RESEARCH ARTICLE

Biphasic haematological response to nitrocellulose thinner vapour exposure and withdrawal in male Wistar rats

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ABSTRACT

Background: Nitrocellulose thinner, a commonly used industrial solvent mixture, contains aromatic hydrocarbons, which are known to have toxicological effects.

Objective: There is a dearth of information on haematological effects due to subchronic exposure and the extent of recovery following withdrawal. In this study, the effects of subchronic inhalation of nitrocellulose thinner vapour (NCTV) and its withdrawal on haematological parameters in male Wistar rats were evaluated.

Methods: Twenty-four male rats divided into 3 groups (n=8) were used for the study. Group I (control, no exposure), Group II (90-day NCTV exposure), Group III (90-day exposure with 90-day withdrawal). Haematological indices (white blood cell (WBC), red blood cell (RBC), platelet (PLT) counts, haemoglobin (HGB), haematocrit (HCT), and erythrocyte indices (MCH and MCHC) were evaluated using automated haematological analysis.

Results: Exposure to NCTV led to a significant ($p < 0.05$) increase in WBC count (19.85 ± 7.64). Following withdrawal, WBC levels reduced (13.56 ± 4.30). In contrast, there was no significant ($p < 0.05$) change in RBC and PLT counts due to exposure, but withdrawal led to a significant ($p < 0.05$) decline in both parameters. Also, MCH and MCHC were not significantly affected due to exposure, but withdrawal led to a significant ($p < 0.05$) increase in both parameters.

Conclusion: Subchronic NCTV exposure induces a biphasic haematological response characterised by transient inflammation and delayed suppression of erythropoiesis and thrombopoiesis. These findings highlight the persistence of toxic effects beyond exposure and underscore the importance of post-exposure monitoring in solvent-exposed populations.

Key words: Haematotoxicity; Nitrocellulose thinner; vapour inhalation; Wistar rats; Withdrawal

Introduction

Nitrocellulose thinner is a widely used industrial solvent which has been used in painting, wood finishing and automotive spraying. This widespread application has raised occupation health concern. Nitrocellulose thinner is a complex mixture of volatile organic compounds (VOCs) the most common of which include toluene, xylene, and various esters, are recognized systemic toxicants (David & Niculescu, 2021; Li et al., 2021). These compounds have lipophilic properties that allow their easy diffusion across biological membranes when inhaled. This thereby results in widespread physiological disturbances in both humans and experimental animals (Khajeh Hoseini et al., 2022; Horvat et al., 2025). In Nigeria, automobile spray painters and other artisans are particularly at risk of chronic exposure, predisposing them to various systemic toxicities, including haematological alterations (Ogbodo et al., 2024).

The haematopoietic system is highly susceptible to toxic insults from VOC exposure. The bone marrow, which supports haematopoietic stem and progenitor cells, is highly vulnerable to solvent-induced genotoxicity and metabolic strain, which the literature frequently associates with oxidative stress pathways (Mathialagan et al., 2020; Vaughan Watson et al., 2021). The disruption of this system may result in myelosuppression and measurable alterations in haematological indices such as red blood cell (RBC) count, haemoglobin concentration (Hb), packed cell volume (PCV), white blood cell (WBC) count, and platelet (PLT) levels (Park

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& Chang,2025). These indices serve as critical biomarkers for evaluating physiological and pathological changes associated with toxicant exposure.

Reports of experimental as well as epidemiological studies have shown that exposure to solvents such as toluene and xylene induces significant changes in both erythrocytic and leukocytic parameters. For instance, leukocytosis is commonly observed following subchronic exposure, reflecting systemic inflammation and immune activation, while reductions in erythrocytic indices may indicate impaired erythropoiesis (Yang et al., 2025; Mank et al., 2026). Also, disrupted thrombopoiesis may be manifested as alterations in platelet counts, even in the absence of obvious clinical manifestations (Šebeková et al., 2024).

Although there has been an increase in reports of VOC toxicity, the reversibility of haematological alterations following cessation of exposure remains poorly understood. The concept of recovery or reversibility is crucial in toxicological and occupational health assessments. While some haematological parameters may return to baseline levels after withdrawal, others may exhibit delayed or persistent alterations, a phenomenon referred to as delayed toxicity (Hamdaoui et al., 2019; Mrong et al., 2021). Such effects may be attributed to long-term structural or epigenetic changes within the bone marrow, thereby affecting haematopoietic stem cell function (Kim et al., 2024).

