

Haematopoietic Protective Effects of *Pterocarpus mildbraedii* Leaf Extract against Gamma-Radiation-Induced Blood Cell Suppression in Wistar Rats

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ABSTRACT

Background: Gamma radiation can cause substantial haematological injury because of the radiosensitivity of the haematopoietic system. *Pterocarpus mildbraedii* possesses reported antioxidant properties, but its protective effects against radiation-induced blood-cell suppression require evaluation. Objective: To evaluate the haematopoietic protective effects of ethanolic *P. mildbraedii* leaf extract against gamma-radiation-induced alterations in peripheral blood parameters in Wistar rats. Methods: Female Wistar rats received *P. mildbraedii* extract orally at 200, 400 or 600 mg/kg for 15 days before whole-body gamma irradiation at 3, 6 or 8 Gy. Blood samples were collected three days after irradiation and analysed for white blood cells (WBC), lymphocytes, MID cells, granulocytes, red blood cells (RBC), haemoglobin (HGB), packed cell volume (PCV) and platelets (PLT). Data were expressed as mean $\pm$ standard deviation and analysed using comparative statistical tests at $p \leq 0.05$. Results: Radiation produced suppression of several haematological parameters, including WBC, lymphocytes, RBC, HGB, PCV and platelets. Extract administration produced variable restoration of these parameters. The clearest significant effect occurred following 200 mg/kg treatment at 6 Gy, where platelet count increased from 274.5 ± 7.78 to 492.5 ± 57.23 ($p = 0.033$). Significant differences were also observed in selected WBC and lymphocyte comparisons. Erythrocytic parameters showed favourable numerical responses, particularly at 200–400 mg/kg. Conclusion: *P. mildbraedii* demonstrated selective haematopoietic protective activity against gamma-radiation-induced blood-cell suppression, with effects dependent on extract dose, radiation dose and blood-cell parameter. Further studies should directly assess bone-marrow function.

Keywords: *Pterocarpus mildbraedii*; gamma radiation; haematopoietic protection; haematological parameters; Wistar rats; radioprotection; blood-cell suppression.

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INTRODUCTION

Ionising radiation is an important component of modern medicine, with applications ranging from diagnostic imaging to the treatment of malignant diseases. Although radiotherapy provides substantial therapeutic benefits, exposure of normal tissues to ionising radiation can produce undesirable biological effects [1,2]. Ionising radiation can damage biological systems through both direct interactions with critical cellular macromolecules and indirect effects mediated by Reactive Oxygen Species (ROS) generated during the radiolysis of water [3]. These reactive species can induce oxidative damage to DNA, proteins and membrane lipids, with DNA strand breaks

and other complex lesions representing important determinants of radiation-induced cellular injury [2, 3]. The severity of radiation injury depends on several factors, including radiation dose, dose rate, exposure pattern, tissue radiosensitivity and the ability of affected cells to repair or replace damaged cellular components [2, 4].

The haematopoietic system is among the biological systems particularly vulnerable to ionising radiation because of the high proliferative activity and radiosensitivity of its stem, progenitor and precursor cell populations [4, 5]. Radiation exposure can suppress

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haematopoiesis through direct injury to haematopoietic stem and progenitor cells, alterations in the bone-marrow microenvironment, apoptosis and redistribution of mature circulating blood cells [4, 5]. Consequently, whole-body or substantial partial-body irradiation can produce clinically important cytopenias, including lymphopenia, anaemia and thrombocytopenia [4, 6]. Peripheral blood abnormalities are therefore useful indicators of systemic radiation injury. Lymphocytes are particularly radiosensitive and may decline rapidly after irradiation, while alterations in granulocytes and platelets can provide additional information about the severity and temporal evolution of haematopoietic injury [6, 7].

Experimental and clinical observations have demonstrated that irradiation can produce marked alterations in peripheral blood indices. The magnitude and pattern of these changes depend on radiation dose, dose rate and the time elapsed after exposure [6, 7]. In particular, depletion of lymphocytes can occur rapidly after whole-body irradiation, whereas reductions in granulocytes and platelets may develop according to the extent of bone-marrow injury and the kinetics of blood-cell production [6, 7]. Such changes have important physiological consequences because leukocyte depletion can compromise immune defence, erythroid suppression can impair oxygen transport, and thrombocytopenia can increase the risk of haemorrhagic complications [4, 6].

Radiation-induced haematological injury is therefore not restricted to leukocyte depletion. Suppression of erythroid and megakaryocytic production can lead to reductions in red blood cell count, haemoglobin concentration, packed cell volume and platelet count [4, 5]. The haematological consequences of ionising radiation reflect a combination of impaired marrow production, apoptosis and altered distribution or survival of mature blood cells [5]. These effects make peripheral haematological indices useful endpoints for evaluating the severity of radiation injury and the efficacy of candidate radioprotective interventions [6].

The development of effective and safe radioprotective agents has consequently remained an important area of experimental research. Although synthetic radioprotective agents have demonstrated protective effects, their broader application may be limited by toxicity and other adverse effects [8, 9]. Amifostine, for example, has established radioprotective activity in selected clinical settings, but its use is associated with adverse effects including hypotension, nausea and vomiting, and its application as a general countermeasure for acute radiation syndrome remains limited [8, 9]. These limitations have stimulated continued investigation into naturally occurring compounds and plant-derived preparations as potential alternatives or complementary radioprotective agents.

Plant-derived preparations are of particular interest because they contain chemically diverse constituents capable of influencing several pathways involved in radiation-induced injury. Natural antioxidants and phytochemicals have been investigated for their ability to

scavenge radiation-generated free radicals, reduce oxidative stress, protect cellular membranes and DNA, modulate inflammatory responses and enhance endogenous antioxidant defence [10-12]. Experimental studies have reported radioprotective effects for several medicinal plants and plant-derived compounds in different models of radiation-induced lethality, oxidative damage, lipid peroxidation and DNA injury [10-12]. However, promising experimental observations should not be interpreted as evidence of established clinical efficacy, because many plant-derived radioprotectors remain at the preclinical stage and require further pharmacological and clinical evaluation [11-14].

