RESEARCH PAPER

The Influence of Different Doses of Melatonin on Bone Marrow Cellularity in Benzene-Induced Pre-Leukemia in Rats

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ABSTRACT

Background: Benzene is a prototypical marrow toxicant that induces pre-leukemic changes through cytotoxic and genotoxic injury. Melatonin exhibits antioxidant and hematoprotective actions, but its dose-response effects on marrow cellularity and genotoxicity under benzene stress require clarification. **Materials and Methods:** Rats were randomized to six groups: control; benzene only (0.2 mL; benzene:2-propanol: distilled water = 1:5:5, tail-vein, every 48 h for 4 weeks); and benzene plus oral melatonin at 5, 10, 20, or 40 mg/kg/day for 4 weeks. Outcomes included bone-marrow micronucleus metrics, polychromatic erythrocyte (%PCE), normochromatic erythrocyte (%NCE), micronucleate polychromatic erythrocyte (%MN-PCE), micronucleate normochromatic erythrocyte (%MN-NCE), PCE/NCE ratio, complete blood counts with differentials and derived ratios, PLT indices, and blast percentage. **Results:** Benzene caused marked erythroid cytotoxicity and genotoxicity, decreasing %PCE and PCE/NCE while increasing %NCE, %MN-PCE, and %MN-NCE (e.g., %MN-PCE 0.5% → 3.7%; PCE/NCE 0.90 → 0.07; ****$p < 0.0001$). Melatonin dose-dependently corrected these changes, with 20 mg/kg yielding near-physiologic values (%MN-PCE 0.7%, %MN-NCE 0.08%, PCE/NCE 0.73) and no additional gain at 40 mg/kg. Benzene reduced WBC, LYM, MON and increased NEU, BAS and circulating blasts (35.0% vs. 0% control; ***$p < 0.001$); melatonin improved leukocyte profiles and suppressed blasts (12.63% at 20 mg/kg; 5.88% at 40 mg/kg; ****$p < 0.0001$). PLT morphology/function were also rescued (MPV, PDW, PLCC), while SII rose at higher doses consistent with platelet rebound. **Conclusions:** Melatonin provides robust, dose-responsive hematoprotection against benzene-evoked pre-leukemic injury, with an efficacy plateau suggesting 20 mg/kg as the optimal dose in this model.

Keywords: Benzene-induced pre-leukemia; Bone marrow cellularity; Melatonin; Blast Cell

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INTRODUCTION

Pre-Leukemia is a group of hematological malignancies characterized by clonal proliferation of abnormal blood-forming cells in the bone marrow and blood. Among these, acute myeloid leukemia (AML) is a rapidly progressing cancer of the myeloid lineage that predominantly affects adults and carries a poor prognosis (Zhou et al., 2024). Globally, the burden of AML has been rising; the incidence has increased significantly over the past decades, reflecting an urgent need for improved preventive and therapeutic strategies (Zhou et al., 2024). AML pathogenesis is characterized by the buildup of immature myeloblasts in the bone marrow, which displace regular hematopoietic cells and cause bone marrow failure (Bispo et al., 2020). Clinically, this marrow replacement is associated with anemia, neutropenia, and thrombocytopenia with or without leukocytosis, owing to the increased burden of leukemic blasts (Rehan et al., 2023). Despite the progress made in treatment, AML outcomes remain unsatisfactory, motivating research into its causes and modifiers. Benzene is a well-established environmental and occupational carcinogen that has been strongly linked to pre-leukemia, particularly AML (Bispo et al., 2020; Zhou et al., 2024). Chronic benzene exposure (e.g., in petrochemical industries or via cigarette smoke) is causally associated with bone marrow toxicity and an increased risk of hematological malignancies (Zhou et al., 2024; Spatari et al., 2021). Benzene's toxic metabolites (such as benzoquinones and phenolic compounds) induce genotoxic damage, oxidative stress, and bone marrow microenvironmental alterations that drive leukemogenesis (Spatari et al., 2021; Abou Elazab et al., 2022). In both humans and experimental animal models, benzene causes a continuum of hematopoietic injury: initial bone marrow hypocellularity and aplastic anemia can progress to myelodysplasia and eventually AML with clonal blast proliferation (Abou Elazab et al., 2022). Rodent studies have established a benzene-induced leukemia model that recapitulates key features of AML, including marked reduction in normal bone marrow cellularity and the appearance of undifferentiated blast cells in marrow and peripheral blood (Rehan et al., 2023; Abou Elazab et al., 2022). These models allow controlled investigation of hematological parameters during leukemogenesis and are useful for testing chemopreventive interventions against benzene's effects.

In the experimental benzene-induced pre-leukemia, several

hematological markers and indices serve as indicators of disease progression and severity. Bone marrow cellularity refers to the proportion of hematopoietic cells filling the marrow space; in leukemia, the marrow typically becomes hypercellular with blasts, although benzene toxicity may initially cause hypocellularity before malignant transformation (Abou Elazab et al., 2022). Blast cell count, the percentage of immature leukemic cells in bone marrow or blood, is a defining feature of AML (typically >20% blasts in marrow) and correlates with disease burden (Rehan et al., 2023). Complete blood count (CBC) parameters are profoundly altered in leukemia and benzene toxicity: red blood cell counts and hemoglobin drop due to anemia, platelets decrease from thrombocytopenia, and white blood cell counts may be aberrantly high or low with shifts in differential counts (Abou Elazab et al., 2022).

Importantly, the balance of leukocyte subsets can be summarized by ratios like the neutrophil-to-lymphocyte ratio (NEU-LYM) ratio and lymphocyte-to-monocyte (LYM-MON) ratio . These CBC-derived indices are increasingly recognized as markers of systemic inflammation and immune status; for instance, a higher NEU-LYM ratio coupled with a lower LYM-MON ratio often portends a poorer prognosis in AML patients (Stefaniuk et al., 2025). Monitoring such parameters, LYM-MON, NEU-LYM ratio, platelet-to-lymphocyte (PLT-LYM) ratio, etc. in experimental models can provide insight into the pre-leukemic process and the host response. In sum, bone marrow cellularity and blast counts reflect the primary malignant process in the marrow, while peripheral blood indices and ratios reflect the secondary hematopoietic and immunological disturbances all are critical endpoints in evaluating potential protective agents against benzene-induced pre-leukemia.

