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Dose-Dependent Hematological Effects of Sodium Cyanate (NaOCN) in Adult Wistar Rats: Gender Variability and Toxicological Thresholds via Probit and Weibull Analysis

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ABSTRACT

Sodium cyanate (NaOCN), historically used for sickle cell disease treatment, poses potential hematological risks due to environmental and occupational exposure. This study investigates the dose-dependent hematological effects of NaOCN (0, 2, 3, 4, 5, 10, 15 and 20 mg/kg bw) in adult Wistar rats (n=48, 24 males, 24 females) over 7 days, focusing on gender variability and toxicological thresholds. Blood samples were analyzed for 17 hematological parameters using a Sysmex XN-1000V analyzer. Two-way MANOVA revealed a significant NaOCN dose-sex interaction (Wilks' Lambda=0.001, $p<0.001$, Partial $\eta^2=0.710$), with significant effects on neutrophils, MCV, MCHC, RDW-CV, RDW-SD, and MPV ($p<0.05$, Partial $\eta^2=0.456–0.691$). Tukey HSD tests identified the 3 mg/kg bw dose as a critical threshold, showing lower MCH, MCHC, and MPV, and higher RDW-CV compared to other doses. Females exhibited greater sensitivity (e.g., WBC EC50: 2.695 vs. male 8.680 mg/kg bw). Probit and Weibull models estimated LD_{50} at ~13.75 mg/kg bw (95% CI: 9.58–17.92) and LD_{90} at ~27.06 mg/kg bw, with females more susceptible (LD_{50}: 12.10 mg/kg bw). Low EC_{50} values for HCT (0.198 mg/kg bw), MCV (0.226 mg/kg bw), and MCHC (0.192 mg/kg bw) indicate high sensitivity at low doses. These findings highlight NaOCN's dose-dependent hematological toxicity, with significant gender differences, likely due to hormonal influence, hemoglobin carbamylation and metabolic variations. The narrow therapeutic window (100% mortality at 20 mg/kg bw) underscores the need for sex-stratified safety assessments in NaOCN applications.

Keywords: Dose-Dependent Effects, Gender Variability, Hematological Toxicity, Sodium Cyanate (NaOCN), Toxicological Thresholds, Wistar Rats

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INTRODUCTION

Sodium cyanate (NaOCN), a cyanide derivative, is utilized industrially to produce primary carbamates by reacting with alcohols in the presence of acidic catalysts (Dong *et al.*, 2020). Structurally, NaOCN contains a cyanate ion (OCN^-) with a carbon atom bound to both nitrogen and oxygen, rendering it less toxic than sodium cyanide but still hazardous (Nautha and Opatz, 2019). Its

environmental accumulation, driven by the widespread use of cyanate-containing fungicides and pesticides in agriculture, poses potential health risks (Elmore *et al*., 2015; Luque-Almagro *et al*., 2016).

Sodium cyanate (NaOCN) is a pharmacological agent with a unique history both in clinical and in physiological areas (Rivera-CH, *et al*., 1996). In pharmacology, NaOCN gained prominence in the 1970s for its potential in treating sickle cell disease by carbamylating the N-terminal valine of hemoglobin S, increasing oxygen affinity and reducing erythrocyte sickling (Cerami and Manning, 1971; Gillette *et al*., 1971; Cerami *et al*., 1973). This carbamylation, however, is not limited to hemoglobin, as NaOCN also affects erythrocyte membrane proteins and reduces Glucose-6-phosphate dehydrogenase (G6PD) activity, potentially altering hematological function (Glader and Conrad, 1972; Tebbi, 2022).

Blood, comprising approximately 45% cells (erythrocytes, leukocytes, thrombocytes) and 55% plasma, is a critical indicator of physiological health (Babeker and Elmansoury, 2013; Owalabi *et al*., 2013; Pessini *et al*., 2020). Erythrocytes transport oxygen, leukocytes combat infections, and thrombocytes facilitate coagulation, making hematological parameters essential for diagnosing disorders and assessing toxicological impacts (Erhabor *et al*., 2021; Mudimba *et al*., 2019; Muriithi *et al*., 2015; Ofem *et al*., 2012). Hematological studies are vital for detecting abnormalities such as anemia, neutropenia, or thrombocytopenia, often induced by toxicants like pesticides, metals, or pharmaceuticals (Dias *et al*., 2023; Duman and Sahan, 2023; Kanu *et al*., 2023; Rohani, 2023; Witeska *et al*., 2023). While NaOCN therapeutic effects are well-documented, its dose-dependent hematological toxicity, particularly in preclinical models like Wistar rats, remains underexplored (Haschek-Hock *et al*., 2022).

In addition to their use in monitoring and diagnosing illnesses and abnormalities (Fielder, 2019), Hematological studies aid in the diagnosis of numerous illnesses and the assessment of the degree of damage to blood and hematopoietic organs like the bone marrow and spleen (Shrivastav and Singh, 2012). According to Maxwell (2018), Hematology addresses a variety of blood-related illnesses, including anemia. Changes in Hematological parameters, either qualitatively or quantitatively, are associated with numerous physiological processes (Claassen *et al*., 2021).

Administration of the chemical compounds at toxic doses often results in changes in blood parameters that are indicative of Hematological disorders such as anemia which is characterized by low hemoglobin content; neutropenia which occurs in cases of reduced production of white blood cells or increased utilization and destruction, or both (Arika *et al*., 2016); thrombocytopenia which precedes a reduction in the platelet count as a result of decrease in platelet production, decline in platelet survival, and dilution of platelet numbers resulting from transfusion of platelet-poor blood; and malignancies such as leukemia, lymphoma and myeloma (Bradbury and Murray, 2013).

Despite its pharmacological promise, NaOCN environmental and occupational exposure risks raise concerns about its safety profile. Previous studies have focused on acute toxicity metrics like LC50 (Lei and Sun, 2018), but there is a paucity of data on subchronic effects, gender-specific responses, and precise toxicological thresholds for hematological parameters (Igbokwe *et al*., 2024). Gender variability is particularly relevant, as physiological differences (e.g., hormonal influences on erythropoiesis) may modulate toxic responses (Patel *et al*., 2024). Moreover, advanced statistical models like Probit and Weibull analyses, which account for non-linear dose-response relationships, have not been extensively applied to NaOCN hematological effects (Lei and Sun, 2018). This study addresses these gaps by examining the dose-dependent hematological effects of NaOCN in adult male and female Wistar rats, assessing gender variability, and determining toxicological thresholds using Probit and Weibull models.

MATERIALS AND METHODS

Experimental Animal

A total of forty-eight (48) adult Wistar rats (24 males, 24 females, aged 8–10 weeks, average body weight 110 ± 10 g) were procured from the Animal Research Facility at HONA Tech Research Centre, Osun, Nigeria. The rats were housed in polycarbonate cages with appropriate bedding under controlled conditions: temperature 23–25°C, relative humidity 30–70%, and a 12-hour light/dark cycle, with ventilation at 10–15 air changes per hour. They were provided ad libitum access to standard chow diet (Mazuri, Land O' Lakes Inc.) and filtered water. The sample size was determined using

G*Power 3.1 (Faul *et al*., 2009). Given that the study measured 17 hematological parameters simultaneously across 16 experimental groups (2 × 8 factorial design: 2 treatment levels × 8 time points), a multivariate analysis of variance (MANOVA) framework was adopted for the power analysis rather than a univariate approach. for a two-way (Sex × Dose) factorial ANOVA, that assume a medium effect size (f=0.25), α = 0.05, and 80% power based on prior studies (Zhang and Hartmann, 2023). ll procedures adhered to the ARRIVE guidelines (Percie du Sert *et al*., 2020) and were approved by the Institutional Animal Ethics Committee of NSUK University (Approval No. NSUKAEC/2024/041). To minimize distress, rats were handled gently, and anesthesia was used during invasive procedures.

Preparation of Sodium cyanate solution

Reagent Preparation and Quality Control

Sodium cyanate (NaOCN, ≥98% purity, Catalog #205-861-8, Sigma-Aldrich, St. Louis, MO, USA) was used for all experiments. Each batch was verified for purity upon receipt using high-performance liquid chromatography (HPLC) with a minimum acceptable purity threshold of 97.5%. The reagent was stored in a desiccator at room temperature (20–25°C) under low humidity conditions (<30% relative humidity) to prevent moisture absorption and degradation.

Solution Preparation Protocol

NaOCN solutions were prepared fresh daily using the following standardized protocol:

1.Solvent preparation: Sterile 0.9% saline solution (pharmaceutical grade, pH 6.5–7.5) was warmed to room temperature (22 ± 2°C) before use.

Dissolution method:

Accurately weighed quantities of NaOCN (using an analytical balance with 0.1 mg precision, Model XS205, Mettler Toledo, Switzerland) were added gradually to pre-measured volumes of saline in amber glass vials (10 mL capacity).