Pterocarpus mildbraedii Harms is an indigenous African tree belonging to the Fabaceae family and is distributed in several West and Central African countries, including Nigeria [15]. Its leaves are traditionally consumed as a cooked vegetable in Nigeria and have also been associated with ethnomedicinal uses [15, 16]. Previous investigations have demonstrated that the leaves contain nutritionally important constituents, minerals and several secondary metabolites [16, 17]. Phytochemical investigations have reported constituents including flavonoids, saponins, tannins, alkaloids, phenolic compounds and other secondary metabolites in the leaves of *P. mildbraedii* [17, 18]

The antioxidant properties of *P. mildbraedii* provide a biological rationale for investigating its possible protective effects against radiation-induced oxidative injury. Usunobun and Igwe reported that *P. mildbraedii* leaf preparations demonstrated concentration-dependent reducing power and free-radical-scavenging activity in vitro, together with the presence of several phytochemical constituents, including flavonoids, saponins, alkaloids and tannins [17]. More recent phytochemical investigations of *Pterocarpus* species occurring in Nigeria have likewise demonstrated the presence of several secondary metabolites in *P. mildbraedii*, including tannins, flavonoids, saponins, phenols, alkaloids and terpenoids [18]. These findings provide a plausible pharmacological basis for investigating the plant as a potential source of biologically active compounds.

The rationale for investigating *P. mildbraedii* in radiation-associated haematological injury is further supported by the broader literature on plant-based radioprotection. Plant-derived compounds have been reported to exert radioprotective effects through multiple mechanisms, including direct scavenging of radiation-generated free radicals, attenuation of lipid peroxidation, enhancement of endogenous antioxidant systems, modulation of inflammatory and apoptotic pathways, and possible effects on DNA repair [10-12]. Nevertheless, evidence obtained from other medicinal plants cannot be directly extrapolated to *P. mildbraedii*, because the phytochemical composition, pharmacological activity, bioavailability and dose-response characteristics of botanical preparations may differ substantially among species and extracts.

Furthermore, radioprotection should not be inferred solely from antioxidant activity or biochemical changes. Preservation of circulating blood-cell populations represents a functionally relevant endpoint because it reflects the systemic haematological consequences of radiation exposure [6, 7]. Examination of WBC, lymphocytes, MID cells, granulocytes, RBC, haemoglobin, PCV and platelets therefore permits assessment of different components of the peripheral blood response following irradiation. Such parameters can provide complementary information regarding leukocyte depletion, erythropoietic suppression and platelet abnormalities associated with radiation-induced haematopoietic injury.

The original experimental study provides an appropriate model for such an investigation. Female Wistar rats were administered ethanolic *P. mildbraedii* leaf extract orally at doses of 200, 400 and 600 mg/kg for 15 days before irradiation. The animals were subsequently exposed to single whole-body radiation doses of 3, 6 or 8 Gy, and blood samples were collected three days after irradiation for haematological assessment. The haematological analysis included WBC, RBC, platelet and related blood-cell parameters. These experimental procedures and observations constitute the primary study-specific basis for the present investigation rather than findings extrapolated from the general radioprotection literature.

Importantly, the available findings indicate that the haematological response to *P. mildbraedii* was not uniformly dose dependent. Significant improvement was observed for selected parameters at particular extract and radiation-dose combinations. For example, platelet count was significantly higher in animals receiving 200 mg/kg extract before 6 Gy irradiation compared with the corresponding radiation-only group ($p = 0.033$). The 400 mg/kg treatment also produced increases in several erythrocytic and leukocytic indices in selected comparisons, although not all differences reached statistical significance.

Similarly, at 3 Gy irradiation, animals pretreated with 400 mg/kg extract showed higher mean RBC, haemoglobin, PCV and platelet values than radiation-only animals, although the individual comparisons did not reach statistical significance. At 600 mg/kg, platelet counts were also numerically higher following 3 Gy irradiation, while other parameters showed variable responses. These findings indicate that the protective response requires careful consideration of both extract dose and radiation dose rather than assuming a simple linear dose-response relationship.

Despite these observations, the haematological findings have not previously been developed as an independent publication focused specifically on radiation-associated blood-cell suppression. The original investigation combined haematological, oxidative, biochemical and histopathological endpoints within a broader radioprotective framework. Separating the haematological component provides an opportunity to examine the blood-

cell response in greater depth and to interpret the findings according to the major functional compartments of peripheral blood: leukocytes, erythrocytes and platelets. Such an approach may provide clearer evidence of the potential of *P. mildbraedii* to preserve haematological homeostasis following whole-body radiation exposure.

Accordingly, the present study focused specifically on the haematopoietic protective effects of ethanolic *P. mildbraedii* leaf extract against gamma-radiation-induced blood-cell suppression in Wistar rats. The study evaluated leukocyte parameters comprising WBC, lymphocytes, MID cells and granulocytes; erythrocytic parameters comprising RBC, haemoglobin and PCV; and platelet count following exposure to 3, 6 and 8 Gy radiation. By comparing extract-pretreated animals with radiation-only and control groups, the study sought to determine the extent to which *P. mildbraedii* could preserve peripheral blood indices following irradiation and to identify extract doses associated with the most favourable haematological response.

The study therefore provides a focused experimental assessment of *P. mildbraedii* as a potential natural candidate for mitigating radiation-associated haematological injury. Rather than interpreting changes in peripheral blood counts as direct evidence of bone-marrow regeneration, the present investigation uses these indices as measurable indicators of the systemic haematological response to radiation. This distinction is important because direct assessment of bone-marrow cellularity or haematopoietic progenitor activity was not included in the original experimental design. The findings should consequently be interpreted as evidence of preservation or restoration of peripheral haematological parameters following radiation exposure.

MATERIALS AND METHODS

Study design

This study was an experimental animal investigation designed to evaluate the effects of ethanolic leaf extract of *Pterocarpus mildbraedii* on peripheral haematological parameters following whole-body gamma-radiation exposure. The study was conducted using adult female Wistar rats. The original experimental design comprised six principal groups (R1–R6), with six rats allocated to each group. R1 served as the untreated control, R2 received radiation only, R3 received extract only, while R4, R5 and R6 received the extract at 200, 400 and 600 mg/kg, respectively, followed by radiation exposure. The radiation-treated groups were further divided according to radiation dose of 3, 6 and 8 Gy, with two animals assigned to each radiation-dose subgroup.

For the present manuscript, the analysis was restricted to haematological outcomes, namely white blood cell count (WBC), lymphocyte count, MID-cell count, granulocyte count, red blood cell count (RBC), haemoglobin concentration (HGB), packed cell volume (PCV) and platelet count (PLT). Biochemical, renal and histopathological outcomes from the original experiment

were excluded because they are outside the specific scope of this article.

