadol (30 mg/kg body weight). Group 1 was not treated within the period of the study, Group 2, 3, 4 and 5 were treated for 7, 14, 21 and 42 days respectively and was sacrificed. Group 6 was withdrawn for 21 days after 21 days of treatment. The animal's Liver and kidney were excised for biochemical analysis. Generated data were analyzed using SPSS package and results expressed as mean ± SEM. Results obtained showed a progressive weight gain, elevation in the activities of ALT, ALP and AST, urea and creatinine and electrolytes level in all the groups treated with tramadol, when compared to the normal control. Groups 4 and 5 demonstrate the highest value compared to other groups treated. Conclusively, the use of tramadol has toxic effects on the liver function and can also be demonstrated by a substantial increase of urea and creatinine concentration, and in electrolyte levels. Tramadol use must be monitored and restricted to prescription only because therapeutic or severe dose can lead to toxicity.

Keywords: Tramadol, nephrotoxicity, hepatotoxicity, wistar rat

INTRODUCTION

Tramadol, a potent analgesic medication used worldwide to treat acute and chronic pain (Grond and Sablotzki 2004; Gillman, 2005; Miotto et al 2017) is also believed to be a sex-enhancing drug (Cicero et al 2005) and has been one of the most commonly prescribed and abused pharmaceuticals in recent years. Illegal drug disorder is one of the most frequent illegal psychoactive chemicals consumed in particular by young individuals It contributes significantly to the worldwide disease burden. Tramadol's overdose was known to cause abrupt liver failure and its products are eliminated through the urine by the kidney, which makes the kidney the primary target organ of toxicity to tramadol, particularly in cases of abuse and overdose. The adverse effects associated with tramadol used in clinics constitute a serious problem for patients and health care providers (Asis et al., 2009). It has been estimated that about 10% of drugs are associated with severe, undesirable side effects (Hussami and Farrington, 2007; Shi et al., 2010). However, this number is probably underestimated, given that drug-induced adverse effects are difficult to detect due to pre-existing medical conditions, multiple drug usage, and lack of diagnostic standards (Asis et al.,

2009). Tramadol-related side effects used in hospitals pose a significant concern for patients and health care providers (Assis and Navarro, 2009). Around 10% of medications are estimated to be associated with serious adverse side effects (Hassanian-Moghaddam et al., 2013; Shin et al., 2017), however, considering that drug-induced adverse effects are difficult to detect due to pre-existing medical conditions, multiple drug use, and lack of diagnostic criteria is likely to be underestimated (Assis and Navarro, 2009). Therefore this research specifically aimed to assess the outcome of tramadol intake on liver enzymes, renal function and electrolytes level in adult wistar rats.

MATERIALS AND METHODS

Chemicals and drugs

Tramadol was purchased from Omena pharmacy, Abraka, Delta State, Nigeria. All other chemicals and drugs used were of analytical grade.

Experimental animal

Sixty (60) adult Wistar rats, comprising 30 males and 30 females, were purchased for this research at the Faculty of Basic Medical sciences Animal house, Delta State University, Abraka, Nigeria, and housed in metabolic cages. They were kept on the animal feed growers' daily mash diet, (a product of Top Feed in Sapele, Delta State. Feed components include: 17.0% protein, 4.5% minimum, fat, 0.96% min. calcium, 3.92% usable min. phosphorus, and 2450kcal energy) and water *ad libitum*.

Drugs preparation and administration

300 mg of tramadol was dissolved in 50 ml of distilled water to yield 6 mg/ml of stock solution of tramadol. The route of administration was oral

Experimental design

Group 1 (n = 5 Male and 5 Female) - Control Group Wister Rats were not treated within the period of the study before sacrificing.
Group 2 (n = 5 Male and 5 Female) – Received 30 mg/kg body weight of tramadol for 7 days and were sacrificed
Group 3 (n = 5 Male and 5 Female) – Received 30 mg/kg body weight of tramadol for 14 days and were sacrificed
Group 4 (n = 5 Male and 5 Female) – Received 30 mg/kg body weight of tramadol for 21 days and were sacrificed
Group 5 (n = 5 Male and 5 Female) – Received 30 mg/kg body weight of tramadol for 42 days and were sacrifice
Group 6 (n = 5 Male and 5 Female) –Withdrawn for 21 days after receiving tramadol 30 mg/kg for 21 days before sacrificing

Sample collection

Each rat was sacrificed by cervical dislocation and was placed on its dorsal surface, a laparotomy was carried out to reveal the internal organs, and blood was collected by cardiac puncture, using 5ml syringes and 23G needle into blood sample containers and centrifuged for 10 minutes at a rate of 4000 rpm, and serum was collected and stored in blood sample containers.. The liver and the kidney were harvested for biochemical analysis.

Determination of body weight

Body weight of experimental animals was checked at week 0 before tramadol administration and last day of drug administration before sacrifice. Percentage weight change was calculated as follows.

$$\text{Percentage weight change (\%)} = \frac{\text{final-initialbodyweight (g)}}{\text{initialbodyweight (g)}} X \frac{100}{1}$$

Biochemical analysis

Biochemical analysis was carried out on the samples collected as follows:

Determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities

Estimation of *Aspartate aminotransferase (AST), alanine aminotransferase (ALT)* and *alkaline phosphatase (ALP) activities* in serum and tissue homogenates was carried out by the method described by Reitman and Frankel (1957) and recommended by The International Federation of Clinical Chemistry (IFCC, 1977).

Estimation of urea and creatinine

Urea and Creatinine Analyzer-2 (Beckman Coulter Inc., USA) in combination with a Hichem Creatine Pak, (Elan Diagnostics, USA) were used to analyse urea and creatinine content of the serum samples (Adekomi, 2010).

Estimation of total protein

Total Protein and albumin *level* in serum and tissue homogenates was carried out by Biuret Method as described by Khalifa (2020)

Estimation of Serum Electrolytes level

Serum Sodium, Potassium, Magnesium and Bicarbonate were determined using a method described by Terri and Sesin (1958) and Grindler and Health (1971).

Statistical analysis

The data were analyzed by comparing the values for individual controls for different treatment groups and the results were expressed as mean values ± standard mean error (mean ± SEM). Using the student's t-test, ANOVA and the results were considered significant at P-values of less than 0.05 ($P<0.05$) using SPSS version 23 software, significant differences between control and experimental groups were measured.

RESULTS

There was a steady increase in the body weight of groups 2, 3, 4 and 5, respectively in male wistar rats relative to the control group and group 6 demonstrated a substantial decrease in the body weight. There was a steady increase in the body weight of groups 2, 3, 4 and 5, respectively in the female wistar rats relative to the control group and group 6 demonstrated a substantial decrease in the body weight. There is an increase in plasma activity of ALT, ALP and AST compared with control rats, however, group 6 demonstrated a significant reduction. There is an increase in plasma activity of ALT, ALP and AST compared with control rats, however, group 6 demonstrated a significant reduction.

DISCUSSION

Overdosing tramadol has been one of the most practices of recent especially in young adult males with a history of drug addiction (Shadnia et al., 2008). The liver and kidney are responsible for the metabolism and excretion of tramadol and a high risk of hepatotoxicity and nephrotoxicity has been recorded (Wu et al., 2001). Opioid medication is commonly believed to affect the way food is used by the body, resulting in changes in the diet and exercise patterns of the patient, which in turn causes body weight to increase or decrease (Mohammed and Mahmoud, 2019). Most of the physical effects of Tramadol are in the brain, so any phase in the body that depends on the central nervous system, such as heart rate, breathing and even thought, becomes sluggish and slow (Guyton and Hall, 2006). The slowing down of these processes results in a decline in the quantity of energy utilized by the body. The body will absorb this extra energy and store it as fat, which results in increased body weight. The administration of tramadol (30 mg/kg) to experimental animals, irrespective of period of administration, shows a steady increase in the body weight of groups 2, 3, 4 and 5, respectively, in both male and female wistar rats relative to the control group (Figures 1a and 1b) and a substantial decrease in the body weight of group 6 animals. Major body weight loss in group 6 (group withdrawal) may be due to its gastrointestinal side effects, including loss of appetite which is one of the significant sign of tramadol withdrawal (Jolobe, 2015). In comparison, Ahmed and Kurkar (2014) recorded no substantial increase in the body weight of tramadol-administered male wistar rats. Different doses and durations of administration, sex differences, eating habits or environmental factors may account for the observed disparity. Organ weight gain was also observed in all groups that received tramadol (30 mg/kg) relative to control group 1, although the increase observed in groups 3 and 5 was comparable to the increase observed in control group 1 (Table 1a and b).

