Original Research Article

REPRODUCTIVE TOXICITY AND BIOMARKER RESPONSE TO A DAILY DOSE OF TRAMADOL IN MALE ALBINO RATS *(Rattus norvegicus)*

ABSTRACT

This study was designed to evaluate the effect of a daily dose of tramadol on selected biomarkers viz: haematological parameters, sperm count, kidney and liver damage in male albino rats. Twenty four wistar rats were divided randomly into two groups: control group and treated groups, the treated group were further divided into four groups and housed in cages. Clean drinking water was served to control (group 1), and 1.6 mg/kg bodyweight of tramadol was administered to group 2 (7days treatment), group 3(14 days treatment), group 4 (21days treatment) and group 5 (21days treatment +7days withdrawal) in addition to a daily standard diet for all groups. Treatment of rats with tramadol caused significant decrease ($P<0.05$) in WBC, platelet and lymph. in group 2, on bicarbonate, AST and protein, it showed significant decrease ($P<0.05$) in group 3, and it showed significance decrease ($P<0.05$) in group 5 on Cl^-, AST, ALT, bicarbonate, AST, PCV, Hb, RBC, WBC, platelet, lymphocytes and sperm count. The results indicates that tramadol has negative effects on the liver which may induce severe liver damage when used for a prolonged period, the results also shows that tramadol can cause anaemia as seen by the observed negative changes in the blood parameters evaluated. Therefore, administration should be with great caution and from a licensed pharmacist or doctor while self prescription or over the counter administration should be avoided considering the associated adverse effects.

1.0 INTRODUCTION

Tramadol is a novel centrally, synthetic, analgesic with both opioid and non-opioid mechanisms responsible for its effects, it is a synthetic analogue of codeine [24]. It is mainly used for the treatment of moderate to severe pain [1]. It has been reported that other than using tramadol for pain relief, it is used for other reasons particularly, using it to relax, to sleep, to get high or to relieve boredom. Tramadol hydrochloride is attractive to drug abusers and people with addiction disorders for its pain relieving and mood altering effects. People abuse tramadol and use the drug non-medically to produce; altered emotional state, feelings of euphoria, physical sedation [2]. A Chinese study, conducted by the National Institute on Drug Dependence, enlisted 219 subjects categorized as opiate addicts with history of tramadol abuse. Study subjects were assessed using an opiate withdrawal scale. The results indicated that tramadol resulted in euphoric effects,

sedative effects, and psychotomimetic effects. 57.1% of tramadol abuse subjects had a craving for tramadol. The National Institute on Drug Dependence, Beijing, concludes that tramadol produced high abuse potential among opiate addicts [3].

Although it is effective at treating mild pain, tramadol is one of the least potent painkillers available. However, tramadol can still be addictive, especially when taken for a long period of time, but rare cases of tramadol dependence have been described in patients without prior substance abuse history [4] and have been reported to have the potential to trigger two dramatic events—seizures and serotonin syndrome [24]. Studies have shown that tramadol affects some major organs of the body such as the liver and kidney which are responsible for the metabolism and excretion leading to high risk of hepatotoxicity and nephrotoxicity [5]. Tramadol's neurotoxicity is speculated to be related to the reuptake inhibition of serotonin and norepinephrine, rather than its opioid effects [6]. [7] and [8] reported in a similar study that erythrocyte indices decreased after intravenous tramadol injection in sheep. [9, 26] in their study on histopathological and Molecular Studies on tramadol mediated hepato-renal toxicity in rats found hydropic degeneration, with congested central veins and necrotic signs in some hepatocytes. The aim of this study is to investigate the effect of tramadol on hepato-renal functions, hematological and sperm parameters in male albino rats, to evaluate its possible effect on humans.

2.0 MATERIALS AND METHODS

2.1 *Study population*

A total of twenty-four (24) male nine (9) weeks old healthy albino rats weighing 250g-350g were used. The animals were housed in a well-constructed animal cage, at 24^{o}C - 26^{o}C. They were fed with a standard diet and drinking water and were acclimatized for 1 week before the commencement of the study.