Solutions were mixed using a vortex mixer (Model VM-300, Gemmy Industrial Corp., Taiwan) at 2500 rpm for 3 minutes to ensure complete dissolution.

Visual inspection confirmed complete dissolution with no particulate matter present.

Concentration calculations: Dosages were calculated using the formula:

Concentration (mg/mL) = [Dosage (mg/kg bw) × Average Body Weight (kg)] / Administration Volume (mL)

Where:

- Dosages: 2, 3, 4, 5, 10, 15, and 20 mg/kg body weight
- Average body weight: 0.11 ± 0.015 kg (measured 24 hours prior to dosing)
- Administration volume: 0.5 mL per rat (equivalent to ~4.5 mL/kg bw)

Labeling: Each vial was labeled with:

- Treatment group identifier (e.g., Group IV: 5 mg/kg bw)
- Exact concentration (mg/mL)
- Preparation date and time
- Expiration time (8 hours from preparation)
- Batch number for traceability

Stability Testing and Storage

Solution stability was verified using the following protocol:

1. UV-Vis spectrophotometry: Absorbance measurements at λ = 230 nm (characteristic peak for cyanate ion) were performed at 0, 2, 4, 6, and 8 hours post-preparation using a spectrophotometer (Model UV-1800, Shimadzu, Japan).
2. Acceptance criteria: Solutions showing >5% deviation in absorbance from baseline (0 hour) were discarded.
3. Storage conditions: Prepared solutions were stored in amber vials at 4°C in darkness to minimize photodegradation and used within 8 hours of preparation.
4. Temperature monitoring: Storage refrigerator temperature was logged continuously and maintained at 4 ± 1°C.

pH Monitoring

The pH of each prepared solution was measured using a calibrated pH meter (Model FE28, Mettler Toledo, Switzerland) and maintained within the range of 7.0–7.4 to ensure physiological compatibility and solution stability. pH measurements were recorded in the preparation log for each batch.

Inclusion and Exclusion Criteria

Inclusion Criteria

1. Adult Wistar rats (8–10 weeks) confirmed healthy via physical examination (e.g., normal fur, activity, and weight).
2. Body weight within 100–120 g to ensure uniformity.
3. Equal distribution of males and females, determined by anogenital distance and confirmed by veterinary inspection.

Exclusion Criteria

1. Rats showing signs of illness (e.g., lethargy, weight loss, abnormal fur) during a 7-day acclimatization period.
2. Pregnant or nursing females, identified by abdominal palpation and weight monitoring.
3. Rats with physical injuries or behavioral abnormalities (e.g., aggression, reduced feeding).

Sodium cyanate administration

After a 7-day acclimatization period, rats were randomly assigned to eight groups (n=6 per group, 3 males and 3 females) using a random number generator to minimize bias. Group I (control) received 0.5 mL sterile saline orally, while experimental groups (II–VIII) received NaOCN at doses of 2, 3, 4, 5, 10, 15, and 20 mg/kg bw, respectively, via oral gavage using a 1 mL syringe with a 20-gauge gavage needle (Table 1). The administration occurred daily at 8:00 AM for 7 consecutive days, following OECD Test Guideline 407 for repeated dose toxicity (Organisation for Economic Co-operation and Development (OECD), 2018). Rats were monitored twice daily (8:00 AM and 4:00 PM) for behavioral changes (e.g., lethargy, aggression), toxicity indicators (e.g., tremors, respiratory distress), and mortality. Body weight was recorded daily to adjust dosages and monitor health.

Randomization and Blinding

Group assignment: Rats were randomly assigned to treatment groups using a computer-generated randomization sequence (random.org) performed by an investigator not involved in animal handling or data collection.

Blinding: Solutions were prepared and labeled by Investigator A (unblinded). Animal dosing, monitoring, and sample analysis were performed by Investigators B and C (blinded to treatment allocation). Code breaking occurred only after statistical analysis completion.

Post-Administration Monitoring

Immediate Observation (0–6 hours)

Following NaOCN administration, rats were monitored continuously for the first 30 minutes, then at 1, 2, 4, and 6 hours for:

i. Behavioral changes (lethargy, hyperactivity, ataxia)
ii. Respiratory distress (labored breathing, gasping)
iii. Convulsions or tremors
iv. Salivation, lacrimation, or piloerection
v. Body temperature (rectal thermometer, recorded at 0, 2, 4, and 6 hours)

Extended Monitoring (6–24 hours)

Animals were checked every 6 hours for:

i. General activity level and responsiveness
ii. Food and water consumption (measured gravimetrically)
iii. Fecal and urinary output (visual assessment)
iv. Body weight (recorded at 24 hours post-administration)

Distress and Humane Endpoints

In accordance with institutional ethical guidelines, the following humane endpoints were pre-established:

Immediate euthanasia criteria

i. Severe respiratory distress (respiratory rate >200 or <50 breaths/min)
ii. Continuous severe convulsions lasting >5 minutes
iii. Loss of righting reflex persisting >10 minutes
iv. Rectal temperature <35°C or >40°C
v. >20% acute body weight loss
vi. Unresponsive to gentle stimulation

Euthanasia method: Rats meeting humane endpoints were euthanized via CO_2 inhalation followed by cervical dislocation (performed by trained personnel), as per American Veterinary Medical Association (AVMA) Guidelines on Euthanasia (2020).

Distress scoring: A modified distress assessment protocol was employed using a cumulative scoring system (0–15 scale), with scores ≥10 triggering veterinary consultation and scores ≥12 meeting euthanasia criteria.

Hematology parameter tests

Blood samples (1 mL) were collected on day 8 via retro-orbital plexus puncture under light isoflurane anesthesia

(2–3% in oxygen) to minimize stress, following Patel *et al*. (2024). Samples were immediately transferred to EDTA-coated tubes and stored at 4°C until analysis within 4 hours. Hematological parameters, including white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), platelet count (PLT), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW-CV, RDW-SD), and differential WBC counts (neutrophils, lymphocytes, eosinophils, monocytes, basophils), were measured using a Sysmex XN-1000V automated hematology analyzer (Sysmex Corporation, Kobe, Japan). The analyzer was calibrated daily using manufacturer-supplied controls, and duplicate measurements were performed to ensure accuracy. Quality control included running standard samples to verify instrument precision (CV < 5%).

Table 1: Showing Sodium cyanate Administration Protocol Table

Group	NaOCN Dose (mg/kg bw)	Administration period (days)	Number of Animals per Group (n)	Sex of rats
I (Control)	0 (Saline)	7	6	3 Female, 3 male
II	2	7	6	3 Female, 3 male
III	3	7	6	3 Female, 3 male
IV	4	7	6	3 Female, 3 male
V	5	7	6	3 Female, 3 male
VI	10	7	6	3 Female, 3 male
VII	15	7	6	3 Female, 3 male
VIII	20	7	6	3 Female, 3 male

Ethical Approval and Monitoring

This study was approved by the Institutional Animal Ethics Committee of NSUK University (Approval No. NSUKAEC/2024/041)., valid from January 15, 2024, to January 14, 2025). All procedures adhered to the National Institutes of Health Guide for Care and Use of Laboratory Animals (8th Edition, 2011) and ARRIVE Guidelines 2.0.

Ethical oversight included:

i. Bi-weekly committee reviews of monitoring records
ii. Mandatory reporting of all animals meeting humane endpoints within 24 hours
iii. Veterinary consultation available 24/7 for distress assessment
iv. Post-study debriefing with all personnel involved in animal handling

All personnel involved in animal procedures completed institutional training in laboratory animal care, handling, and welfare assessment before study commencement.

Outlier and Missing Data Management

1. Outlier detection: Values exceeding mean ± 3 SD were flagged and investigated for technical errors (e.g., hemolysis, clotting, instrument malfunction).
2. Technical outliers: Data points resulting from confirmed technical errors were excluded and repeated if sample volume permitted.
3. Biological outliers: Values confirmed as biologically extreme but not due to technical error were retained in analysis with justification documented.
4. Missing data: Reasons for missing data points were documented (e.g., insufficient sample volume, animal mortality, sample hemolysis). No imputation methods were used; analyses were performed on available complete data sets only.
5. Reporting: All exclusions and missing data were reported in results with transparent justification.

Data Recording and Storage

1. All observations were recorded in real-time using pre-formatted data collection sheets.
2. Data were entered into electronic databases (Microsoft Excel 2021) by two independent operators with cross-verification.
3. Discrepancies were resolved by reviewing original records.
4. All raw data, preparation logs, and monitoring records were retained for audit purposes.