Experimental animals

A total of 48 female Wistar rats weighing approximately 200–250 g were used in the original study. Thirty-six animals constituted the main experimental cohort, while 12 animals were used for the acute toxicity study. The experimental rats were obtained from the animal house of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. The animals were acclimatised for 14 days before commencement of the experiment and maintained under standard laboratory conditions with free access to water and commercial rat feed. A 12-h light/12-h dark cycle was maintained throughout the experimental period.

Plant material and authentication

Leaves of *Pterocarpus mildbraedii* Harms were obtained from a local farm in Nnewichi, Nnewi North Local Government Area, Anambra State, Nigeria. The plant material was authenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka, and assigned the herbarium identification number NAU Herbarium 199A.

Preparation of the plant extract

The collected leaves were cleaned, cut into smaller pieces and air-dried at room temperature until completely dry. The dried leaves were subsequently pulverised into a fine powder. Approximately 797 g of powdered plant material was obtained and subjected to extraction with 96% ethanol using a Soxhlet extraction procedure at 40 °C. The extract was filtered and concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator. The resulting dried extract weighed 42.18 g and was stored in a tightly closed container under refrigeration until required for administration.

Acute toxicity assessment

The acute toxicity of the ethanolic leaf extract was assessed using the method of Lorke. The toxicity study was conducted in two phases. In the first phase, rats received oral doses of 10, 100 and 1,000 mg/kg. In the absence of mortality or major signs of toxicity, a second phase was conducted using doses of 1,600, 2,900 and 5,000 mg/kg. Animals were observed for behavioural and physical signs of toxicity, including salivation, paw licking, writhing, hyperactivity, respiratory distress, muscle paralysis, changes in body weight and mortality. Observation was continued for up to 72 h following administration. No mortality or observable signs of acute toxicity were recorded at the highest tested dose; consequently, the median lethal dose (LD₅₀) was considered to be greater than 5,000 mg/kg.

Extract administration and experimental grouping

The experimental animals were allocated into six principal groups of six rats each. R1 served as the untreated control and received neither extract nor radiation. R2 served as the radiation-only group. R3 received extract alone, while R4, R5 and R6 received 200, 400 and 600 mg/kg of *P. mildbraedii* extract,

respectively, in combination with radiation. The radiation-treated groups were subdivided into D, E and F subgroups corresponding to 3, 6 and 8 Gy, respectively. Each subgroup contained two rats.

The extract was administered orally by gavage once daily for 15 consecutive days. The doses used in the main experiment were 200, 400 and 600 mg/kg body weight. These doses were selected following the acute toxicity assessment and represented the experimental dose range used in the original study.

Gamma-radiation exposure

Irradiation was performed at the Radiotherapy Unit of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State, Nigeria. Radiation exposure was performed 24 h after the final administration of the ethanolic *P. mildbraedii* extract. The animals were irradiated without anaesthesia and secured in the prone position to minimise movement during exposure.

Whole-body irradiation was performed using an Elekta Precise linear accelerator. A field size of 26 × 29 cm and a depth of 3 cm were used, with a single-field source-to-surface-distance technique. Three single-fraction radiation doses—3, 6 and 8 Gy—were administered at a dose rate of 400 cGy/min using 6-MV photon energy. The corresponding monitor units were 298 MU for 3 Gy, 597 MU for 6 Gy and 796 MU for 8 Gy. No part of the animals was shielded during irradiation.

Blood sample collection

Blood samples were collected three days after irradiation. Blood was obtained by retro-orbital bleeding and transferred into ethylenediaminetetraacetic acid (EDTA) sample tubes for haematological analysis. The timing of blood collection was maintained across the experimental groups to permit comparison of the peripheral haematological response following radiation exposure.

Haematological assessment

Haematological parameters were determined using a Mingray BC-3200 automated haematology analyser. EDTA-anticoagulated blood samples were analysed for WBC, RBC, platelet count and related haematological indices. Haemoglobin concentration was determined using the analyser's colorimetric method, while RBC, WBC and platelet counts were determined using the electrical impedance principle.

The erythrocytic parameters included RBC count, haemoglobin concentration and PCV. The analyser calculated erythrocytic indices from the RBC histogram, while PCV was calculated from RBC and mean corpuscular volume according to the analyser's standard procedure.

The leukocyte parameters evaluated in this article comprised total WBC count, lymphocyte count, MID-cell count and granulocyte count. These parameters were selected to provide an assessment of the leukocytic response to radiation and the extent to which extract pretreatment altered this response.

Assessment of haematological protection

The effect of *P. mildbraedii* pretreatment was assessed by comparing haematological parameters in extract-plus-radiation groups with their corresponding radiation-only groups at each radiation dose. Particular attention was given to the direction and magnitude of changes in WBC, lymphocytes, MID cells, granulocytes, RBC, HGB, PCV and PLT.

Where appropriate, percentage restoration of a haematological parameter was calculated relative to the radiation-only and untreated control groups using the following equation:

$$\text{Protection (\%)} = \frac{(\text{Extract + radiation}) - (\text{Radiation only})}{(\text{Control}) - (\text{Radiation only})} \times 100$$

For parameters expected to decrease following irradiation, higher positive restoration values indicate greater recovery towards the untreated control value. This analysis was intended to provide an additional quantitative description of the magnitude of haematological recovery beyond conventional statistical comparisons.

Ethical considerations

Ethical approval for the original animal experiment was obtained from the Research and Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, and the Research and Ethics Committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. Animal handling and experimentation were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. The

investigator also underwent animal-handling training organised by the Nnamdi Azikiwe University Animal Research Ethics Committee.

Statistical analysis

The original study analysed the data using Statistical Package for the Social Sciences (SPSS), version 20. Descriptive results were expressed as mean $\pm$ standard deviation, while inferential analyses included Student's *t*-test, analysis of variance (ANOVA) and Dunnett's multiple-comparison test. Statistical significance was set at $p \leq 0.05$.

For the present manuscript, the statistical presentation will be reorganised around the predefined haematological domains. Leukocyte parameters will comprise WBC, lymphocytes, MID cells and granulocytes; erythrocytic parameters will comprise RBC, HGB and PCV; and platelet count will be analysed separately. The reported comparisons will retain the original radiation-dose structure rather than pooling the 3, 6 and 8 Gy subgroups, because each radiation dose represented a distinct experimental exposure. The original design assigned two animals to each radiation-dose subgroup within the radiation-treated groups.

