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Bone Marrow Protective Potential of Syzygium Cumini Ethanol Extract Against Chemotherapy-Induced

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ABSTRACT

Purpose

To evaluate whether Syzygium cumini ethanol extract (SCEE) mitigates cisplatin-induced myelosuppression in Wistar rats by restoring haematological indices, normalising coagulation times, reducing oxidative stress, and preserving bone-marrow histoarchitecture.

Methodology

Randomised, controlled in-vivo study (n=24; 4 groups, 6 rats/group). Myelotoxicity was induced with cisplatin (5 mg/kg, i.p.) on Days '1, 3, 5, and 7'. Interventions: SCEE 200 or 400 mg/kg p.o. for 21 days versus normal and toxic controls. Primary readouts at Days 1, 7, 14, 21 included CBC (RBC, WBC, platelets), haemoglobin, haematocrit, differential leukocyte counts, bleeding and clotting times. Mechanistic assays in bone marrow quantified lipid peroxidation (MDA/TBARS) and antioxidant enzymes (CAT, SOD, GPx, GR, GST). Histopathology (H&E) assessed cellularity/fat infiltration. Statistics: one-way ANOVA with Dunnett's post hoc test; data as mean ± SEM.

Results

Cisplatin significantly depressed haematological parameters and prolonged bleeding/clotting times, with concomitant marrow hypocellularity and fat infiltration, elevated MDA, and reductions in CAT, SOD, GPx, GR, and GST. SCEE produced dose-dependent recovery across endpoints. The 400 mg/kg arm delivered near-baseline restoration of RBC, WBC, platelets, haemoglobin, and haematocrit; normalised bleeding/clotting times; significantly lowered MDA; and re-established antioxidant enzyme activities. Histology corroborated functional recovery with improved cellularity and reduced fat infiltration approaching the normal profile.

Conclusion

SCEE demonstrates robust haematoprotective efficacy against cisplatin-driven myelosuppression, plausibly via antioxidant reinforcement and attenuation of lipid peroxidation. These preclinical data de-risk SCEE as a plant-based adjunct to maintain marrow function during platinum chemotherapy. Translation will require bioactive standardisation, dose optimisation, pharmacokinetics, and clinical validation.

Keywords: Cisplatin-induced myelosuppression, bone marrow toxicity, Syzygium cumini ethanol extract, Haematoprotective effect, Antioxidant phytochemicals, Wistar rat model.

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1. INTRODUCTION

A platinum-based chemotherapy drug called cisplatin is widely employed in the treatment of various solid tumours, including those of the testis, ovary, lung, and bladder[1]. While its antineoplastic efficacy is well-established, its clinical utility is frequently limited by severe adverse effects, notably dose-dependent myelosuppression[2]. Bone marrow toxicity resulting from cisplatin administration leads to significant reductions in erythrocytes, leukocytes, and platelets, increasing susceptibility to infection, anaemia, and haemorrhage, and often necessitating dose reduction or treatment cessation[3].

The underlying mechanism of cisplatin-induced haematotoxicity involves oxidative stress, mitochondrial dysfunction, and apoptosis of rapidly proliferating hematopoietic progenitor cells[4]. Such effects compromise the integrity of haematological parameters, including RBC, WBC, platelet counts, haemoglobin, and haematocrit, which are essential indicators of marrow function and patient prognosis. Additionally, alterations in leukocyte profiles reflect immune suppression, while changes in bleeding and clotting times serve as functional markers of platelet activity and haemostatic balance. Together, these parameters provide a comprehensive evaluation of chemotherapy-induced bone marrow injury[5].

Oxidative stress also contributes substantially to marrow toxicity, as cisplatin administration elevates lipid peroxidation while depleting endogenous antioxidant defences. Major enzymatic antioxidants including ‘catalase, glutathione reductase (GR), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-S-transferase (GST)’ are adversely affected during chemotherapy. Assessing lipid peroxidation markers like malondialdehyde (MDA) and antioxidant enzyme activities is therefore critical to elucidate the mechanisms of damage and the protective potential of candidate agents. Complementary to these biochemical markers, histopathological evaluation of bone marrow cellularity and architecture provides direct evidence of structural damage, including hypocellularity and fat infiltration, which are hallmarks of cisplatin-induced myelosuppression[6].

In light of these challenges, there is an urgent need for adjunct therapies that can mitigate haematological toxicity without compromising the anticancer activity of cisplatin. Natural products with documented antioxidant and cytoprotective activities are being increasingly explored for this purpose. Syzygium cumini, commonly referred to as Indian blackberry or jamun, is a medicinal plant traditionally employed in the management of various ailments. Earlier studies have reported the presence of flavonoids, phenolic acids, glycosides, and tannins in the leaves, contributing to their potent antioxidant and anti-inflammatory effects[7]. These characteristics imply a possible function for S. cumini in the protection of hematopoietic tissues against chemotherapeutic damage.