Quality Assurance and Data Management

Analyzer Calibration

Biochemical analyzer (Model BS-240, Mindray, China):

1. Daily calibration using manufacturer-supplied calibrators before each analytical session
2. Quality control samples (normal and pathological ranges) run at the beginning, middle, and end of each batch
3. Acceptance criteria: QC values within ±2 SD of target values
4. Recalibration performed if QC failures occurred

Hematology analyzer (Model BC-5150, Mindray, China):

1. Daily calibration using manufacturer-supplied calibrators
2. Three-level quality control (low, normal, high) performed before sample analysis
3. Monthly maintenance and verification with commercial control materials

UV-Vis Spectrophotometer:

i. Wavelength accuracy verified weekly using holmium oxide filter
ii. Baseline correction performed before each measurement session

Statistical analysis

Data were analyzed using SPSS 25.0 (IBM, USA), Python 3.13 (with pandas, numpy, scipy), and R 4.4.5 (with Rcmdr, doseResponse packages). The sample size MANOVA special effects and interactions was conducted using G*Power 3.1.9.7. Normality was assessed using the Shapiro-Wilk test ($p < 0.05$) after removing outliers identified via boxplot inspection. Homoscedasticity (equality of variances) was assessed using Levene's test ($p < 0.05$). Normally distributed parameters were analyzed using parametric tests, while non-normally distributed parameters were analyzed using non-parametric tests. Means, standard deviations, and coefficients of variation (CV%) were calculated for all parameters. To determine toxicological thresholds (LC50, LC90) for hematological effects (e.g., significant reductions in RBC, HGB), Probit and Weibull dose-response models were fitted using the R package 'drc' (Ritz *et al*., 2015). Model goodness-of-fit was assessed using Akaike Information Criterion (AIC) and chi-square tests. One-way multivariate ANOVA (MANOVA) was used to evaluate the combined effect of NaOCN doses on multiple hematological parameters ($p < 0.05$). Two-way MANOVA assessed dose and sex interactions, with post-hoc Tukey's HSD tests for significant effects. Effect sizes were reported using partial eta-squared (η^2). All analyses were conducted at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Power and Statistical Validity

A post-hoc power analysis was conducted using the MANOVA special effects and interactions module in G*Power (Wilks' U, O'Brien–Shieh algorithm) with the following parameters as illustrate in Table 2: total sample size ($N = 48$), number of groups ($k = 16$), number of predictors (2), and number of response variables (17). The observed multivariate effect size was $f^2(U) = 1.57$, the significance criterion was set at $\alpha = 0.05$, and the noncentrality parameter (λ) was 150.72. The critical F value was 1.7936 with numerator degrees of freedom (df_1) = 34 and denominator degrees of freedom (df_2) = 32. The achieved power ($1 - \beta$) was 0.9999841, indicating that the study was adequately powered to detect the observed effects across all 17 hematological parameters with a total sample size of 48. This substantially exceeds the conventionally

accepted minimum power threshold of 0.80 (Cohen, 1988), confirming that the sample size of 48 was sufficient to detect the observed treatment effects with a negligible probability of a Type II error. The high achieved power, combined with the large multivariate effect size, supports the statistical reliability and generalizability of the findings reported in this study.

Table 2: MANOVA special effects and interactions module post-hoc power analysis

Parameter	Value
Effect size f^2(U)	1.57
Cohen's benchmark for large f	0.40
α (significance level)	0.05
Total sample size (N)	48
Number of groups	16
Number of predictors	2
Number of response variables	17
Noncentrality parameter (λ)	150.72
Critical F (34, 32)	1.7936
Wilks' U	0.1514
Achieved power (1 − β)	0.9999841
Minimum acceptable power	0.80

Descriptive Analysis of the parameters across NaOCN Dose Groups

The Table 3 summarizes the normality (Shapiro-Wilk test) and homoscedasticity (Levene's test) assessments for 18 hematological parameters measured in Wistar rats exposed to varying doses of sodium cyanate (NaOCN). These tests determine whether parametric or non-parametric statistical tests are appropriate for analyzing dose and sex effects. Normality is assessed by the minimum Shapiro-Wilk p-value across NaOCN dose and sex groups, with $p \geq 0.05$ indicating normal distribution. Homoscedasticity is assessed by Levene's test, with $p \geq 0.05$ indicating equal variances (homoscedasticity) and $p < 0.05$ indicating unequal variances (heteroscedasticity). The parameter Bas (basophils) lacks data due to insufficient measurable values.

Furthermore, from Table 3, WBC (p = 0.138), Lym (p = 0.060), RBC (p = 0.091), HGB (p = 0.074), HCT (p = 0.125), MCH (p = 0.405), RDW_SD (p = 0.815), PLT (p = 0.212), PCT (p = 0.191). These parameters are normally distributed across at least some dose-sex groups, suggesting potential suitability for parametric tests, pending variance assessment. Neu (p = 0.002), Mon (p = 0.006), Eos (p = 0.000), MCV (p = 0.002), MCHC (p = 0.000), RDW_CV (p = 0.004), MPV (p = 0.049), PDW (p = 0.002). These parameters exhibit non-normal distributions, requiring non-parametric tests.

Neu (F = 1.5390, p = 0.1954), Mon (F = 2.2883, p = 0.0589), Eos (F = 1.8998, p = 0.1124), RBC (F = 0.7486, p = 0.6148), HGB (F = 0.3822, p = 0.8852), HCT (F = 0.6045, p = 0.7246), MCV (F = 0.8671, p = 0.5286), MCH (F = 0.6184, p = 0.7141), MCHC (F = 1.9298, p = 0.1033), RDW_CV (F = 1.2392, p = 0.3104), RDW_SD (F = 1.6300, p = 0.1681), PLT (F = 0.3971, p = 0.8757), PDW (F = 0.7512, p = 0.6127), PCT (F = 0.2803, p = 0.9424). These parameters have equal variances across NaOCN dose groups, satisfying a key assumption for parametric tests. WBC (F = 3.1848, p = 0.0134), Lym (F = 2.5589, p = 0.0381), MPV (F = 6.1186, p = 0.0002). These parameters exhibit unequal variances, suggesting dose-specific variability and requiring robust or non-parametric tests.

Table 3: Normality and Homoscedasticity Assessment of Hematological Parameters Across NaOCN Dose Groups

Parameter	Shapiro-Wilk Min P-value	Normality Status	Levene's F-statistic	Levene's P-value	Variance Status
WBC	0.138	Normal	3.1848	0.0134	Unequal
Neu	0.002	Non-normal	1.5390	0.1954	Equal
Lym	0.060	Normal	2.5589	0.0381	Unequal
Mon	0.006	Non-normal	2.2883	0.0589	Equal
Eos	0.000	Non-normal	1.8998	0.1124	Equal
Bas	NA	NA	NA	NA	NA
RBC	0.091	Normal	0.7486	0.6148	Equal
HGB	0.074	Normal	0.3822	0.8852	Equal
HCT	0.125	Normal	0.6045	0.7246	Equal
MCV	0.002	Non-normal	0.8671	0.5286	Equal
MCH	0.405	Normal	0.6184	0.7141	Equal
MCHC	0.000	Non-normal	1.9298	0.1033	Equal
RDW_CV	0.004	Non-normal	1.2392	0.3104	Equal
RDW_SD	0.815	Normal	1.6300	0.1681	Equal
PLT	0.212	Normal	0.3971	0.8757	Equal
MPV	0.049	Non-normal	6.1186	0.0002	Unequal
PDW	0.002	Non-normal	0.7512	0.6127	Equal
PCT	0.191	Normal	0.2803	0.9424	Equal

The Table 4 reports mean hematological parameter values for Wistar rats (n=3 males, n=3 females per group) in control (0 mg/kg bw, saline) and experimental groups (2, 3, 4, 5, 10, 15 mg/kg bw NaOCN) after 7 days of administration. Data for the 20 mg/kg bw group were unavailable due to 100% mortality (6/6 rats) before the 7-day period ended. Measured parameters include white blood cell count (WBC), neutrophils (Neu), lymphocytes (Lym), monocytes (Mon), eosinophils (Eos), basophils (Bas), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW-CV, RDW-SD), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT).

The WBC (Control: Male 11.88, Female 11.67 × 10^9/L) from Table 4, for the experimental males, decreases at 3–5 mg/kg bw (7.55–8.36 × 10^9/L), increases at 10 mg/kg bw (14.88 × 10^9/L), and returns to near control at 15 mg/kg bw (10.29 × 10^9/L). Suggests a biphasic response, with suppression at low doses and stimulation at higher doses. For the females rats, decreases across all doses (7.71–9.74 × 10^9/L), most pronounced at 15 mg/kg bw (7.71 × 10^9/L). This indicates consistent immunosuppression with gender variability in which the females show greater WBC suppression (Calabro *et al*., 2023). The effect of NaOCN for the WBC and Lymphocytes, dose-dependent decreases in males (e.g., Lym: 10.43 to 6.56 × 10^9/L) suggest immunosuppression, possibly due to NaOCN interference with leukocyte production (Charoensappakit *et al*., 2025).