Due to the important role of the liver in drug metabolism that predisposes it to toxic injury, most medications have been linked to hepatotoxicity (Atici et al., 2005). In the present study, regardless of gender or duration of administration, liver function was compromised in the groups of experimental animals treated with tramadol, as reflected by elevation in plasma

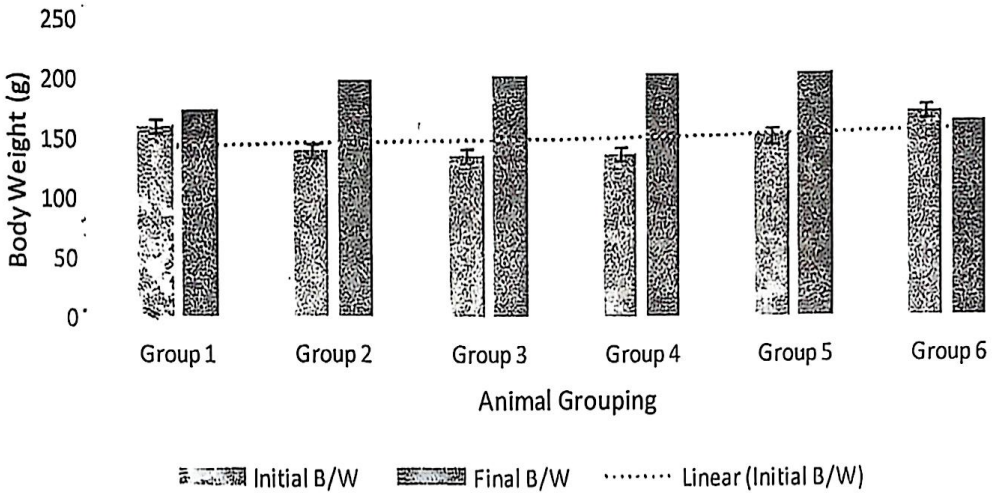

Figure 1a. Effects of Tramadol consumption on body weight in male Wistar rat.

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. $P<0.05$. $^{a}P<0.05$ indicate significant increase and $^{b}P>0.05$ indicate no significant difference
KEY: Group 1 = Normal Untreated rats, Group 2 = Received tranadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks.

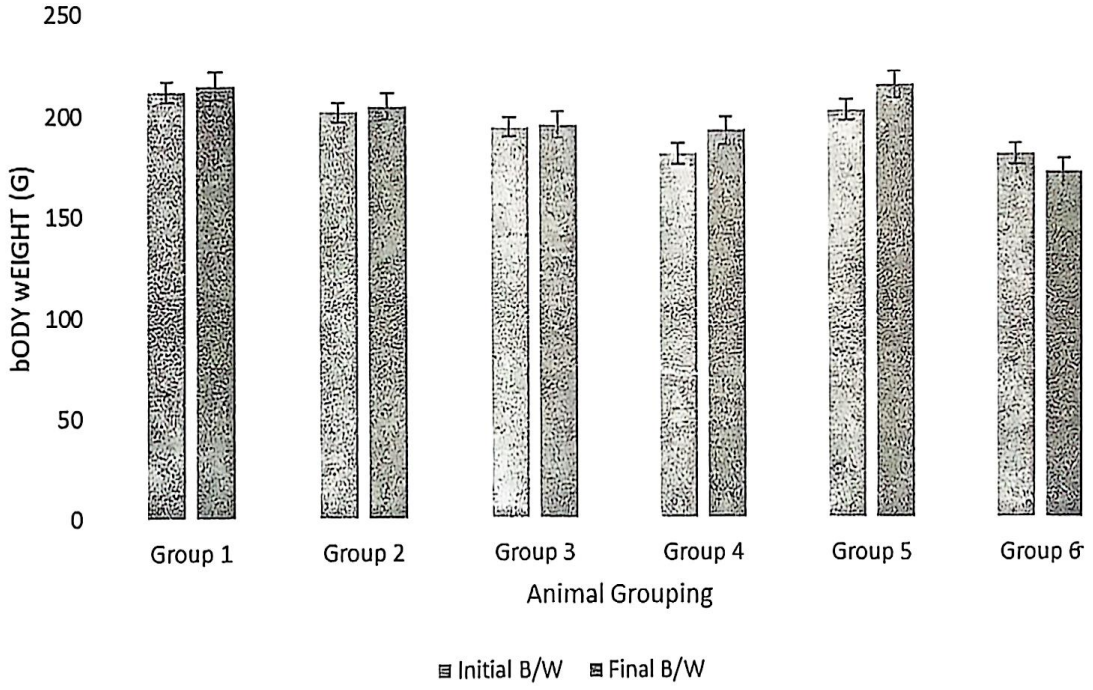

Figure 1b. Effects of Tramadol consumption on body weight in female Wistar rat.

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range testS (Ojieh, 2020) significantly at $P<0.05$.

$^{a}P<0.05$ indicate significant increase and $^{b}P>0.05$ indicate no significant difference.
KEY: Group 1 = Normal Untreated rats, Group 2 = Received tranadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks.

Table 1a. Acute and chronic effects of Tramadol consumption on organ weight of male Wistar rat

GROUP	Liver weight (gm)	Relative Liver weight (%)	Kidney weight (gm)	Relative Kidney weight (%)
1	5.97±0.54	3.10±0.16	1.05±0.17	0.54±0.06
2	4.49±0.20	3.21±0.06	0.88±0.04	0.63±0.03
3	4.74±0.35	3.09±0.23	0.81±0.16	0.53±0.12
4	3.67±0.46	2.56±0.08	0.42±0.04	0.30±0.02
5	5.49±0.36	3.23±0.11	1.17±0.13	0.69±0.08
6	4.80±0.14	2.93±0.07	1.12±0.06	0.69±0.04

Values are expressed as mean± SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05. ^{a}P<0.05 indicate significant increase and ^{b}P>0.05 indicate no significant difference

KEY: GROUP 1 = Normal Untreated rats, GROUP 2 = one week treatment with tranadol 30mg/kg.

GROUP 3 = Received tramadol 30 mg/kg for two weeks, GROUP 4 = Received tramadol 30 mg/kg for three weeks, GROUP 5 = Received tramadol 30 mg/kg for six weeks and GROUP 6 = withdraw from receiving tramadol 30 mg/kg after three weeks

Table 1b. Acute and chronic effects of Tramadol consumption on organ weight of female Wistar rat

GROUP	Liver weight (gm)	Relative Liver weight (%)	Kidney weight (gm)	Relative Kidney weight (%)
1	6.67±0.43	3.14±0.31	1.20±0.21	0.56±0.09
2	5.23±0.12	2.83±0.05	1.21±0.06	0.65±0.02
3	4.60±0.54	2.33±0.25	0.56±0.03	0.28±0.01
4	5.49±0.15	2.86±0.15	0.59±0.04	0.31±0.01
5	6.62±0.48	3.60±0.16	1.47±0.05	0.80±0.03
6	7.18±0.86	3.71±0.17	1.41±0.19	0.72±0.04

Values are expressed as mean± SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05. ^{a}P <0.05 indicate significant increase and ^{b}P>0.05 indicate no significant difference

KEY: GROUP 1 = Normal Untreated rats, GROUP 2 = one week treatment with tranadol 30mg/kg.