Therefore, the present study investigates the effects of subchronic exposure to nitrocellulose thinner vapour (NCTV) on selected haematological indices in male Wistar rats. Furthermore, it evaluates the extent of recovery following a 90-day withdrawal period, with the aim of providing insights into the reversibility and long-term implications of solvent-induced haematotoxicity.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Ethylene-diaminetetraacetic acid (EDTA) sample bottles were used for blood collection.

Nitrocellulose Thinner

Nitrocellulose thinner (NCT) of the Abro® brand was purchased from a chemical retail outlet located at Timber Market, Marian Road, Calabar, Cross River State, Nigeria.

Experimental Animals and Study Design

Twenty-four (24) male Wistar rats weighing 100–110 g were obtained from the Animal House, Department of Biochemistry, University of Calabar, Nigeria. The animals were housed in standard laboratory cages under controlled environmental conditions (temperature: 25 ± 2°C; relative humidity: 50–62%; 12 h light/12 h dark cycle) and allowed free access to standard feed and water *ad libitum*.

The study was conducted over a period of approximately six (6) months, comprising a two-week acclimatization period, a 90-day exposure phase, and a 90-day withdrawal (recovery) phase.

The animals were randomly assigned into three groups (n = 8 per group):

- **Group I (Control):** No exposure to NCTV
- **Group II (Exposure):** Exposed to NCTV for 90 days
- **Group III (Recovery):** Exposed to NCTV for 90 days followed by a 90-day withdrawal period

Haematological analyses were conducted at the end of the exposure and recovery periods, respectively.

Ethical Approval

All experimental procedures were carried out in accordance with internationally accepted guidelines for the care and use of laboratory animals. Ethical approval was obtained from the Faculty Research Animal Ethics Committee (FAREC-FBMS), Faculty of Basic Medical Sciences, University of Calabar, Nigeria (Approval No.: 418BCM2628; Approval Date: 5 March 2023). Efforts were made to minimize animal suffering and to reduce the number of animals used.

Mode of Exposure

Exposure to nitrocellulose thinner vapour (NCTV) was carried out using a modified whole-body inhalation method as previously described by Uboh et al. (2005). The exposure chambers consisted of locally fabricated glass-enclosed compartments (150 cm × 90 cm × 210 cm).

Two highly perforated 1000 mL containers, each containing 500 mL of nitrocellulose thinner, were placed within the chamber to allow passive volatilization. Animals were exposed to the vapour for 4 hours daily (9:00 am to 1:00 pm).

The chambers were adequately ventilated to ensure uniform vapour distribution and to prevent excessive accumulation. After daily exposure, animals were transferred to a fume-free section of the animal facility.

Collection of blood samples

Twenty-four (24) hours after the final exposure and starvation, the rats were anaesthetized with chloroform. Blood was collected by cardiac puncture into well-labelled EDTA-treated sample bottles for haematological assays.

Estimation of haematological indices

The collected blood samples in EDTA tubes were immediately gently inverted to ensure proper mixing and prevent coagulation. Automated haematological analysis was performed within two hours of collection to determine the red blood cell (RBC) count, haemoglobin (Hb) concentration,

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packed cell volume (PCV), total white blood cell (WBC) count and platelet (PLT) levels. These haematological parameters were determined using the Sysmex Automated Haematological Analyzer (KX-21N).

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

Results

Impact of withdrawal of NCTV exposure on haematological parameters of male Wistar rats

The effects of subchronic exposure to nitrocellulose thinner vapour (NCTV) and subsequent withdrawal on haematological parameters are presented in Tables 1–3.

