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Effect of chronic tramadol administration on haematological parameters of Wistar Albino rats (*Rattus norvegicus*)

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Abstract

Chronic tramadol misuse represents a significant public health challenge, with emerging evidence suggesting sex-specific physiological impacts that remain insufficiently characterized. This investigation examined the haematological consequences of prolonged tramadol administration (100 mg/kg/day orally for 28 days) in mature Wistar albino rats, with particular emphasis on identifying potential sex-based variations. The experimental design employed twenty-eight rats (14 males, 14 females), systematically stratified by sex and subsequently divided into treatment and control cohorts (n = 7 per subgroup). Haematological analysis of blood samples quantified erythrocytic indices (RBC count, Hb concentration, PCV), total leukocyte count, and differential leukocyte profiles using automated analyzers complemented by microscopic verification. Statistical evaluation (Two-Way ANOVA with Tukey's HSD post-hoc test, $p < 0.05$) revealed significant sex-treatment interactions across multiple haematological parameters. The tramadol-exposed groups demonstrated marked alterations in erythrocytic parameters relative to respective controls. Male rats showed reductions in PCV (27%), RBC (25%), and Hb (27%), while female group displayed more pronounced decreases in PCV (38%) and Hb (38%), with moderate RBC decline (14%). Notably, female rats manifested distinct microcytic hypochromic anaemia, evidenced by significant decreases in MCV (44.90 ± 4.67 fL versus control 62.66 ± 2.84 fL) and MCH (14.91 ± 1.51 pg versus 21.09 ± 1.26 pg). Leukocyte profiling identified a consistent neutropenia (~16% reduction) across sexes, with female rats showing reactive lymphocytosis (22% increase). No significant alterations were observed in monocyte, eosinophil, or basophil. These findings indicate heightened female susceptibility to tramadol-induced haematopoietic suppression, potentially mediated through estrogen-enhanced opioid receptor sensitivity or hormonal modulation of erythropoietic pathways. The results corroborate clinical observations of tramadol-associated anaemia and immune dysregulation, emphasizing necessity for sex-specific therapeutic monitoring in chronic users. Public health initiatives should prioritize prevalent safety misconceptions, particularly among female populations, while future research should investigate underlying molecular mechanisms and potential recovery following cessation of use.

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Introduction

Since the approval of tramadol use by the Food and Drug Agency of the United States of America in 1995, tramadol has become a widely prescribed analgesic for moderate to severe pain. Unlike classical opioids, its dual mechanism combining weak μ-opioid receptor activation with inhibition of serotonin/norepinephrine reuptake offers comparable pain relief with a lower risk of respiratory depression (Grond & Sablotzki, 2004). However, its perceived safety has fueled widespread non-medical use, particularly among young adults seeking euphoric effects. The World Health Organization now identifies tramadol misuse as a growing public health threat, with long-term physiological consequences, especially on blood health, remaining poorly understood (WHO, 2018).

Emerging animal and clinical studies highlight concerning links between chronic tramadol exposure and disruption of blood cell production. Reported effects include anaemia (reduced red blood cell and haemoglobin concentration), erratic white blood cell counts, and abnormal platelet activity (Bakare, 2019), which may increase the risks of infection, clotting disorders, or haemorrhage (Al-Rejaie *et al.,* 2016). Proposed explanations range from oxidative stress damaging bone marrow to immune system interference (Elshamy *et al.,* 2022), though precise pathways remain unclear. These haematological disturbances could compound health risks in chronic users, particularly given evidence of sex-based differences in opioid metabolism (Becker & Koob, 2016).

This study evaluates prolonged tramadol exposure in Wistar albino rats, a species chosen for their translational relevance to human physiology. By analyzing blood cell counts, haemoglobin, haematocrit, and immune cell profiles in treated versus control groups, we aim to clarify the haematological risks. The results will inform clinical monitoring guidelines for long-term users and enhance public health strategies to address tramadol misuse.

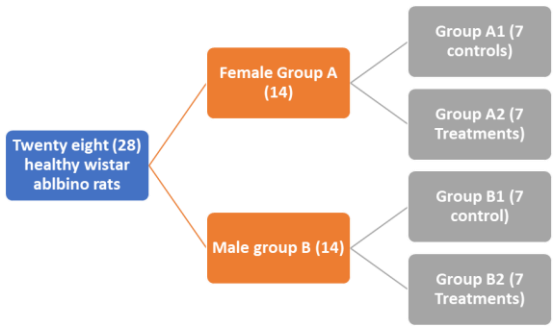

Figure 1: Experimental design

Materials and Methods

Ethical approval

Approval was secured from the Faculty of Veterinary Medicine's Animal Use and Ethics Committee, University of Maiduguri prior to experimentation. (Approval Number: AUP R003/2025).