Accordingly, the present investigation aimed to examine the preventive effect of Syzygium cumini ethanol extract (SCEE) in relation to cisplatin-induced bone marrow injury in Wistar rats. Specifically, the study aimed to evaluate the extract’s impact on haematological parameters, leukocyte profiles, and bone marrow histoarchitecture. Secondary objectives included the assessment of bleeding and clotting times as indicators of hematopoietic recovery, determination of lipid peroxidation and antioxidant enzyme activities as mechanistic markers of oxidative stress, and the preliminary identification of phytoconstituents through qualitative screening. This investigation seeks to contribute to the development of plant-based supportive therapies for reducing chemotherapy-associated haematological complications.

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2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Collection site: rear compound, East West College of Pharmacy. Botanical authentication: Dr. V. Rama Rao, Research Officer (Botany), CCRAS. Authentication ID: SMPU/CARI/BNG/2022–2023/2330.

2.2 Preparation of Extract

The freshly collected Syzygium cumini leaves were shade-dried, milled, and sieved to obtain a coarse powder. An aliquot of approximately 250 g of the powdered material was initially defatted using ' petroleum ether' (40–70 °C)through continuous hot extraction in a 'Soxhlet apparatus'. The spent marc was then dried in a hot-air oven at '90 °C', and the defatted material collected. Subsequently, the coarse powder was extracted with methanol for 72 h (Soxhlet), and the methanolic extract was retained for further analysis[8].

2.3 Phytochemical Screening

Preliminary phytochemical profiling of SCEE was performed in accordance with Khandelwal's standardized methods[9].

2.4 Animals and Ethical Approval

Wistar rats of either sex (150–200 g; 8–16 weeks old) were employed for the study. All experimental procedures were reviewed and approved by the 'Institutional Animal Ethics Committee', East West College of Pharmacy (IAEC Approval No. EWCP/CPCSEA/IAEC/VII/2023/04) and were conducted in compliance with CPCSEA guidelines (Registration No. 2037/PO/Re/S/18/CPCSEA).

2.5 Chemicals and Drugs

Cisplatin (5 mg/kg, intraperitoneally) was employed to induce bone marrow toxicity in experimental animals. Normal saline (0.9% NaCl solution) was administered to the control group as a baseline comparator. A 2% Tween 20 solution, used as a vehicle in the toxic control group, was obtained from a certified chemical supplier. Ethanol of analytical grade was utilized for the preparation of the plant extract. Analytical-grade chemicals and reagents, acquired from approved suppliers, were used throughout.

2.6 Experimental Design

Table 1: The cohort was randomized into four groups with six animals in each.

Group	Treatment Description
Group I – Normal Control	Normal saline (2 mL/kg, oral)
Group II – Toxic Control	Cisplatin (5 mg/kg, i.p., 4 days) + 2% Tween 20 for 1st to 21st days.
Group III – SCEE Medium Dose	Cisplatin + SCEE (200 mg/kg, oral, 21 days)
Group IV – SCEE High Dose	Cisplatin + SCEE (400 mg/kg, oral, 21 days)

2.7 Induction of Cisplatin induces myelosuppression

SCEE was evaluated for chemoprotective efficacy against cisplatin-induced myelotoxicity. Myelosuppression was induced in Wistar rats by intraperitoneal cisplatin (5 mg/kg) administered on days '1, 3, 5, and 7' [10].

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2.8 Haematological and Histopathological Evaluation

Baseline blood was drawn under light diethyl-ether anaesthesia before extract/cisplatin dosing on Day 1. Subsequent samples were collected 1 h post-dose on Days 7, 14, and 21. Haematology included CBC (RBC, WBC, platelets), differential leukocyte count, haemoglobin, haematocrit, bleeding time, and clotting time, per established methods[11].

Bone marrow and brain tissues from all experimental groups were collected and fixed in '10%' neutral buffered formalin (pH 7.2) for '24 hours'. Tissues were dehydrated through graded alcohols (methyl, ethyl, absolute), embedded in paraffin, and sectioned at '4 µm' on a rotary microtome. Sections were mounted on clean slides, stained with 'haematoxylin–eosin (H&E)', and examined by light microscopy for histopathology using standard procedures[12].

2.9 Lipid Peroxidation Assay in Bone Marrow

Lipid peroxidation in rat bone marrow was assessed by quantifying malondialdehyde (MDA), a marker of thiobarbituric acid reactive substances (TBARS)[13].

2.10 Antioxidant Enzyme Assays in Bone Marrow

2.10.1 Catalase–Peroxidase (CAP) Assay

Catalase activity in rat bone marrow was assayed by mixing '0.1 mL' homogenate with '2.5 mL 50 mM phosphate buffer (pH 5.0) and 0.4 mL 5.9 mM H_2O_2', then tracking the fall in A_{240} over 1 min. One unit equaled a 0.01 absorbance decrease per minute at 240 nm (1-cm pathlength)[14].

2.10.2 Superoxide Dismutase (SOD) Assay

SOD activity in rat bone marrow was assayed by adding 0.3 mL of the post-centrifugation supernatant (1,500 ×g, 10 min; then 10,000 ×g, 15 min) to a reaction mix containing '0.1 mL phenazine methosulfate (186 µmol) and 1.2 mL 0.052 mM sodium pyrophosphate buffer (pH 7.0)'. The reaction was initiated with '0.2 mL NADH' (780 µmol), allowed to proceed for 1 min, and quenched with '1.0 mL glacial acetic acid'. The resulting chromogen was read at '560 nm', and activity expressed as units per mg protein[15].

