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Hematological and Biochemical Changes in Blood, Liver and Kidney Tissues under the Effect of Tramadol Treatment

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Abstract

This study aimed to investigate the effects of tramadol administration on some haematological and biochemical indices in rats. Tramadol was administered orally to rats for 28 days at a dose of 10 mg/kg body weight/day, 50 mg/kg body weight/day and 100 mg/kg body weight/day. Twenty-four hours after the last tramadol, blood, liver and kidney were removed from the animals after an overnight fast and analysed for their haematological and biochemical parameters. Results obtained revealed that tramadol administration significantly reduced the levels of white blood cells (WBC), red blood cell (RBC), haemoglobin and platelet count (PLT) while its resulted in non-significant changes in other haematological parameters examined when compared with control rats. Tramadol intake significantly increased plasma levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatinine and urea while its reduced total protein levels. Hepatic and renal thiobarbituric acid reactive substances (TBARS) levels were significantly increased by tramadol administration while levels of endogenous antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were reduced. This study confirmed the risk of increased oxidative stress, hepatotoxicity and nephrotoxicity due to tramadol administration. Although tramadol is reported to be effective in pain management, its toxicity should be kept in mind.

Keywords: Tramadol; Haematological; Oxidative stress; Hepatotoxicity; Nephrotoxicity

Introduction

Tramadol is a synthetic centrally acting analgesic with effects similar to those of codeine and 10 times less than morphine [1]. Tramadol has a wide range of applications mostly in the treatment of moderate to severe, acute or chronic pain [2]. It is an effective analgesic in acute ureteric spasm, postoperative, musculoskeletal and cancer pain [3,4]. The analgesic effect of tramadol is mediated by three mechanisms: mu opioid binding, nor-epinephrine and serotonin reuptake inhibition [5,6].

Tramadol is metabolized mainly in the liver by cytochrome P450 (CYP2D6), cytochrome P4503A (CYP3A4) and cytochrome P450 isozyme (CYP2B6), being O-and N-demethylated to five different metabolites, followed by conjugation with glucuronic acid and sulphate [7]. Tramadol is responsible for life-threatening poisonings, resulting in consciousness impairment, seizures, agitation and respiratory depression [8]. Like other opioids, central apnea has been attributed to the ingestion of elevated doses of tramadol [9]. Therefore, tramadol toxic effects should be kept in mind during long term therapy especially in large doses [10].

Although, opioids are effective in the treatment of pain but activation of μ-opioid receptors (MOR) by opioids are known to cause side effects such as central nervous system (CNS) depression, nausea, dependence and addiction [11,12]. Presently, addicts abuse tramadol every day by using the drug without doctor prescriptions. In the USA and Europe, there have been an upsurge in tramadol use following withdrawal of dextropropoxyphene from the market thus raising the risk of increased poisonings and deaths attributed to this drug [13]. Similarly, in Nigeria, the rate of tramadol abuse has been on the increase among Nigerian youths in recent time [14]. The main factor responsible for this could be link to off-label use of tramadol as on-demand treatment for premature ejaculation (PE) [15,16]. However, wide-spread use of tramadol is associated with toxicity and a recent study showed that tramadol cause brain, heart and lung toxicity [17]. Therefore, there is need to conduct a study that would examine the toxicity of tramadol in the liver where tramadol is metabolized and, in the kidney, where tramadol metabolites are excreted, thus making liver and kidney the primary toxicity targets for tramadol. Therefore, in the present work, we performed an *in vivo* study, using male Wistar rats, to analyse oxidative stress, biochemical and haematological alterations, at the liver and kidney levels, deriving from exposure to a broad range of tramadol.

Materials and Methods

Reagents

Thiobarbituric acid (TBA), nicotinamide adenine dinucleotide reduced (NADH) and tramadol hydrochloride were obtained from Sigma–Aldrich Chemical Co. Ltd. (England). Nitrobluetetrazolium (NBT), 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) are product of Fluka (Buchs, Switzerland). All other chemicals used were analytical grade.

Experimental design

Twenty (20) male wistar strain albino rats were divided into four groups of five rats each according to their weight. Group I labelled control received saline solution orally for 28 days. Group II received tramadol dose at 10 mg/kg/day body weight of rat. Group III received

tramadol dose at 50 mg/kg/day. Group IV received tramadol dose at 100 mg/kg/day. Tramadol was constituted in saline solution and administered through the oral route with the use of oral gavage. During the experiment, the animals were allowed free access to food and distilled water. After 28 days of tramadol treatment and after an overnight fast, animals were sacrificed by cardiac puncture under light ether anaesthesia into ethylene diamine tetra-acetic acid (EDTA) sample bottles for haematological analysis and heparinised sample bottles for biochemical analysis. Liver and kidney were removed from the animals for biochemical analyses. Blood samples in heparinized bottles were centrifuged to separate plasma and red blood cells. All samples were stored at -20°C until analysed.