Melatonin, an endogenous indoleamine hormone produced mainly by the pineal gland, shows promise as a hematoprotective, chemopreventive agent with therapeutic potential. In addition to being well-known as a circadian rhythm regulator, melatonin exhibits many biological activities that may prevent oncogenic and toxic processes. It is a potent antioxidant (Monteiro et al., 2024), scavenging free radicals directly and enhancing antioxidant enzymes, thus reducing tissue oxidative damage. At the same time, melatonin demonstrates substantial anti-inflammatory and immunomodulatory effects, as it inhibits pro-inflammatory signaling (e.g., NF-κB pathways) and modulates immune cell activity (Sieminski et al., 2024; Cao et al., 2025). Since benzene is known to induce oxidative stress and inflammation in a bone marrow microenvironment (Spatari et al., 2021), such properties are highly relevant. Melatonin has shown oncostatic activities in different cancer models: inhibiting cancer cell proliferation, inducing apoptotic cell death, and impairing tumor angiogenesis and metastasis (Cao et al., 2025). In hematological malignancies particularly, melatonin has been demonstrated to promote apoptosis of leukemic cells and improve their chemotherapy-induced sensitivity, while protecting normal hematopoietic cells from damage (Mafi et al., 2023; Mojarradgandoukmolla, S. and Akan, H., 2022; Yang et al., 2020). Interestingly, clinical and preclinical investigations have proved that melatonin supplementation can alleviate the hematologic side effects of chemotherapy agents, reducing therapy-induced thrombocytopenia and improving recovery of the bone marrow after toxic insults (Yang et al., 2020). This two-fold action enhancing anti-tumor efficacy and protection of the normal blood cells that support oncological therapy highlights melatonin as a possible adjunct therapy in the field of oncology. Although melatonin has been shown at high levels to have antioxidant, anti-inflammatory, and anti-cancer activities, its role in benzene-associated leukemogenic diseases associated with induced hematopathies has yet to be fully appreciated. Benzene-containing AML models present an alternative situation of chemically mediated deoxyribonucleic acid (DNA) damage, oxidative stress, and altered hematopoiesis where melatonin's protective effects have not been fully elucidated. However, so far there is no evidence of traditional prophylactic therapies for the hematotoxicity of benzene, and no specific drug has been approved to protect the bone marrow function in exposed individuals (Abou Elazab et al., 2022). This knowledge gap leads to a research into whether melatonin might influence relevant pre-leukemia biomarkers under benzene exposure. This study sought to fill this gap by studying the dose-dependent impact of melatonin on hematological and bone marrow properties in a rat model subjected to benzene-induced pre-leukemia. Specifically, we aimed to investigate whether different amounts of melatonin could be administered at varying amounts to maintain bone marrow cellularity, decrease leukemic blast accumulation, and normalize peripheral blood counts and ratios in benzene-treated rats. In this study, we aim to establish melatonin as a potent chemopreventive adjuvant for toxicant-induced leukemia and to potentially identify a potent strategy for protecting hematopoietic health from benzene's leukemogenic insult.

2. Materials and Methods

2.1 Animals

Experiments were conducted on 8-10-week-old rats whose weight ranged from 180 to 220 grams. They were kept under normal laboratory conditions consisting of a 12-hour illumination-dark cycle along with unlimited feeding and drinking facilities (Mojarradgandoukmolla, S., et al. 2024). All the methods strictly followed the widely accepted ethics in the use and treatment of animals, and the study protocol was approved by the Institutional Animal Ethics Committee of Cihan University-Erbil, Approval Number (CUE-REC/2025/ 10).

2.2 Chemicals

A benzene working solution was prepared by mixing benzene (Chem Lab, Belgium), 2-propanol, and distilled water in a 1:5:5 (v/v) ratio. Volume of all fluids were measured and combined into a sealed container and mixed thoroughly until one uniform phase of all liquids was obtained. The melatonin powder (Bioven Ingredients, India) was weighed exactly on an electronic balance, dissolved in distilled water to produce a stock solution and diluted in order to yield dosing preparations equivalent to 5, 10, 20, and 40 mg/kg body weight. For each preparation the mixture was vortex mixed to achieve complete homogeneity and individual dosing volumes were calculated for each animal based on its current body mass.

2.3 Induction of Pre-leukemia with Benzene

Pre-leukemic changes were brought on by the multiple intravenous administration of a benzene solution (benzene:2-propanol: distilled water, 1:5:5, v/v). Each rat

received 0.2 mL via the lateral tail vein every 48 h for 4 weeks. This regimen was taken with an eye towards the potential to assess longitudinal normal hematological variability with early genotoxic alterations that are indicative of pre-leukemic conditions. Throughout, animals were monitored for physiological and behavioral changes (Elazab et al., 2022).

2.4 Experimental Design

Rats were randomized into six experimental arms (G1-G6) to examine the dose-response of Melatonin against benzene-evoked pre-leukemic alterations. G1 (control) received oral distilled water throughout the 4-week study. G2 (benzene only) underwent induction as outlined in Section 2.3: 0.2 mL of a benzene:2-propanol: distilled water mixture (1:5:5, v/v) delivered via the tail vein every 48 h for four weeks. Groups G3-G6 followed the same benzene protocol as G2 and additionally received once-daily oral Melatonin for 4 weeks at 5, 10, 20, or 40 mg/kg/day, respectively. All other husbandry, handling, and procedural conditions were standardized across groups.

2.5 Bone Marrow Smear Preparation

Rats that underwent treatment for four weeks were euthanized through cervical dislocation. The bone marrow was collected using phosphate-buffered saline (PBS, pH 7.2) supplemented with 50% fetal bovine serum (FBS) and washed well. The suspension was subjected to centrifugation at 3,000 rpm for approximately 10 min and subsequently the pellet was resuspended in PBS to create smears. To each specimen a minimum of 5 slides were then made. Smears were also air-dried for 10 min, fixed in methanol, and stained with Giemsa and May Grünwald for 20 min. Slides were then washed out and allowed to dry thoroughly, and the slides were viewed with a light microscope (Asita & Molise, 2011).