2.10.3 Glutathione-S-transferase (GST) assay

GST activity was assayed in rat bone marrow using a '2.0 mL' reaction: 1.475 mL 0.1 M phosphate buffer (pH 6.5), '0.2 m' 1 mM GSH, '0.025 mL' 1 mM CDNB, and '0.3 mL' tissue homogenate. The increase in A_{340} was tracked, and activity reported as nmol CDNB–GSH conjugate formed min^{-1} mg^{-1} protein ($\varepsilon_{340} = 9.6 \times 10^3$ $M^{-1}\cdot cm^{-1}$)'[16].

2.10.4 Glutathione Reductase (GRD) Assay

GRD in bone-marrow homogenates was assayed per Carlberg–Mannervik. The '2.0 mL' reaction comprised '1.65 mL' 0.1 M phosphate buffer (pH 7.6), '0.10 mL' 0.5 mM EDTA, '0.05 mL' 1 mM GSSG, 0.10 'mL 0.1' mM NADPH, and '0.10 mL' sample. The fall in A_{340} at '25°C' was recorded, and activity calculated as nmol NADPH oxidized min^{-1} mg^{-1} protein using ε_{340} = '6.22×10^3 $M^{-1}\cdot cm^{-1}$'[17].

2.10.5 Glutathione Peroxidase (GPx) Assay

GPx in bone-marrow homogenates was assayed per Mohandas et al. A '2.0 mL' reaction contained 0.1 M phosphate buffer (pH 7.4; 1.49 mL), EDTA (1 mM; 0.10 mL), sodium azide (1 mM; 0.10 mL), glutathione reductase (1 IU/mL; 0.05 mL), GSH (1 mM; 0.05 mL), NADPH (0.2 mM; 0.10 mL), H_2O_2 (0.25 mM; 0.01 mL), and sample (0.10 mL). NADPH consumption was monitored at '340 nm' (25 °C). Activity was reported as 'nmol NADPH' oxidized $min^{-1}mg^{-1}$ protein using ε_{340} = '6.22×10^3 M^{-1} cm^{-1}'[18].

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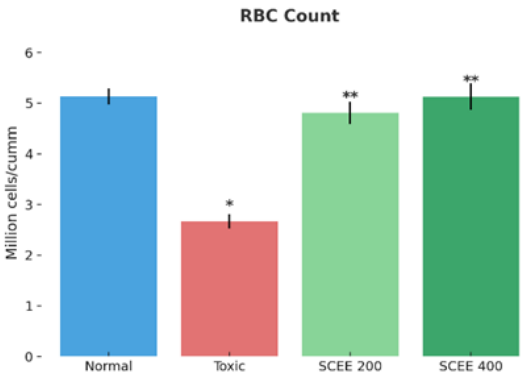
2.8 Statistical Analysis
Data are presented as mean ± SEM. Statistical analysis used 'one-way ANOVA' in 'GraphPad Prism', with 'Dunnett's post hoc test' for treatment-versus-control comparisons.

3. RESULTS
3.1 Extractive Values
The SCEE yielded a dark brown solid mass with a percentage yield of 0.6% w/w, while the petroleum ether extract yielded 0.36% w/w. These values indicate a higher efficiency of ethanol as a solvent under Soxhlet extraction conditions.

3.2 Preliminary Phytochemical Investigation
Qualitative phytochemical screening of the SCEE confirmed the presence of several bioactive constituents, including Triterpenoids, glycosides, alkaloids, flavonoids, proteins, amino acids, carbohydrates, tannins, and phenolic compounds. These phytoconstituents are widely recognized for their pharmacological properties, particularly antioxidant, anti-inflammatory, and cytoprotective activities, which may underlie the observed protective effects in the present study.

3.3 Effect on Hematological Parameters
Cisplatin administration resulted in a marked suppression of hematological parameters, including significant reductions in RBC, WBC, hemoglobin, platelet count, and hematocrit, indicating pronounced bone marrow toxicity. Additionally, cisplatin caused substantial alterations in leukocyte profiles, evidenced by decreased neutrophil, lymphocyte, eosinophil, and basophil counts, along with prolonged bleeding and clotting times. Treatment with the SCEE effectively reversed these changes in a dose-dependent manner. The high-dose SCEE group demonstrated near-complete restoration of all hematological indices and coagulation parameters toward normal levels, suggesting strong hematoprotective and marrow-reviving potential of the extract.