Given the small number of animals within individual radiation-dose subgroups, statistically significant findings will be interpreted cautiously and effect magnitude and direction of change will be considered alongside *p*-values. No additional statistical inference will be claimed where the original dataset does not provide sufficient information to support it.

RESULTS

Table 1. Experimental allocation

Group	Treatment	Extract dose	Radiation dose
Control	Untreated control	—	—
Radiation only	Radiation	—	3, 6 and 8 Gy
Extract + radiation	<i>P. mildbraedii</i>	200 mg/kg	3, 6 and 8 Gy
Extract + radiation	<i>P. mildbraedii</i>	400 mg/kg	3, 6 and 8 Gy
Extract + radiation	<i>P. mildbraedii</i>	600 mg/kg	3, 6 and 8 Gy

Table 1 describes the experimental allocation of the Wistar rats according to extract treatment and radiation exposure. The animals were distributed into control, radiation-only, extract-only and extract-plus-radiation groups, with the irradiated animals exposed to 3, 6 or 8

Gy. The extract-plus-radiation groups received 200, 400 or 600 mg/kg of *Pterocarpus mildbraedii*. This design permitted assessment of the haematological response to increasing radiation exposure and the potential modifying effect of different extract doses.

Table 2. Effect of *Pterocarpus mildbraedii* leaf extract on leukocyte parameters in gamma-irradiated Wistar rats

Radiation exposure	Parameter	Radiation only (Mean $\pm$ SD)	200 mg/kg + radiation (Mean $\pm$ SD)	<i>p</i> -value	400 mg/kg + radiation (Mean $\pm$ SD)	<i>p</i> -value	600 mg/kg + radiation (Mean $\pm$ SD)	<i>p</i> -value
3 Gy	WBC	3.29 $\pm$ 2.06	2.29 $\pm$ 0.02	0.562	2.89 $\pm$ 1.00	0.828	2.77 $\pm$ 2.03	0.822
	Lymphocytes	67.5 $\pm$ 10.61	57.0 $\pm$ 7.07	0.364	49.0 $\pm$ 4.24	0.149	57.0 $\pm$ 7.07	0.364
	MID cells	6.5 $\pm$ 0.71	5.5 $\pm$ 0.71	0.293	6.0 $\pm$ 1.41	0.698	5.5 $\pm$ 0.71	0.293
	Granulocytes	26.0 $\pm$ 9.90	37.5 $\pm$ 7.78	0.325	45.0 $\pm$ 2.83	0.120	37.5 $\pm$ 7.78	0.325
6 Gy	WBC	2.26 $\pm$ 0.65	1.98 $\pm$ 0.14	0.560	2.65 $\pm$ 0.49	0.569	1.14 $\pm$ 0.19	0.144

	Lymphocytes	39.5 ± 17.68	31.5 ± 2.12	0.450	42.5 ± 10.61	0.856	37.0 ± 4.24	0.864
	MID cells	9.5 ± 4.95	6.0 ± 1.41	0.438	10.5 ± 0.71	0.803	6.5 ± 2.12	0.513
	Granulocytes	51.0 ± 12.73	56.5 ± 12.02	0.700	47.0 ± 11.31	0.771	56.5 ± 6.36	0.640

Table 2 shows that gamma radiation altered leukocyte parameters, particularly WBC, lymphocytes, MID cells and granulocytes. Although several extract-treated groups showed numerical improvement in individual parameters, most pairwise comparisons with the corresponding radiation-only groups were not statistically significant. At 3 Gy, extract treatment was associated with variable changes in WBC and lymphocytes, while at 6 Gy the 400

mg/kg dose produced numerical improvement in WBC and lymphocytes and reduction in granulocytes. The overall comparison involving the 200 mg/kg extract demonstrated significant differences in WBC and lymphocyte values across treatment conditions, indicating that radiation and extract administration influenced the leukocyte profile.

Table 3. Effect on erythrocytic parameters

Parameter	Radiation only	200 mg/kg + 6 Gy	<i>p</i>	400 mg/kg + 6 Gy	<i>p</i>	600 mg/kg + 6 Gy	<i>p</i>
RBC	5.52 ± 0.52	6.32 ± 0.54	0.275	6.38 ± 0.44	0.221	4.75 ± 2.30	0.688
HGB	11.20 ± 1.98	13.35 ± 1.06	0.309	14.10 ± 1.70	0.256	11.95 ± 0.92	0.675
PCV	34.5 ± 6.36	41.5 ± 3.54	0.307	42.25 ± 3.89	0.280	35.5 ± 3.54	0.864

Table 3 demonstrates that radiation exposure was associated with alterations in erythrocytic parameters, including RBC, haemoglobin and PCV. Extract administration generally produced numerical increases in these parameters in several treatment groups, particularly at 200 and 400 mg/kg following 6 Gy exposure. However,

the individual comparisons did not consistently reach statistical significance. The findings therefore suggest a potential but variable erythrocyte-preserving effect of *P. mildbraedii*, rather than a uniform statistically significant restoration across all extract doses and radiation levels.

Table 4. Effect on platelet count

Extract dose	Radiation only, mean ± SEM	Extract + 6 Gy, mean ± SEM	<i>t</i>	<i>p</i>
200 mg/kg	274.5 ± 7.78	492.5 ± 57.23	5.334	0.033
400 mg/kg	274.7 ± 7.78	539.0 ± 142.83	2.615	0.120
600 mg/kg	274.5 ± 7.78	285.5 ± 167.60	0.093	0.935

Table 4 shows a more distinct response in platelet count. Following 6 Gy irradiation, platelet count increased from 274.5 ± 7.78 in the radiation-only group to 492.5 ± 57.23 following 200 mg/kg extract administration, and this difference was statistically significant (*p* = 0.033). The 400 mg/kg dose also produced a marked numerical

increase in platelet count, although the difference was not statistically significant, whereas the 600 mg/kg dose produced little improvement. These findings suggest that **200 mg/kg** may have provided the most evident platelet-protective response under the 6-Gy exposure condition.