2.6 Micronucleus Assay

The bone-marrow micronucleus test was used to evaluate benzene-induced genotoxicity damage. From femurs, marrow was flushed, washed once with 50% FBS and rinsed with PBS, then centrifuged at 3000 rpm for 10 min. A small aliquot of the cell suspension was placed on a sterile glass slide with a spreading drop and drawn into a thin film. Smears were air dried at room temperature, fixed in absolute methanol for 10 min, and stained sequentially with May–Grünwald and Giemsa (1:1, v/v) for 20 min, followed by a phosphate-buffer rinse and air-drying. Slides were viewed with a 100× oil-immersion objective (total magnification 1000×). For each rat, 1000 PCEs were scored to identify MN-PCEs, and their frequency was displayed as MN-PCEs per 1000 PCEs. MN-NCEs were also counted per 1000 NCEs. Erythroid maturation was evaluated by calculating %PCE and %NCE as PCE or NCE count /(PCE+NCE)×100, and the PCE:NCE ratio was derived to compare the relative abundance of the two cell types. Procedures followed were established according to hematology guidelines (Sen et al., 2010).

2.7 Complete Blood Count (CBC)

The whole blood was retrieved and analysed on an automated hematology analyzer for a complete blood count (CBC) as reported by He et al. The panel included total WBC with automated differential NEU, LYM, MON, EOS, and BAS, as well as red-cell parameters: hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Platelet metrics included PLT count and platelet indices: MPV, PDW, PCT, P-LCR, and P-LCC. We calculated derived indicators from these, namely LYM/MON, NEU/LYM, NEU/MON, MPV/PLT, PLT/LYM, WBC/MPV, RDW/PLT ratio, and the SII. Auto-generated differential leukocyte percentages gave us a more complete picture of leukocyte distribution and immune status (He et al., 2017; Mojarrad, S. et al. 2020).

2.8 Peripheral Blood Smear

Peripheral blood smears were taken at the end of studies from these samples that had been anticoagulated with Ethylenediaminetetraacetic acid (EDTA). Films were air-dried in a desiccator, fixed in absolute methanol, and stained according to the May-Grünwald-Giemsa (MGG) method as per a standard procedure for hematology. The blast cell morphology was examined on the basis of classical characteristics such as high nuclear-to-cytoplasmic ratio, nucleolar prominence, finely granular chromatin with sharp margins, and intensely basophilic cytoplasm. A 100-cell differential in oil immersion (total magnification 1000×) was conducted to quantify number of leukocyte subsets and calculate blast percentage. The tests were conducted by two independent observers, in order to enhance accuracy and to control for inter-observer variation. The protocols were according to recognized hematology guidelines (Dai et al., 2021) for clinical care and laboratory studies.

2.9 Statistical Analysis

Statistical analyses were performed using one-way ANOVA, followed by Tukey's post-hoc testing for pairwise comparisons. Data are presented as mean ± SEM. Assumptions of normality and homogeneity of variances were evaluated before making inferences. Results were shown as bar graphs in GraphPad Prism (version 9.0.0). Statistical significance was defined by a p-value < 0.05.

3. Results

3.1 Micronucleus Assay

Benzene and melatonin were shown in **Figure 1** as effective at different doses (5, 10, 20, and 40 mg/kg), as displayed the percentages of %PCE and %NCE. Benzene exposure resulted in a decrease in %PCE (Control 50% → Benzene 9%) and an increase in %NCE (Control 56% → Benzene 93%) compared to the control group (****$p < 0.0001$), exhibiting notable bone marrow cytotoxicity. Melatonin treatment restores %PCE and decreases %NCE significantly in all doses tested, with 20 mg/kg being more effective with a higher recovery: 56%, 61%, than the standard benzene (****$p < 0.0001$). The 40 mg/kg dose was the same as the 20 mg/kg (ns) and protective efficacy was plateaued. Benzene and melatonin effect on the genotoxic endpoints %MN-PCE, %MN-NCE, and PCE/NCE are depicted in

Figure 2. Benzene massively raised %MN-PCE (Control 0.5% → Benzene 3.7%) and %MN-NCE (Control 0.05% → Benzene 0.34%), and decreased the PCE/NCE ratio (Control 0.90 → Benzene 0.07) (****p < 0.0001), signaling strong genotoxic and cytotoxic stress. Melatonin had a dose-based correction, with the greatest enhancement at 20 mg/kg %MN-PCE 0.7%, %MN-NCE 0.08%, PCE/NCE 0.73 being observed and 40 mg/kg produced no significant excess than 20 mg/kg (ns). These effects validate melatonin's strong genoprotective action.

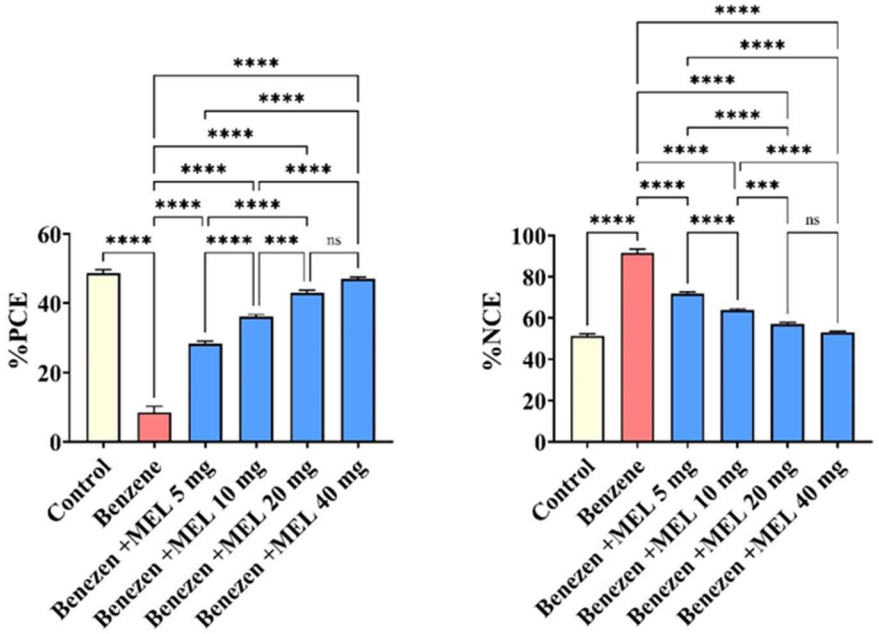

Figure 1: Effects of different doses of Melatonin on %PCE and %NCE in Benzene-Exposed Rat.