The RBC increased in males (8.46 × 10^12/L at 4 mg/kg bw vs. 7.32 × 10^12/L) and females (8.38 × 10^12/L at 3 mg/kg bw vs. 5.55 × 10^12/L). Comparing to the Reference (Table 5) Male 8.20–9.50, Female 7.80–9.00 × 10^12/L), The RBC of control Males (7.32) and females (5.55) were below reference, suggesting baseline anemia. the experimental group males within range at 4 mg/kg bw (8.46) and 15 mg/kg bw (8.14); females within range at 3 mg/kg bw (8.38) and 15 mg/kg bw (7.58).

Hemoglobin and hematocrit increased, with females showing higher HCT (0.51 vs. 0.42 at 15 mg/kg bw). Platelet counts increased in females (1052.67 × 10^9/L at 15 mg/kg bw vs. 751 × 10^9/L), while males showed variable responses. MCV and MCHC increased at low doses, with females maintaining higher MCV (66.67 vs. 51.93 fL at 15 mg/kg bw). Compared to reference intervals (Table 5), WBC, HGB, and MCV exceeded normal ranges, confirming NaOCN-induced abnormalities, with females more affected. The alterations in MCH and MCHC; for example, with female was in 285.33 - 350.67 g/dL range across the doses largely fall below the reference data for Wistar rats, suggest that NaOCN exposure affects erythrocyte morphology and hemoglobin content, likely through oxidative stress-induced changes in red blood cell membrane integrity, hemoglobin denaturation, baseline anemia or strain/method differences (Oloro and Obeagu, 2022). NaOCN, a cyanate compound, is known to cause carbamoylation of hemoglobin and structural proteins, which can reduce red cell lifespan and modify hemoglobin-binding characteristics (Tebbi, 2022).

For the platelet count comparing with the Reference (male 573–998, Female 591–836 × 10^9/L), the control group males (581.67) and females (751) within range, while the experimental group males within range (685.67–968); females exceed at 4–15 mg/kg bw (931–1052.67), confirming mild thrombocytosis and female sensitivity. Mild thrombocytosis is reactive, where platelets are elevated due to an underlying, temporary, or chronic condition such as: acute infection (bacterial, viral, or parasitic), inflammation, iron deficiency etc. (Rokkam *et al*., 2024). The platelet count typically normalizes once the root cause is treated (Bleeker and Hogan, 2011). The MPV Reference (Male 6.70–8.00, Female 7.00–7.80 fL) compared to the control group males (8.20) and females (8.40) exceed reference. The experimental groups males within/exceed at 2–10 mg/kg bw (8.03–8.43), below at 15 mg/kg bw (6.63); females within/exceed at 2–15 mg/kg bw (7.13–8.60). Confirms female sensitivity and suggest reactive thrombocytosis or stress thrombopoiesis, potentially as a compensatory response to systemic oxidative stress (Camara-Lemarro *et al*., 2017). Such changes might precede or reflect early endothelial irritation or platelet turnover (Al-Tameemi and Noori, 2022) due to NaOCN.

Furthermore, the RBC, HGB, HCT increases at low to mid-doses (2–5 mg/kg bw) suggest compensatory erythropoiesis (Pillai *et al*., 2023; Zivot *et al*., 2018), likely due to NaOCN's carbamylation of hemoglobin, enhancing oxygen affinity (Baer, 1992). Declines at higher doses (10–15 mg/kg bw) in males indicate potential erythrocyte damage, while females maintain elevated HCT, supporting. In the platelets values, female thrombocytosis at 15 mg/kg bw (1052.67 × 10^9/L) vs. male decreases (856 × 10^9/L) highlights gender-specific thrombopoiesis, consistent with lower female EC50 for PLT (2.510 mg/kg bw vs. Male: 3.358 mg/kg bw). The erythrocyte indices increased MCV and MCHC at low doses suggest macrocytosis and improved hemoglobin concentration (Kauffmann and Evans, 2022), possibly compensatory responses to NaOCN effects.

Table 4: Hematological Parameters Across Control and Experimental Groups Treated with Varying Doses of NaOCN (2–15 mg/kg bw)

Parameters	Control		2 mg/kg bw		3 mg/kg bw		4 mg/kg bw		5 mg/kg bw		10 mg/kg bw		15 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
WBC (10^9/L)	11.88	11.67	12.21	8.42	7.55	9.74	8.36	9.00	7.98	7.91	14.88	8.73	10.29	7.71
Neutrophils(10^9/L)	1.38	0.92	0.44	2.44	3.11	1.63	1.2	0.81	0.92	0.35	1.18	2.15	3.69	0.42
Lymphocytes(10^9/L)	10.43	10.66	11.71	5.91	4.4	7.99	7.03	8.11	6.97	7.52	7.4	4.95	6.56	7.26
Monocytes(10^9/L)	0.03	0.05	0.04	0.04	0.003	0	0.06	0.07	0.09	0.03	0.04	0.01	0.01	0.03
Eosinophils(10^9/L)	0.04	0.02	0.02	0.03	0	0.02	0	0.01	0.003	0.01	0.01	0.03	0.02	0.007
Basophils(10^9/L)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
RBCells (10^{12}/L)	7.32	5.55	5.75	7.58	7.84	8.38	8.46	7.42	6.34	5.39	6.82	5.47	8.14	7.58
Hemoglobin (g/L)	132.67	110.33	113.67	145.33	160.33	178.5	118.67	141	120.33	105.67	131.33	105.67	159.33	149
Hematocrit (%)	0.42	0.38	0.40	0.42	0.42	0.41	0.49	0.49	0.43	0.36	0.46	0.33	0.42	0.51
MCV (fL)	57.87	68.37	68.67	56.37	53.57	65.23	63.3	66.57	68.17	67.17	67.7	56.27	51.93	66.67
MCH (pg)	18.13	19.63	19.8	19.2	20.43	21.25	18.8	19	18.9	19.67	19.27	19.6	19.53	19.7
MCHC (g/dL)	314.67	288	288	347	382	345.67	299.33	285.33	277.33	293	285.67	350.67	376.67	295.67
RCD Width – CV	0.23	0.27	0.28	0.21	0.17	0.23	0.23	0.27	0.27	0.28	0.29	0.22	0.18	0.27
RCD Width – SD	55.9	78.23	79.87	50.83	37.23	62.3	61.13	74.43	76.4	77.53	80.43	50.43	39.07	74.17
Platelet (10^9/L)	581.67	751	695.67	823.67	968	596.5	696	931	809.67	864	685.67	328	856	1052.67
MPV (fL)	8.2	8.4	8.43	7.77	7.07	7.57	8.03	7.97	8.1	8.5	8.27	7.13	6.63	8.6
PD Width (fL)	15.97	16.17	16.23	15.73	15.5	16.2	16	16.33	16.47	16.17	16.1	15.2	15.5	16.4
Plateletcrit (m/L)	5.11	6.3	5.86	6.44	6.87	2.99	5.58	7.44	6.56	4.00	5.56	2.3	5.67	5.49

Table 5: Reference Interval for Hematological Parameters of Wistar rats

Parameters	Reference Interval	
	Male	Female
WBC (10^9/L)	3.40-9.50	2.20-5.90
Neutrophils(10^9/L)	0.40-1.50	0.30-1.00
Lymphocytes(10^9/L)	2.60-7.80	1.70-4.80
Monocytes(10^9/L)	0.10-0.40	0.10-0.40
Eosinophils(10^9/L)	0-0.20	0-0.10
Basophils(10^9/L)	0-0.04	0-0.04
RBCells (10^{12}/L)	8.20-9.50	7.80-9.00
Hemoglobin (g/L)	91-103	88-101
Hematocrit (%)	42-48	42-48
MCV (fL)	48-54	48-57
MCH (pg)	16.10-19.30	17.70-19.30
MCHC (g/dL)	340-361	324-359
RCD Width – CV	-	-
RCD Width – SD	-	-
Platelet (10^9/L)	573-998	591-836
MPV (fL)	6.70-8.00	7.00-7.80
PD Width (fL)	-	-
Plateletcrit (m/L)	-	-

Reference data for Wistar rats are from Liberati *et al.* (2004), Boehm *et al.* (2007), Kampfmann *et al.*, (2012), He *et al.* (2017).

The Table 6 presents result from a one-way multivariate analysis of variance (MANOVA) and subsequent univariate ANOVAs to assess the effect of NaOCN dose (0, 2, 3, 4, 5, 10, 15 mg/kg bw) on 17 hematological parameters. The MANOVA assesses the overall dose effect across all parameters (Wilks' Lambda), while univariate ANOVAs test individual parameters.