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activity of ALT, ALP and AST compared with control rats (Figures 2a and 2b). This finding is similar to previous studies which documented significant increases in serum ALT, AST and ALP levels in rats following long-term use of tramadol (Wu et al., 2001; Atici et al., 2005). The increased plasma activity of ALT, AST and ALP found in this study is an indication of liver damage, as the liver is an organ that detoxifies the body's toxic and chemical substances (Vozarova et al., 2002). Acute cell necrosis or damage to the liver cell membrane can accompany the increased secretion of these liver enzymes, leaking the enzymes into the blood circulation (Loughrey et al., 2003). Similar studies have also shown that the postoperative effects of morphine and tramadol on liver histopathology in rabbits that experienced isoflurane anesthesia, hepatocyte degeneration, central vein dilatation, and mononuclear cell infiltration were more severe in the morphine and tramadol community than in the control group (ZuhtuUtku et al., 2006). In addition, ZuhtuUtku et al. (2006), inusoidal dilatation and degeneration of the hepatocyte cell membrane were more frequent in the tramadol community than in the morphine group. These findings indicate that morphine and tramadol can lead to some liver tissue changes (ZuhtuUtku et al., 2006).

Due to the vital function of the kidney, almost every drug is associated with nephrotoxicity (Matthiessen et al., 1998; Tolman, 1998). Drug metabolites are excreted in the

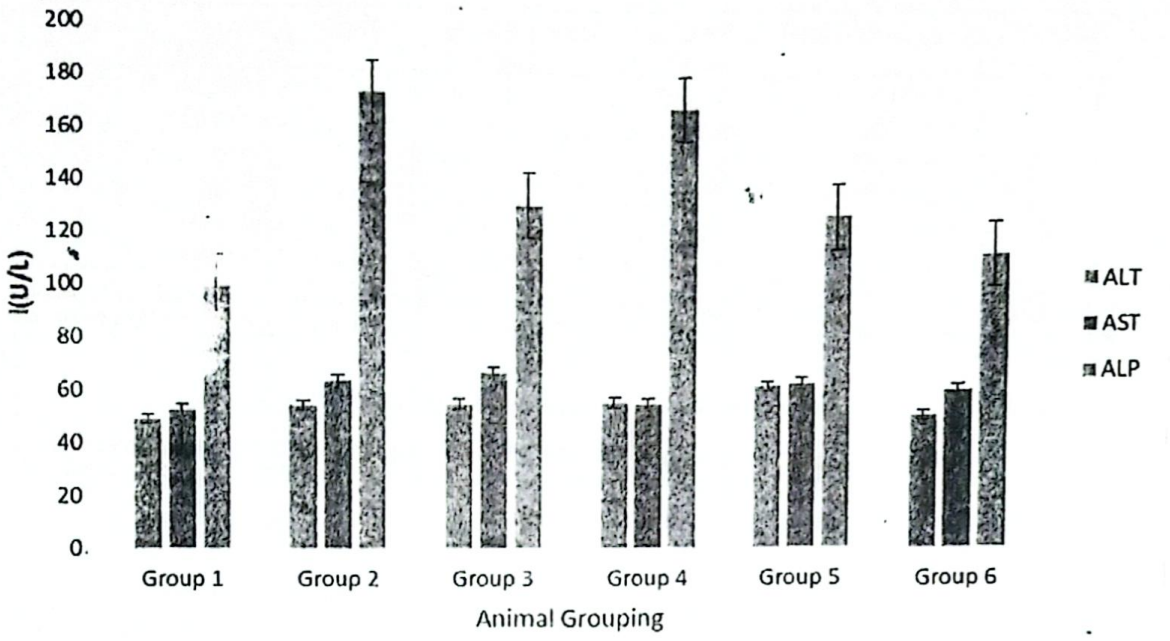

Figure 2a. Effects of tramadol consumption on *Aspartate aminotransferase (AST), alanine aminotransferase (ALT)* and *alkaline phosphatase (ALP)* activities in male wistar rats.

Values are expressed as mean ±SEM. P<0.05. [a]P<0.05 indicate significant increase and [b]P>0.05 indicate no significant difference

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tranadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks

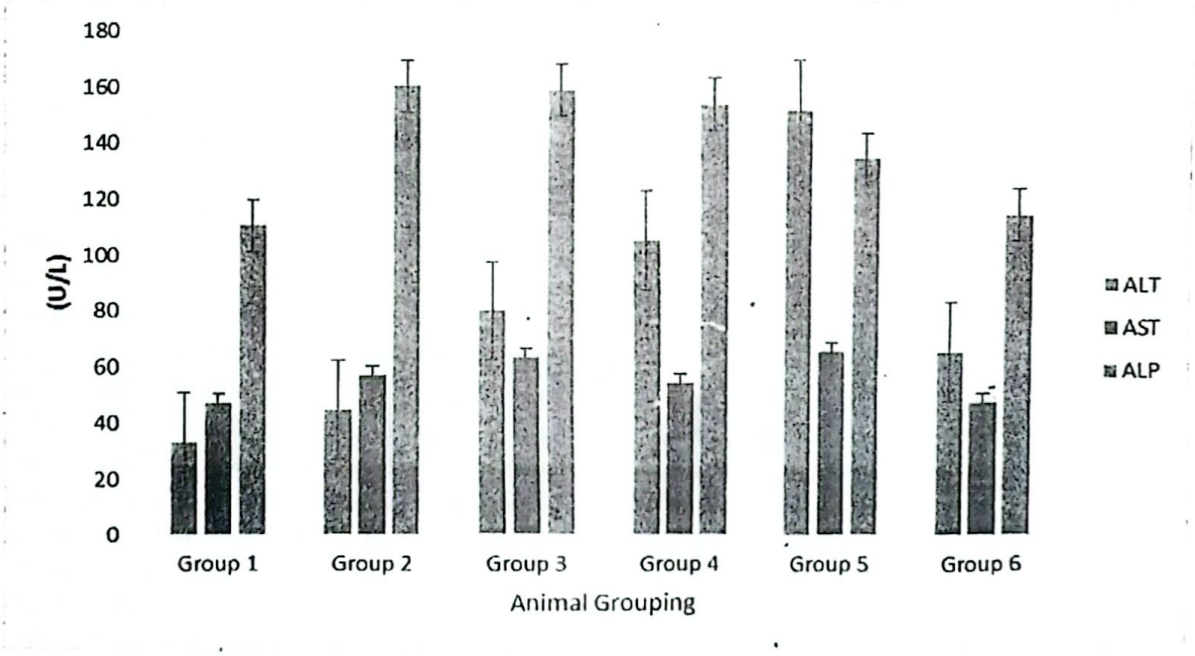

Figure 2b. Effects of tramadol consumption on *Aspartate aminotransferase (AST), alanine aminotransferase (ALT)* and *alkaline phosphatase (ALP)* activities in female wistar rats.

Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tramadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks

kidneys and some may cause cellular damage that may result in kidney dysfunction (Singhal et al., 1998). Creatinine and urea levels in blood are common biochemical parameters used for assessing the function of the renal system (Sembulingam and Sembulingam, 2010). The amount of plasma creatinine is used to assess the rate of glomerular filtration while the nephrotoxic profile of xenobiotics is an indicative calculation of urea (El-Wessemy, 2008). In this study, renal dysfunction in rats treated with tramadol was demonstrated by a substantial increase in the concentration of urea and creatinine in comparison with the control group (Figures 3a and b).