Table 1. *Effects of NCT Exposure and Withdrawal on Primary Heamatological parameters*

Parameters	Control	NCTV 90-day Exposure	NCTV 90-day withdrawal
WBC (10^3/µL)	11.12 ± 2.68	19.85 ± 7.64 *	13.56 ± 4.30 [a]
RBC (10^6/µL)	8.45 ± 1.24	8.52 ± 0.56	7.54 ± 0.90 *,[a]
HGB (g/dL)	14.10 ± 1.90	14.05 ± 1.04	13.98 ± 1.56
HCT (%)	50.68 ± 3.70	50.52 ± 5.30	50.35 ± 3.84

Values are expressed as mean ± SD (n = 8)
** Significantly different from control (p < 0.05)*
[a] Significantly different from NCTV 90-day exposure group (p < 0.05)

As shown in Table 1, exposure to NCTV for 90 days resulted in a significant increase ($p < 0.05$) in white blood cell (WBC) count (19.85 ± 7.64) compared with the control group, with values rising by approximately two-fold. Following withdrawal, WBC levels decreased (13.56 ± 4.30) significantly ($p < 0.05$) relative to the NCTV-exposed group, returning toward control values, with no significant difference ($p > 0.05$) observed when compared with the control group

In contrast, no significant changes ($p > 0.05$) were observed in red blood cell (RBC) count, haemoglobin (HGB), or haematocrit (HCT) following 90-day NCTV exposure compared with control values. However, after withdrawal, a significant reduction ($p < 0.05$) in RBC count was recorded compared with both the control and the NCTV-exposed groups. The changes observed in HGB and HCT following withdrawal were not statistically significant ($p > 0.05$).

Table 2. *Effects of NCTV Exposure and Withdrawal on Platelet Count of Wistar rats*

Parameter	Control	NCTV 90-day Exposure	NCTV 90-day Withdrawal
PLT 10^3/µL	668.50 ± 156.80	658.12 ± 139.70	456.45 ± 24.60*,[a]

Values are expressed as mean ± SD (n = 8)
** Significantly different from control (p < 0.05)*
[a] Significantly different from NCTV 90-day exposure group (p < 0.05)

Table 3. *Effects of NCTV Exposure and Withdrawal on Red Blood Cell Indices*

Parameters	Control	NCTV 90-day Exposure	NCTV 90-day Withdrawal
MCV (fL)	58.95 ± 4.10	58.02 ±6.40	56.05 ± 3.09
MCH (pg)	16.52 ± 0.90	16.42 ± 0.30	18.45 ± 1.00*,[a]
MCHC (g/dL)	27.45 ± 1.30	28.22 ± 2.70	33.05 ± 1.10*,[a]

Values are expressed as mean ± SD (n = 8)
** Significantly different from control (p < 0.05)*
[a] Significantly different from NCTV 90-day exposure group (p < 0.05)

Platelet (PLT) counts (Table 2) were not significantly altered ($p > 0.05$) after 90-day NCTV exposure when compared with the control group. However, a significant decrease ($p < 0.05$) in PLT count was observed in the withdrawal group relative to both the control and the NCTV-exposed groups.

The red blood cell indices are presented in Table 3. No significant differences ($p > 0.05$) were observed in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), or mean corpuscular haemoglobin concentration (MCHC) following NCTV exposure compared with control values. However, in the withdrawal group, MCH and MCHC values were significantly increased ($p < 0.05$) compared with both the control and NCTV-exposed groups, while MCV remained unchanged.

Exposure to NCTV did not cause any statistically significant ($p > 0.05$) changes in the red blood cells (RBC) count, haemoglobin (HGB) level and haematocrit (HCT) to the rats in the NCTV 90-day exposure when compared, with those obtained for rats in the control group. While withdrawal from exposure caused a significant ($p < 0.05$) reduction in RBC for the NCTV withdrawal group compared with the control and the NCTV 90-day exposure group, respectively, the changes recorded for the HGB level and the HCT were not significant ($p > 0.05$) when compared to the control.

Discussion

The haematopoietic system is highly sensitive to toxicological insults and serves as a critical indicator of systemic physiological status. In the present study, subchronic inhalation of nitrocellulose thinner vapour (NCTV) produced a biphasic haematological response. First during exposure,

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there was an inflammatory activation. However, the withdrawal phase showed a suppression of both the erythropoietic and thrombopoietic pathways. These findings suggest that even after the cessation of exposure, the toxic effects within the bone marrow are not immediately terminated. This phenomenon is consistent with reports of xenobiotic-induced haematological alterations following toxicant exposure (Carmona-Aparicio et al., 2025).