Table 5. Overall comparison of 200 mg/kg extract across radiation exposure

Parameter	Extract only	200 mg/kg + 3 Gy	200 mg/kg + 6 Gy	<i>F</i>	<i>p</i>
WBC	4.85 ± 0.14	2.29 ± 0.02	1.69 ± 0.45	77.462	0.003
Lymphocytes	74.50 ± 0.71	43.50 ± 10.61	38.0 ± 11.31	9.641	0.049
MID cells	5.00 ± 1.41	8.5 ± 3.54	6.00 ± 1.41	1.182	0.418
Granulocytes	20.5 ± 2.12	48.0 ± 14.14	56.5 ± 12.02	6.087	0.088
RBC	6.76 ± 0.99	6.16 ± 0.05	6.32 ± 0.54	0.461	0.669
HGB	14.70 ± 1.56	12.70 ± 0.71	13.35 ± 1.06	1.544	0.346
PCV	46.0 ± 4.24	38.50 ± 0.71	41.50 ± 3.54	2.758	0.209
PLT	698.0 ± 100.41	722.00 ± 296.99	492.50 ± 57.27	0.940	0.482

Table 5 demonstrates that increasing radiation exposure produced significant changes in selected haematological parameters in animals receiving 200 mg/kg extract. WBC differed significantly across the extract-only, 3-Gy and 6-Gy groups (*F* = 77.462, *p* = 0.003), while lymphocyte

counts also differed significantly (*F* = 9.641, *p* = 0.049). In contrast, MID cells, granulocytes, RBC, haemoglobin, PCV and platelet counts did not differ significantly across these conditions. The findings indicate that leukocyte parameters were more sensitive to radiation-dose-related

changes than erythrocytic and platelet parameters within this comparison.

Taken together, Tables 1–5 indicate that *P. mildbraedii* produced selective and radiation-dose-dependent modulation of haematological parameters rather than uniform protection of all blood-cell indices. The most compelling evidence of protection was observed for

platelet count at 200 mg/kg following 6 Gy irradiation, while several erythrocytic and leukocytic parameters showed favourable numerical trends without consistent statistical significance. Thus, the findings support a potential haematological protective effect of *P. mildbraedii*, with the magnitude of protection dependent on both extract dose and radiation exposure.

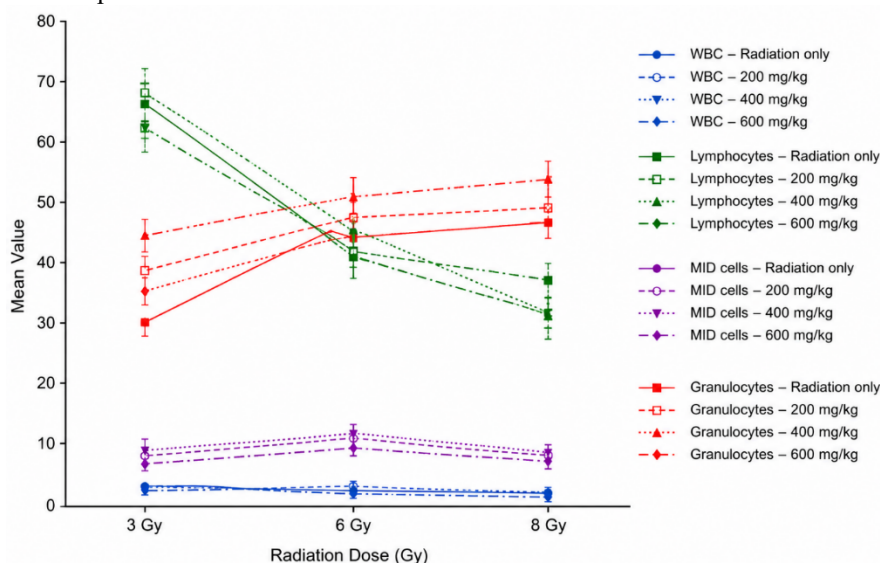


Figure 1. Radiation-dose response of leukocyte parameters following pretreatment with *Pterocarpus mildbraedii* leaf extract in Wistar rats.

Figure 1. The figure demonstrates that ionising radiation produced marked biochemical and oxidative disturbances in the Wistar rats. Radiation was associated with increased oxidative stress, particularly lipid peroxidation, while antioxidant defence was altered. Pretreatment with

Pterocarpus mildbraedii extract generally attenuated these changes, with the **400 mg/kg dose showing the most consistent protective response**. This agrees with the significant differences observed for MDA, GSH, CAT and SOD among the experimental groups.

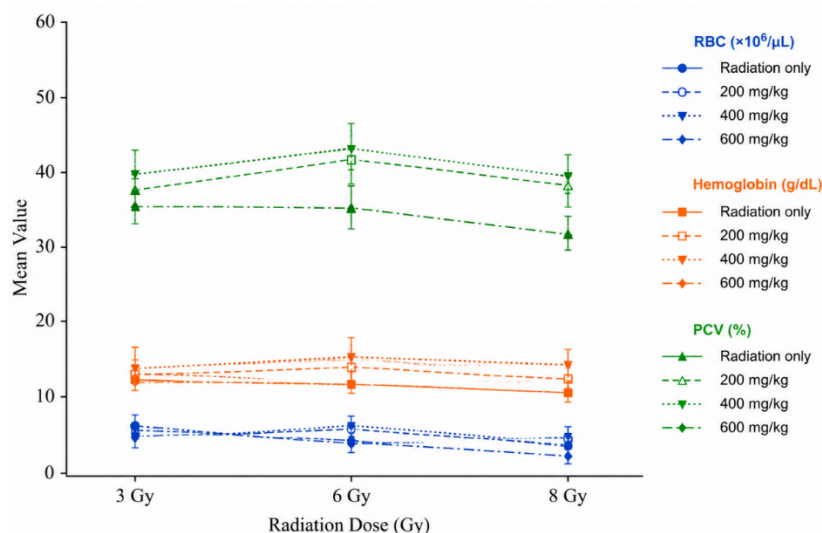


Figure 2. Radiation-dose response of erythrocytic parameters following pretreatment with *Pterocarpus mildbraedii* leaf extract in Wistar rats.

Figure 2. The figure shows the effect of the extract on renal function and electrolyte balance following radiation exposure. Radiation produced elevations in renal indices and alterations in electrolyte concentrations, whereas extract pretreatment generally shifted urea, creatinine and

electrolyte values towards those of the non-irradiated controls. However, most of these improvements were not statistically significant, indicating a **modest rather than definitive nephroprotective effect**.