A



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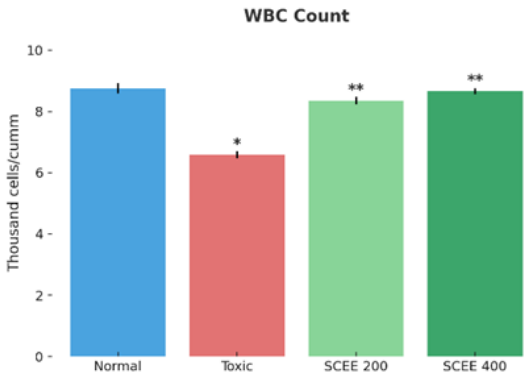

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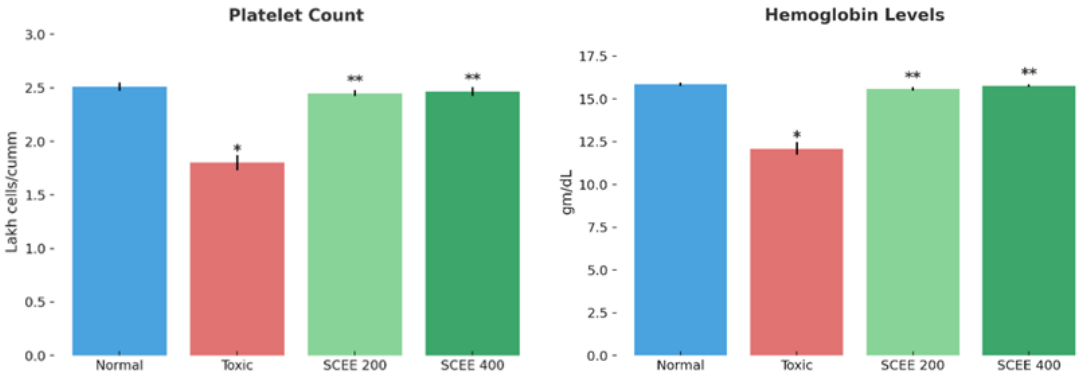

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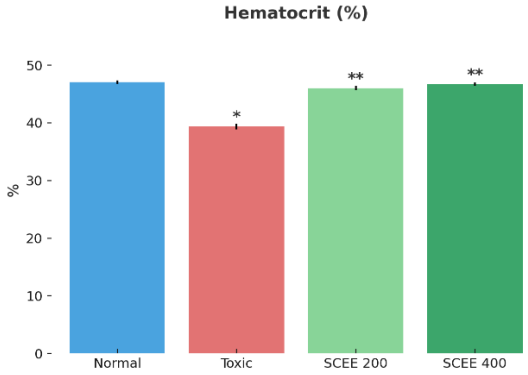


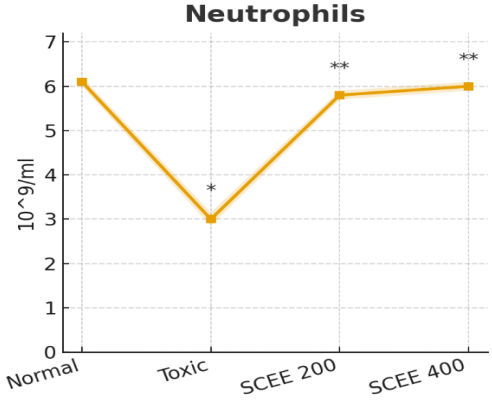
Values are mean ±SEM, 'n=6' symbols represent statistical significance are * $p < 0.05$-Toxic vs Normal, ** $p \leq 0.01$-Extract treated vs toxic

Figure 1. Haematological parameters in response to cisplatin and SCEE treatment, showing changes in '(A) RBC count, (B) WBC count, (C) platelet count, (D) haemoglobin level, and (E) haematocrit (%)'.

3.4 Differential Leukocyte Count & Coagulation Parameters

Neutrophils, lymphocytes, eosinophils, and basophils were significantly reduced in the toxic control group, reflecting haematological suppression. Following treatment with the SCEE, these leukocyte parameters were restored Demonstrated dose dependence; the high-dose group approached normal-control levels. Similarly, cisplatin-induced prolongation of bleeding and clotting times was effectively corrected by SCEE administration, with the higher dose restoring coagulation times to near-normal levels. These findings further support the extract's hematopoietic and haemostatic protective effects.

A



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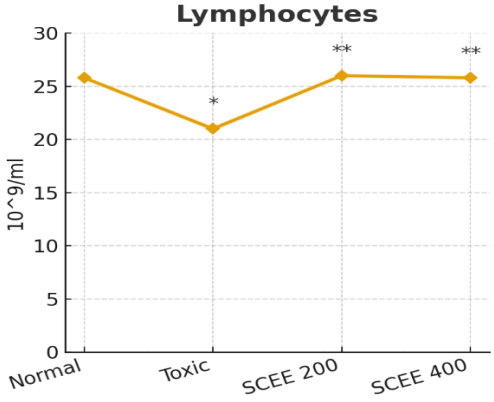