The significant elevation in white blood cell (WBC) count observed following 90-day exposure is indicative of systemic inflammation. NCTV contains aromatic hydrocarbons such as toluene and xylene, which are components of the benzene–toluene–xylene (BTX) group known to induce oxidative stress and inflammatory responses in exposed individuals (Zhang et al., 2022). Occupational and experimental studies have shown that BTX exposure is associated with alterations in WBC and lymphocyte profiles. These alterations reflect immune activation and hypersensitivity manifestations (Cordiano et al., 2022). A recent study further confirms that BTEX exposure is associated with significant alterations in haematological markers, reinforcing the clinical relevance of these findings (Parsarad et al., 2025). The leukocytosis observed in this study is therefore consistent with solvent-induced inflammatory responses. Importantly, the subsequent normalization of WBC levels following withdrawal suggests that while the primary inflammatory stimulus is transient and reversible upon removal of the toxicant, the underlying haematopoietic suppression may persist.

Interestingly, RBC and PLT counts showed no significant changes during the active exposure phase, dropping only after toxicant withdrawal. This delayed onset points to a latent haematotoxic effect, which could stem from cumulative damage to the haematopoietic system over the 90-day period. The stable blood counts observed during initial exposure were likely sustained by systemic physiological compensation, which temporarily offset early toxicity to maintain normal circulating cell levels.

This trend suggests that while circulating cell levels are initially maintained during active exposure, the post-exposure phase reveals systemic strain that eventually limits normal erythropoiesis and thrombopoiesis. Specifically, the significant reduction in platelet counts during withdrawal points to an impairment in platelet production kinetics. Aromatic hydrocarbons are known to affect the broader haematopoietic framework responsible for regulating cellular development (Stone et al., 2022). This explains why thrombocytopenia became evident only after exposure ceased, indicating that the toxic impact on platelet production is cumulative and delayed rather than acute (Noh, 2021). Because platelets play an important role in overall vascular health, their prolonged suppression during withdrawal could have systemic consequences extending beyond basic haemostasis (Kim & Lee, 2025).

Interestingly, the elevation of Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) during the withdrawal phase indicates a compensatory erythrocytic response. Despite a reduction in RBC count, haemoglobin (HGB) and haematocrit (HCT) remained relatively stable, suggesting that individual erythrocytes contained increased haemoglobin content to maintain oxygen-carrying capacity. Alternatively, increased MCHC may reflect alterations in erythrocyte membrane integrity or cellular dehydration associated with oxidative stress, as observed in experimental models of BTX-induced toxicity (Sayed et al., 2023) and in human populations exposed to BTX where oxidative stress plays a mediating role (Zhou et al., 2024). Therefore, the delayed erythrocytic and thrombopoietic suppression observed during withdrawal likely stems from persistent oxidative damage inflicted upon the hematopoietic stem cell microenvironment during the active exposure phase.

Collectively, the observed pattern of reduced RBC and PLT counts alongside increased MCH and MCHC during withdrawal highlights the persistence of haematological alterations beyond the exposure period. These findings support the concept of delayed toxicity; whereby adverse effects continue to evolve even after removal of the causative agent. From an occupational health perspective, this suggests that haematological assessments conducted during active exposure may underestimate the true extent of toxicity, emphasizing the need for post-exposure monitoring in solvent-exposed populations. While the present study is limited by the lack of bone marrow biopsy and peripheral blood smear morphological data, these parameters represent crucial avenues for future follow-up studies to fully elucidate the structural dimensions of this delayed myelotoxicity.

Conclusion

Subchronic inhalation of nitrocellulose thinner vapour (NCTV) induces a distinct biphasic haematological response, characterized by an initial inflammatory phase marked by leukocytosis, followed by a latent toxic phase during withdrawal with significant reductions in red blood cell and platelet counts. The concurrent elevation of MCH and MCHC suggests a compensatory, yet insufficient, erythrocytic adaptation to underlying haematopoietic suppression.

These findings indicate that NCTV-induced myelotoxicity persists and evolves beyond active exposure, such that assessments conducted solely during exposure may underestimate the true extent of haematological damage. This delayed toxicity is intimately coupled with systemic oxidative stress pathways, which continue to compromise cellular integrity post-exposure. Accordingly, extended post-exposure

monitoring is essential for accurately evaluating solvent-induced toxicity in occupational settings.

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