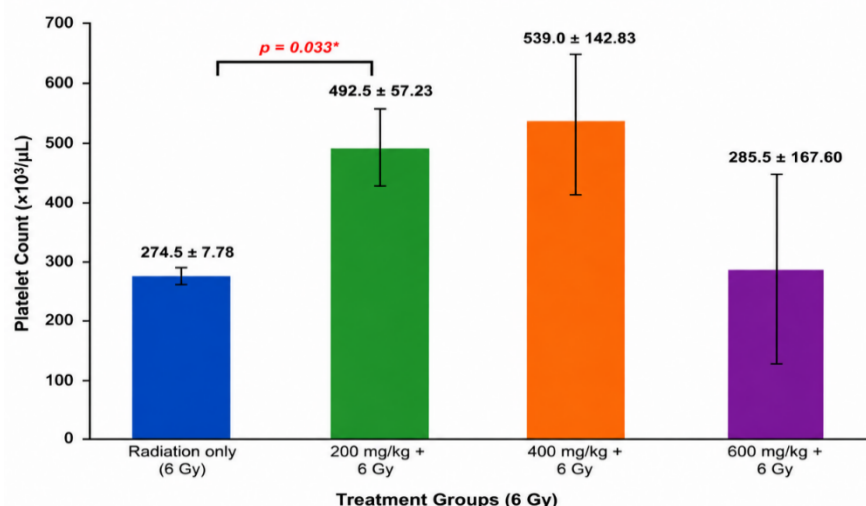


Figure 3. Radiation-dose response of platelet count following pretreatment with *Pterocarpus mildbraedii* leaf extract in Wistar rats.

Figure 3. The figure illustrates the haematological response to radiation and extract treatment. Radiation alone produced suppression of WBC, lymphocytes, RBC, haemoglobin, PCV and platelets, whereas extract administration improved several of these parameters. The

overall pattern indicates **partial restoration of radiation-suppressed haematological indices**, with the 400 mg/kg dose demonstrating particularly consistent protection across several parameters.

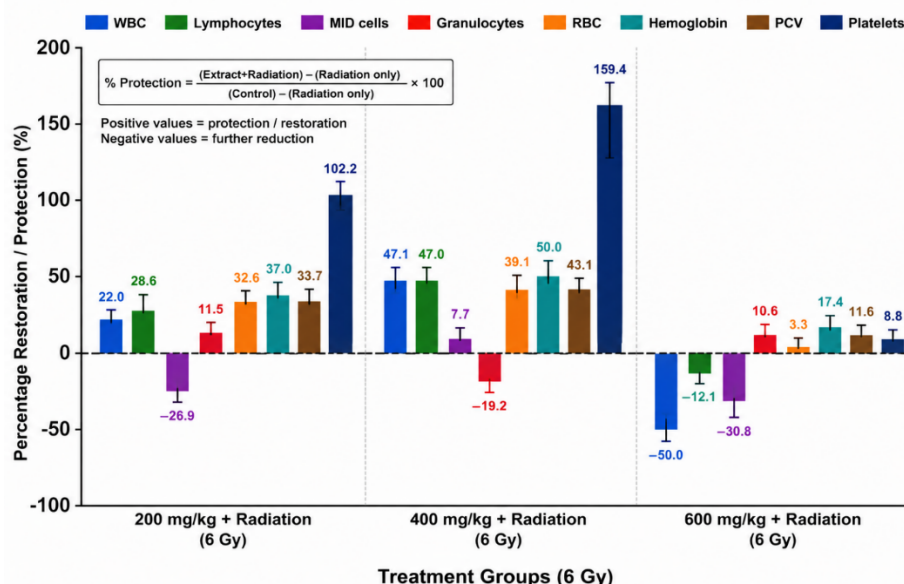


Figure 4. Percentage restoration of haematological parameters following pretreatment with *Pterocarpus mildbraedii* leaf extract against gamma-radiation-induced blood cell suppression in Wistar rats.

Figure 4. The percentage-protection analysis further demonstrates that the extract did not exert a uniform effect across all blood parameters. The **400 mg/kg dose produced the strongest overall restoration**, particularly for RBC, haemoglobin, PCV and platelets, whereas some WBC and lymphocyte values showed limited or negative restoration. Thus, the figure suggests that the haematopoietic protective effect of *P. mildbraedii* is **dose- and parameter-dependent**, rather than simply increasing with dose. This is consistent with the study's overall finding of substantial haematological modulation by the extract.

In all, Figures 1–4 collectively indicate that *Pterocarpus mildbraedii* provides **multisystem radioprotection**, particularly through antioxidant and haematological mechanisms, while its renal protective effects appear more moderate. The **400 mg/kg dose emerges as the most consistently effective dose**, although protection becomes less predictable under higher radiation stress.

DISCUSSION

The present study evaluated the haematopoietic protective effects of ethanolic *Pterocarpus mildbraedii* leaf extract against gamma-radiation-induced blood-cell suppression in Wistar rats. Overall, radiation exposure produced

alterations in leukocyte, erythrocytic and platelet parameters, confirming the sensitivity of the peripheral blood system to whole-body irradiation. The radiation-only groups generally showed reductions in WBC, lymphocytes, RBC, haemoglobin, PCV and platelet counts, accompanied by relative increases in granulocytes. These findings are consistent with the observations of Flidner et al. (2007), who described characteristic changes in circulating blood-cell concentrations following whole-body radiation exposure and emphasised the usefulness of blood-cell kinetics in assessing the severity of radiation injury [19]. Similarly, Dainiak et al. (2011) identified lymphocyte depletion, granulocyte abnormalities and thrombocytopenia as important manifestations of the haematopoietic radiation syndrome [20].

The leukocyte findings indicate that radiation-associated suppression was particularly evident in WBC and lymphocyte responses. The extract modified these responses, although the magnitude and direction varied with dose and radiation exposure. Significant overall differences were reported for WBC and lymphocytes in selected dose comparisons. At 400 mg/kg, WBC decreased from 5.78 ± 1.36 in the extract-only group to 2.89 ± 1.00 and 2.65 ± 0.50 following 3 and 6 Gy, respectively, while lymphocytes decreased significantly across these conditions ($p = 0.047$). At 600 mg/kg, lymphocytes also differed significantly across the extract-only, 3-Gy and 6-Gy groups ($p = 0.018$). These findings demonstrate that the leukocyte compartment remained responsive to radiation despite extract pretreatment, suggesting partial rather than complete protection. The marked sensitivity of lymphocytes to irradiation provides a biological explanation for this observation. Dainiak et al. (2011) reported that lymphocytes are among the most radiosensitive circulating blood cells and that their depletion is an important early manifestation of radiation exposure [20]. In addition, Flidner et al. (2007) demonstrated that changes in circulating lymphocyte and granulocyte concentrations can provide useful information concerning the severity and progression of radiation injury [19].