Experimental design

Twenty-eight healthy adult Wistar albino rats (14 males, 14 females) were acclimatized under standardized laboratory conditions. Rats were stratified by sex into four groups (n=7 per group):

- *Male treatment group:* Received daily tramadol (100 mg, orally) (Thakur *et al*., 2009).
- *Male control group*: Administered equivalent saline volumes.
- *Female treatment group:* Received daily tramadol (100 mg, orally).
- *Female control group:* Administered equivalent saline volumes (Figure 1).

The 28-day dosing period was followed by terminal blood collection via cardiac puncture under anaesthesia. Haematological analysis was conducted using automated analyzers Sysmex XN-1000 ®, with differential leukocyte counts performed manually via microscopy. Statistical comparisons focused on treatment effects and sex disparities.

Monitoring: The rats were monitored for general health, behavioural changes, and side effects throughout the treatment period were recorded.

Source of the drug: The drug (Tramadol hydrochloride 50 mg/ml) was obtained from Novalab Healthcare Private Limited, India.

Housing and feeding: The rats were housed in standard laboratory cages under controlled environmental conditions at the Laboratory Animals' House of the Department of Veterinary Physiology and Biochemistry, University of Maiduguri, Nigeria. 12:12-hour light: dark cycle, with *ad libitum* access to standard rat chow and water (Krohn *et al.,* 2003).

Tramadol administration: Tramadol was administered orally at a daily dose 100mg/kg follows;

$$\text{Dose} = \frac{Dossage\left(\frac{mg}{kg}\right) \times Rat's\ Bodyweight\ (Kg)}{Drug\ conc.\ (mg/mL)}$$

The dose is selected based on previous studies indicating its efficacy and relevance for simulating chronic overuse (El-Sherbiny & Khalaf, 2016). The treatment period lasts for 4 weeks, with daily administration to ensure consistent exposure.

Sample collection and storage

At the end of the treatment period, blood samples were collected via direct cardiac puncture under anaesthesia induced by ketamine (80 mg/kg) and xylazine (10 mg/kg) (Parasuraman *et al.,* 2010). Approximately 2 mL of blood is drawn from each rat and transferred into EDTA tubes for haematological analysis to prevent clotting. The samples are stored at 4°C and processed within two hours to maintain integrity (Weiss & Wardrop, 2010).

Sample analysis

Haematological parameters were analyzed using an automated haematology analyzer (Sysmex XN-1000 ®). The following parameters were measured;

Red Blood Cell (RBC) Count
White Blood Cell (WBC) Count
Haemoglobin (Hb) Concentration
Haematocrit or Park Cell Volume (PCV %)
Differential Leukocyte Count

The following data were manually calculated using the values of the RBCs, PCV and the (Hb);

Mean Corpuscular Volume (MCV): indicates the average volume of red blood cells (RBCs) and is expressed in femtoliters (fL) (Dacie & Lewis, 2011).

$$\text{MCV} = \frac{PCV\ (\%)}{RBC\ count} \times 10$$

where:

Haematocrit (PCV) is expressed as a percentage (%).

RBC is expressed as the number of cells per microliter (million cells/µL).

Mean Corpuscular Haemoglobin (MCH): indicates the average amount of haemoglobin per red blood cell and is expressed in picograms (pg) (Dacie & Lewis, 2011).

$$\text{MCH} = \frac{Hemoglobin\ (Hb)}{RBC\ count} \times 10$$

where:

Haemoglobin (Hb) is expressed in grams per deciliter (g/dL).

RBC is expressed as the number of cells per microliter (million cells/µL).

Mean Corpuscular Haemoglobin Concentration (MCHC): indicates the average concentration of haemoglobin in a given volume of packed red blood cells and is expressed in grams per deciliter (g/dL) (Dacie & Lewis, 2011).

$$\text{MCHC} = \frac{Hemoglobin\ (Hb)}{PCV\ (\%)} \times 100$$

where:

Haemoglobin (Hb) is expressed in grams per deciliter (g/dL).

