



**EFFECT OF TRAMADOL ON BILIRUBIN, PROTEIN SYNTHESIS AND LIVER ENZYMES (AST, ALT) IN ANIMAL MODELS**

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**Abstract:** Demand of tramadol in southern Nigeria has been on the increase and this demand has been shown to be made by youths. Uses include improved sexual performance and as lasting energy source for those who are labourers. The consequent misuse of tramadol at self-recommended doses for sexual improvement prompted the assessment of the effect of tramadol at these doses on hepatic function of male and female *Oryctolagus cuniculus* (European rabbits). Twenty seven healthy rabbits were used, weighing 1.0 to 2.0kg. These were divided into four groups of male and female controls and male and female tramadol treated groups. Tramadol treated groups were further divided into oral and intramuscularly administered groups. Animals were housed under laboratory conditions with commercially prepared feed and water *ad libitum* and one dark one light cycle throughout the experiment. Blood samples were collected at the end of the experiment and biochemical assays performed: Transaminases (ALT and AST), alkaline phosphatase (ALP), Bilirubin (Total and Direct) (TB and DB respectively), serum total protein (TP) and albumin levels (ALB). Tramadol treated groups were compared with control groups at the end of the study as well as within group comparison. Decrease activity was observed for AST ( $p < 0.01$ ) and ALP ( $p < 0.0001$ ). TP showed decreased levels ( $p < 0.01$ ) and TB and DB also showed decreased levels ( $p < 0.05$ ). ALT and ALB showed no change ( $p > 0.05$ ). This study suggest effective conjugative function of the liver with subsequent effective maintenance of hepatocyte integrity, thus, the ability of the liver to effectively metabolize tramadol at the doses been considered.

**Key words:** Tramadol, Bilirubin, AST, ALT, Animal Models

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**Introduction:** Tramadol is a common over the counter drug sold in pharmaceutical and retail medicine outlets in Nigeria. It is an opioid pain medication use in treating moderate to moderately severe pain. Tramadol exist as two isomers: (1R, 2R) and (1S, 2S)-tramadol (Brayfield, 2013). It has usually an hour pain relief onset and accomplishes this through two

mechanisms of action (Katz, 1996),  $\mu$ -opioid receptor binding, although more weakly than morphine, and inhibition of the reuptake of serotonin and norepinephrine (Leppert, 2009). Tramadol administration in the management of pain on a long term basis and as an acceptable alternative for persons with drug seeking behavior is controversial (Abdelraouf *et al.*, 2015).

The role of the liver in detoxification and drug metabolism has previously been identified. This function increases the risk of these organs to toxic injuries, thus, all drugs been associated with hepatotoxicity following the essential role of the liver in drug metabolism (Abdelraouf *et al.*, 2015). With high oral availability, tramadol peaks at two hours in blood following oral doses, it is metabolized in the liver by cytochrome p450 and biotransformed to O- and N-desmethyl-tramadol. O-desmethyl-tramadol has been indicated to be of most significance as it has 200 times the  $\mu$ -affinity of (+)-tramadol and an elimination half-life of nine hours when compared with tramadol itself known to have six hours half-life (Samer *et al.*, 2013).

The main metabolic pathway of N- and O-demethylation and conjugation of O-demethylated compounds have been previously described. Eleven metabolites have been identified, five from phase I (M1 to M5) and six from phase II (glucuronides and sulfates of M1, M4 and M5) reactions. Tramadol is also rapidly metabolized in animals in animals than in man (Lintz and Colleagues, 1981).

The enzyme P450 (CYP) 2D6 has been suggested to be responsible for catalyzing O-demethylation of tramadol to M1 metabolite as a result of the inhibition by quinidine (selective CYP2D6 inhibitor) (Subrahyan *et al.*, 2001). Polymorphisms have been shown by the gene coding of CYP2D6 and the existence of different alleles results in functionally different enzymes (Lledo, 1993). Variations in tramadol biotransformation have been seen in phenotypic populations which depend on the genotype of CYP2D6 (Abdel-Rahman *et al.*, 2002).