From Table 6, Wilks' Lambda = 0.007, F = 1.763, df = 6, 35, p = 0.002, Partial Eta Squared = 0.564. The low Wilks' Lambda and significant p-value (p = 0.002) indicate a strong overall effect of NaOCN dose on the combined hematological parameters. The partial eta squared (0.564) suggests that 56.4% of the variance in the multivariate response is explained by dose, a large effect size (Cohen, 1988). Large effect sizes (>0.14, Cohen, 1988) from Table 5 for MCH (0.410), MCHC (0.420), MPV (0.380), and RDW-CV (0.279) indicate strong dose effects. Moderate effect sizes (0.06–0.14) for WBC (0.146), Lym (0.134), and HGB (0.142) suggest potential dose effects not detected due to low power. Small effect sizes (<0.06) for Mon (0.069), HCT (0.067), and PCT (0.102) indicate weak or negligible dose effects, consistent with low baseline counts. This finding shows that Sodium cyanate (NaOCN) administration induced a significant dose-dependent changes in the hematological parameters of adult Wistar rats

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Table 6: One-way Multivariate Analysis of variance (ANOVA) for the effect of NaOCN dose on the Hematological parameters

WBC-White Blood Cell, RBC- Red Blood Cells, MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Hemoglobin,

Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Wilks' Lambda	F	df	Sig.	Partial Eta Squared
WBC	89.061	6	14.844	.996	.443	.146					
Neutrophils	17.723	6	2.954	1.343	.265	.187					
Lymphocytes	83.838	6	13.973	.903	.504	.134					
Monocytes	.016	6	.003	.433	.851	.069					
Eosinophils	.005	6	.001	1.046	.413	.152					
RBC	15.072	6	2.512	.747	.616	.113					
Hemoglobin	7233.476	6	1205.579	.966	.462	.142					
Hematocrit	.032	6	.005	.422	.860	.067					
MCV	316.958	6	52.826	.984	.451	.144					
MCH	26.630	6	4.438	4.057	.003	.410	0.007	1.763	6, 35	0.002	0.564
MCHC	26560.143	6	4426.690	4.225	.003	.420					
RCDW–CV	.021	6	.003	2.257	.060	.279					
RCDW– SD	2716.156	6	452.693	1.690	.153	.225					
Platelet	736239.619	6	122706.603	1.495	.209	.204					
MPV	8.975	6	1.496	3.569	.007	.380					
PDW	18.226	6	3.038	1.406	.240	.194					
Plateletcrit	25.844	6	4.307	.663	.680	.102					

MCHC- Mean Corpuscular Hemoglobin Concentration, RCDW-CV- Red Cell Distribution Width – Coefficient of Variation, RCDW-SD- Red Cell Distribution Width–Standard Deviation, MPV- Mean Platelet Volume, PDW- Platelet Distribution Width

The Table 7 reports significant pairwise comparisons from Tukey HSD post-hoc tests following significant univariate ANOVAs (Table 6) for mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean platelet volume (MPV), and marginally significant RDW-CV ($p = 0.060$). For MCH, 3 mg/kg bw (20.43 pg) was significantly higher than control (18.88 pg, $p = 0.003$), 2 mg/kg bw (19.00 pg, $p = 0.045$), 4 mg/kg bw (18.90 pg, $p = 0.003$), 5 mg/kg bw (19.29 pg, $p = 0.018$), and 10 mg/kg bw (19.44 pg, $p = 0.034$). For MCHC, 3 mg/kg bw (363.5 g/dL) exceeded control (301.335 g/dL, $p = 0.029$), 4 mg/kg bw (292.33 g/dL, $p = 0.008$), and 5 mg/kg bw (284.665 g/dL, $p = 0.003$). RDW-CV at 3 mg/kg bw (0.20) was lower than 5 mg/kg bw (0.275, $p = 0.033$). MPV was lower at 3 mg/kg bw (7.37 fL, $p = 0.004$) and 15 mg/kg bw (7.615 fL, $p = 0.037$) compared to control (8.30 fL).

The MANOVA results in Table 8 indicate a significant interaction between NaOCN dose and sex on hematological parameters, with a strong overall effect (Wilks' Lambda, $p < 0.001$, Partial $\eta^2 = 0.710$). Specifically, Neutrophils, MCV, MCHC, RCDW-CV, RCDW-SD, and MPV show significant sex-specific responses to NaOCN dose, with large effect sizes (Partial $\eta^2 = 0.456–0.691$). These findings suggest that NaOCN affects immune function (Neutrophils), red blood cell characteristics (MCV, MCHC, RCDW), and platelet size (MPV) differently in males and females, which could have implications for toxicology, pharmacology, or clinical applications.

The Turkey post-hoc test from Table 9 shows that the significant differences, particularly the lower MCH and MCHC at 3 mg compared to other doses, suggest that this dose may impair hemoglobin synthesis or red blood cell quality. Higher doses (4 mg, 5 mg) appear to restore or enhance these parameters, but the decline at 15 mg (e.g., MCHC lower than 4 mg and 5 mg) suggests a potential toxic or suppressive effect at high doses. These effects could relate to anemia, altered erythropoiesis, or hemoglobin metabolism, with sex-specific differences possibly due to hormonal or metabolic factors. Furthermore, the higher RDW-CV abs RDW-SD at 3 mg and 15 mg indicates increased red blood cell size variability, which could reflect stress on erythropoiesis or

bone marrow function. The lower RDW-CV at 4 mg, 5 mg, and 10 mg suggests more uniform red blood cell sizes at these doses. The lower MPV at 3 mg and higher MPV at 4 mg, 10 mg, and 15 mg suggest that NaOCN dose affects platelet size, potentially influencing clotting or inflammatory responses. Larger platelets (higher MPV) are often more reactive, which could have implications for thrombosis or vascular health. The sex-specific interaction (from the MANOVA, Partial η^2 = 0.684 for MCHC, 0.655 for RDW-CV, 0.691 for MPV) indicates that these effects are modulated by sex, possibly due to differences in metabolism, hormonal regulation, or baseline hematological profiles.

Table 7: Significant Tukey HSD Post-Hoc Comparisons for Hematological Parameters

Dependent Variable	Comparison	Mean Diff.	Std. Error	Sig.	95% CI (Lower - Upper)
MCH (pg)	Control vs 3 mg	-2.533*	0.604	0.003	(-4.4209, -0.6457)
	2 mg vs 3 mg	-1.9167*	0.604	0.045	(-3.8043, -.0291)
	3 mg vs 4 mg	2.5167*	0.604	0.003	(0.6291, 4.4043)
	3 mg vs 5 mg	2.1333*	0.604	0.018	(0.2457, 4.0209)
	3 mg vs 10 mg	1.9833*	0.604	0.034	(0.0957, 3.8709)
MCHC(g/dL)	Control vs 3 mg	-62.500*	18.688	0.029	(-120.9184, -4.0816)
	3 mg vs 4 mg	71.5000*	18.688	0.008	(13.0816, 129.9184)
	3 mg vs 5 mg	78.6667*	18.688	0.003	(20.2483, 137.0851)
RDW-CV	3 mg vs 5 mg	-0.0745*	0.0226	0.033	(-0.1452, -0.0038)
MPV (fL)	Control vs 3 mg	1.5167*	0.3738	0.004	(0.3482, 2.6851)
	15 mg vs Control	1.2167*	0.3738	0.037	(0.0482, 2.3851)

MCH-Mean Corpuscular Hemoglobin, MCHC- Mean Corpuscular Hemoglobin Concentration RDW-CV - Red Cell Distribution Width – Coefficient of Variation, MPV- Mean Platelet Volume, *Mean difference is significant at the 0.05 level.

Table 8: Two-way Multivariate Analysis of variance (ANOVA) for the effect of NaOCN dose on the Hematological parameters sex-wise

	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Wilks' lambda	F	df	Sig.	Partial Eta Squared
NaOCN * Sex	WBC	74.805	6	12.468	.820	.564	.150	0.001	2.269	6, 96	0.000	0.710
	Neutrophils	34.783	6	5.797	3.906	.006	.456					
	Lymphocytes	171.260	6	28.543	2.248	.068	.325					
	Monocytes	.027	6	.004	.713	.642	.133					
	Eosinophils	.004	6	.001	.837	.552	.152					
	RBC	18.803	6	3.134	.919	.496	.165					
	Hemoglobin	5941.286	6	990.214	.749	.616	.138					
	Hematocrit	.078	6	.013	1.002	.444	.177					
	MCV	1084.631	6	180.772	6.810	.000	.593					
	MCH	6.843	6	1.140	1.165	.353	.200					
	MCHC	25037.476	6	4172.913	10.106	.000	.684					
	RCDW–CV	.034	6	.006	8.873	.000	.655					
	RCDW– SD	6112.550	6	1018.758	9.657	.000	.674					
	Platelet	854130.667	6	142355.111	1.978	.103	.298					
	MPV	10.140	6	1.690	10.453	.000	.691					
	PDW	22.700	6	3.783	2.050	.092	.305					
	Plateletcrit (Thrombocrit)	47.741	6	7.957	1.301	.289	.218					

The 3 mg dose consistently emerges as a critical point, showing lower MCH, MCHC, and MPV, and higher RDW-CV and RDW-SD compared to other doses. This suggests a unique biological effect at this dose, possibly a threshold where NaOCN disrupts hematological homeostasis. Higher doses (4 mg, 5 mg, 10 mg) often

show opposite effects (e.g., higher MCH, MCHC, MPV, lower RDW-CV), indicating a non-linear dose-response relationship. The 15 mg dose shows a decline in some parameters (e.g., MCHC, higher RDW-CV), suggesting potential toxicity or diminished efficacy at higher doses.