Biochemical analyses

Determination of urea, creatinine, total protein and ALP, AST activities in plasma: Plasma concentrations of alkaline phosphatase (ALP), aspartate aminotransferase (AST), urea, creatinine and total protein were determined using enzymatic kits (CYPRESS® Diagnostics, Langdorp, Belgium) according to the manufacturer's instructions.

Preparation of liver and kidney homogenates: Prior to biochemical analyses, the liver and kidney samples were cut into small pieces and homogenized in Phosphate buffer saline (PBS) with a homogenizer to give a 10% (w/v) liver and kidney homogenate. The homogenates were then centrifuged at 12,000 rpm for 15 min. The supernatant obtained was used for the assay of superoxide dismutase, catalase, gluthathione, thiobarbituric acid reactive substances (TBARS) content, and protein estimation.

Determination of hepatic and renal antioxidant enzyme activities and MDA levels: Hepatic and renal superoxide dismutase (SOD) activities were assayed in the tissue homogenates by the method of Kakkar et al. [18] at 560 nm. One unit of enzyme activity was defined as that amount of enzyme which caused 50% inhibition of nitrobluetetrazolium reduction/mg protein. Catalase (CAT) activity was determined at room temperature by using the method of Aebi [19] and the absorbance of the sample was measured at 240 nm in a UV spectrophotometer. The concentration of reduced glutathione (GSH) in liver and kidney homogenates was measured, as described by Jollow et al. [20]. The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid-reactive product malondialdehyde (MDA), using the method of Draper and Hadley, [21]. All of the enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry et al., [22], using bovine serum albumin (BSA) as a standard.

Haematological study

Freshly collected blood samples in EDTA bottles were analysed for haematological assay using an automatic haematological assay analyser (ERMA PCE 210, ERMA, Japan). Different tested haematological parameters were as follows: White Blood Cell (WBC), Red Blood Cells (RBC), Haemoglobin (HGB), Haematocrit (HCT), Red cells (RDW%), Red cells Distribution Width (RDW), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelet (PLT), Mean Platelet Volume (MPV), Mean Corpuscular Volume (MCV), Platelet crit (PCT), Platelet distribution width (PDW).

Statistical analysis

Results are expressed as mean ± S.E.M. The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Turkey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and p-value<0.05 were considered statistically significant.

Results

Effect of tramadol administration on creatinine, urea and total protein

Administration of tramadol at 10 mg/kg, 50 mg/kg and 100 mg/kg doses significantly increased plasma creatinine concentration by 93.98%, 148.19% and 361.45% respectively and increased plasma urea concentration by 95.29%, 183.69% and 210.80% respectively while total proteins levels were decreased by 30.35%, 57.79% and 48.02% respectively by administration of 10 mg/kg, 50 mg/kg and 100 mg/kg doses of tramadol when compared with the normal rats (Table 1).

Parameters	Creatinine (mg/dL)	Urea (mg/dL)	Total protein (g/dL)
Control	0.83 ± 0.08	23.79 ± 1.64	8.60 ± 0.73
10 mg/kg/day tramadol	1.61 ± 0.25**	46.46 ± 3.94**	5.99 ± 0.36**
50 mg/kg/day tramadol	2.06 ± 0.32**	67.49 ± 3.01**	3.63 ± 0.49**
100 mg/kg/day tramadol	3.83 ± 0.49**	73.94 ± 4.32**	4.47 ± 0.68**
Each value represents the mean of five rats. **=significantly different from control (p<0.05).			

Table 1: Effect of tramadol administration on creatinine, urea and total protein.

Effect of tramadol administration on ALP and AST activities

Administration of tramadol at 10 mg/kg, 50 mg/kg and 100 mg/kg doses significantly increased the activity of ALP by 39.72%, 74.45% and 142.91% respectively and AST activity by 90.09%, 204.77% and 181.36% respectively when compared with the normal rats (Figure 1).