Pack cells volume (PCV) is expressed as a percentage (%).

Sex-based difference expressed in (%)

Quantitative sex differences in tramadol-induced haematological alterations were analyzed by comparing percentage changes between treated male and female groups. The relative sex difference for each parameter was calculated (Verghese *et al.,* 2006).

Data analysis

Results are reported as mean ± SD. Statistical comparisons used was Two-Way ANOVA and post-hoc (Tukey's HSD test) ($p < 0.05$) via SPSS v20.

Results

Prolonged tramadol exposure induced marked haematological changes in Wistar rats, with pronounced sex-specific disparities. Key parameters, packed cell volume (PCV), red blood cell (RBC) count, haemoglobin (Hb) concentration, and leukocyte profiles were systematically analyzed across four cohorts: female control (A1), male control (B1), female treatment (A2), and male treatment (B2).

Control Groups (A1 & B1): Haematological values were stable in untreated rats. Females (Group A1) showed a PCV of 45.71 ± 3.01%, RBC count of 7.35 ± 0.22 ×10^6/µL, and Hb of 15.20 ± 1.00 g/dL. Males (Group B1) showed PCV (47.00 ± 1.07%), RBC counts (7.33 ± 0.41 ×10^6/µL and HB (15.67 ± 0.36 g/dL), (Tables 2 and 3). *Tramadol-Treated Females (Group A2):* PCV plummeted by 38% (28.29 ± 3.65% as against 45.71 ± 3.01% in controls), accompanied by a 14% RBC decline (6.29 ×10^6/µL versus 7.35 ± 0.22 ×10^6/µL in the control group) and a 38% Hb reduction (9.40 g/dL compared to 15.20 g/dL in the control) (Table 1). Mean corpuscular haemoglobin (MCH) and volume (MCV) also dropped sharply to 14.91 pg and 44.90 fL, as against 21.09 pg and 62.66 fL in the treatment and control groups, respectively (Table 4).

Table 1: Data for the female control group (A1)

Parameters	A1	A2	A3	A4	A5	A6	A7	Mean ± SD
PCV (%)	40	43	47	46	49	49	46	45.71 ± 3.01
RBC ($\times10^6$/µL)	7.05	7.0	7.4	7.6	7.6	7.4	7.4	7.35 ± 0.22
WBC ($\times10^3$/µL)	48	45	53	48	54	53	50	50.14 ± 3.09
HB (g/dL)	13.3	14.3	15.6	15.3	16.3	16.3	15.3	15.20 ± 1.00
MCH (pg)	18.6	20.4	21.6	22.8	21.46	22.07	20.72	21.09 ± 1.26
MCV (fL)	56.7	61.4	63.5	64.2	64.5	66.2	62.1	62.66 ± 2.84
MCHC (g/dL)	33.8	33.3	33.3	33.3	33.37	33.37	33.33	33.40 ± 0.17

PCV = packed cell volume; RBC = red blood cell count; WBC = white blood cell count; Hb = haemoglobin; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; MCHC = mean corpuscular haemoglobin concentration. Values A1–A7 represent individual animal measurements within the female control group (group a1), with mean ± SD denoting the group average and standard deviation. Units: PCV (%), RBC ($\times10^6$ cells per microliter), WBC ($\times10^3$ cells per microliter), Hb (grams per deciliter), MCH (picograms), MCV (femtoliters), MCHC (grams per deciliter).

Table 2: Data for male control group (B1)

Parameters	B1	B2	B3	B4	B5	B6	B7	Mean ± SD
PCV (%)	45	47	47	48	46	48	48	47.00 ± 1.07
RBC ($\times10^6$/µL)	7.3	7.1	6.8	6.9	7.4	7.8	8.0	7.33 ± 0.41
WBC ($\times10^3$/µL)	43	46	45	51	49	49	54	48.14 ± 3.48
HB (g/dL)	15.0	15.67	15.67	16.0	15.33	16.0	16.0	15.67 ± 0.36
MCH (pg)	20.55	22.07	23.01	21.62	22.22	20.51	20.0	21.43 ± 1.02
MCV (fL)	61.6	66.2	69.1	69.5	62.1	61.5	60.0	64.29 ± 3.63
MCHC (g/dL)	33.33	33.33	33.33	33.33	33.33	33.33	33.33	33.33 ± 0.00

PCV = packed cell volume; RBC = red blood cell count; WBC = white blood cell count; Hb = haemoglobin; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; MCHC = mean corpuscular haemoglobin concentration. Values A1–A7 represent individual animal measurements within the female control group (group a1), with mean ± SD denoting the group average and standard deviation. Units: PCV (%), RBC ($\times10^6$ cells per microliter), WBC ($\times10^3$ cells per microliter), Hb (grams per deciliter), MCH (picograms), MCV (femtoliters), MCHC (grams per deciliter).