Table 9: Significant Tukey HSD Post-Hoc Comparisons for Hematological Parameters based on NaOCN effects sex-wise

Dependent Variable	Comparison	Mean Diff.	Std. Error	Sig.	95% CI (Lower - Upper)
MCH (pg)	Control vs 3 mg	-2.533*	0.571	0.002	(-4.3457, -0.7210)
	2 mg vs 3 mg	-1.9167*	0.571	0.033	(-3.7290, -0.1043)
	3 mg vs 4 mg	2.5167*	0.571	0.002	(0.7043, 4.3290)
	3 mg vs 5 mg	2.1333*	0.571	0.013	(0.3210, 3.9457)
	3 mg vs 10 mg	1.9833*	0.571	0.025	(0.1710, 3.7957)
	15 mg vs 3 mg	-1.8000*	0.571	0.050	(-0.0124, 3.6124)
MCHC(g/dL)	Control vs 3 mg	-62.500*	11.732	0.000	(25.2842, 99.7158)
	2 mg vs 3 mg	-46.333*	11.732	0.008	(9.1175, 83.5492)
	3 mg vs 4 mg	71.5000*	11.732	0.000	(34.2842, 108.7158)
	3 mg vs 5 mg	78.6667*	11.732	0.000	(41.4508, 115.8825)
	3 mg vs 10 mg	45.6667*	11.732	0.009	(8.4508, 82.8825)
	4 mg vs 15 mg	43.8333*	11.732	0.013	(-81.0492, -6.6175)
	5 mg vs 15 mg	51.0000*	11.732	0.003	(-88.2158, -13.7842)
RDW-CV	Control vs 3 mg	0.0537*	0.015	0.015	(-0.0998, -0.0076)
	3 mg vs 4 mg	-0.0500*	0.015	0.027	(-0.0961, -0.0039)
	3 mg vs 5 mg	-0.0745*	0.015	0.000	(-0.1206, -0.0284)
	3 mg vs 10 mg	-0.0553*	0.015	0.011	(-0.1014, -0.0092)
	5 mg vs 15 mg	0.0490*	0.015	0.032	(0.0029, 0.0951)
RDW-SD	3 mg vs 5 mg	-27.200*	5.930	0.001	(-46.0107, -8.3893)
	5 mg vs 15 mg	20.350*	5.930	0.027	(1.5393, 39.1607)
MPV (fL)	Control vs 4 mg	0.8333*	0.232	0.019	((0.3970, 1.8697)
	Control vs 10 mg	1.1333*	0.232	0.001	(1.5393, 39.1607)
	Control vs 15 mg	1.2167*	0.232	0.000	(0.4803, 1.9530)
	3mg vs Control	-1.5167*	0.232	0.000	(-2.2530, -0.7803)
	3mg vs 2mg	-0.7833*	0.232	0.031	(-1.5197, -0.0470)
	3mg vs 5 mg	-0.9833*	0.232	0.004	(-1.7197, -0.2470)

MCH-Mean Corpuscular Hemoglobin, MCHC- Mean Corpuscular Hemoglobin Concentration RDW-CV - Red Cell Distribution Width – Coefficient of Variation, MPV- Mean Platelet Volume, *Mean difference is significant at the 0.05 level.

Dose-Response Analysis and Toxicological Thresholds

Probit and Weibull dose-response models Estimated Effective Concentration 50 (EC_{50}) values for hematological parameters affected by NaOCN exposure in Wistar rats (Table 10). White blood cell count (WBC) showed moderate sensitivity with a Probit EC_{50} of 4.213 mg/kg bw (AIC = 58.087) and Weibull EC^{50} of 4.382 mg/kg bw (AIC = 58.325) for all rats, with females more sensitive (Probit EC_{50} = 2.695 mg/kg bw) than males (Probit EC50 = 8.680 mg/kg bw). Hematocrit (HCT) exhibited high sensitivity (Probit EC_{50} = 0.198 mg/kg bw, AIC = 55.763), though the overall estimate (96.593 mg/kg bw) was unreliable due to model instability. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) also showed high sensitivity (Probit EC_{50} = 0.226 and 0.192 mg/kg bw, respectively), particularly in females (MCV: 0.107 mg/kg bw). Red blood cell count (RBC) had a moderate EC_{50} (Probit = 12.599 mg/kg bw, AIC = 50.417), with males more sensitive (5.854 mg/kg bw). Platelet count (PLT) and mean platelet volume (MPV) showed moderate sensitivity (PLT: 4.390 mg/kg bw; MPV: 10.496 mg/kg bw), with gender differences (e.g., MPV Female: 1.872 mg/kg bw). Models for eosinophils (Eos), monocytes (Mon, Male), hemoglobin (HGB, Male), and RBC (Female) failed due to insufficient response variability, reflecting low prevalence of abnormal values.

Table 10: Estimated Effective Concentration 50 (EC_{50}) of Sodium Cyanate (NaOCN) on Hematological Parameters in Wistar Rats Using Probit and Weibull Models Analysis

Parameter	Sex	Probit_EC_{50}	Probit_AIC	Weibull_EC_{50}	Weibull_AIC
WBC	All	4.213	58.087	4.382	58.325
WBC	Male	8.680	29.744	8.746	29.805
WBC	Female	2.695	29.446	2.812	29.644
Neu	All	0.261	55.604	0.198	55.599
Neu	Male	26.358	23.871	23.490	23.888
Neu	Female	2.506	30.728	2.540	30.333
Lym	All	3.866	59.570	3.986	59.653
Lym	Male	11.615	33.053	NA	33.057
Lym	Female	3.762	28.009	3.941	27.890
Mon	All	295176.508	25.388	NA	25.409
Mon	Male	NA	NA	NA	NA
Mon	Female	48.851	20.814	61.976	20.839
Eos	All	NA	NA	NA	NA
Eos	Male	NA	NA	NA	NA
Eos	Female	NA	NA	NA	NA
RBC	All	12.599	50.417	12.009	50.493
RBC	Male	5.854	27.515	5.861	27.563
RBC	Female	NA	24.443	NA	24.445
HGB	All	0.948	11.639	0.955	11.640
HGB	Male	NA	NA	NA	NA
HGB	Female	1.046	7.819	1.034	7.820
HCT	All	96.593	55.763	78.232	55.764
HCT	Male	4.319	32.738	4.273	32.710
HCT	Female	NA	21.224	NA	21.224
MCV	All	0.226	53.841	0.160	53.852
MCV	Male	0.669	31.725	0.590	31.712
MCV	Female	0.107	24.188	0.007	24.261
MCH	All	1.890	62.016	1.239	62.028
MCH	Male	0.228	33.062	NA	33.063
MCH	Female	1.531	32.402	1.513	32.391
MCHC	All	0.192	33.614	0.111	33.660
MCHC	Male	1.046	7.819	1.034	7.820
MCHC	Female	14.878	19.997	13.629	20.021
PLT	All	4.390	61.557	4.390	61.575
PLT	Male	2.510	30.711	2.567	30.882
PLT	Female	3.358	26.289	3.220	25.489
MPV	All	10.496	61.099	11.707	61.129
MPV	Male	4.565	30.476	4.871	30.778
MPV	Female	1.872	32.178	1.698	32.237

AIC = Akaike Information Criterion (lower indicates better fit).

The EC_{50} estimates confirm NaOCN's dose-dependent hematological effects, with low EC_{50} values for HCT (0.198 mg/kg bw), MCV (0.226 mg/kg bw), and MCHC (0.192 mg/kg bw) indicating high sensitivity at low

doses, this could be associated with likely carbamylation-induced changes in erythrocyte function (Pieniążek and Gwoździński, 2003; Smykiewicz *et al*., 2025). Moderate EC_{50} values for WBC (4.213 mg/kg bw) and PLT (4.390 mg/kg bw) suggest immune and platelet effects at mid-range doses, consistent with NaOCN impact on leukocyte production and thrombopoiesis (Jesri *et al*., 2005; Kuter and Gernsheimer, 2009).

Gender variability was evident, with females showing greater sensitivity for WBC (2.695 vs. 8.680 mg/kg bw), Neu (2.506 vs. 26.358 mg/kg bw), MCV (0.107 vs. 0.669 mg/kg bw), and MPV (1.872 vs. 4.565 mg/kg bw), possibly due to sex-specific differences in hemoglobin metabolism or immune response (Arnold *et al*., 2022; Feng *et al*., 2024). Males were more sensitive for RBC (5.854 mg/kg bw) and PLT (2.510 vs. 3.358 mg/kg bw). Model failures for Eos, Mon (Male), and HGB (Male) highlight limitations in analyzing rare cell types, as eosinophils and monocytes have low baseline counts in rats (Delwatta *et al*., 2018). Unrealistic EC_{50} values (e.g., Mon All: 295,177 mg/kg bw, HCT All: 96.593 mg/kg bw etc.) suggest that the tested dose range (0–20 mg/kg bw) was insufficient to capture full dose-response curves for some parameters. The small sample size (n=3 per sex per dose) likely contributed to model instability, as seen in high AIC values (e.g., HCT All: 55.763) and failed fits (Brewer *et al*., 2016; Olofsen and Dahan, 2013).