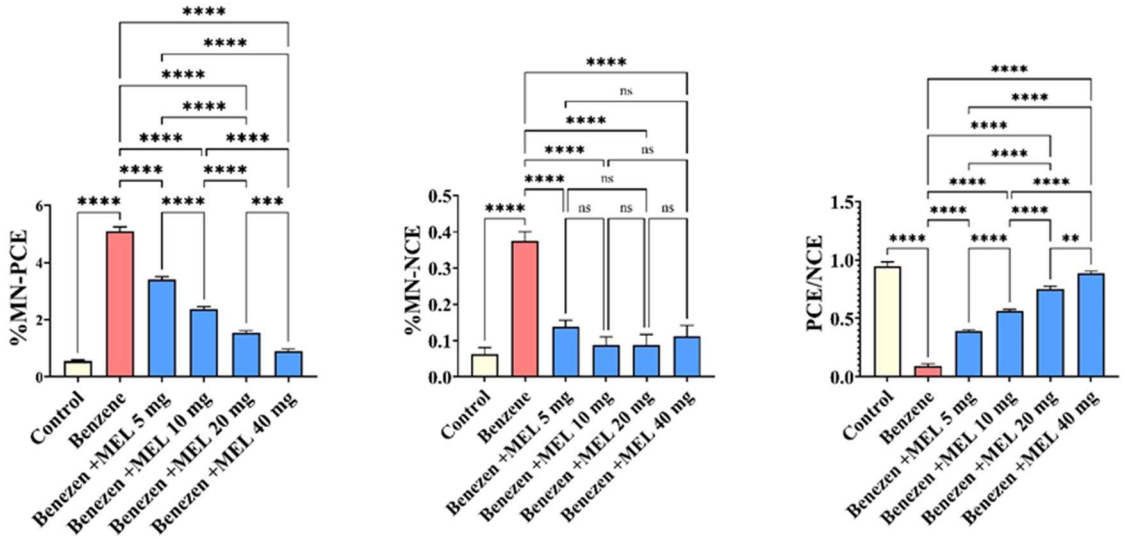

Figure 2: Effects of different doses of Melatonin on %MN-PCE, %MN-NCE, and PCE/NCE ratio in Benzene-Exposed Rat.

3.2 Alterations in Differential Leukocyte Count

Table 1 depicts the effect of benzene and different doses of melatonin on differential leukocyte counts and blast cell proportions in rats. Benzene showed significant reduction in total WBC count, LYM, MON, and EOS while increasing NEU, BAS, and circulating blast cells compared to the control group (***p < 0.001), indicating severe

immunotoxicity and leukemic transformation. Melatonin co-treatment resulted in dose-dependent improvement of these parameters. MEL at 10 mg/kg significantly increased WBCs, LYM, and MON and decreased NEU and BAS levels relative to benzene alone. MEL concentration at 20 mg/kg also led to a substantial increase in WBCs (9.60±1.01), the recovery of LYM as close to control (6.573±0.8634), and a significant reduction in %Blast Cells (12.63±0.62), indicating significant preservation. The maximum reduction in blast cells (5.875 ± 0.789, ***p < 0.001) was achieved with 40 mg/kg as well as the most uniform recovery in WBC subtypes. This may reveal that melatonin diminishes benzene-induced hematological injury, both at doses of 20 and 40 mg/kg, with the latter dose best suppressing aberrant blastogenesis and repopulating the immune cell populations.

Table 1: Effects of different doses of Melatonin on differential leukocyte count and blast cells in Benzene-Exposed Rat.

Parameters	Control	Benzene	Benezen +MEL 5 mg	Benezen +MEL 10 mg	Benezen +MEL 20 mg	Benezen +MEL 40 mg
WBCs count (Cells x 10^3 /µL)	11.93 ± 0.9967	7.535 ± 0.7221*	7.813 ± 0.8643	9.133 ± 0.9942	9.603 ± 1.013	8.483 ± 0.6534
NEU count (Cells x 10^3 /µL)	3.074 ± 0.3156	4.042 ± 0.7045	4.114 ± 0.4943	2.652 ± 0.3321	2.653 ± 0.3334	4.544 ± 0.4823
LYM count (Cells x 10^3 /µL)	7.936 ±0.8087	5.535 ± 0.5483	5.746 ± 0.5663	5.032 ± 0.6323	6.573 ± 0.8634	7.073 ± 0.7156
MON count (Cells x 10^3 /µL)	0.8045 ± 0.1967	0.4165 ± 0.0532***	0.6943 ± 0.0423	3.463 ± 0.4821***###	0.4241 ± 0.1024	0.6074 ± 0.0623
EOS count (Cells x 10^3 /µL)	0.1065 ± 0.0334	0.1354 ± 0.0489	0.0221 ± 0.0121	0.2034 ± 0.0321###	0.0356 ± 0.0000	0.0242 ± 0.0122
BAS count (Cells x 10^3 /µL)	0.0154 ± 0.0083	0.1265 ± 0.0478**	0.0252 ± 0.0043	0.1534 ± 0.0411#	0.0653 ± 0.0323	0.0223 ± 0.0023
%Blast Cells	0.000 ± 0.000	35.00 ± 1.402***	28.00±0.0.8452***###	19.75±0.7500***###	12.63±0.6250***###	5.875±0.7892***

Star sign (*) denotes that Benzene is compared with the control and treatment groups, while # denotes that Melatonin low doses are compared with High dose treatment.