2.2 *Experimental setup*

A complete randomized design (CRD) was used for this research. The animals were assigned into 5 groups in triplicates as follows; Group 1: control did not receive any treatment, Group 2: received 1.6 mg/kg body weight of tramadol through oral administration, using 1 ml syringe. They were exposed for 1 week before they were sacrificed. Group 3: received 1.6 mg/kg body weight of tramadol through oral administration, using 1 ml syringe. They were exposed for 2 weeks before they were sacrificed. Group 4: received 1.6 mg/kg body weight of tramadol through oral administration, using 1 ml syringe. They were exposed for 3 weeks before they were sacrificed. Group 5: received 1.6 mg/kg body weight of tramadol through oral administration, using 1 ml syringe. They were treated for 3 weeks and no treatment was given to them during the fourth week before they were sacrificed. Tramadol treatment was administered orally between 7 days and 21 days.

2.3 *Biochemical Analysis*

Standard procedures were ensured during the collection of the blood, sperm and liver samples prior to biochemical analysis. The serum electrolytes were determined using ISO 4000 Automated electrolyte analyser. SFRI, France. Biuret method was used to determine the level of total protein in the samples according to the method of Flack and Woollen [10], while the plasma activity of alkaline phosphatase (ALP) was determined using Radox kit (colorimetric method) of [11]. The plasma activity of aspartate transaminase was determined using Reitman and Frankel method [12]. The red blood cells (RBC) and total white blood cells (WBC) counts were determined by the improved Neubauer hemocytometer method. The hemoglobin (Hb) concentration was determined using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the micro-haematocrit method. Schilling method of leucocyte count was used to determine the lymphocyte count of the white blood cells, the sperm count were determined using the hemacytomer method.

2.4 *Method of Data Analysis*

Data were analyzed using Tukey test at a level of 5% probability, using Assitat Software Version 7.7 en (2017).

3.0 RESULTS

3.1 Effects of tramadol on haematological parameters

The result in Table 1 shows the summary of effect of tramadol on some blood parameters; it shows the mean value and standard deviation (STDEV) for each of the parameters. The result for red blood cell (RBC), packed cell volume (PCV), and hemoglobin (Hb), in rats treated with tramadol for 7 days (week 1) showed that there was no significant difference ($p>0.05$) compared to the control, while for white blood cell (WBC), platelet, and lymphocytes, there were significant difference ($p<0.05$) in them. RBC, PCV, Hb, WBC, platelet and lymphocytes showed non-significant difference ($p>0.05$) in rats treated with tramadol orally for 14 days (2^{nd} week) and 21 days (3^{rd} week) compare to the control. RBC, PCV, Hb, WBC, platelet and lymphocytes showed significant difference ($p<0.05$) in rats treated with tramadol for 21 days + 7 days withdrawal (4^{th} week) compared to the control.

The result also showed non-significant differences ($p>0.05$) in PCV, platelet and Hb in rats treated with tramadol orally for 7 days, while there were significant difference ($p<0.05$) in RBC, WBC and lymphocytes of rats treated with tramadol orally for 7 days, compare to weekly average control. Treatment showed non-significant difference ($p>0.05$) in RBC,WBC, PCV, Lymph, Platelet and Hb in rats treated with tramadol orally for 14 days and 21 days compare to weekly average control. Treatment effect on WBC and PCV showed non-significance difference ($p>0.05$) in rats treated with tramadol orally for 21 days+ 7 days withdrawal, while treatment showed significance difference ($p<0.05$) in RBC, Hb, platelets and lymphocyte in rats treated with tramadol orally 21 days + 7 days withdrawal, all compare to the weekly average control (table 1).

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Table 1: Effects on hematological parameters in rats treated orally with 1.6 mg/kg body weight of tramadol for7 days, 14 days, 21 days and 21 days + 7 days withdrawal.