Lethal Dose Analysis

Probit and Weibull dose-response models estimated the lethal doses (LD_{50} and LD_{90}) for NaOCN in Wistar rats after 7 days of administration (**Table 11**). The Probit model estimated an LD_{50} of 13.75 mg/kg bw (95% CI: 9.58-17.92) and LD_{90} of 27.06 mg/kg bw (95% CI: 11.03-43.09), with an AIC of 16.59. The Weibull model yielded similar results, with an LD_{50} of 13.13mg/kg bw (95% CI: 7.09-19.17) and LD_{90} of 47.67mg/kg bw (95% CI: 100.89-100.87), with an AIC of 17.88. The dose-response curve (Fig. 1) shows a steep increase in mortality from 0% at 4 mg/kg bw to 100% at 20 mg/kg bw, indicating a narrow toxicity window.

Table 11: LD_{50} and LD_{90} Estimates for NaOCN in Wistar Rats Using Probit and Weibull Models

Model	LD_{50} (mg/kg bw)	95% CI (LD_{50})	LD_{90} (mg/kg bw)	95% CI **(**LD_{90})	AIC
Probit	13.75	9.58-17.92	27.06	11.03-43.09	16.59
Weibull	13.13	7.09-19.17	47.67	10.89-21.87	17.88

LD_{50} and LD_{90} represent doses causing 50% and 90% mortality, respectively. CI = confidence interval. AIC = Akaike Information Criterion (lower indicates better fit).

The LD_{50} of approximately 13.75 mg/kg bw indicates moderate acute toxicity of NaOCN in Wistar rats, consistent with its hematological effects observed at lower doses (e.g., HCT EC_{50} = 0.198 mg/kg bw) (Smykiewicz *et al*., 2025). The LD_{90} (27.06 mg/kg bw) aligns with 100% mortality at 20 mg/kg bw, suggesting a steep dose-response curve and a narrow safety margin. Mortality increases with dose, likely due to NaOCN's disruption of hemoglobin function and subsequent hypoxia (Allen *et al*., 2009; Storz and Moriyama, 2008). Compared to EC_{50} values for hematological parameters (e.g., WBC: 4.213 mg/kg bw), the higher LD_{50} indicates that sublethal hematological changes precede mortality, critical for establishing safe therapeutic doses (Adel *et al*., 2016; Borgert *et al*., 2021).

The similar AIC values for Probit (16.59) and Weibull (17.88) models suggest both adequately describe NaOCN's lethality, with Probit slightly preferred due to lower AIC (Burnham and Anderson, 2002; Chave *et al*., 2005; da Silva Scaranello *et al*., 2012). The small sample size (n=6 per group) may widen confidence intervals.

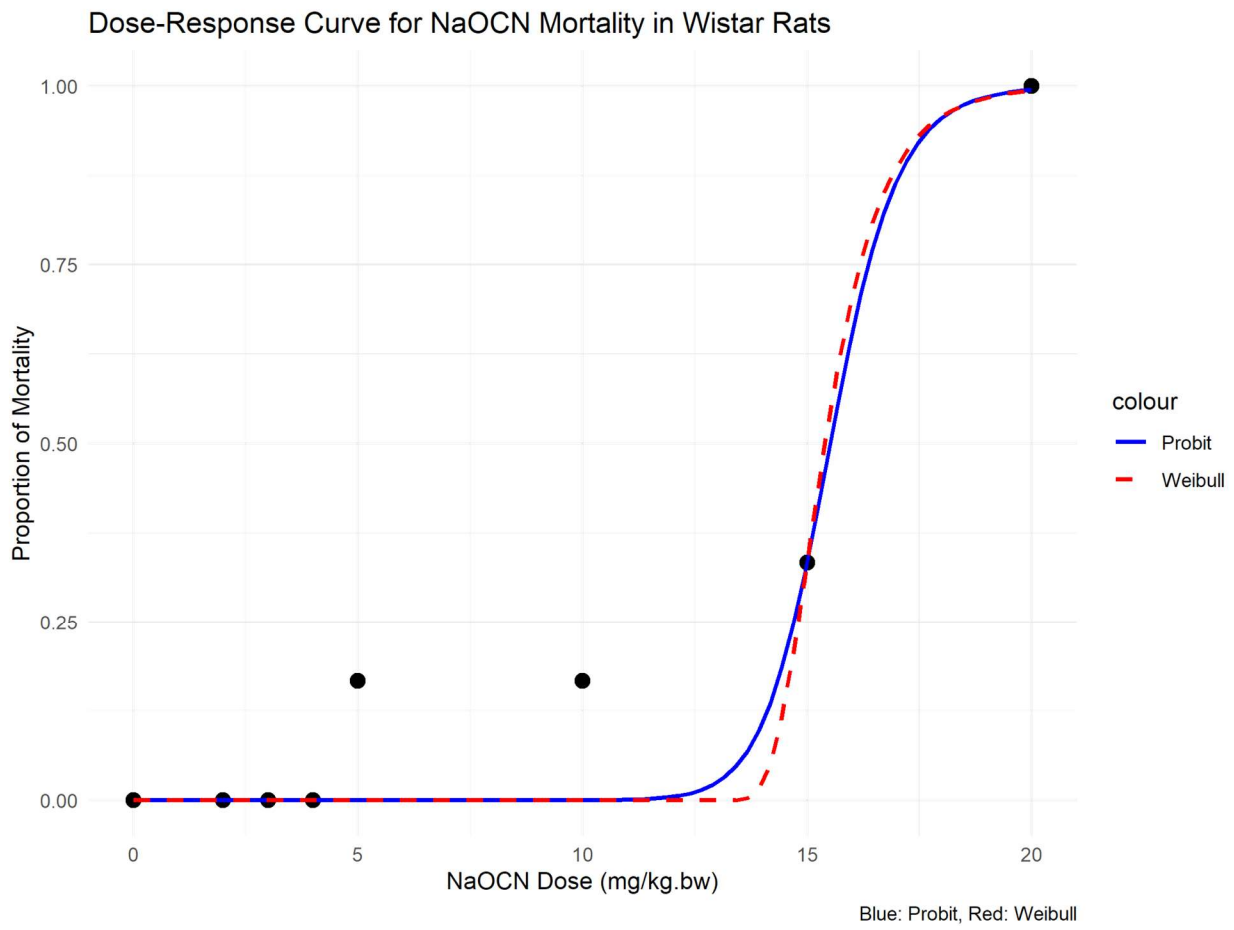

Fig. 1: Dose-response curve for NaOCN mortality in Wistar rats, showing Probit (blue) and Weibull (red) fits

Sex-specific analysis from Table 12 revealed females were more sensitive (Probit LD_{50}: 12.10 mg/kg bw, 95% CI: 5.77-18.44) than males (LD_{50}: 15.27 mg/kg bw, 95% CI: 10.03–20.51). Dose-response curves (Fig. 2) show a steep mortality increase from 0% at 4 mg/kg bw to 100% at 20 mg/kg bw, with females showing earlier mortality (5–10 mg/kg bw). Female sensitivity (LD_{50}: 12.10 mg/kg bw) suggests sex-specific metabolic or immune differences, aligning with prior EC_{50} findings (e.g., WBC Female: 2.695 mg/kg bw)

Table 12: Sex-Specific LD_{50} and LD_{90} Estimates for NaOCN in Wistar Rats Using Probit and Weibull Models

Sex	Model	LD_{50} (mg/kg bw)	95% CI (LD_{50})	LD_{90} (mg/kg bw)	95% CI (LD_{90})	AIC
Male	Probit	15.27	10.03-20.51	16.16	-6.64-38.95	5.62
	Weibull	15.23	12.84-17.62	16.19	3.78-28.60	5.62
Female	Probit	12.10	5.77-18.44	29.99	-1.13-61.11	13.32
	Weibull	11.16	3.86-18.46	45.08	-29.28-119.44	13.31

LD_{50} and LD_{90} represent doses causing 50% and 90% mortality, respectively. CI = confidence interval. AIC = Akaike Information Criterion (lower indicates better fit).