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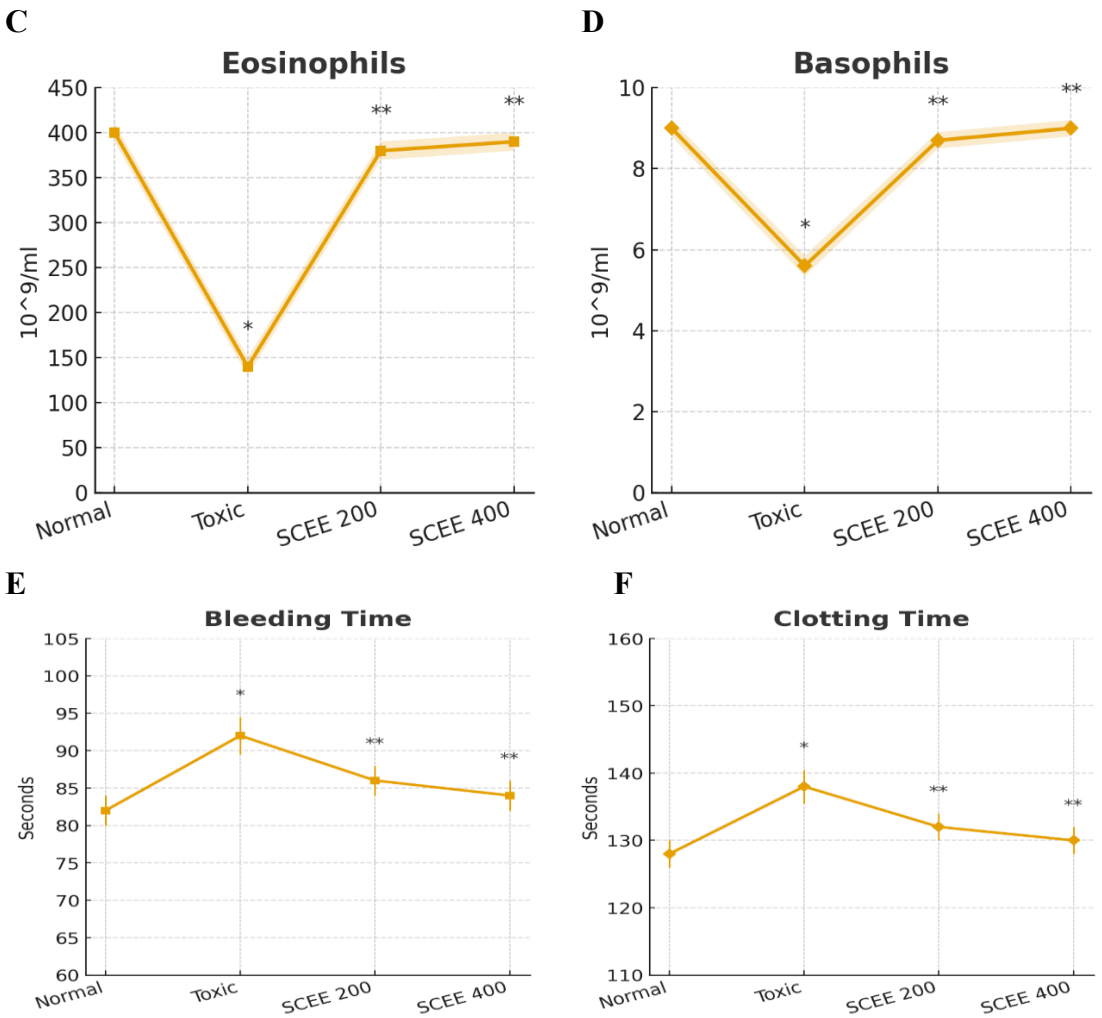

Values are mean ±SEM, 'n=6' symbols represent statistical significance are * $p < 0.05$-Toxic vs Normal, ** $p < 0.01$-Extract treated vs toxic

Figure 2. Immune cell profiles and coagulation parameters in response to cisplatin and SCEE treatment, showing changes in '(A) neutrophils, (B) lymphocytes, (C) eosinophils, (D) basophils, (E) bleeding time, (F) clotting time'.

3.4 Lipid Peroxidation in Bone Marrow

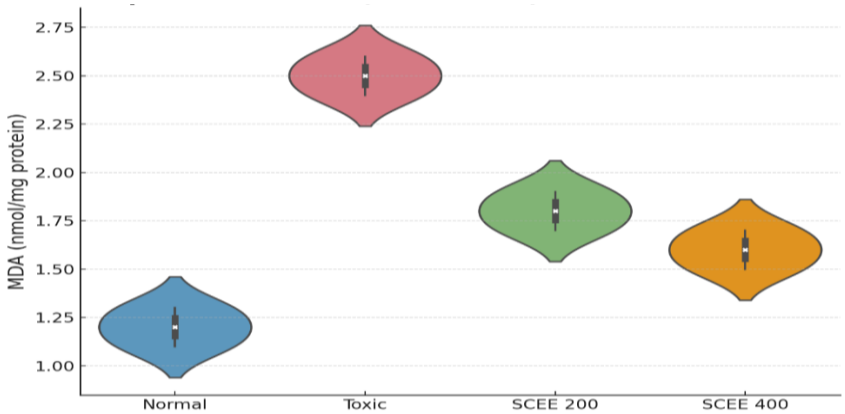

Figure 3. The distribution of malondialdehyde (MDA; nmol/mg protein) in Normal, Toxic, SCEE '200 mg/kg', and SCEE '400 mg/kg groups'; central marker denotes the median and bars indicate the interquartile range.