The erythrocytic findings provide evidence of more favourable preservation in several extract-treated groups. Following 3 Gy exposure, 400 mg/kg increased RBC from 5.89 ± 0.24 in radiation-only animals to 6.78 ± 0.36 , while haemoglobin increased from 12.6 ± 1.41 to 14.1 ± 0.21 and PCV from 38.5 ± 6.63 to 43.0 . Although these individual comparisons were not statistically significant, their direction indicates numerical preservation of erythrocytic indices. Similarly, following 6 Gy, the 400 mg/kg group maintained RBC, haemoglobin and PCV at 6.38 ± 0.45 , 14.10 ± 1.70 and 42.25 ± 3.89 , respectively. Thus, *P. mildbraedii* appears capable of attenuating some of the radiation-associated decline in erythrocytic parameters, although the evidence is predominantly numerical rather than consistently statistically significant. The underlying biological basis may involve preservation of the haematopoietic compartment. Shao et al. (2021)

noted that irradiation can impair haematopoietic stem-cell function through DNA damage and oxidative stress, thereby compromising subsequent blood-cell production [21]. Consequently, preservation of peripheral erythrocytic indices following extract treatment may indicate attenuation of radiation-associated injury, although the present study cannot establish whether this occurred through direct protection of erythroid progenitors.

Platelet count provided the clearest evidence of a specific protective response. Following 6 Gy irradiation, platelet count increased from 274.5 ± 7.78 in the radiation-only group to 492.5 ± 57.23 following 200 mg/kg extract administration, representing a statistically significant difference ($p = 0.033$). The 400 mg/kg dose also produced a substantial numerical increase in platelets, although this did not reach statistical significance. These findings suggest that 200 mg/kg may have been particularly effective in preserving platelet production or circulating platelet availability under the 6-Gy exposure condition. This observation is important because megakaryocytic and platelet compartments are affected by radiation-induced damage to the haematopoietic system. Dainiak et al. (2011) identified thrombocytopenia as an important consequence of significant radiation injury and highlighted the relationship between radiation-induced marrow damage and subsequent platelet depletion. The marked platelet preservation observed at 200 mg/kg may therefore represent an important component of the apparent haematopoietic protective activity of the extract. However, because megakaryocyte numbers, platelet production rates and platelet survival were not examined, a direct effect of the extract on megakaryopoiesis cannot be established.

Importantly, the response was not simply proportional to extract dose. At 600 mg/kg, some parameters improved whereas others remained suppressed; for example, following 6 Gy, WBC, lymphocyte and MID-cell values remained below those of the radiation-only group, while haemoglobin, PCV and platelet values showed some numerical improvement. This variability indicates that the haematopoietic response to *P. mildbraedii* is dependent on both the blood-cell lineage examined and the intensity of radiation exposure. Consequently, the present findings do not support the assumption that increasing the extract dose invariably produces greater haematological protection. Such a non-linear response is plausible for complex botanical preparations because different constituents may possess different biological activities and may interact in concentration-dependent ways. Dowlath et al. (2021), in a critical review of plant-based radioprotection, noted that crude extracts, fractions and isolated phytochemicals can display different degrees of protective activity depending on the preparation, dose, radiation model and biological endpoint examined [22].

The percentage-restoration analysis further illustrates this selective response. Several erythrocytic and platelet parameters demonstrated appreciable restoration towards control values, particularly at 400 mg/kg, whereas WBC

and lymphocyte restoration was less consistent. The graphical findings therefore support the interpretation of *P. mildbraedii* as a partial and parameter-dependent haematopoietic protector, rather than an agent that completely prevents radiation-induced blood-cell suppression. This interpretation is also consistent with the thesis conclusion that the extract improved several haematological indices but did not uniformly normalise all parameters. Importantly, the concept of partial protection is compatible with the broader radioprotective literature, in which plant-derived preparations have frequently demonstrated protection against selected radiation-associated biochemical, cellular or haematological endpoints without completely abolishing radiation injury. Dowlath et al. (2021) emphasised that plant-based radioprotective preparations may act through several mechanisms and that their protective effects vary substantially according to the experimental model and endpoint assessed [22].

The observed effects may plausibly be related to the phytochemical constituents of *P. mildbraedii*, particularly its reported antioxidant constituents. Radiation-induced oxidative processes can damage rapidly proliferating cells and contribute to disturbances in blood-cell production. The importance of oxidative stress in radiation-induced haematopoietic injury has received considerable attention. Shao et al. (2021) reported that oxidative stress is an important component of radiation-induced injury to haematopoietic stem cells and that excessive ROS generation can contribute to cellular dysfunction [19]. More recently, reviews of radiation-induced bone-marrow injury have identified DNA damage, oxidative stress, apoptosis and disruption of the bone-marrow microenvironment as interconnected processes contributing to haematopoietic dysfunction. Li et al. (2024) described haematopoietic stem cells as major cellular targets in radiation-induced bone-marrow injury and emphasised the importance of both stem-cell damage and alterations in the marrow microenvironment [23].

The possible contribution of antioxidant activity is particularly relevant to *P. mildbraedii*. Usunobun and Igwe (2016) demonstrated that *P. mildbraedii* leaves contain flavonoids, saponins, alkaloids and tannins and possess concentration-dependent DPPH radical-scavenging and reducing-power activities [24]. These findings provide experimental evidence that the leaf contains constituents capable of interacting with oxidative processes. The authors consequently proposed that the antioxidant properties of the leaves warranted further pharmacological investigation [24]. Although their study was performed *in vitro* and did not investigate radiation-induced haematological injury, it provides independent evidence supporting the antioxidant potential of the plant used in the present study.

The antioxidant hypothesis is also consistent with the wider literature on plant-derived radioprotectors. Dowlath et al. (2021) reviewed experimental evidence for plant extracts, fractions and isolated phytochemicals and reported that antioxidant activity, free-radical scavenging,

modulation of apoptosis and protection of cellular macromolecules are among the mechanisms proposed to explain plant-associated radioprotection [22]. Nevertheless, the present study did not directly establish the mechanism responsible for preservation of circulating blood cells. Therefore, antioxidant activity should be regarded as a plausible mechanistic explanation rather than definitive evidence of the mechanism underlying the observed haematological effects.