Effect of tramadol administration on TBARS levels

Hepatic TBARS levels of rats treated with 10 mg/kg, 50 mg/kg and 100 mg/kg tramadol doses were dose-dependently significantly increased by 58.25%, 201.84% and 252.25% respectively when compared with the normal rats. Similarly, administration of tramadol at 10 mg/kg, 50 mg/kg and 100 mg/kg doses, significantly increases renal TBARS levels by 57.59%, 129.00% and 127.18% respectively when compared with the control rats (Figure 2).

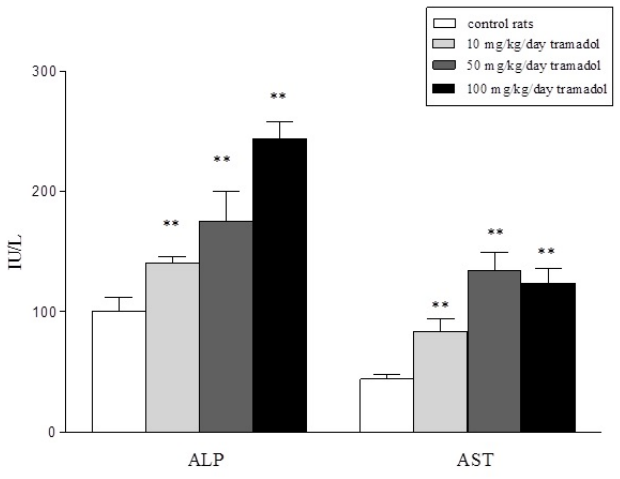

Figure 1: Effect of tramadol administration on plasma ALP and AST levels of rats. Values are mean ± SEM (n=5). ** = significantly different from control (p<0.05).

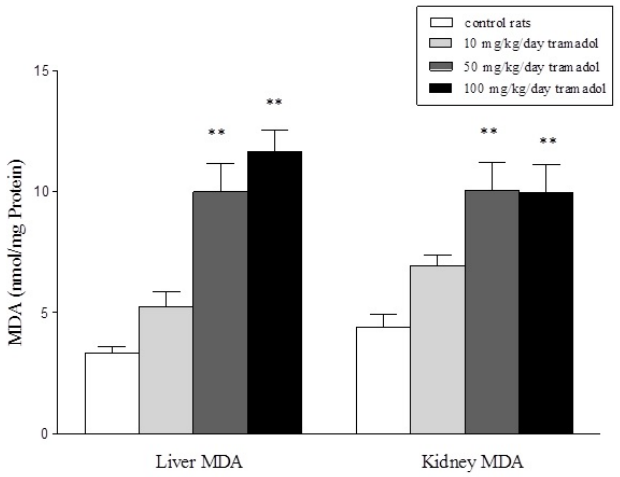

Figure 2: Effect of tramadol administration on hepatic and renal TBARS levels of rats. Values are mean ± SEM (n=5). ** = significantly different from control (p<0.05).

Effect of tramadol administration on SOD activity

Administration of tramadol at 10 mg/kg, 50 mg/kg and 100 mg/kg doses significantly reduced hepatic SOD levels by 39.06%, 59.66% and 51.21% respectively and reduced renal SOD levels by 37.70%, 48.28% and 60.37% respectively when compared with normal rats (Figure 3).

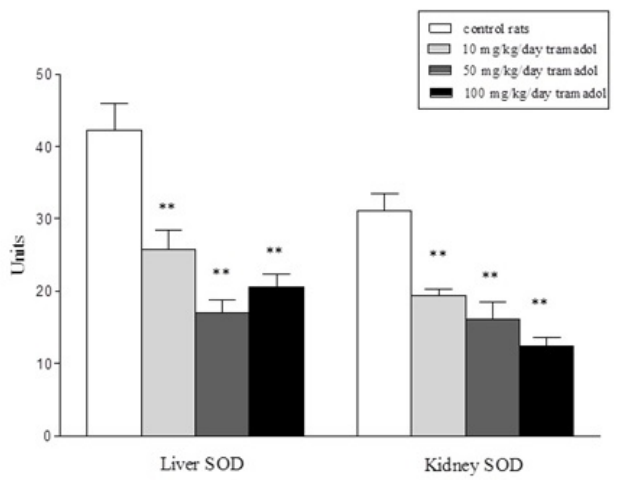

Figure 3: Effect of tramadol administration on hepatic and renal SOD levels of rats. Values are mean ± SEM (n=5). ** = significantly different from control (p<0.05).

Effect of tramadol administration on catalase activity

Administration of tramadol at 10 mg/kg, 50 mg/kg and 100 mg/kg doses significantly reduced hepatic catalase levels by 28.89%, 65.33% and 80.00% respectively and reduced renal catalase levels by 45.92%, 51.52% and 62.24% respectively when compared with normal rats (Figure 4).