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3.6 Determination of antioxidant enzymes in bone marrow samples

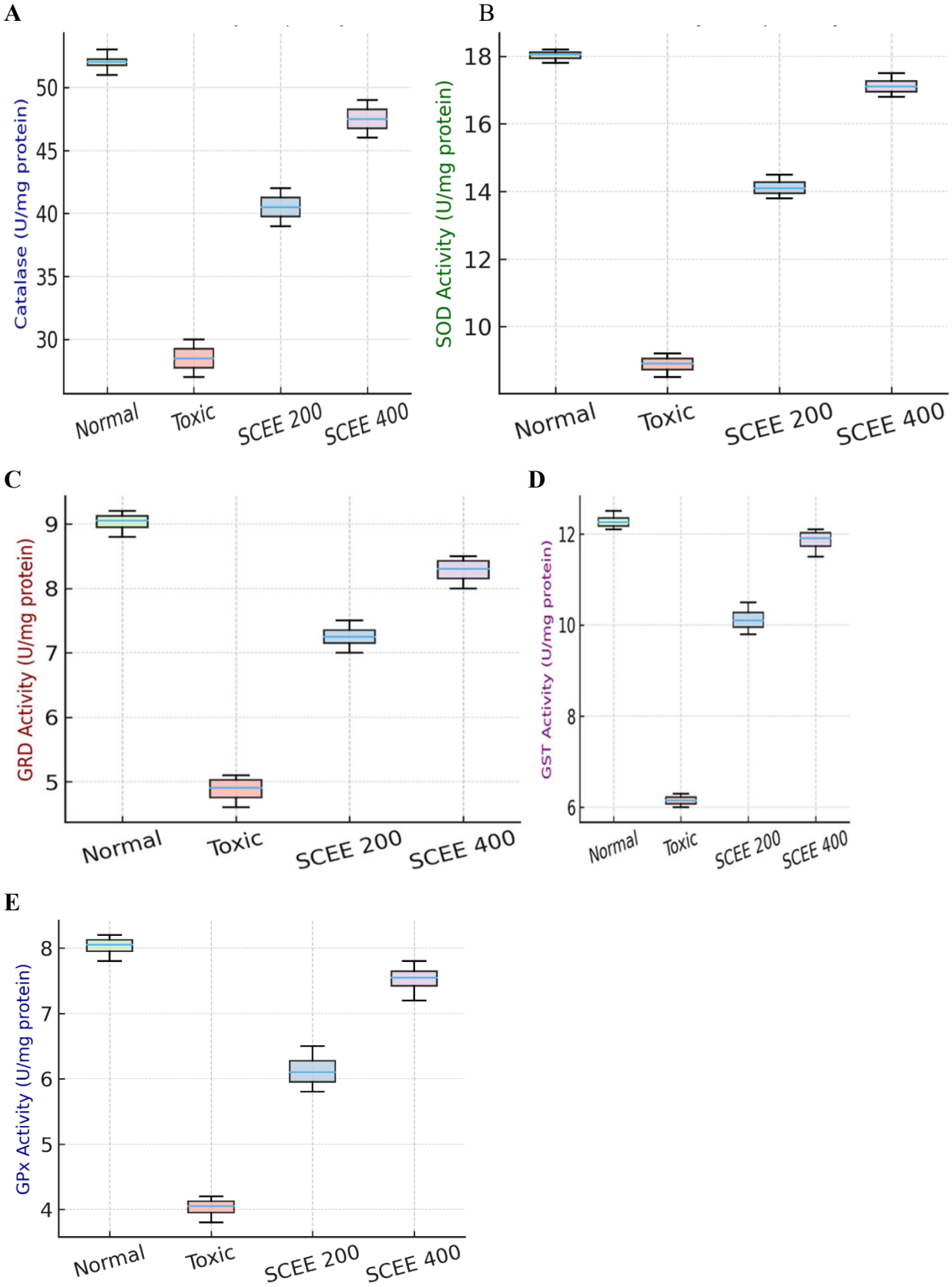

Figure 4. Antioxidant enzyme activities (U/mg protein): '(A) CAT, (B) SOD, (C) GR, (D) GST, (E) GPx'.

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3.7 Histopathology of Bone Marrow

The histopathological analysis of bone marrow showed significant cisplatin-induced damage, with the toxic control group (B) showing reduced cellularity and increased fat infiltration, indicative of myelosuppression. However, treatment with SCEE, particularly at the '200 mg/kg' (C) and '400 mg/kg' (D) doses, demonstrates a dose-dependent restoration of bone marrow structure. The SCEE-treated groups show improved cellularity and reduced fat infiltration, with the highest dose restoring bone marrow architecture to near-normal levels, like the normal control group (A). These findings suggest that SCEE has strong hematopoietic and marrow-reviving potential, effectively mitigating the bone marrow damage caused by cisplatin.