Suggestions are that CYP2D6-mediated metabolite (M1) is to a lesser extent formed while a more pronounced production of non-CYP2D6 product (M2) is formed in subjects with one versus two functional alleles (Abdel-Rahman *et al.*, 2002).

Tramadol consumption in southern Nigeria has been on the increase following demand by youths and cases of abuse reported in Northern Nigeria (Osadolor and Omo-Erhabor, 2016). The demand of this potent opioid analgesic include, lasting energy by laborers (Pharmanews, 2015) and as sexual performance drug by youths (Daily trust, 2014). Kano state in Nigeria has been pointed out to have the highest drug abuse rates by NDLEA. This is based on the number of seizures, arrests of addicts and convictions of arrested dealers (Osadolor and Omo-Erhabor, 2016). The poor regulations and consequent misuse of tramadol at self-recommended doses for sexual improvement prompted the assessment of the effects of tramadol at these doses on hepatic function in male and female *Oryctolagus cuniculus* (European rabbits).

#### Materials and Methods

**Dosing and Experimental Animals:** Twenty seven European rabbits weighing 1.0 to 2.0 kg purchased from local market in Benin City were used for this study. The drug tramadol HCl (Ampoule) 100mg/2ml manufactured by Gland Pharma Limited, India and Nkoyo tramadol (Tramadol capsule B.P. 100mg) manufactured by Mancare Pharma Pvt. Ltd. Vasai, India were administered intramuscular and oral respectively. Rabbits were left in the animal's house for two week before experimentation to adapt and acclimatize to laboratory condition under the following conditions of natural light and dark cycle and given free access to commercial balanced diet and tap water *ad libitum* all over the experimental period. Animals were divided into four groups consisting male and female controls, and male and female tramadol treated groups. Tramadol treated groups were further divided into oral and

intramuscular (IM) groups. IM groups were inoculated with 15mg/kg b.wt./day for thirty days while orally groups were administered 25mg/kg b.wt./day for thirty days.

**Measurement Biochemical Indices:** Blood samples were collected from tramadol treated groups (25mg/kg b.wt./dy for oral group and 15mg/kg b.wt./day for IM group) and control groups at the end of the experiment into plain sample bottles and serum transferred into a second plain bottle after allowing to clot. Determination of enzyme activities were carried out on fresh serum samples while other samples were stored at -70°C until ready for assay. The determination of total protein, albumin, AST, ALT, serum alkaline phosphatase and bilirubin were done using SelectraProS auto analyzer as described by Mohadeseh and Abdolali (2015).

**Statistics:** GraphPad Prism (version 6.04; GraphPad Software, USA) was used for statistical analysis. Error bars were reported as means ± SEM. Statistical significance was determined using Student t-Test, one-way ANOVA to compare treated groups and controls, and oral administered groups with intramuscular administered groups with controls. Statistical significance levels of  $p < 0.05$  was used.

**Ethics:** This study was approved by Ministry of Health, King Square, Benin City, Edo State.

**Results:** Data revealed that liver enzymes activity following administration of tramadol at 25mg/kg b.wt./dy for oral group and 15mg/kg b.wt./day for IM group showed significant decreases for AST for Mean±SEM (39.30±4.267, N=10) and ALP ( $p < 0.0001$ ) for Mean±SEM (186.7±19.49, N=10) (Fig 1). ALT levels showed no significance ( $p > 0.05$ ) for Mean±SEM (113.9±5.180, N=10) (Fig 1). While this decrease was same in significance for Oral and IM groups when compared with Control for ALP (F-values (2, 24) = 27.91), AST showed only significant changes when Oral group was compared with Control group ( $p < 0.05$ , F-value (2, 24) = 3.852) (Fig 2). Furthermore, data comparison of Male Control

groups with Male tramadol treated groups as well as Female Control groups with Female Tramadol treated groups showed significance for ALP ( $p < 0.0001$  and  $p < 0.001$  respectively) (Fig 3).