WBCs count: White Blood Cell count; NEU: Neutrophils; LYM: Lymphocytes; MON: Monocytes; EOS: Eosinophils; BAS: Basophils

3.3 Differential Leukocyte Ratios

Benzene-induced immunotoxicity and the modulatory effects of melatonin upon CBC-derived leukocyte ratios were evaluated: LYM/MON, NEU/LYM and NEU/MON, respectively **Figure 3**. Compared to the control group, benzene markedly decreased the LYM/MON ratio (****p<0.0001), indicating lymphocytic suppression compared to monocytic counts. Melatonin treatment restored this ratio in a dose-dependent fashion; 20 mg/kg and 40 mg/kg doses obtained significantly greater changes from **p<0.01 to ****p<0.0001 than benzene alone. In contrast, the NEU/LYM ratio was increased in the benzene group, which is expected for inflammatory stress, though the expression does not reach statistical significance among groups (ns). Benzene exposure rat NEU/MON ratio was high (**p<0.01), whereas this result was observed for all melatonin-treated groups, most pronounced at moderate (20 and 40 mg/kg) doses, although not statistically pronounced (ns). The overall effect of melatonin, particularly when the medications were administered at 20 mg and 40 mg, partially normalized the LYM/MON and NEU/MON ratios between the groups, suggesting attenuation of benzene-induced immunological disturbance and myeloid imbalance.

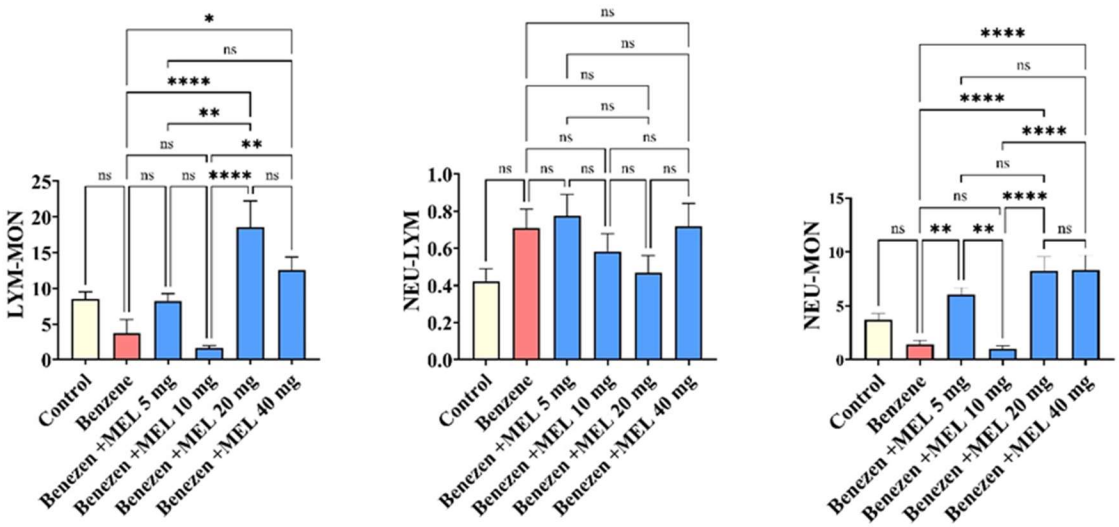

Figure 3: Effects of different doses of Melatonin on Leukocyte Ratios (LYM/MON, NEU/LYM, and NEU/MON) in Benzene-Exposed Rats.

3.4 Platelet Count and Indices

Table 2 shows how benzene and variations in melatonin levels affected PLT count and corresponding indices of rats. PDW decrease (PDW=9.055 ± 0.4154) was significant for benzene versus control (PDW=12.53 ± 0.6065), which showed that its size was highly variable (***p<0.001). There was a reduction in MPV and %PCT after benzene treatment, indicating reduced platelet activity and volume. The overall PLT did not differ, but melatonin co-administration provided dose-dependent enhancement of several parameters. It was observed that melatonin significantly increased PDW, %PCT, and PLT count with the two dose concentrations of 20 mg/kg and 40 mg/kg, to 538.6±27.64 beyond control values with the 40 mg/kg. The WBC/MPV ratio, substantially reduced by benzene (*p<0.05), exhibited a stepwise normalization to melatonin. Systemic Immune-Inflammation Index (SII), which was elevated in the benzene group, showed a further increase at higher melatonin doses. Changes in %PLCR and PLCC values are shown in **Figure 4.** Benzene decreased %PLCR significantly (***p<0.001) as compared with control, indicative of diminished proportion of large reactive platelets. While treatment with melatonin resulted in modest increases in %PLCR between doses, none were significant. On the other hand, PLCC decreased in the benzene group (*p<0.05) while 10 mg/kg and 40 mg/kg of melatonin markedly recovered PLCC levels (**p<0.01 and ***p<0.001, respectively), indicating a partial recovery of large platelet concentration in absolute numbers. These results confirm melatonin as protective from the effects of benzene on thrombocytic development, especially at higher doses.

Table 2: Effects of different doses of Melatonin on platelet count and its indices In Benzene-Exposed Rat

Parameters	Control	Benzene	Benezen +MEL 5 mg	Benezen +MEL 10 mg	Benezen +MEL 20 mg	Benezen +MEL 40 mg
PLT count (Cells x 10^3 /µL)	435.6 ± 41.51	424.6 ± 61.74	373.1 ± 72.86	388.6 ± 67.59	435.6 ± 33.48	538.6 ± 27.64
MPV count (fL)	9.384 ± 0.2665	6.47 ± 0.0843***	6.864 ± 0.2343	6.866 ± 0.2176	7.053 ± 0.2345#	6.155 ± 0.0554
PDW count (fL)	12.53 ± 0.6065	9.055 ± 0.4154***	10.49 ± 0.6233	9.124 ± 0.3256###	9.813 ± 0.4943#	12.08 ± 0.1754***
%PCT	0.4056 ± 0.0454	0.2776 ± 0.0343	0.2534 ± 0.0578	0.2553 ± 0.0442	0.3032 ± 0.0234	0.3357 ± 0.0187
MPV/PLT	0.0225 ± 0.0035	0.0188 ± 0.0034	0.0225 ± 0.0048	0.0274 ± 0.0103	0.0157 ± 0.0028	0.0165 ± 0.0011
PLT/LYM	59.24 ± 7.686	76.47 ± 8.275	62.95 ± 8.474	81.69 ± 17.78	75.97 ± 11.55	81.05 ± 8.455

Star sign (*) denotes Melatonin low doses are compared with High dose treatment.