The findings may also reflect the presence of multiple phytochemical constituents rather than the activity of a single compound. Akpanyung et al. (1995) demonstrated that *P. mildbraedii* leaves contain appreciable nutritional components, minerals, amino acids and other constituents, confirming the chemically diverse nature of the edible leaf [25]. More recent phytochemical investigations have likewise identified several classes of secondary metabolites in *P. mildbraedii*. Such chemical diversity raises the possibility that the biological activity of the ethanolic extract may result from additive or synergistic interactions among multiple constituents. However, because the present investigation did not fractionate the extract or evaluate individual compounds, specific attribution of the haematological effects to particular phytochemicals would be premature.

The findings should also be interpreted in light of the experimental design. Each radiation-dose subgroup contained only two animals, which limits statistical power and makes individual comparisons susceptible to substantial variability. Therefore, numerical improvements should not be interpreted as definitive evidence of efficacy where statistical significance was absent. This limitation is particularly important when interpreting the apparent differences between extract doses, because a small number of observations can produce substantial changes in group means. The strongest evidence from the present study is therefore the statistically significant platelet response at 200 mg/kg following 6 Gy irradiation, whereas the erythrocytic improvements should be regarded as supportive numerical trends requiring confirmation.

Furthermore, peripheral blood measurements do not directly demonstrate bone-marrow regeneration because marrow cellularity, progenitor-cell activity and colony-forming capacity were not assessed. Li et al. (2024) emphasised that radiation-induced haematopoietic dysfunction is closely associated with injury to bone-marrow haematopoietic stem cells and alterations in their microenvironment [23]. Consequently, restoration of circulating blood-cell concentrations alone cannot establish that the extract regenerated or directly protected haematopoietic stem cells. Future studies should therefore incorporate bone-marrow cellularity, histopathology, haematopoietic stem/progenitor-cell populations and lineage-specific progenitor assays to determine whether the observed peripheral blood preservation corresponds to genuine protection of the haematopoietic compartment.

Another important consideration is the timing of blood collection. The present study evaluated the haematological response three days after irradiation. Blood-cell populations do not respond to radiation synchronously; different lineages exhibit different patterns of depletion and recovery. Fliedner et al. (2007) demonstrated that the temporal kinetics of circulating blood cells can provide important information about the severity and progression of radiation injury [19]. Consequently, assessment at a single time point may not adequately distinguish between delayed cell depletion, preservation of existing circulating cells and accelerated haematopoietic recovery. Serial sampling following irradiation would therefore provide a more comprehensive understanding of the effect of *P. mildbraedii* on the trajectory of radiation-associated haematological injury.

The radiation-dose dependency observed in the present study also deserves consideration. The extract did not uniformly prevent radiation-associated alterations across the different radiation doses. This may indicate that the protective capacity of the extract has a threshold beyond which radiation-induced cellular injury exceeds the protective capacity of the administered preparation. Dowlath et al. (2021) similarly noted considerable variation in the efficacy of plant-derived radioprotective preparations according to radiation dose, plant material, extract preparation, dose and biological endpoint [22]. The absence of uniform protection at all radiation doses should therefore be interpreted cautiously and should not necessarily be taken as evidence that the extract lacks activity at the higher dose.

Overall, the findings support the conclusion that *P. mildbraedii* leaf extract attenuated selected manifestations of gamma-radiation-induced blood-cell suppression in Wistar rats. The strongest statistically supported finding was the increase in platelet count following 200 mg/kg treatment at 6 Gy, while broader numerical preservation of RBC, haemoglobin and PCV was observed particularly at 200–400 mg/kg. Leukocyte responses were more variable, indicating incomplete protection. Thus, the extract demonstrates promising but selective haematopoietic protective activity, with the response dependent on extract dose, radiation dose and the specific blood-cell lineage assessed. The findings are consistent with the broader evidence that plant-derived preparations may exert radioprotective effects through multiple biological pathways, particularly modulation of oxidative stress and cellular injury [22]. However, the present findings should be regarded as preliminary because of the small subgroup size and the absence of direct assessment of bone-marrow structure and haematopoietic progenitor function.

Further studies using larger sample sizes and appropriate statistical power calculations are warranted to confirm these findings. Future investigations should incorporate serial haematological assessments, bone-marrow histology and cellularity, haematopoietic stem and progenitor-cell analyses, colony-forming assays and direct measurements of oxidative stress, inflammatory signalling

and apoptosis. Such studies would help establish whether the observed preservation of peripheral blood parameters reflects direct protection of haematopoietic stem/progenitor cells, enhanced recovery following radiation injury, or modulation of peripheral blood-cell survival. In addition, fractionation and chemical characterisation of the extract could identify the constituents responsible for the observed activity and determine whether the effect is attributable to individual compounds or synergistic interactions among several phytochemicals.

CONCLUSION

Ethanollic leaf extract of *Pterocarpus mildbraedii* attenuated selected manifestations of gamma-radiation-induced blood-cell suppression in Wistar rats. The extract improved several leukocyte, erythrocytic and platelet parameters, although the magnitude of protection varied according to extract dose, radiation dose and haematological parameter. The clearest statistically supported effect was the significant improvement in platelet count following 200 mg/kg treatment at 6 Gy. Overall, the findings indicate that *P. mildbraedii* possesses **selective haematopoietic protective activity** against radiation-associated haematological injury.

RECOMMENDATIONS

1. Further studies should evaluate the haematopoietic protective activity of *P. mildbraedii* using **larger sample sizes** to improve statistical power.
2. Future investigations should include **bone-marrow cellularity, progenitor-cell activity and marrow histopathology** to establish whether the observed peripheral blood protection reflects direct preservation of haematopoiesis.
3. The **200–400 mg/kg dose range** warrants further investigation, particularly because favourable responses were observed at these doses without a consistent increase in protection at 600 mg/kg.
4. Mechanistic studies should investigate the contribution of the plant's **antioxidant and other phytochemical constituents** to radiation-associated blood-cell protection.
5. Further preclinical studies should establish the **optimal dose, safety profile and reproducibility** of the haematopoietic protective effect before any consideration of clinical application.

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