The LD_{50} estimates (Male: ~15.2 mg/kg bw, Female: ~11.2–12.1 mg/kg bw) confirm NaOCN moderate acute toxicity in Wistar rats, with females more susceptible. This aligns with prior EC_{50} findings, where females showed lower EC_{50} values for hematological parameters (e.g., WBC: 2.695 mg/kg bw vs. Male: 8.680 mg/kg bw; Neu: 2.506 vs. 26.358 mg/kg bw). The higher female sensitivity may reflect sex-specific differences in hemoglobin carbamylation or metabolic clearance, potentially influenced by hormonal factors (Murphy,

2014; Shi *et al.*, 2015). The LD_{50} values are higher than most EC_{50} values (e.g., HCT: 0.198 mg/kg bw, MCV: 0.226 mg/kg bw), indicating that hematological abnormalities precede lethality, critical for therapeutic safety in sickle cell disease applications (Demirci *et al.*, 2018; McGann *et al.*, 2013).

The LD_{90} estimates are less reliable due to wide CI and unrealistic values (e.g., Female Probit: 29.99 mg/kg bw), as 100% mortality occurs at 20 mg/kg bw for both sexes. The steep dose-response curve (0% at 4 mg/kg bw to 100% at 20 mg/kg bw) suggests a narrow therapeutic window, consistent with NaOCN mechanism of disrupting hemoglobin function and causing hypoxia (Cerami *et al.*, 1973; Glader and Conrad, 1972). The earlier female mortality (1/3 at 5–10 mg/kg bw vs. 0/3 for males) underscores gender-specific risks, necessitating sex-stratified safety assessments.

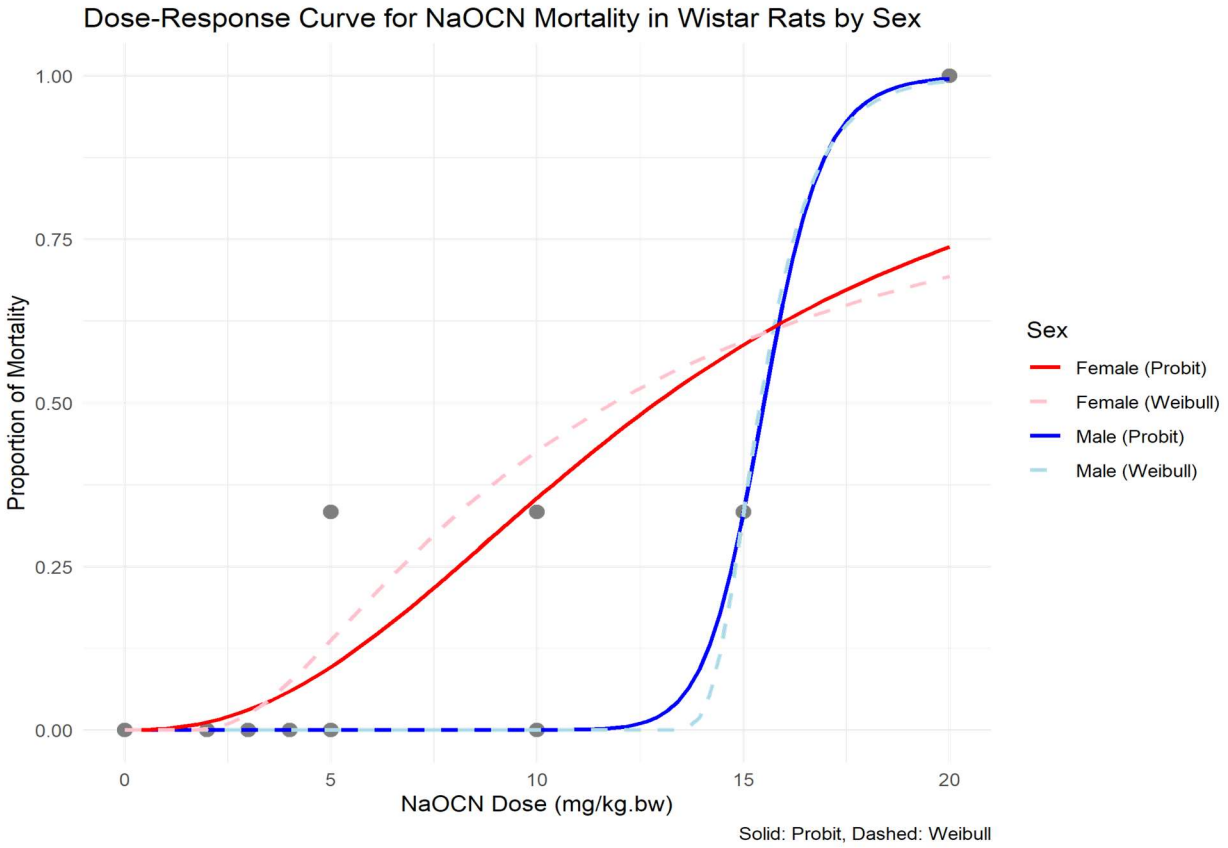

Fig.2: Dose-response curve for NaOCN mortality in Wistar rats by sex

CONCLUSION

This study elucidates the dose-dependent hematological effects of sodium cyanate (NaOCN) in adult Wistar rats, revealing significant gender variability and establishing toxicological thresholds critical for its safe use, particularly in pharmacological contexts like sickle cell disease treatment. The two-way MANOVA demonstrated a robust NaOCN dose-sex interaction (Wilks' Lambda=0.001, $p<0.001$, Partial $\eta^2=0.710$), indicating that NaOCN's impact on hematological parameters varies significantly between males and females. Notably, neutrophils, MCV, MCHC, RDW-CV, RDW-SD, and MPV exhibited significant sex-specific responses ($p<0.05$, Partial $\eta^2=0.456–0.691$), with the 3 mg/kg bw dose emerging as a critical empirical inflection point. At this dose, MCH, MCHC, and MPV were lower, and RDW-CV and RDW-SD were higher compared to control and other doses (e.g., 4 mg, 5 mg), suggesting disrupted hemoglobin synthesis, erythrocyte morphology, and platelet function. Higher doses (4–10 mg/kg bw) often reversed these effects, while 15 mg/kg bw indicated potential toxicity (e.g., lower MCHC, higher RDW-CV), consistent with a non-linear dose-response relationship.

Females displayed greater sensitivity, with lower EC_{50} values for WBC (2.695 vs. 8.680 mg/kg bw), neutrophils (2.506 vs. 26.358 mg/kg bw), MCV (0.107 vs. 0.669 mg/kg bw), and MPV (1.872 vs. 4.565 mg/kg bw), likely due to sex-specific differences in hemoglobin

metabolism or immune response, which could be suggessted to be influenced by hormonal factors. Probit and Weibull models estimated an LD_{50} of ~13.75 mg/kg bw and LD_{90} of ~27.06 mg/kg bw, with females more susceptible (LD_{50}: 12.10 vs. male 15.27 mg/kg bw). The steep dose-response curve (0% mortality at 4 mg/kg bw to 100% at 20 mg/kg bw) highlights a narrow therapeutic window, where sublethal hematological changes (e.g., HCT EC_{50}: 0.198 mg/kg bw) precede lethality, emphasizing the importance of low-dose monitoring.

These findings suggest that NaOCN's carbamylation of hemoglobin and erythrocyte membrane proteins induces dose-dependent hematological abnormalities, including macrocytosis, altered hemoglobin concentration, and reactive thrombocytosis, particularly in females. Future research should explore sex-specific mechanisms, such as hormonal influences on erythropoiesis or thrombopoiesis, and conduct longitudinal studies to assess chronic exposure effects. Given NaOCN's environmental and occupational exposure risks, these results advocate for sex-stratified toxicological assessments and cautious dose optimization in therapeutic applications to mitigate hematological toxicity while maximizing efficacy.

AUTHORS' CONTRIBUTION

All authors made substantial contributions to the conception, design, and execution of the study. Data collection was carried out collaboratively across the various research locations. Each author participated in the critical review of the manuscript and approved the final version for submission, taking collective responsibility for the integrity and accuracy of the work.

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CONFLICT OF INTEREST

The authors declare that there are no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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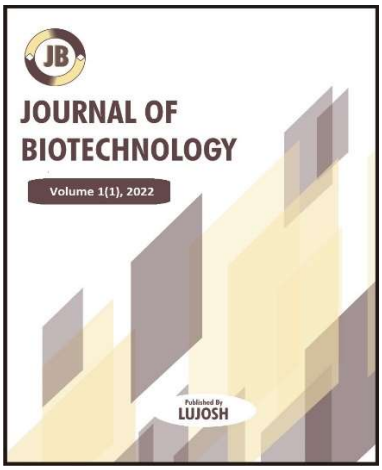

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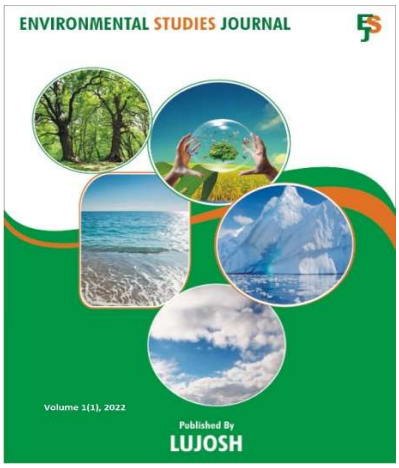

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