WBC/MPV	1.285 ± 0.1154*	1.184 ± 0.1243	1.145 ± 0.1167	1.352 ± 0.1643	1.388 ± 0.1654	1.385 ± 0.1167
RDW/PLT	0.0913 ± 0.0083	0.1134 ± 0.01563	0.1464 ± 0.0342	0.1543 ± 0.0543	0.0885 ± 0.0091	0.0738 ± 0.0045
SII	179.3 ± 25.07	290 ± 219.1	404.6 ± 80.97	210.2 ± 75.74	727.1 ± 123.7	780.6 ± 167.7

PLT: Platelet count; MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; PCT: Plateletcrit; MPV/PLT: Mean Platelet Volume to Platelet Ratio; PLT/LYM: Platelet to Lymphocyte Ratio; WBC/MPV: White Blood Cell to Mean Platelet Volume Ratio; RDW/PLT: Red Cell Distribution Width to Platelet Ratio; SII: Systemic Immune-Inflammation Index.

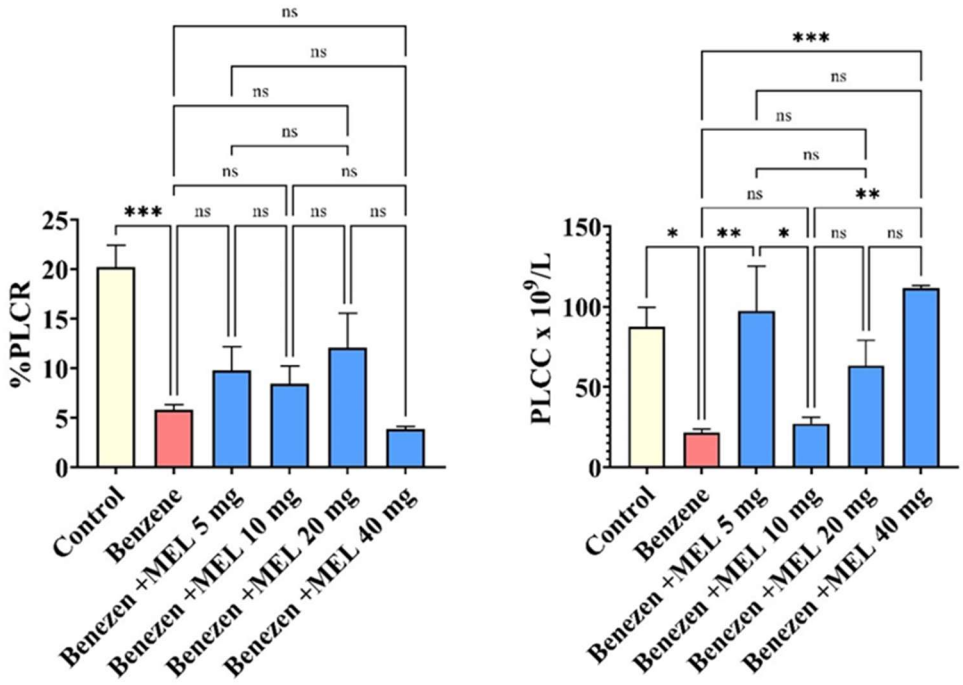

Figure 4: Effects of different doses of Melatonin on Platelet Indices (%PLCR and PLCC) in Benzene-Exposed Rats.

3.5 Microscopic Examination of Blood Cells

The percentage size of blast cells found in peripheral blood between the five experimental groups is shown in **Figure 5.** Benzene exposure resulted in an increased circulating blast cell count (****p<0.0001) compared to the control group, suggesting that leukemogenic activity and hematopoietic dysregulation were implicated. Melatonin 5, 10, 20, and 40mg/kg treatment in a dose-dependent manner reduced blast cell percentages (****p<0.0001 for all comparisons with the benzene group). Most reductions occurred in the 20mg and 40mg dosage levels with the 40mg group demonstrating almost total normalizing of blast cell count in comparison to control. There were also significant differences between all treatment groups, confirming the graded therapeutic effect of increasing melatonin levels.

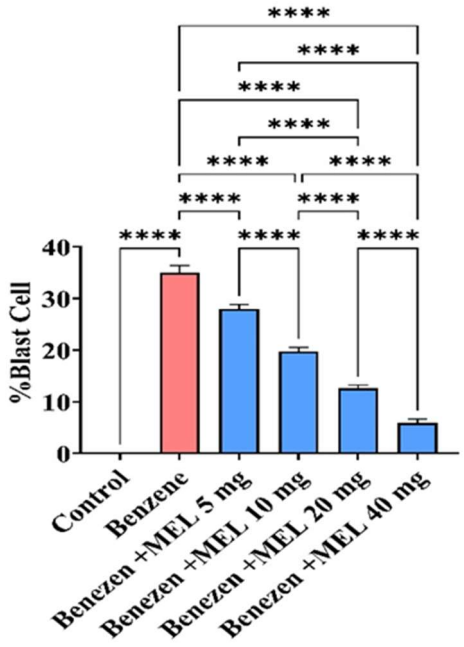

Figure 5: Effects of different doses of Mealtonin on the Percentage of Blast Cells in Peripheral Blood Smear of Benzene-Induced Leukemia Rat.