	Treatment	Treatment	PCV (%)	Hb (g/dl)	RBC($X10^{12}$)	WBC($X10^{9}$)	Platelet($X10^{9}$)	Lymph. ($X10^{9}$)
I	7 days treatment	Control	26.6 ± 1.5^{a}	9.0 ± 0.3^{a}	4.36 ± 0.15^{a}	6.90 ± 2.5^{a}	270.00 ± 0.0^{b}	70.00 ± 2.0^{a}
		Test	$28.6\pm1.5^{a,A}$	$9.5\pm0.5^{a,A}$	$4.40\pm0.1^{a,B}$	$4.30\pm0.5^{b,B}$	$315.00\pm15.0^{a,B}$	$57.50\pm2.5^{b,B}$
ii	14 days treatment	Control	32.6 ± 2.9^{a}	9.9 ± 0.9^{a}	5.56±0.7a	9.86 ± 5.6^{a}	335.66 ± 105^{a}	84.40 ± 1.4^{a}
		Test	$29.1\pm2.4^{a,A}$	$8.9\pm0.8^{a,AB}$	$5.06\pm0.6^{a,AB}$	$7.00\pm0.1^{a,AB}$	$390.66\pm94.5^{a,AB}$	$84.30\pm4.7^{a,A}$
iii	21 days treatment	Control	32.8 ± 3.9^{a}	10.3 ± 1.2^{a}	6.04 ± 0.6^{a}	7.46 ± 2.8^{a}	423.00 ± 108^{a}	78.20 ± 1.4^{a}
		Test	$31.3\pm2.4^{a,A}$	$9.7\pm0.9^{a,A}$	$5.81\pm0.3^{a,A}$	$6.00\pm2.3^{a,AB}$	$377.00\pm99.0^{a,AB}$	$69.10\pm13.1^{a,AB}$
iv	21 days + 7 days withdrawal	Control	39.1 ± 2.4^{a}	13.8 ± 0.5^{a}	6.90 ± 1.6^{a}	6.26 ± 0.05^{b}	416.66 ± 3.5^{b}	84.00 ± 0.7^{a}
		Test	$25.5\pm2.1^{b,A}$	$7.1\pm0.3^{b,B}$	$4.30\pm0.1^{b,B}$	$8.00\pm0.6^{a,AB}$	$550.66\pm26.5^{a,A}$	$56.43\pm2.25^{b,B}$
V	Average weekly control	Control	30.63 ± 4.18^{A}	9.75 ± 2.02^{A}	5.31 ± 1.1^{AB}	8.77 ± 3.54^{A}	343 ± 86.48^{B}	77.53 ± 3.18^{A}

$^{a-b}$ Different letters in the same column indicate significance difference ($p<0.05$) within the week

$^{A-B}$Different letters in the same column indicate significance difference ($p<0.05$) across the week

3.2 Effects of tramadol on kidney and liver parameters

Sodium (Na^+) , Chloride (Cl^-) , alanine amino transferase (ALT), bicarbonate, aspartate alanin transferase (AST) and potassium (K) results were non-significantly different ($p>0.05$) in rats treated with tramadol orally for 7 days and 21 days compare to their control. Sodium (NA+), alanine amino transferase (ALT), potassium (K) and Chloride (Cl^-) were not significantly difference ($p>0.05$), while bicarbonate and aspartate amino transferase (AST) showed significance difference ($p<0.05$), in rats treated with tramadol orally for 14 days, compare to the control. In rats treated for 21 days, chloride (Cl^-), alanine amino transferase (ALT), bicarbonate and aspartate alanin transferase (AST) showed significance difference ($p<0.05$) while sodium (Na^+) and potassium (K) showed significant difference, compared to the control. NA+, ALT, AST, CL, protein, bicarbonate and K+ showed non-significance difference ($p>0.05$)in rats treated with tramadol orally for 7days, compare to average weekly control.Bicarbonate was significantly difference($p<0.05$) while Na^+, ALT, AST, Cl^-, Protein, and K^+ showed non-significance difference ($p>0.05$)in rats treated with tramadol orally for 14 days, compare to average weekly control. Treatment on Bicarbonate showed significance difference ($p>0.05$) while treatment on CL, Protein, Na^+, K^+, AST and ALT showed non-significance difference ($p>0.05$) in rats treated with tramadol orally for 21 days, compare to the weekly average control. In rats treated with tramadol orally for 21 days + 7 days withdrawal, Bicarbonate, AST and ALT showed significance difference ($p>0.05$) while CL, Protein, $Na^+$and K^+ showed no significant difference ($p>0.05$), compare to weekly average control.

143 Table 2: Effects on kidney and liver parameters in rats treated orally with 1.6 mg/kg body weight of tramadol for 7 days, 14 days, 21 days and 21
144 days + 7 days withdrawal.