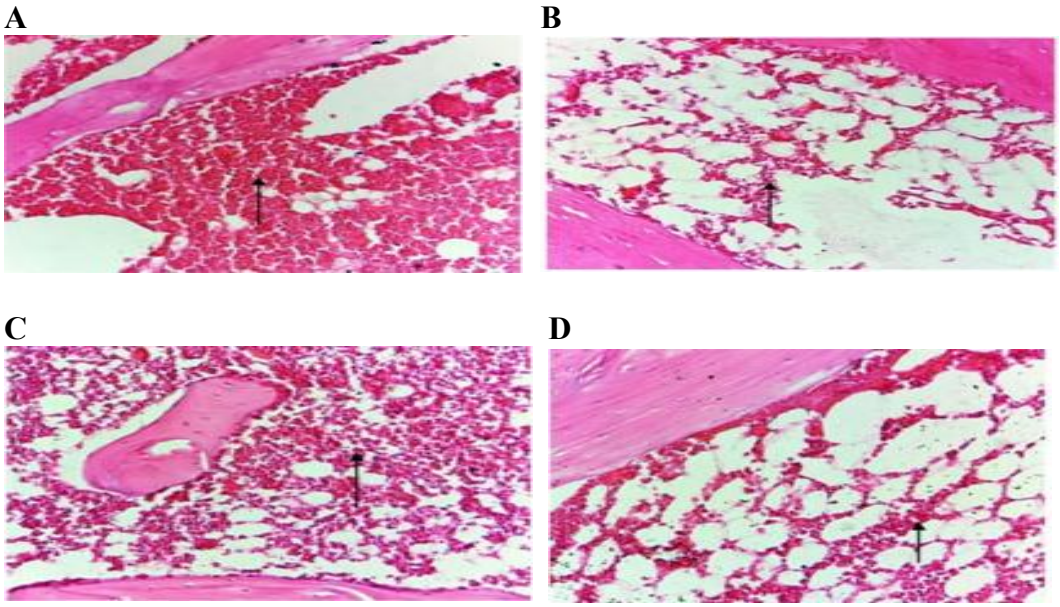

Figure 5. Histopathology of bone marrow in different treatment groups showing normal architecture in the control group (A), cisplatin-induced damage with reduced cellularity and increased fat infiltration in the toxic group (B), and dose-dependent restoration of bone marrow structure with improved cellularity and reduced fat infiltration in the SCEE '200 mg/kg' (C) and SCEE '400 mg/kg' treatment groups (D).

4. DISCUSSION:

Cisplatin remains one of the most widely used chemotherapeutic agents for solid tumours, but its dose-dependent bone marrow suppression severely restricts clinical application. In the present study, cisplatin treatment caused significant reductions in haematological indices such as RBC, WBC, platelet counts, haemoglobin, and haematocrit, in addition to prolongation of bleeding and clotting times. These findings are consistent with earlier reports that describe cisplatin-induced myelosuppression as a major dose-limiting toxicity, arising primarily from oxidative stress, mitochondrial dysfunction, and apoptosis of hematopoietic progenitors.

Importantly, treatment with SCEE significantly and dose-dependently reversed these haematological alterations. At the '400 mg/kg', haematological parameters, immune cell profiles, and coagulation indices approached near-normal levels. These protective effects can be explained by the extract's ability to enhance endogenous antioxidant defence systems. Cisplatin-induced toxicity is strongly associated with elevated lipid peroxidation, as evidenced by higher MDA levels, coupled with suppression of antioxidant enzymes. In this study, SCEE administration restored catalase, SOD, GPx, GR, and GST activities, thereby reducing oxidative stress burden. Restoration of these enzymes directly correlates with reduced apoptosis of bone marrow progenitors and Preliminary profiling of SCEE detected flavonoids,

phenolics, tannins, glycosides, and alkaloids—classes widely associated with antioxidant, anti-inflammatory, and cytoprotective activities. Flavonoids and phenolic compounds, in particular, are known to scavenge free radicals and inhibit lipid peroxidation, thereby preserving hematopoietic stem cell viability. These findings align with other studies in which natural extracts, such as green tea polyphenols, curcumin, and quercetin, demonstrated similar protective effects against chemotherapy-induced haematotoxicity. Thus, the haematoprotective action of SCEE can be attributed to synergistic interactions of its diverse phytoconstituents.

Histopathological evaluation further confirmed the extract's marrow-revitalizing potential. Cisplatin-treated groups showed hypocellularity and fat infiltration, hallmarks of myelosuppression, whereas SCEE treatment restored bone marrow architecture in a 'dose-dependent manner'. The higher dose restored cellularity comparable to the control group, underscoring its marrow-protective efficacy.

The clinical relevance of these findings is significant. Bone marrow suppression during chemotherapy predisposes patients to anaemia, infections, and haemorrhagic complications, often necessitating dose reduction or discontinuation of therapy. The ability of SCEE to restore haematological balance without interfering with cisplatin's anticancer activity highlights its potential as a supportive therapy to improve patient outcomes and quality of life during chemotherapy.

Nevertheless, this study has certain limitations. The data are derived from an animal model, and extrapolation to humans requires caution. Moreover, the specific bioactive molecules responsible for the protective effect were not isolated. Future work should focus on phytoconstituent standardization, bioactive compound identification, dose optimization, and validation in clinical models to fully establish SCEE's therapeutic potential.

5. CONCLUSION:

The present study clearly demonstrates the haematoprotective potential of SCEE against Cisplatin-Driven Myelosuppression: Wistar Rat Model. Cisplatin administration resulted in profound hematological suppression, leukocyte alterations, coagulation abnormalities, and marrow architectural damage. SCEE treatment, particularly at the 400 mg/kg, significantly and dose-dependently reversed these effects. The extract improved hematological values, restored antioxidant enzyme activity, reduced lipid peroxidation, normalized coagulation parameters, and preserved bone marrow histoarchitecture.