4. Discussion

In this study, the benzene-treated control met with the expected pattern of hematopoietic damage: depressed erythropoiesis, leukopenia with neutrophil predominance, and circulating blasts, mirroring AML-like disease progression. These findings are in agreement with previous reports demonstrating chronic benzene induction of bone marrow suppression and subsequent pre-leukemic transformation in rodents (Zhao et al., 2021). In our benzene-constrained rats, a high prevalence of micronucleated erythrocytes was present using the bone marrow micronucleus assay, with a smaller PCE/NCE ratio, further reflecting that significant genotoxic stress and impaired erythropoietic activity were exerted upon them. These results are in line with the clastogenic activity of benzene in other studies. Melatonin co-therapeutic use significantly improved these pathologic changes. Melatonin-treated groups found a restoration of the PCE/NCE ratio and a dramatic decrease in micronucleus formation compared with their benzene control, which indicated that melatonin served to shield erythroid precursors from damage by deoxyribonucleic acid (DNA) (Colares et al., 2022; Golabi-Habashi et al., 2021). This genoprotective effect is consistent with melatonin's well-characterized antioxidant activity, by scavenging reactive oxygen species and promoting DNA repair pathways in hematopoietic tissues (Cruciani et al., 2022; Mafi et al., 2023). Indeed, melatonin also protects megakaryocytes and enhances thrombopoiesis during treatment (Yang et al., 2020) and prevents treatment-induced genotoxicity damage and apoptosis in bone marrow cells. Melatonin prevented benzene oxidative genotoxicity and maintained the proliferative potential of the bone marrow as observed by a higher percentage of PCE and overall cellularity in the treated rat. In addition, melatonin reversed benzene-enhanced changes in total leukocyte and LYM counts. Melatonin-treated animals had significantly higher WBC and LYM content and a normalization of NEU:LYM ratios compared with the benzene-only animals, signaling the recovery of immune-cell production. This is consistent with previous studies demonstrating the stimulation of hematopoiesis and immune reconstitution post toxic insults by melatonin (Amini et al., 2020; Kumar et al., 2021). Melatonin may induce granulocyte colony-stimulating factors and other cytokines, which are critical for bone marrow progenitor cell survival and proliferation (Kumar et al., 2021), responsible for the enhanced leukocyte counts noted. In immunosuppressed circumstances, there is also evidence that melatonin antagonizes LYM apoptosis and increases peripheral LYM numbers (Atasever et al., 2025; Mafi et al., 2023). In other words, our findings confirm that melatonin retains leukocyte populations under benzene toxicity. Melatonin markedly decreased the number of benzene-induced blast cells in the bloodstream. Only benzene resulted in 34% blasts, while the majority of co-treated rats with melatonin had much lower blast percentages, especially at high doses. That is, melatonin inhibited the clonal expansion of aberrant myeloid progenitors, thereby blocking the leukemic process. A robust anti-pre-leukemic action of melatonin is also well established by previous studies, which demonstrated that melatonin was responsible for differentiation and apoptosis of leukemia cells and improved chemotherapy potency (Liu et al., 2023; Lomovskiy et al., 2023). The oncostatic actions of melatonin are stimulation of the mitochondrial apoptotic pathway in cancerous cells and a decrease of pro-survival signaling (Mafi et al., 2023; Ye et al., 2024). In our in vivo model these effects almost certainly corresponded to a reduction in blasts from benzene transformation in response to melatonin. Such a finding is particularly relevant especially since benzene leukemogenicity is described by the expansion of blast cells with resulting acute leukemia (Zhao et al., 2021) and our effectiveness in inhibiting blastogenesis suggests a prevention effect of melatonin in this study. Interestingly, 20 mg/kg melatonin was as effective as 40 mg/kg for most endpoints. The 20 mg/kg dose improved the PCE/NCE ratio, micronuclei, and blood counts but gained no added benefit with 40 mg/kg. This plateau effect indicates that an intermediate dose of melatonin is adequate to fully participate in the protective actions, consistent with reported other dose-optimization efforts (Zhang et al., 2023). Melatonin may not induce further hematologic effect at higher concentrations as receptor-mediated pathways are saturated or peak antioxidant and antiapoptotic activity is already achieved at 20 mg/kg. Importantly, both doses of melatonin were safe and effective, and even the highest dose was not detrimental to blood parameters compared to control. This aligns with the high safety margin of melatonin reported in published literature (Yusoff et al., 2023). Mechanistically, the robust protection seen is probably due to several complementary actions of melatonin. Melatonin is a strong free radical scavenger and ferroptosis inhibitor in hematopoietic cells (Sun et al., 2022; Bao et al., 2025). Benzene is toxic due to its oxidative DNA damage and possibly iron-catalyzed pathways to cell death in bone marrow (Bao et al., 2025), and melatonin antioxidant activity acts as direct countermeasure to these mechanisms. Melatonin alleviates the oxidative stress leading to DNA breaks and micronuclei