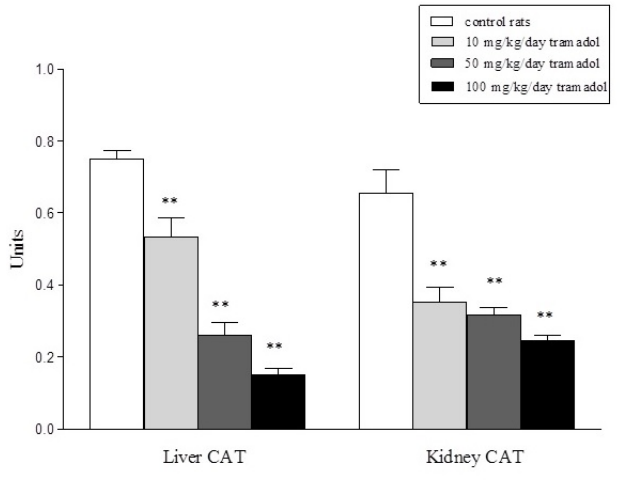

Figure 4: Effect of tramadol administration on hepatic and renal catalase levels of rats. Values are mean ± SEM (n=5). ** = significantly different from control (p<0.05).

Effect of tramadol administration on GSH activity

Hepatic GSH levels of rats treated with 10 mg/kg, 50 mg/kg and 100 mg/kg tramadol doses were significantly reduced by 37.04%, 50.86%

and 58.89% respectively when compared with the normal rats. Similarly, administration of tramadol at 10 mg/kg, 50 mg/kg and 100 mg/kg doses significantly reduced renal GSH levels by 33.06%, 49.04% and 59.07% respectively when compared with the control rats (Figure 5).