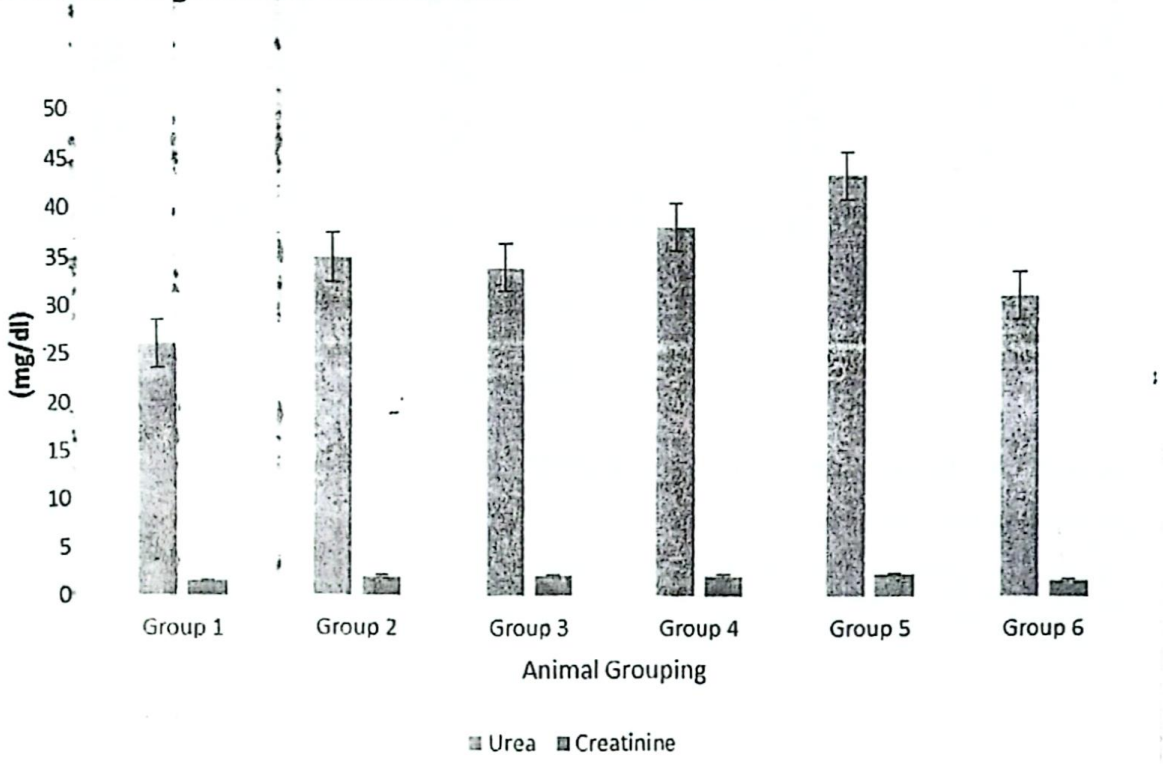

Figure 3a. Effects of tramadol consumption on Urea and Creatinine in male wistar rats.

Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tramadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks

This finding is supported of previous reported by Atici et al. (2005) 'that ingestion of opioid drugs had significant increase in urea, creatinine, electrolytes and testicular biomarkers' (El-Gaafarawi, 2006; Noori and Mahboobe, 2010) and is an indication of renal toxicity that causes the rate of glomerular filtration to decrease, leading to the accumulation of creatinine and urea in the blood. Groups 4 and 5 demonstrated significant increase in relative to other treated groups, irrespective of gender, but no substantial increase was observed in group 6 compared to groups 3, 4 and 5, respectively.

It has been recognized that protein synthesis is not only affected by impaired hepatic activity, but also by the availability of amino acids, catabolic states, cytokine behaviour, hormones and/or congenital deficiency states (Robert, 2012). In this analysis, serum total protein was substantially reduced (Table 2a and b), Serum total protein, in general, consists of albumin and globulin portions (Osadolor and Omo-Erhabor, 2017). A decrease in total serum protein may result from a decrease in any portion of total serum protein levels (Guyton and Hall, 2006). The decrease observed in this study may have been due to the impact of tramadol on some

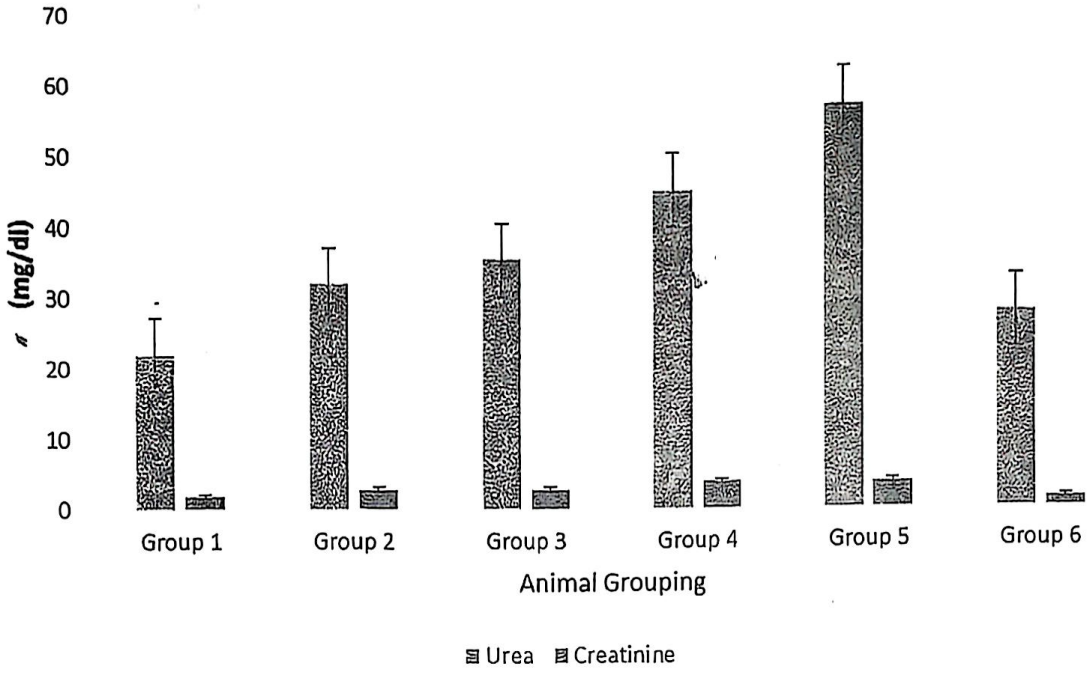

Figure 3b. Effects of tramadol consumption on Urea and Creatinine in female wistar rats.

Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tramadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks

Table 2a. The short and long term effects of Tramadol consumption on serum proteins level of male Wistar rat

Group	Total Protein	Albumin
1	10.59 ± 1.36^{a}	4.10 ± 0.38^{b}
2	23.63 ± 6.57^{c}	5.93 ± 0.44^{b}
3	19.33 ± 2.16^{c}	5.91 ± 0.73^{b}
4	54.53 ± 4.41^{c}	7.49 ± 0.21^{b}
5	38.93 ± 4.25^{c}	4.80 ± 0.54^{b}
6	47.42 ± 4.69^{c}	5.43 ± 0.33^{b}

Values are expressed as mean± SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at $P<0.05$. $^{a}P<0.05$ indicate significant increase and $^{b}P>0.05$ indicate no significant difference

KEY: GROUP 1 = Normal Untreated rats, GROUP 2 = one week treatment with tranadol 30mg/kg.

GROUP 3 = Received tramadol 30 mg/kg for two weeks, GROUP 4 = Received tramadol 30 mg/kg for three weeks, GROUP 5 = Received tramadol 30 mg/kg for six weeks and GROUP 6 = withdraw from receiving tramadol 30 mg/kg after three weeks

of the globulin fractions, thereby indicating that long-term use of tramadol may be harmful to immune responses (Osadolor and Omo-Erhabor, 2017). While Zhihen and colleagues (Zhihen et al., 2006) suggested potential immune-enhancing effects of tramadol, Sacerdote and colleagues (Sacerdote et al., 2007) reported tramadol utility in the care of patients who may be contraindicated by immunosuppression. It is recognized that the liver has a reserve capacity,

Table 2b. The short and long term effects of Tramadol consumption on serum proteins level of female Wistar rat

Group	Total Protein	Albumin
1	15.38±0.88	6.88±0.76
2	19.06±0.22	9.06±0.22
3	21.77±4.29	8.63±1.09
4	28.43±8.75	8.23±1.50
5	30.12±0.18	10.05±0.15
6	36.78±2.39	9.21±1.53

Values are expressed as mean±SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at $P<0.05$. $^{a}P<0.05$ indicate significant increase and $^{b}P>0.05$ indicate no significant difference

KEY: GROUP 1 = Normal Untreated rats, GROUP 2 = one week treatment with tranadol 30mg/kg.