These protective effects are attributed to the phytoconstituents of SCEE, including flavonoids and phenolic compounds, which confer antioxidant and cytoprotective properties. Collectively, the findings suggest that SCEE could serve as a natural adjunct therapy to mitigate chemotherapy-associated haematotoxicity.

Further research involving bioactive compound isolation, pharmacokinetic profiling, and clinical validation is warranted to translate these promising preclinical results into effective supportive care strategies for patients undergoing chemotherapy.

6. REFERENCE

1. Dasari, S., & Tchounwou, P. B. (2014). Mechanistic overview of cisplatin's anticancer action. European Journal of Pharmacology, 740, 364–378. https://doi.org/10.1016/j.ejphar.2014.07.025
2. Gold, J. M., & Raja, A. (2023). Cisplatin: Pharmacological and clinical insights. In StatPearls. StatPearls Publishing.
3. Matsui, K., Masuda, N., Uchida, Y., et al. (1996). Predictors of bone marrow suppression during cisplatin-based chemotherapy for non-small cell lung carcinoma. Japanese Journal of Cancer Research, 87, 781–786. https://doi.org/10.1111/j.1349-7006.1996.tb00292.x
4. Marullo, R., Werner, E., Degtyareva, N., et al. (2013). Mitochondrial redox imbalance mediates the cytotoxic response to cisplatin exposure. PLOS ONE, 8, e81162. https://doi.org/10.1371/journal.pone.0081162

5. Hart, L., Ogbonnaya, A., Boykin, K., et al. (2023). Chemotherapy-induced myelosuppression burden among patients with advanced small-cell lung cancer: Evidence from community practice. Cancer Medicine, 12, 10020–1030 (n.p.). https://doi.org/10.1002/cam4.5738
6. Ognjanović, B. I., Djordjević, N. Z., Matić, M. M., et al. (2012). Selenium mitigates lipid peroxidation and oxidative kidney injury triggered by cisplatin. International Journal of Molecular Sciences, 13, 1790–1803. https://doi.org/10.3390/ijms13021790
7. Liu, Y., Dai, S., Xu, Y., et al. (2024). Herbal synergism of Jixueteng and Yinyanghuo reduces cisplatin-induced myelosuppression: Integrative network and experimental validation. Integrative Cancer Therapies, 23, 15347354241237969. https://doi.org/10.1177/15347354241237969
8. Patel, H. (2011). Cardioprotective potential of Delonix regia flower extract against isoproterenol- and doxorubicin-induced toxicity in animal models. (n.p.).
9. Pandhi, S., & Poonia, A. (2019). Comparative phytochemical screening of jamun seed extracts obtained through varied extraction procedures. The Pharma Innovation Journal, 8, (n.p.).
10. Karale, S., & Kamath, J. V. (2017). Evaluation of daidzein's role in preventing cisplatin-associated hepatic and haematologic toxicity in rats. Indian Journal of Pharmacology, 49, (n.p.).
11. Sushmitha, D. H., Ramesh, C., Sowmya, B. A., et al. (2022). Protective influence of Cedrus deodara methanolic extract on cisplatin-induced bone marrow suppression in Wistar rats. Journal of Complementary and Integrative Medicine, 19, 975–985. https://doi.org/10.1515/jcim-2021-0515
12. Bancroft, J., & Gamble, M. (2008). Theory and practice of histological techniques (6th ed.). Churchill Livingstone.
13. Ayatollahi, M., Hesami, Z., Jamshidzadeh, B., & Gramizadeh, B. (2014). Antioxidative role of bone marrow mesenchymal stem cells against CCl_4-induced liver damage in rats. International Journal of Organ Transplantation Medicine, 5, (n.p.).
14. Hadwan, M. H., Hassan, A., Alta'ee, A. H., Mohammed, R. M., Hadwan, A. M., Al-Kawaz, H. S., & Al-Talebi, Z. A. (2024). Optimised method for catalase activity determination in biological specimens. Bulletin of the National Research Centre, 48, 34. https://doi.org/10.1186/s42269-024-01189-z
15. Ramesh, K. S. V., Swetha, P., Kumar, P. M., et al. (2019). Assessment of salivary and crevicular superoxide dismutase activity in smokers and non-smokers with periodontitis. Nigerian Medical Journal, 60, 133–137. https://doi.org/10.4103/nmj.nmj_56_19
16. Thyagaraju, K., Hemavathi, B., & Vasundhara, K. S. (2005). Comparative analysis of rat brain and testis glutathione transferases under phenobarbital and β-methylcholanthrene stress. Journal of Zhejiang University Science B, 6, 759–765. https://doi.org/10.1631/jzus.2005.B0759
17. Singh, L., Rai, J., & Sharma, S. C. (2024). Role of Hal5 gene in improving ethanol tolerance in Saccharomyces cerevisiae. BBA Advances, 6, 100123. https://doi.org/10.1016/j.bbadva.2024.100123
18. Sattar, A. A., Matin, A. A., Hadwan, M. H., et al. (2024). Streamlined procedure for estimating glutathione peroxidase activity. Bulletin of the National Research Centre, 48(1), 1–17. https://doi.org/10.1186/s42269-024-01250-x

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