	Treatment	Treatment	Na+ (M/mol)	K+ (M/mol)	Cl- (M/mol)	Bicarb. (M/mol)	AST U/L	ALT (U/L)	Protein (g/L)
I	7 days treatment	Control	134.0±2.0^{a}	4.06±0.3^{a}	100.6±4.5^{a}	23.6±0.5^{a}	17.6±3.5^{a}	10.6±1.5^{a}	66.04±12.2^{a}
		Test	137.6±7.5^{a,A}	4.73±0.5^{a,A}	94.6±2.5^{a,A}	22.6±1.5^{a,B}	22.0±3.0^{a,B}	10.0±1.0^{a,B}	66.88±11.0^{a,A}
II	14 days treatment	Control	157.6±5.0^{a}	7.26±0.3^{a}	109.6±18.5^{a}	23.6±1.5^{b}	34.6±3.5^{a}	10.0±2.0^{a}	72.31±2.4^{a}
		Test	140.0±5.0^{a,A}	4.30±2.6^{a,A}	94.6±2.5^{a,A}	29.6±0.5^{a,A}	23.0±2.0^{b,B}	9.3±1.5^{a,B}	61.93±2.4^{b,A}
III	21 days treatment	Control	136.6±10.5^{a}	5.00±0.6^{a}	86.6±4.5^{a}	24.6±3.5^{a}	23.6±5.5^{a}	11.0±4.0^{a}	69.26±2.3^{a}
		Test	142.6±7.5^{a,A}	5.20±0.1^{a,A}	91.6±5.5^{a,A}	28.0±0.0^{a,A}	17.0±1.0^{a,B}	9.6±0.5^{a,B}	73.20±6.9^{a,A}
IV	21 days + 7 days	Control	149.6±0.5^{a}	106.0±1.0^{a}	23.0±1.0^{a}	23.0±1.0^{b}	13.0±1.0^{b}	73.27±2.3^{a}	5.10±0.1^{a}
	withdrawal	Test	153.0±4.0^{a,A}	5.20±0.1^{a,A}	97.6±1.5^{b,A}	16.6±1.5^{b,C}	42.0±0.0^{a,A}	25.0±1.0^{a,A}	62.19±6.6^{a,A}
V	AVERAGE	Control	153.0±4.0^{a,A}	5.20±0.1^{a,A}	97.6±1.5^{b,A}	16.6±1.5^{b,C}	42.0±0.0^{a,A}	25.0±1.0^{a,A}	62.19±6.6^{a,A}

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146 $^{a-b}$ Different letters in the same column indicate significance difference ($p<0.05$) within the week

147 $^{A-B}$Diffferent letters in the same column indicate significance difference ($p<0.05$) across the week

3.3 Effects of tramadol on sperm count

Treatment on sperm count showed non-significant difference ($p>0.05$) in rats treated with tramadol orally for 7days, 14 days, and 21 days compare to the control. Treatment on sperm count also showed significance difference in rats treated with tramadol orally for 21 days + 7 days withdrawal, compare to the control. Sperm count showed non-significance difference in rats treated with tramadol orally for 7days, 14 days, 21 days and 21 days + 7 days withdrawal, compare to average weekly control.

Table 3: Effect on sperm count in rats treated orally with 1.6 mg/kg body weight of tramadol for 7 days, 14 days, 21 days and 21 days + 7 days withdrawal

	Treatment	Treatment	Sperm count($x10^6$)
I	7 days treatment	Control	575.00 ± 25.0^{a}
		Test	$375.00\pm125^{a,B}$
II	14 days treatment	Control	575.00 ± 25.0^{a}
		Test	$625.00\pm25.0^{a,A}$
III	21 days treatment	Control	475.00 ± 175.0^{a}
		Test	$550.00\pm151.5^{a,AB}$
IV	21 days + 7 days withdrawal	Control	650.00 ± 50.0^{a}
		Test	$475.00\pm25.0^{b,AB}$
V	Average weekly control	Average control	541.7 ± 102.3^{AB}

$^{a-b}$ Different letters in the same column indicate significance difference ($p<0.05$) within the week

$^{A-B}$Different letters in the same column indicate significance difference ($p<0.05$) across the week

4.0 DISCUSSION

The values obtained for RBC, PCV and Hb showed no significant difference ($P>0.05$) in 7, 14, and 21 days treated groups, but showed significant difference ($P<0.05$) in those treated for 21 days +7days withdrawal. This is an indication that there was no destruction of red blood cells and no change in the rate of production of RBC (erythropoiesis). It also shows that tramadol does not have the potential to stimulate erythropoietin release from the kidneys, which is the humoral regulator of RBC production. The non-significant ($P>0.05$) effect of treatment of rats with tramadol also indicate that there were no change in the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since RBC and haemoglobin (Hb) are very important in transferring respiratory gases. This is contrary to the result gotten by [13] which showed a marked decrease in erythrocytic variables in rats. This difference may be because in the study, tramadol was injected into the blood stream directly.