GROUP 3 = Received tramadol 30 mg/kg for two weeks, GROUP 4 = Received tramadol 30 mg/kg for three weeks, GROUP 5 = Received tramadol 30 mg/kg for six weeks and GROUP 6 = withdraw from receiving tramadol 30 mg/kg after three weeks

thereby avoiding the type of protein concentration decreasing and seen only in severe liver damage. Liver proteins also have relatively long half-lives, which range from 19 to 21 days for albumin (Osadolor and Omo-Erhabor, 2017), and may also have accounted for the findings in this report. In acute hepatic dysfunction, little changes in plasma protein concentrations have been documented (Robert, 2012). Laila and El (2012) also reported changes in serum protein in a study on' hepatic DNA damage and serum protein pattern abnormality due to long-term use of tramadol in rats.

Electrolyte imbalance may result from being dehydrated or overhydrated, taking certain medications, having certain heart, kidney, or liver problems, receiving inappropriate amounts of intravenous fluids or feeding (Guyton and Hall, 2006). The effects of tramadol intake on electrolyte levels of male and female wistar rats were estimated in Figure 4a and h. Significantly higher than average electrolyte levels (sodium magnesium, potassium and chloride) were found in all groups treated with tramadol (30 mg/kg), regardless of sex and duration of administration, compared with normal control at $p<0.05$.

Group 4 and 5 demonstrated significant increase compared to other treated group However, no significant changes were observed in group 6 (withdrawal's group) as opposed to groups 3, 4 and 5. This is in agreement with Nehad et al. (2013) on the effects of tramadol hydrochloride on the hematological and biochemical profiles of domestic male rabbits, which reveals an increase in electrolyte serum levels: (Na+), (K+), (Ca2+) and (Po43+) and may be affected by tissue damage due to hypoxia and asthmatic drugs. The study by Ghoneim et al. (2014) and Nna and Osim (2016) on oxidative stress markers during and after removal of tramadol administration also revealed that repeated use of tramadol raises the levels of electrolytes (Na+), (K+), (Ca2+) (Cl-) and (Po43+).

CONCLUSION

This study proves that tramadol use has toxic effects on the function of the liver as reflected by elevation in the activities of ALT, ALP and AST in the plasma of wistar rats. It also confirms that tramadol administration may cause nephrotoxicity evidence by the significant increase in urea and creatinine concentration and also the increase in electrolytes level, which could result in high blood pressure and hypertension in users. Therefore tramadol use must be under supervision and its use should be limited to prescription only, avoiding indiscriminate and

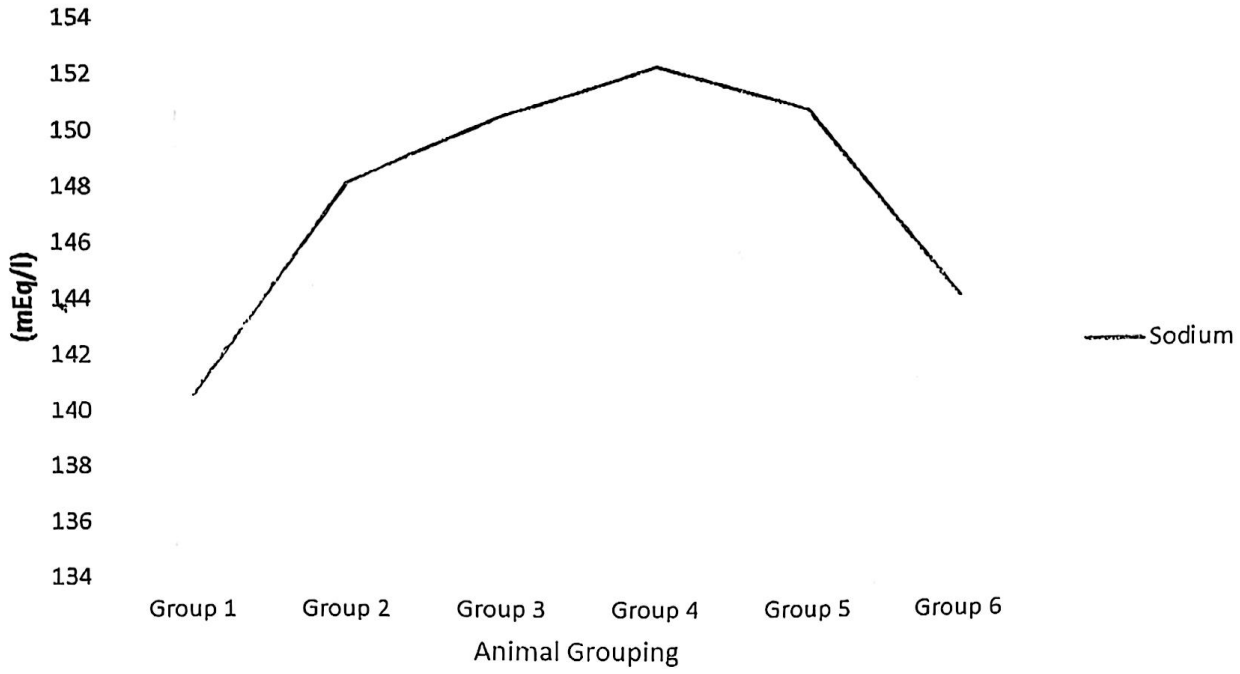

Figure 4a. Effects of tramadol consumption on Sodium ion in male wistar rats.

Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tramadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks.

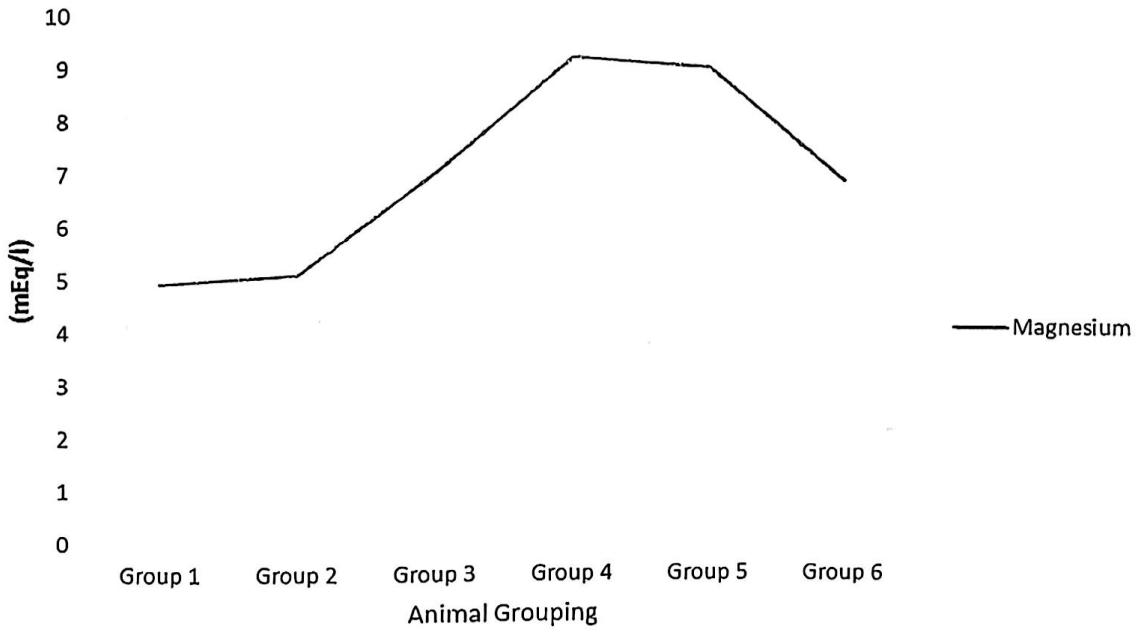

Figure 4b. Effects of tramadol consumption on Magnesium Ion in male wistar rats.

Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tranadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks.

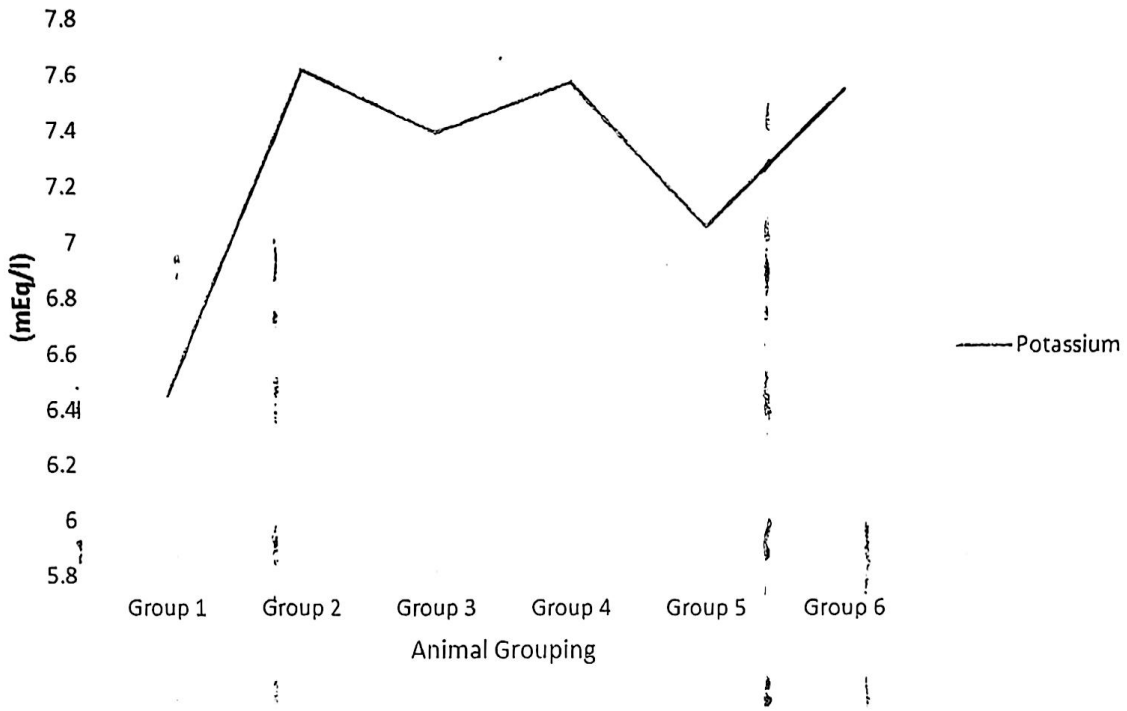

Figure 4c. Effects of tramadol consumption on Potassium ion in male wistar rats.

Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tranadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks

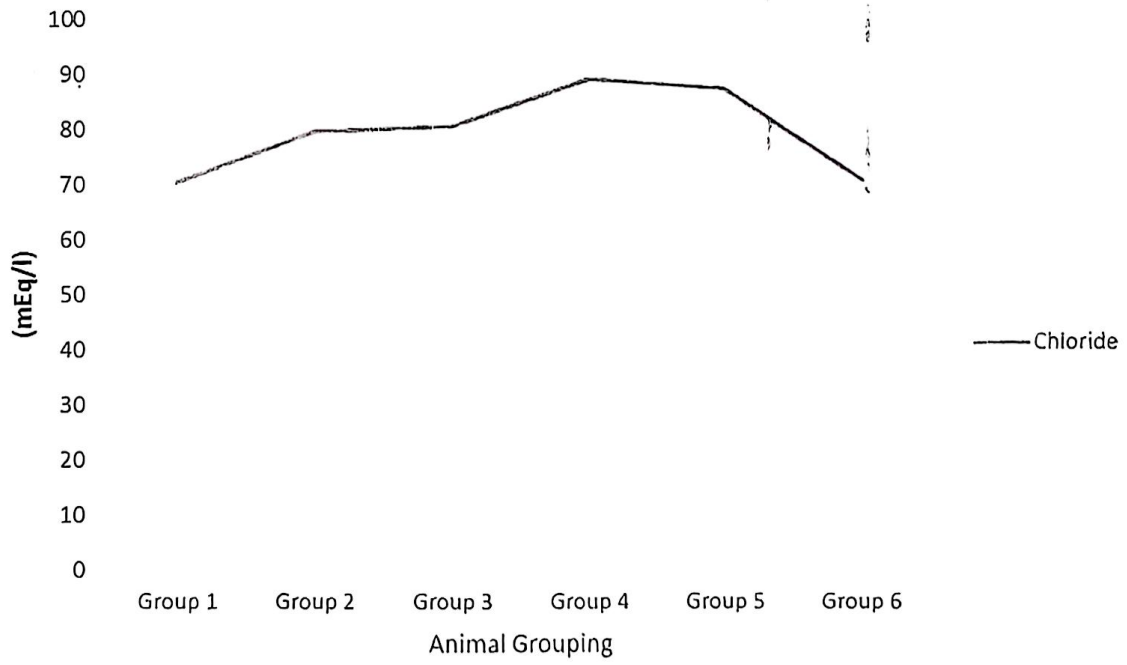

Figure 4d. Effects of tramadol consumption on Chloride ion in male wistar rats.

Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tranadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks

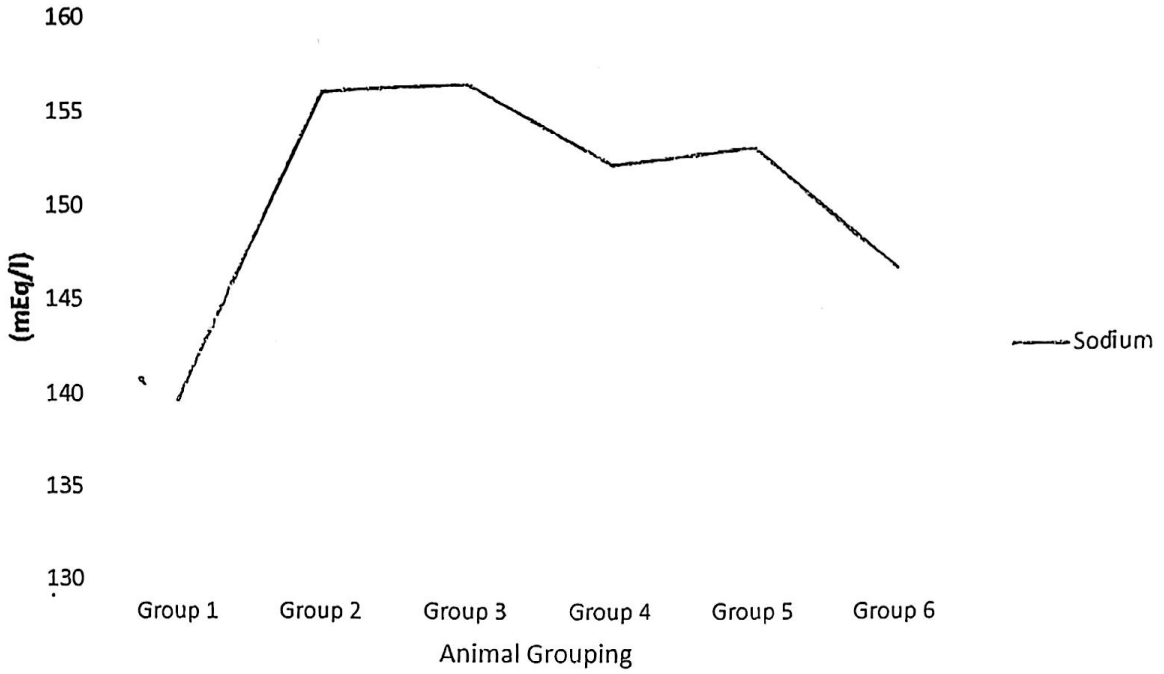

Figure 4e. Effects of tramadol consumption on Sodium ion in female wistar rats.

Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tranadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks.

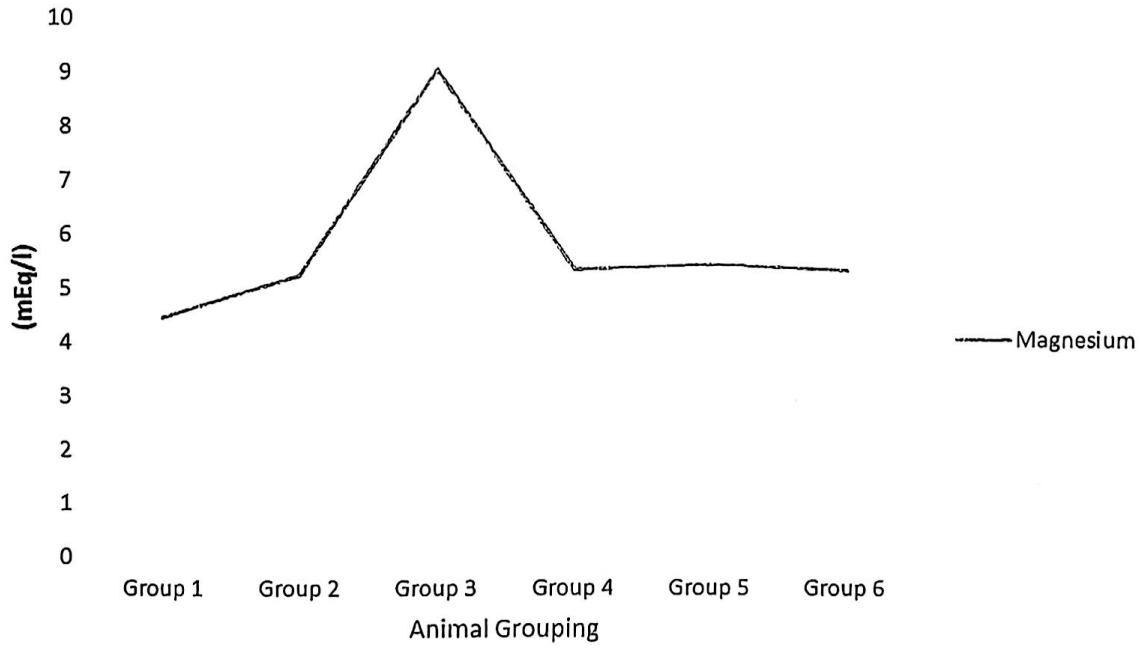

Figure 4f. Effects of tramadol consumption on Magnesium Ion in female wistar rats.

Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tranadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks.

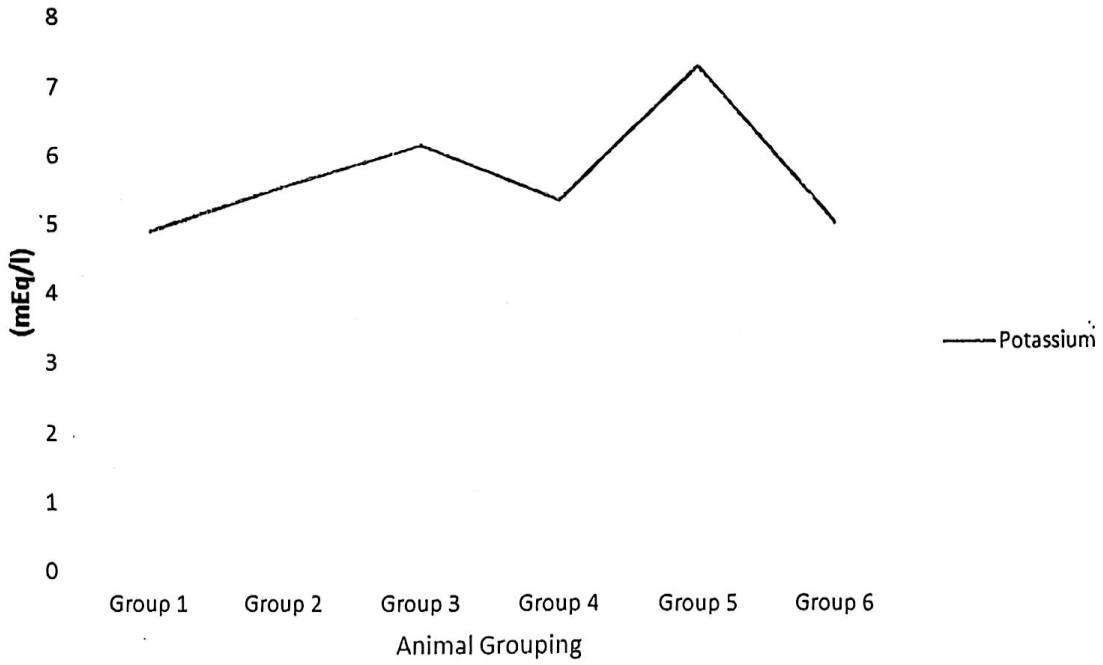

Figure 4g. Effects of tramadol consumption on Potassium ion in female wistar rats.

Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tranadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks.

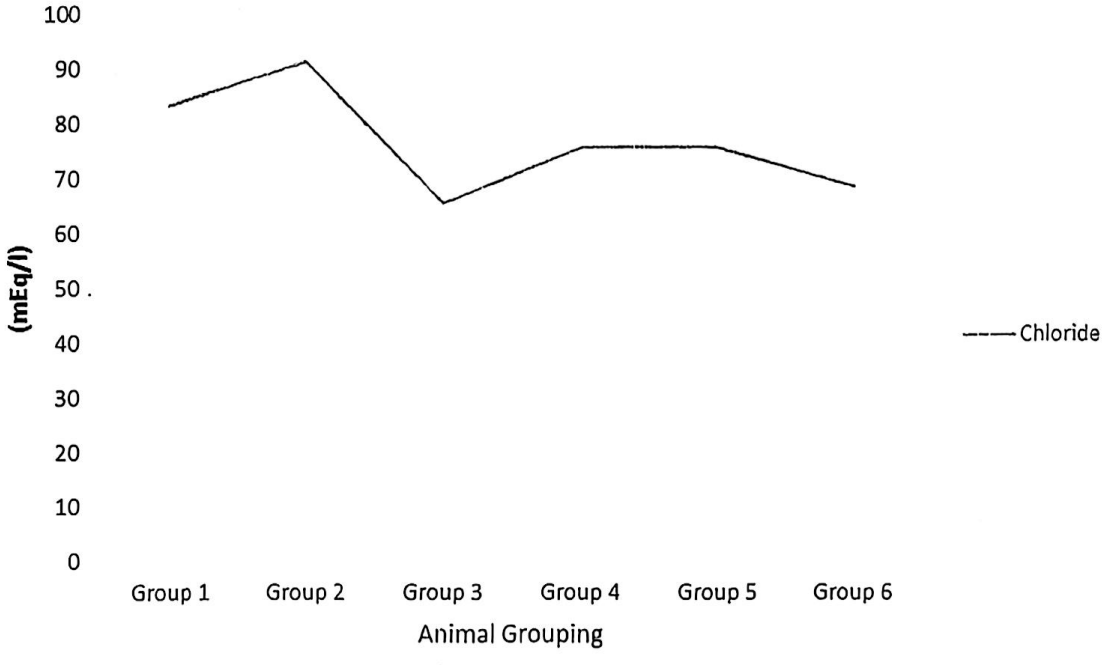

Figure 4h. Effects of tramadol consumption on Chloride ion in female wistar rats.
Values are expressed as mean± SEM.

KEY: Group 1 = Normal Untreated rats, Group 2 = Received tranadol 30mg/kg for one week; Group 3 = Received tramadol 30 mg/kg for two weeks, Group 4 = Received tramadol 30 mg/kg for three weeks, Group 5 = Received tramadol 30 mg/kg for six weeks and Group 6 = withdrawn from receiving tramadol 30 mg/kg after three weeks.

prolong use since therapeutic dose or the extreme dose may lead to organs toxicity and damage.