Significant reduction in total bilirubin level was observed ( $p < 0.05$ ) for Mean±SEM (4.840±0.109, N=10) for control groups and (4.206±0.209, N=10) for tramadol treated groups (Fig 4). IM and Oral comparisons with control as well as male and female group comparison of data showed no significance (Fig 5 and 6 respectively).

Serum protein analysis revealed significant reduction in total protein levels when control groups for Mean±SEM (80.90±1.997, N=10) were compared with tramadol treated groups for Mean±SEM (73.29±1.512, N=17) ( $p < 0.01$ ) (Fig 7). This reduction did not reflect in the albumin portion ( $p > 0.05$ ) (Fig 7). Further analysis of data showed no significant relationship for albumin when comparisons were made with control, oral and IM groups (for  $p > 0.05$ , F-value (2, 24) = 1.645) as well as male and female group comparison (Fig 8 and 9 respectively). Significant reduction was observed for total protein when oral groups were compared with control groups ( $p < 0.05$ , F-value (2, 24) = 4.897) (Fig 8).

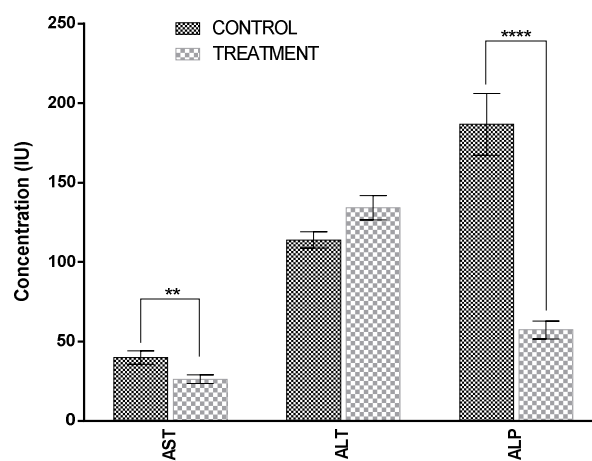


Fig 1. Estimation of Aspartateaminotransferase, Alanineaminotransferase and Alkaline Phosphatase activities of Control groups with Tramadol treated groups. Error bars represent Mean ± SEM. (\*\* $P < 0.01$ , \*\*\*\* $P < 0.0001$ )

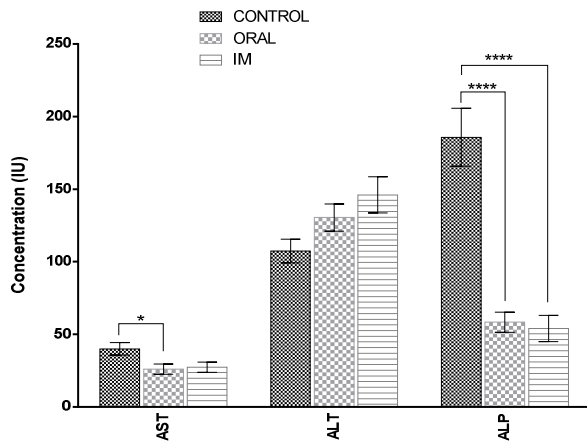


Fig 2. Aspartateaminotransferase, Alanineaminotransferase and Alkaline Phosphatase activities of Control groups with Oral and IM Tramadol treated groups. Error bars represent Mean  $\pm$  SEM. (\* $p < 0.05$ , \*\*\*\* $p < 0.0001$ ).  $F(2, 24) = 3.852$ (AST),  $2.918$ (ALT),  $27.91$ (ALP)