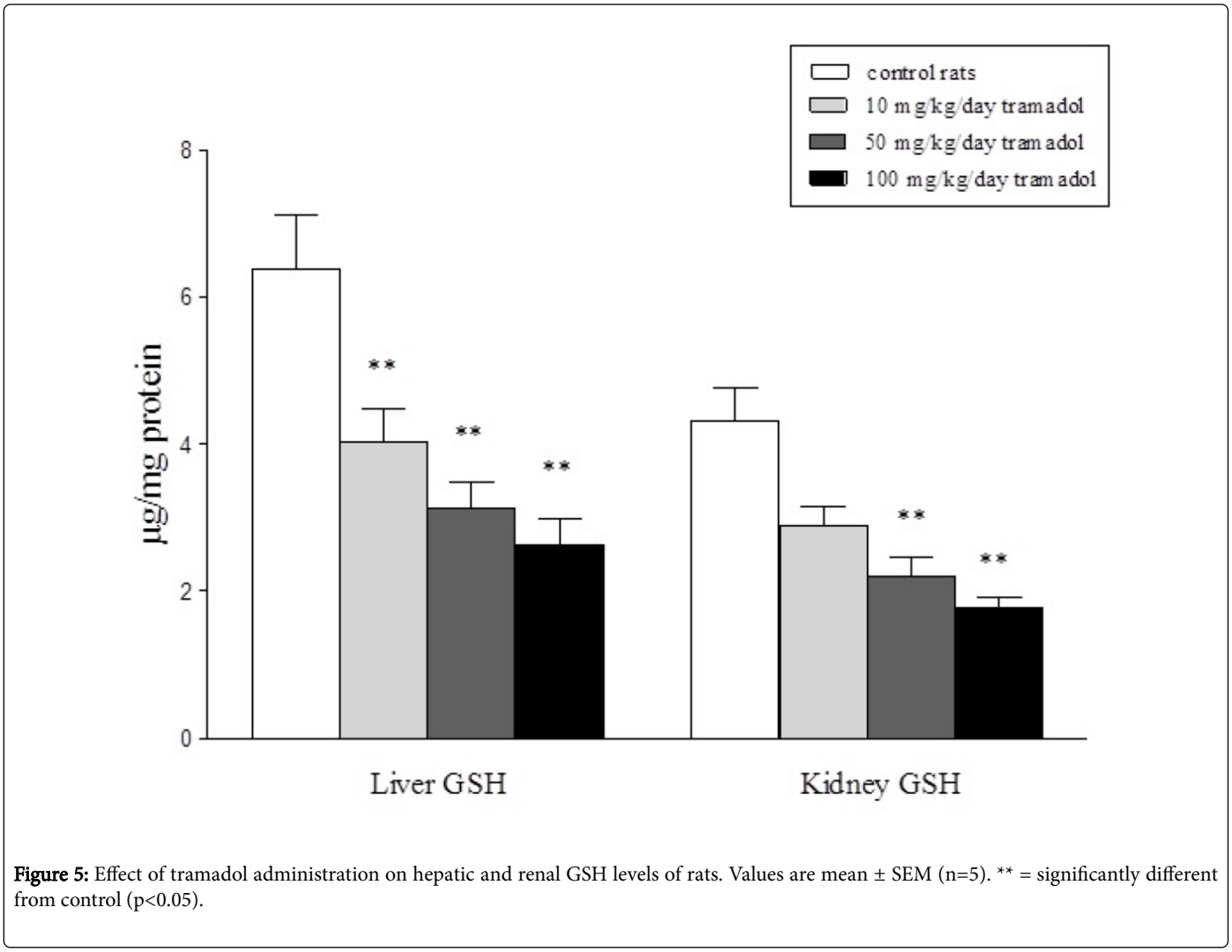

Figure 5: Effect of tramadol administration on hepatic and renal GSH levels of rats. Values are mean ± SEM (n=5). ** = significantly different from control ($p<0.05$).

Haematological Parameters

The effects of tramadol administration on haematological parameter were depicted in Table 2. No significant changes in the parameters of HCT, MCV, MCH, RDW-SD, MPV, PDW and PTC were found when compared with control animals. However, administration of tramadol significantly lowered ($p<0.05$) white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin and platelet (PLT), while the value of Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) were increased when compared with control animals.

Parameter	Control	10 mg/kg tramadol	50 mg/kg tramadol	100 mg/kg tramadol
WBC (× 109/L)	8.30 ± 0.16	8.73 ± 0.32	5.40 ± 0.06**	4.84 ± 0.36**
HGB (g/dl)	10.58 ± 0.39	9.26 ± 0.33	9.06 ± 0.36**	8.98 ± 0.26**
RBC (× 10^{12}/L)	6.92 ± 0.31	5.32 ± 0.22**	5.19 ± 0.20**	5.11 ± 0.17**
HCT (%)	31.64 ± 0.97	28.74 ± 1.16	27.76 ± 0.97	29.00 ± 1.00
MCV (fl)	55.50 ± 0.87	56.46 ± 1.42	53.90 ± 0.34	54.22 ± 0.94
MCH (pg)	15.28 ± 0.31	17.36 ± 0.15	17.48 ± 0.27	17.58 ± 0.32

MCHC (g/L)	275.64 ± 1.93	309.26 ± 9.78	325.00 ± 5.27**	321.40 ± 5.46**
RDW-CV (%)	17.22 ± 0.31	17.06 ± 0.48	13.60 ± 0.39**	13.78 ± 0.27**
RDW-SD (fl)	31.82 ± 0.15	31.00 ± 0.59	27.88 ± 0.58	27.52 ± 0.62
PLT (× 10^9/L)	583.00 ± 1.26	571.26 ± 20.88	536.20 ± 11.82**	521.00 ± 7.88**
MPV (fl)	7.00 ± 0.06	6.80 ± 0.04	7.30 ± 0.10	7.28 ± 0.06
PDW	16.08 ± 0.32	16.02 ± 0.13	15.88 ± 0.22	15.62 ± 0.21
PTC (%)	0.39 ± 0.00	0.56 ± 0.03	0.43 ± 0.02	0.40 ± 0.01
Each value represents the mean of five rats. ** =significantly different from control ($p<0.05$).				

Table 2: Effect of tramadol administration on haematological parameters of rats.

Discussion

Tramadol is a synthetic analogue of codeine which is centrally acting analgesic for treatment of moderate to severe, acute or chronic pain [2,23]. In this study we studied tramadol as a drug, not as analgesics because there is alarming increase in tramadol abuse among Nigerian youths due to the believe that tramadol could lengthen the duration of intercourse before ejaculation in men whom have problems with premature ejaculation and causes mood changes. This study evaluated the toxicity of tramadol on systemic body organs because of people addition to the drug.