The result revealed no significant increase ($P>0.05$) on WBC, platelet and lymphocyte, in 14 and 21 days tramadol treated groups and revealed significance increase ($P<0.05$) in 7 days and 21day +7 days withdrawal groups. The non-significant ($P>0.05$) change in lymphocyte count suggests that the acquired immune responses of the body have not been compromised by tramadol, this results agree with [14] who suggested the use of tramadol over morphine due to the immunosuppressive effect of morphine against tramadol. Tramadol have been reported to have immune enhancing effect [15], it has also been reported that tramadol could increase lymphocyte proliferation in vivo and in vitro [16], but from their study it appears that its immune enhancing effect might be subject to the presence of a pathological condition or conditions such as post operation recovery [17;18]. The effect on the lymphocyte or immune response may also be concentration dependent considering that a similar study carried out by [19] had a significant increase in lymphocyte but at a dose of 50 mg/kg and 100 mg/kg Also, the non-significant change in the platelet count caused by tramadol could be

187 an indication that it does not have the potential to stimulate thromboplastin production with
188 the hemostatic capability of the blood maintaining the *status quo* since platelets mediate in
189 the blood–clotting mechanism. The significant increase ($P<0.05$) in RBC of rats in the group
190 that received tramadol for 21days+7days withdrawal might be the consequence of reduced
191 feed intake and repeated tramadol use. It has also been reported that tramadol has the ability
192 to inhibit erythropoiesis and in the process decrease the RBC count [20, 19] therefore when it
193 was withdrawn from the rats the body recovered and the RBC increased significantly. There
194 was significance decrease on protein in rats treated with tramadol for 14 days, but non-
195 significant change in rats treated for 7days, 21days and 21days+7days.
196 ALT and Chloride levels showed no significance increase in 7, 14 and 21 days tramadol
197 treated groups and showed significance increase in 21day +7 days withdrawal group. The
198 increase in the level of ALT indicated the malfunctioning and damage of liver tissues. A
199 significant elevated level of ALT has been found in rats receiving morphine and tramadol for
200 a long time compared to control group [7]. This result also agrees with [21 and 19] who also
201 reported an increase in ALT levels. These results were comparable with the reports of
202 increased ALT, AST activities in rats after acute and long term administration of morphine
203 like agent levo-alpha- acetylmethadol HCL (LAAM) and in chronic heroin users [22].
204 Similar to the results of [23] who recorded a significant increase in the ALT and AST
205 activities in rats after administration of 40 mg/kg bodyweight and 80 mg/kg bodyweight
206 tramadol than control treatment. Cellular injury may persist as indicated by increased AST
207 and ALT, level. The findings of this study are in agreement with those of [7] who reported
208 that the levels of ALT and AST is significantly higher in rats exposed to acute and gradual
209 increasing doses of morphine till reaching dependency when compared to the control group.

210 Result of this study showed no significant difference ($P>0.05$) of sperm count in 7, 14 and 21
211 days tramadol treated groups, but showed significance difference ($P<0.05$) of sperm count in

21days +7days withdrawal group. The significant increase ($P<0.05$) proved that tramadol can be a potential source of sperm reduction in male due to constant intake and dependency. This is similar to the report of [25], who stated that treatment of rats with paracetamol also caused significant decrease in sperm motility and sperm count but did not produce any pathological lesions on the testes.

5.0 CONCLUSION

Tramadol was observed in this study to have specific negative effects on the studied parameters in rats after prolonged use. Man belongs to the class mammalia like rats and man is the primary end user of tramadol, since the effects studied in rats showed certain detrimental effects and man has a similar physiological response like rats though advanced, it is advised that both medical and non-medical prolonged uses of tramadol should consider these effects before use.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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