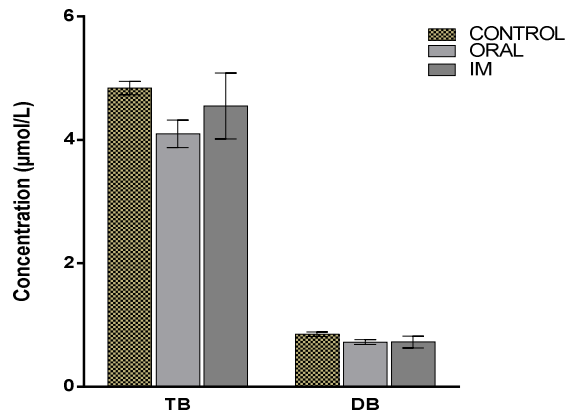


Fig 5. Bilirubin (Total and Direct) Levels of Control groups with Oral and IM Tramadol treated groups. Error bars represent Mean  $\pm$  SEM. ( $F(2, 24) = 3.081$ (TB) and  $2.775$ (DB).

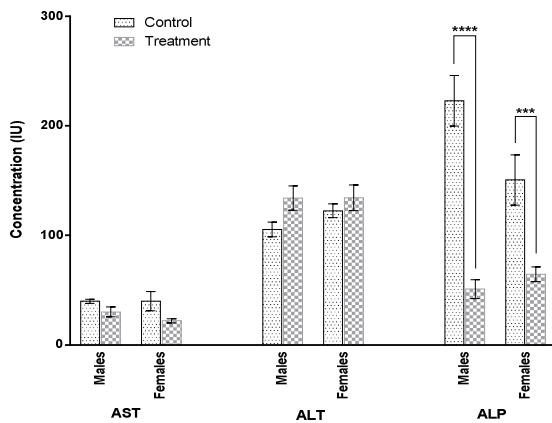


Fig 3. Estimation of Aspartateaminotransferase, Alanineaminotransferase and Alkaline Phosphatase activities of Male and Female Controls with Male and Female Tramadol treated groups respectively. Error bars represent Mean  $\pm$  SEM. (\*\*\*\* $P < 0.001$ , \*\*\*\* $p < 0.0001$ )

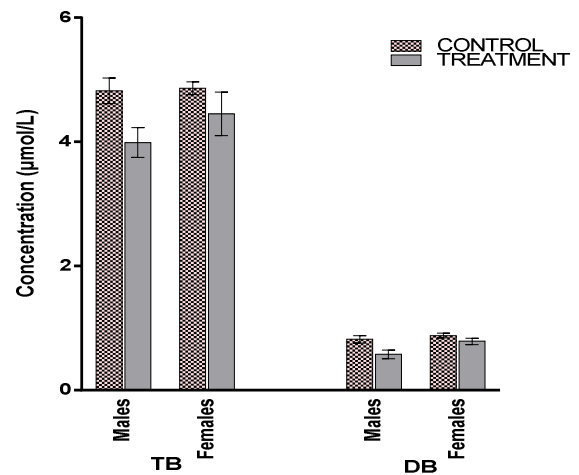


Fig 6. Estimation of Bilirubin (Total and Direct) Levels of Male and Female Controls with Male and Female Tramadol treated groups respectively. Error bars represent Mean  $\pm$  SEM.

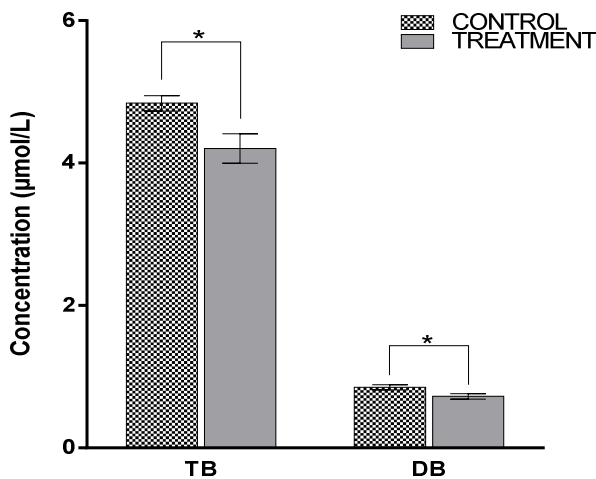


Fig 4. Estimation of Bilirubin (Total and Direct) Levels of Control groups with Tramadol treated groups. Error bars represent Mean  $\pm$  SEM. (\* $P < 0.05$ ).