Table 3: The mean and standard deviation of the data for the erythrocytic values

Parameters	Female Control (A)	Male Control (B)	Male Treatment (C)	Female Treatment (D)
PCV (%)	45.71 ± 3.01	47.00 ± 1.07	34.14 ± 5.08*	28.29 ± 3.65*
RBC (10^6/µL)	7.35 ± 0.22	7.33 ± 0.41	5.53 ± 0.21*	6.29 ± 0.29*
HB (G/DL)	15.20 ± 1.00	15.67 ± 0.36	11.50 ± 1.64*	9.40 ± 1.22*
MCH (PG)	21.09 ± 1.26	21.43 ± 1.02	20.77 ± 2.75*	14.91 ± 1.51*
MCV (FL)	62.66 ± 2.84	64.29 ± 3.63	61.85 ± 8.35*	44.90 ± 4.67*
MCHC (G/DL)	33.40 ± 0.17	33.33 ± 0.00	33.31 ± 0.01	33.31 ± 0.01

The asterisk (*) indicates a significant difference from the control group (P < 0.05).

Tramadol-Treated Males (Group B2): Tramadol-treated male rats showed significant but less pronounced declines in erythrocytic parameters compared to females. PCV decreased by 27% (from 47.00% in controls), RBC count fell by 25% (5.53 $\times10^6$/µL vs. control 7.33 ± 0.41 $\times10^6$/µL), and Hb levels dropped by 27% (11.50 g/dL vs. control 15.67 ± 0.36 g/dL). Notably, MCH declined by 3.1% (20.77 pg vs. control 21.43 pg) and MCV by 3.8% (61.85 fL vs. control 64.29 fL) (Table 5).

Sex-Based Comparisons: Females demonstrated greater vulnerability to tramadol-induced anaemia, with PCV, RBC, and Hb reductions exceeding those of

Table 4: Data for the female treatment group (A2)

Parameters	1	2	3	4	5	6	7	Mean ± SD
PCV (%)	32	34	23	28	28	29	24	28.29 ± 3.65
RBC ($\times10^6/\mu L$)	6.7	6.5	6.0	6.5	6.4	5.9	6.0	6.29 ± 0.29
WBC ($\times10^3/\mu L$)	34	33	44	38	45	46	33	39.00 ± 5.45
HB (g/dL)	10.6	11.3	7.6	9.3	9.3	9.67	8.0	9.40 ± 1.22
MCH (pg)	15.9	17.2	12.7	14.2	14.4	16.38	13.56	14.91 ± 1.51
MCV (fL)	47.76	52.3	38.33	43.07	43.75	49.1	40.0	44.90 ± 4.67
MCHC (g/dL)	33.3	33.3	33.3	33.3	33.3	33.33	33.33	33.31 ± 0.01

PCV = packed cell volume; RBC = red blood cell count; WBC = white blood cell count; Hb = haemoglobin; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; MCHC = mean corpuscular haemoglobin concentration. Values A1–A7 represent individual animal measurements within the female control group (group A1), with mean ± SD denoting the group average and standard deviation. Units: PCV (%), RBC ($\times10^6$ cells per microliter), WBC ($\times10^3$ cells per microliter), Hb (grams per deciliter), MCH (picograms), MCV (femtoliters), MCHC (grams per deciliter).