in bone marrow stem cells by upregulating antioxidant enzymes and preserving mitochondrial function (Cruciani et al., 2022; Atasever et al., 2025). Furthermore and in consideration of the full magnitude of the results, benzene raised %NCE and raised both MN-PCE and MN-NCE, while melatonin decreased both endpoint values, returning %NCE to physiological levels, thus confirming genoprotection at both immature and mature erythroid phases; benzene also depressed MON as well as modified EOS and BAS in association with changes in LYM and NEU, by contrast melatonin returned leukocyte composition to normal which was also reflected in partial normalization of LYM/MON and NEU/MON ratios (both in parallel with pre-existing improvement in NEU) (Babatola, 2023). PLT scores were not compromised: PDW, MPV, and PCT were all enhanced following melatonin with a recovery of PLT along with improvements in the quality of PLT measures (increased relative abundance of large platelet %PLCR and PLCC) in accordance with restored megakaryocytic function. Composite indices monitored these core changes MPV/PLT, PLT/LYM, WBC/MPV, and RDW/PLT overall shifted towards physiologic parameters following melatonin, with SII, a combined NEU, PLT, and LYM index, remaining numerically elevated at higher doses of melatonin, consistent with platelet recovery occurring when there was still recovery of lymphocytes, rendering the SII in question, in this case, relevant only to the obvious marrow-protective and genotoxicity-limiting effects reported at both endpoints (Golabi-Habashi et al., 2021). Melatonin's anti-inflammatory and immunomodulatory action also likely encouraged healing of hematopoietic injury. Benzene sensitization produces pro-inflammatory cytokines and bone marrow niche dysfunction; melatonin blocks the activation and cytokine release of nuclear factor kappa-light-chain-enhancer of activated B cells, thus providing a favorable environment for the survival of stem cells and hematopoiesis (Han et al., 2023; Mafi et al., 2023). In agreement, melatonin maintained a generally unaltered marrow structure and cell fate in our melatonin-treated rats. Additionally, melatonin was demonstrated to aid the quiescence and self-renewal of hematopoietic stem cells under stress (Cruciani et al., 2022) and accounts for the faster-than-usual return of the blood count observed. Maybe the most important point from our data is that melatonin not only shields tissue well from damage, but also helps to regenerate blood cells after benzene damage. Melatonin arms, for example, had higher PLT counts [using recovered marrow megakaryocytes and literature]. Melatonin has also been reported to enhance thrombopoiesis and PLT recovery in myelosuppressed models (Yang et al., 2020) and we agree with that trend. Taken together, these multiple actions produced significant differences, as rats treated with melatonin remained more similar to physiological hematopoiesis in the absence of benzene. Other antioxidants and treatments (such as lactoferrin) have demonstrated partial protection in analogous benzene models (Elazab et al., 2022), however, the effect of melatonin in our experiment returning almost all hematologic indices to the control state is particularly impressive. Melatonin's unique properties of inhibition of DNA damage and induction of progenitor cell viability, and simultaneously inhibiting of malignant transformation, as well as reducing DNA damage, in combination with its ability to modulate survival and limiting malignant transformation, make melatonin effective against the hematotoxicity of benzene, making it an advanced candidate as a potential therapy against hematotoxic benzene, especially in developing countries. Melatonin significantly protective effects were observed without interfering with baseline hematopoiesis at rest and blood-related hematopoiesis in control animal models, which indicated its homeostatic, and not hyper-stimulating modulation but rather, homeostatic control effects, rather than stimulation. This is also consistent with clinical evidence that melatonin can be administered at pharmacological doses in low-toxic concentrations but little if any therapeutic toxicity and benefit the bone marrow activity for those with treatment-induced cytopenias has been observed which could benefit the bone marrow (Yang et al., 2020). Finally, our results provide strong evidence that melatonin acts in a significant way to attenuate the harmful effect of benzene on the bone marrow and leukemogenic pathways. Administration of Melatonin (particularly after administration of 10-20 mg/kg) decreased oxidative genomic damage in marrow cells, preserved the accumulation of normal blood elements and arrested the proliferation of pre-leukemic blasts. These observations are consistent with a model where melatonin can act in four main ways to mediate the pathogenesis of benzene - quenching free radicals and stabilizing the genome, supporting the hematopoietic microenvironment, selectively killing nascent (malignant) cells, and ultimately preventing the proliferation of new cells from developing as the former. Due to the absence of targeted agents for benzene toxicity therapy, melatonin potentially could provide an important niche as a prophylactic medicine for those who might be at increased risk of benzene exposure (Elazab et al., 2022). Furthermore, the ease and low cost of its administration, ease of use, and safety profile also favour translational trials. There is also evidence to benefit from testing the impact of melatonin on long-term trajectories like progression to overt leukemia or bone marrow failure. In spite of these, the present findings are extremely promising and suggest that taping melatonin has the potential to substantially reduce benzene's harmful influence in the bone marrow. Melatonin-treated subjects obtained a better and relatively intact status compared to those who were exposed only to benzene, indicating that melatonin may be able to boost the health of the blood and decrease the pre-diagnosed risk of the disease among those with exposure to benzene. Collectively, our study demonstrated that the addition of melatonin may provide a potential option to protect the bone marrow from benzene and similar hematotoxic drugs, ultimately decreasing the incidence (or degree) of environment-induced leukemia.

CONCLUSION

In conclusion, the results show that the co-administration of melatonin induced significant and dose-dependent hematoprotective effects in rats against benzene-induced bone marrow toxicity. All the concentrations of melatonin combined (5-40 mg/kg/day) counteracted benzene's hematotoxicity and improved cellularity in the bone marrow and cytogenetic injury relative to benzene alone. Of note, the dose of melatonin (20 mg/kg) was the most efficacious and returned the %PCE of the bone marrow to normal and decreased the level of %NCE. At this dose, melatonin also

substantially reduced the occurrence of %MN-PCE and %MN-NCE, normalizing the PCE/NCE ratio, supporting potent protection against benzene-induced genotoxicity. More potent doses did not produce increasing benefit on these erythroid parameters, demonstrating a plateau effect at 20 mg/kg. Melatonin also inhibited benzene's leukemic changes in the white cell form at dose-dependent levels. Lymphocyte aberrant effects induced by benzene were partially reversed with total white blood cell counts growing toward control levels, LYM- and MON- counts improving in conjunction with abnormal NEU/MON and LYM/MON ratios. Melatonin treatment additionally drastically reduced the number of circulating blast cells, a signature of leukemic evolution, as 20 mg/kg was able to halve the blast percentage. 40 mg/kg nearly abolished these uncontrolled cells. Likewise, PLT indices showed improvement, but incremental, with the melatonin dose: PLT counts increased and PDW and PCT normalized, with the majority of improvements being sustained with 20 mg/kg. These protective effects appeared in all the major hematopoietic lineages, indicating melatonin's ability to protect against the near-normal erythropoiesis, leukopoiesis, and thrombopoiesis arising from toxic insult. Altogether, these results suggest that melatonin maintained hematopoietic homeostasis even in the presence of benzene, preventing or dampening nascent changes in the leukemic response. What that suggests is that melatonin not only protected progenitors of hematopoietics against benzene toxicity but also spurred the recovery of normal blood cell production. Out of the tested doses, 20 mg/kg was the most effective dose in the model that delivered broad-spectrum hematoprotection and near-complete remodeling of bone marrow cellularity and functions.

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