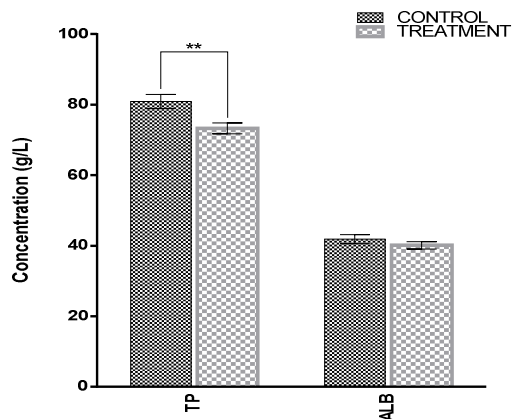


Fig 7. Estimation of Total Protein and Albumin levels of Control groups with Tramadol treated groups. Error bars represent Mean  $\pm$  SEM. (\*\* $P < 0.01$ ).

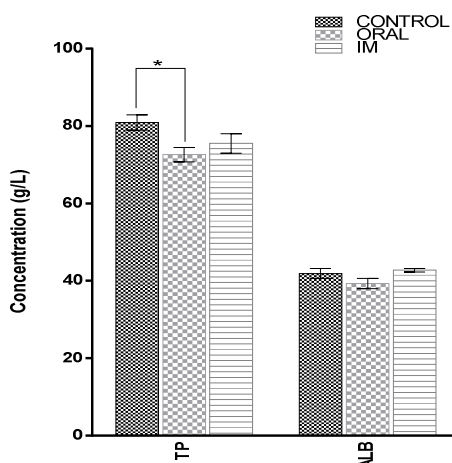


Fig 8. Total Protein and Albumin levels of Control groups with Oral and IM Tramadol treated groups. Error bars represent Mean  $\pm$  SEM. (\* $P < 0.05$ ),  $F(2, 24) = 4.897$  (TP) and 1.645 (ALB).

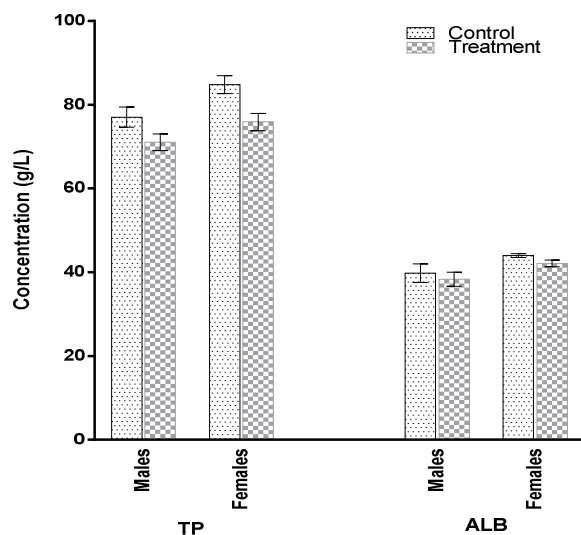


Fig 9. Estimation of Total Protein and Albumin levels of Male and Female Controls with Male and Female Tramadol treated groups respectively. Error bars represent Mean  $\pm$  SEM.

**Discussion:** The place of the liver in the metabolism and detoxification of drugs and xenobiotics has long been established. It is the primary site for the synthesis of plasma proteins. Synthesis is by the rough endoplasmic reticulum and subsequent release into the hepatic sinusoid. Protein synthesis have been known not only to be affected by impaired hepatic function but also by amino acid availability, catabolic states, actions of cytokines, hormones and/or congenital deficiency states (Robert, 2012). The integrity of the liver is usually measured by one of the