ETHICAL APPROVAL

The protocol of the experiments in this study was examined and approved by the Research, Ethics and Grants Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. This research was performed in accordance with the ethical standards on the care and use of animals as laid down by Helsinki 1964 (World Medical Association, 2013).

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

REFERENCES

Abdelraouf AE, Noor ESAT, Hend NE and Ikram MH. (2015). Tramadol-Induced Liver and Kidney Toxicity among Abusere in Gaza Strip, Palestine. *JJBS.* 8(2):133-137.

Adekomi DA. (2010). Madagascar Periwinkle (*Catharanthus roseus*) Enhances Kidney and Liver Functions in Wistar Rats. *International Journal of Biomedical and Health Sciences.* 6(4):245-254.

Ahmed MA. and Kurkar A. (2014). Effects of opioid (tramadol) treatment on testicular functions in adult male rats: The role of nitric oxide and oxidative stress. *Clinical Experimental Pharmacology Physiology.* 41:317–23.

Assis DN, and Navarro VJ. (2009). Human drug hepatotoxicity: a contemporary clinical perspective. *Expert Opinion Drug Metabolism Toxicology.* 5:463–473.

Atici S, Cinel I, Doruk N and Eskandari G. (2005). Oral U. Liver and kidney toxicity in chonic use of opioids: and experimental long term treatment model. *Journal Biosciences.* 30(2):245-252.

Bechara A, Casabe A, Bonis W, Helen A and Bertolino M. (2010). Recreational use of phosphodiesterase type 5 inhibitors by healthy young men. *J Sex Med.* 7:3736–3742.

Cicero TJ, Inciardi JA, Adams EH, Geller A, Senay EC, Woody GE and Munoz SA. (2005). Rates of abuse of tramadol remaine unchanged with the introduction of new braned and generic products: Results of an abuse monitoring system, 1994-2004. Pharmacoeoldemiol. *Drug Safety.* 14(12):851-859.

El-Gaafarawi II. (2006). Biochemical effects of some analgesics, *Egypt. Journal Hospital. Medication.* 21:16-32.

El-Wessemy AM. (2008). Histopathological and ultra-structural studies on the side effects of the analgesic drug tramadol on the liver of albinomice, Egypt. *J Zoology.* 50:423-442.

Ghoneim FM, Khalaf HA, Elsamanoudy AZ and Helaly AN. (2014). Effect of chronic usage of tramadol on motor cerebral cortex and testicular tissues of adult male albino rats and the effect of its withdrawal: histological, immunohistochemical and biochemical study. *International Journal of Clinical and Experimental Pathology.* 7(11):7323–7341.

Gillman P. (2005). Monoamine oxidase inhibitors, opioid analgesics and serotonin toxicity. *British Journal of Anaesthesia.* 95(4):434-441.

Grond S. and Sablotzki A. (2004). Clinical pharmacology of tramadol, *Clinical. Pharmacokinetics.* 43: 879–923.

Guyton AC. and Hall JE. (2006). Textbook of medical physiology (11th Ed.), W.B. Saunders, Philadelphia, PA. 1007.

Hassanian-Moghaddam H, Farajidana H, Sarjami S, Owliaey H. (2013). Tramadol-induced apnea. *The American Journal of Emergency Medicine.* 31(1):26-31.

Jolobe O. (2015). Tramadol-related hypoglycaemia and tramadol-induced anorexia. *Br Journal of Hospital Medication.* 76(4):249-256.

Laila Abd, and El Kawy. (2012). Hepatic DNA damage and abnormality in serum protein

pattern due to long term use of tramadol in rat. *Egyptian Journal Hospital Medicine*. 49:810-826.

Loughrey MB, Loughrey CM, Johnston S and O'Rourke D. (2003). Fatal hepatic failure following accidental tramadol overdose. *Forensic Sci. Int.* 134:232-235.

Matthiessen T, Wöhrmann T, Coogan TP and Uragg H. (1998). The experimental toxicology of tramadol: An overview. *Toxicology. Lett.* 95:63-71.

Miotto, Karen; Cho, Arthur K.; Khalil, Mohamed A.; Blanco, Kirsten; Sasaki, Jun D. and Rawson, Richard. (2017). Trends in Tramadol. *Anesthesia and Analgesia.* 124(1):44–51.

Mohammed Hanaa and Mahmoud Ayman. (2019). Chronic exposure to the opioid tramadol induces oxidative damage, inflammation and apoptosis, and alters cerebral monoamine neurotransmitters in rats. *Biomedicine and Pharmacotherapy.* 110:239-247.

Nehad R. Elyazji, Ismail Abdel-Aziz, Ayoub Aldalou and Osama Shahwan. (2013). The effects of tramadol hydrochloride administration on the hematological and biochemical profiles of domestic male rabbits. *IUG Journal of Natural and Engineering Studies.* 21(2):51-65.

Nna VU, Ani EJ, Ofutet EO, Ofem OE, Iroh CE and Osim EE. (2014). Recurrent side effects following chronic recreational use of sexual stimulants among male subjects in Calabar, Cross River State, Nigeria. *Der Pharmacia Lettre.* 6:56–61.

Nna VU. and Osim EE. (2016). Testicular toxicity following separate and combined administration of PDE5 inhibitors and opioid: Assessment of recovery following their withdrawal, Andrologia; 2016

Noori S. and Mahboobe T. (2010). Antioxidant effect of carnosine pre-treatment on cisplatin-induced renal oxidative stress in rats. *Indian. Journa of Clinical Biochemistry.* 25:86-91.

Osadolor HB and Omo-Erhabor JA. (2017). Effect of Tramadol on Bilirubin, Protein Syntheses and Liver Enzymes (AST, ALT) in Animal models. *Journal of Harmonized Research in Medical and Health Sci.* 4(2):67-73.

Robert, DD. (2012). Liver disease. (5th Ed.)Tietz textbook of clinical chemistry and molecular diagnostics. Elsevier Saunders. 1637-1693.

Sacerdote P, Bianchi M, Manfredi B and Panerai AE. (2007). Effect of tramadol on immune response and nociceptive thresholds in mice. *Pain.* 72(3):325-330.

Sembulingam K. and Prema Sembulingam. (2010). Essentials of Medical Physiology (5th Edition) Jaypee Brothers Medical Publishers Ltd, St. Louis, USA.

Shadnia S, Soltaninejad K, Heydari K, Sasanian G and Abdollahi M. (2008). Tramadol intoxication: a review of 114 cases. *Human Experimental Toxicology.* 3:201–205.

Shin HW, Ju BJ, Jang YK, You HS, Kang H and Park JY. (2017). Effect of tramadol as an adjuvant to local anesthetics for brachial plexus block: A systematic review and meta-analysis. PLoS One.

Singhal PC, Sharma P, Sanwal V, Prassad A and Kapasi A. (1998). Morphine modulates proliferation of kidney fibroblasts. *Kidney International.* 53:350-357.

Tolman KG. (1998). Hepatotoxicity of non-narcotic analgesics. *American Journal of Medicine.* 105:13S-19S.

Vozarova B, Stefan N, Lindsay SR, Saremi A and Pratley ER. (2002). High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes.* 51:1889-1895.

World Medical Association (2013). "*Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects*". *JAMA.* 310 (20): 2191–2194

Wu WN, Mcknown LA, Gauthier AD, Jones WJ and Raffa RB. (2001). Metabolism of analgesic tramadol hydrochloride in rat and dog. *Xenobiotical.* 31:423-441.

Zhihen L, Feng G and Yuke T. (2006). Effects of morphine, fentanyl and tramadol on human immune response. *Journal of Huazhong University Science and Technology*. 26(4):478-481.

Zuhtuutku S, Hakan D and Fazli E. (2006). Histopathologic changes in liver induced by morphine and tramadol. *The Pain Clinic*. 18:321-325.