The role of the liver and the kidneys in tramadol metabolism and excretion predisposes them to toxic injury [24]. Almost every drug has been associated with hepatotoxicity due to the essential role of liver in drug metabolism [25,26]. Metabolites of drugs are excreted in the kidneys and some may cause cellular damage which could lead to kidney dysfunction [27]. In the present study, the liver functions were impaired in tramadol-treated group as reflected by elevation in the activities of ALP and AST in the plasma when compared with the control rats, this finding is similar to previous studies that reported significant increase in the levels of serum ALT, AST and LDH (lactate dehydrogenase) in rats after long term usage of tramadol [28,29]. The liver is an organ that detoxified toxic elements and chemical drugs in the body, the increased in the activities of AST and ALP in plasma in this study are indicative of liver damage [30]. The increased secretion of these liver enzymes may be accompanied by acute cell necrosis, therefore, the increased plasma level of these enzymes in rats treated with tramadol could be due to necrosis or damage to liver cell membrane which leak the enzymes into the blood circulation [31].

Blood creatinine and urea levels are common biochemical parameters used to determine renal functions. The level of plasma creatinine is used to determined glomerular filtration rate while urea is used to determine the nephrotoxic profile of xenobiotics [32]. In this study, impairment of the renal functions in tramadol-treated rats was indicated by a significant increase in urea and creatinine concentration in the plasma, as compared to the control group. This observation is in support of previous studies [29,33,34] and it is an indication of renal toxicity which cause decrease in glomerular filtration rate leading to the build-up of creatinine and urea in the blood.

A large amount of polyunsaturated fatty acids found in all the biological membranes is susceptible to peroxidative attacks by oxidants resulting in lipid peroxidation. So, lipid peroxidation production was used as a marker of oxidant-induced cell injury. In our study, we recorded a significant increase in hepatic and renal malonaldehyde (MDA) levels in tramadol-treated group when compared with the control. It was reported that elevated MDA is indicating an increase of free radical generation and it is considered a useful measure of oxidative stress status [35]. Our results are in support of earlier studies [36,37] who reported an increase in the MDA level in tramadol-treated animals.

The toxic effect of tramadol administration leads to a large population of unquenched free radicals leading to the state of oxidative stress. This is evidence in inhibition in the activities of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) in liver and kidney of rats in this study. SOD, CAT and GSH are important antioxidant enzymes which played a pivotal role in scavenging of oxidative free radicals [38]. The inhibition of these antioxidant enzymes observed in this study could be linked to exhaustion of these enzymes as a result of oxidative stress caused by tramadol administration.

Free radicals and reactive oxygen species are generated by chemicals and pollutants such as factory waste, toxic gases and they are known to disrupt haematological parameters in organisms [39]. The deviation from normal haematological parameters levels represents the presence of toxicity or disease conditions [40]. In this study, tramadol administration caused a significant reduction in red blood cell counts (RBC), white blood cells, Haemoglobin, and PLT. The observed decrease in the number of RBCs suggest that tramadol administration resulted in blood loss due to serious gastrointestinal tract bleeding, red blood cell haemolysis and poor iron absorption in the intestine. White blood cells fight infections, defend the body against foreign organisms' invasion and produce antibodies in immune response [41]. Animals with low WBC are at high risk of disease infection, while high WBC results in high resistance to diseases [41]. The reduction of WBC by tramadol observed in this study suggest that tramadol use supresses the immune system and this could expose individuals that use the drug to infectious disease.

Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates [42]. Since haemoglobin is contained only in red blood cells, a low number of RBCs would lead to low level of haemoglobin [43] as observed in this study. This finding is in support of earlier study which reported reduction in haemoglobin concentration in morphine dependent people [44]. Blood platelets are involved in blood clotting and its low level will prolong the process of clot-formation resulting in excessive

blood loss during injury. A decreased number of platelet (thrombocytopenia) by tramadol in this study is in supports of pervious work which observed that morphine administration induced thrombocytopenia [45]. Tramadol administration also resulted in increased in the levels of mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCHC) while the changes observed in other haematological parameters such as HCT, RDW%, RDW, MCV, MPV, PCT, PDW in this study were largely found to be non-significant, an observation that may be different if tramadol administration period was much longer than 28 days used for this study. We strongly believe that disruption in haematological parameters observed in this study may be due to increased population of unquenched free radicals caused by tramadol administration.

Conclusion

Our results evidence that tramadol administration may cause hepatotoxicity, nephrotoxicity and haematoxicity, its use should therefore be limited to prescription only. Our findings underlined the need to avoid indiscriminately and prolong use of tramadol, since prolonged daily use of the drug either at a therapeutic dose or the extreme dose may lead to damage accumulation.

Authors' Contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Conflict of Interests

Authors have declared that no competing interests exist.

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