following ways: concentration measurement of substances produced by it, serum content measurement of substances known to change in concentration resulting from hepatic damage, measurement of substance concentration from liver cells as a result of injury, liver's ability to perform a metabolic task e.g. conjugation or detoxification and/or measurement of enzyme activity and substrate content of organelles and cells (Robert, 2012). The effects on synthetic function, hepatic integrity and test for cholestasis by tramadol were determined. Serum total protein was significantly reduced in this study (Fig. 7) although not resulting from reduction in albumin. Albumin is a common index of hepatocyte ability to carry out synthetic function. In general, serum total protein is made up of albumin and globulin portions. A reduction in total serum protein could result from a reduction in any portion constituting total protein levels in serum. The reduction seen could have resulted from an effect of tramadol on any of the globulin fraction, thus, proposing long term usage of tramadol could be detrimental to the immune responses. This study however did not evaluate serum globulin level. Although, Zhihen and colleagues (2006) proposed possible immune enhancing effect of tramadol, while Sacerdote and colleagues (1997) suggest its usefulness in treatment of patients where immunosuppression may be contraindicated.

The liver is known to have a reserve capacity, thus, preventing protein concentration from decreasing with such decrease seen only in extensive liver damage. Also, liver proteins have relatively long half-lives which for albumin is between 19 to 21 days and may have also accounted for the results gotten in this study. Little changes in plasma protein concentrations have been reported in acute hepatic dysfunction (Robert, 2012). Changes in serum protein have also been reported by Laila (2012) in a study on "hepatic DNA damage and abnormality in serum protein pattern due to long term use of tramadol in rats".

Determination of hepatic integrity is possible by estimation of liver enzymes which are involved in intermediary metabolism. They are stored in hepatocytes and excess released into plasma on acute hepatic damage. Serum aminotransferase are sensitive test of hepatic injury and measurements extremely useful in recognition and differentiation of liver damage. Although found in other organs of the body, exclusion of non-hepatic conditions which may increase aminotransferase such as myocardial injury, circulatory congestion e.t.c. are necessary. This study revealed low levels AST which is in contrast to Atici and colleagues (2005), Gaafarawi (2006) and Obde and colleagues (2015) who all reported increased AST and ALT levels on tramadol administration. The effect of tramadol on AST as revealed by this study is in line with the U.S. Department of Health and Human Services which reported "serum aminotransferase levels could be elevated in a small proportion of patients receiving tramadol, particularly with high doses". This study however showed no significant change in ALT levels.

A lower level of ALP was noticed in this study (Fig. 1, Fig 2 and Fig 3) contradicting the findings of Obde and colleagues (2015) and ruling out possible cholestasis resulting from impaired liberation of ALP. Abdelraouf and colleagues (2015) reported no significant difference in ALP levels in a Palestinian study which compared tramadol abusers and control subjects. Low levels of ALT, AST and ALP are normally found in the blood with increases indicative of compromised hepatic integrity and impaired ALP liberation. This study thus suggest maintenance of hepatocyte integrity on administration of tramadol at the said doses which may be compromised at increased doses and/or prolong exposures greater than the duration of this study.

Bilirubin is the orange-yellow pigment derived from heme, mainly as a product of red blood cell turnover. It is extracted and biotransformed in the liver and excreted in bile and urine. Its

estimation gives information about the conjugative function of the liver. Results from this study showed a significant reduction in total and direct bilirubin values when treated groups were compared with control groups. Indicating that the conjugative function of the liver is unaffected by tramadol. This finding is supported by Abdelraouf et al. (2015) who demonstrated no significant effect of tramadol between abusers and control groups except for abusers who had used tramadol for more than five years. The liver has also been known to be instrumental in the synthesis (testosterone and estrogen) and regulation of hormone (sex hormones) levels and elimination when necessary. Damage to this organ resulting from tramadol toxicity will further disrupt metabolic processes carried out by the liver and possible effects on hormone synthesis and regulation.

**Conclusion:** The resulting effect on the liver by tramadol suggest maintenance of hepatocyte integrity, effective conjugative function of the liver and effective synthesis of plasma proteins on administration of tramadol at the said doses which may be compromised at increased doses and/or prolong exposures greater than the duration of this study.

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