Table 5: Data for the male treatment group (B2)

Parameters	1	2	3	4	5	6	7	Mean ± SD
PCV (%)	43	36	31	27	32	39	31	34.14 ± 5.08
RBC ($\times10^6/\mu L$)	5.7	5.2	5.3	5.5	5.7	5.8	5.5	5.53 ± 0.21
WBC ($\times10^3/\mu L$)	30	28	33	24	29	31	28	29.00 ± 2.62
HB (g/dL)	14.3	12.0	10.3	9.0	10.6	13.0	11.33	11.50 ± 1.64
MCH (pg)	25.1	23.0	19.3	16.3	18.7	22.41	20.61	20.77 ± 2.75
MCV (fL)	75.44	69.4	58.4	49.8	56.3	67.2	56.4	61.85 ± 8.35
MCHC (g/dL)	33.3	33.3	33.3	33.3	33.3	33.33	33.33	33.31 ± 0.01

PCV = packed cell volume; RBC = red blood cell count; WBC = white blood cell count; Hb = haemoglobin; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume; MCHC = mean corpuscular haemoglobin concentration. Values A1–A7 represent individual animal measurements within the female control group (group a1), with mean ± SD denoting the group average and standard deviation. Units: PCV (%), RBC ($\times10^6$ cells per microliter), WBC ($\times10^3$ cells per microliter), Hb (grams per deciliter), MCH (picograms), MCV (femtoliters), MCHC (grams per deciliter).

males by 11%, 10%, and 11%, respectively. Conversely, leukocyte counts remained stable across sexes, with no significant tramadol-driven shifts in total WBCs or differential counts.

Discussion

The results reveal that 28 days of tramadol use destabilizes key haematological parameters in Wistar rats, with notable signs of anaemia and immune dysregulation. These outcomes are consistent with clinical reports of tramadol-induced blood abnormalities in humans (Gounden *et al.,* 2014; Prasad *et al.,* 2019a; Prasad *et al.,* 2019b). A reduction in packed cell volume (PCV) and haemoglobin levels was observed, reflecting a significant decline in red blood cell health. This was accompanied by decreases in mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV), suggesting the development of microcytic, hypochromic anaemia likely driven by impaired iron metabolism or defective red blood cell (RBC) production (Habib *et al.,* 2011; Nazifi *et al.,* 2014b). Interestingly, mean corpuscular haemoglobin concentration (MCHC) remained relatively stable, implying that tramadol primarily alters RBC quantity and morphology rather than intracellular haemoglobin content (Nazifi *et al.,* 2014a). Immune alterations also emerged as a critical outcome, with neutropenia (~16% reduction in neutrophils) appearing consistently across groups, aligning with documented opioid-induced suppression of bone marrow function (Ahmed *et al.,* 2017). At the same time, adaptive changes in lymphocyte counts were observed, which may reflect compensatory immune responses influenced by hormonal regulation of haematopoietic differentiation (Quatrini, 2021). Three primary mechanisms may underlie these haematological disruptions: (1) bone marrow toxicity, where tramadol metabolites impair haematopoietic stem cell proliferation (Akinyemi *et al.,* 2015); (2) oxidative

stress, as opioids elevate reactive oxygen species that promote RBC haemolysis and suppress erythropoiesis (Goudarzi *et al.,* 2017); and (3) hormonal interference, given that sex steroids can modulate opioid metabolism and receptor interactions, thereby influencing haematological outcomes (Connelly *et al.,* 2018).

While this 28-day exposure model provides important insights, it may not fully capture the prolonged misuse patterns seen in human populations. Furthermore, the absence of molecular data on oxidative stress markers or cytokine profiles limits the ability to delineate precise mechanistic pathways.

These findings highlight the need for vigilant clinical monitoring of chronic tramadol users, particularly through regular haematological screening (e.g., RBC indices, neutrophil counts) to detect early signs of anaemia or immune alterations. Public health initiatives should aim to correct misconceptions about tramadol's safety while emphasizing biological factors that contribute to individual susceptibility. Future research should examine how hormonal fluctuations and treatment duration shape tramadol's haematotoxicity, whether these effects reverse after discontinuation, and how tramadol compares to other opioids in terms of haematological safety. Such knowledge will be essential in informing safer, more personalized approaches to pain management.

In conclusion, chronic use of tramadol causes blood-related changes in Wistar rats, with more noticeable effects in females. These include signs of anaemia like reduced PCV, haemoglobin, and MCV, likely linked to the influence of estrogen. The 11% greater drop in PCV and haemoglobin in females points to estrogen possibly making the blood effects of tramadol worse. Changes in white blood cells, such as neutropenia and differences in lymphocyte response between sexes, show that tramadol also affects the immune system, though other blood cells seem to stay the same. These study results support earlier human findings of blood issues related to tramadol and show the value of using rats to study how opioids can affect the blood.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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