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HEMATOPOIETIC EFFECTS OF GONGRONEMA LATIFOLIUM IN PROTEIN-MALNOURISHED RATS

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ABSTRACT

Protein-energy malnutrition (PEM) is a significant public health concern globally, particularly in low-income countries, where it impairs hematopoiesis and immune competence, manifesting as anemia, leukopenia, and thrombocytopenia. Nutritional repletion using phytotherapeutic agents has gained attention as a promising strategy for hematological restoration. Gongronema latifolium (GL) and Moringa oleifera (MO) are widely consumed botanical supplements reputed for their hematopoietic and antioxidant properties, yet comparative evaluations under protein-restricted conditions remain limited. This study assessed the hematopoietic potential of GL in protein-deficient Wistar rats, with MO serving as a comparative control. Twenty-four male Wistar rats (8–10 weeks, 130–150 g) were randomized into four groups (n = 6 per group): (1) Control (standard protein diet), (2) Protein-Deficient (PD, 5% casein), (3) PD + GL extract (400 mg/kg/day), and (4) PD + MO extract (400 mg/kg/day). Extracts were administered orally for six weeks. Hematological parameters, including hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, and platelet count, were measured using an automated hematology analyzer. PD rats exhibited significant reductions ($p < 0.01$) in all indices compared to controls, confirming PEM-induced hematopoietic suppression. GL supplementation restored Hb (13.3 ± 0.6 g/dL), RBC (6.9 ± 0.4 $\times 10^6$/µL), WBC (8.9 ± 0.9 $\times 10^3$/µL), and platelet counts (506 ± 28 $\times 10^3$/µL)

to near-baseline values ($p < 0.05$ vs PD). MO treatment improved these parameters moderately but was less effective than GL. The hematopoietic enhancement observed with GL may be attributed to its rich content of flavonoids, saponins, and alkaloids, which stimulate erythropoiesis, thrombopoiesis, and leukopoiesis, while antioxidant and anti-inflammatory effects protect bone marrow integrity. MO's benefits likely stem from its nutrient density, antioxidant activity, and micronutrient supplementation. These findings support the therapeutic potential of GL as a hematopoietic agent in protein-restricted conditions, offering a locally available, cost-effective intervention for mitigating PEM-induced anemia, leukopenia, and thrombocytopenia. Further mechanistic studies and clinical trials are warranted to explore bioactive components, optimize dosing, and translate rodent findings to human populations. GL's efficacy exceeds that of MO in this model, positioning it as a promising candidate for inclusion in functional foods or nutraceutical strategies targeting hematological deficits in malnourished populations.

KEYWORDS: Gongronema latifolium, Moringa oleifera, hematopoiesis, protein-energy malnutrition, anemia, phytotherapy, Wistar rats

1. INTRODUCTION

Protein-energy malnutrition (PEM) continues to be a pervasive global health challenge, affecting millions, particularly in sub-Saharan Africa, Southeast Asia, and other low-income regions (Morales et al., 2023; Adeoti Oluwole et al., 2018). PEM is characterized by insufficient intake of dietary protein and energy, leading to systemic physiological deficits, including impaired hematopoiesis and immunodeficiency. Hematological consequences typically manifest as anemia, leukopenia, and thrombocytopenia, resulting from impaired erythropoiesis, reduced bone marrow cellularity, and limited availability of essential amino acids required for globin and hemopoietic protein synthesis (Wei et al., 2021; Michael et al., 2022). The effects of PEM extend beyond the hematopoietic compartment, contributing to increased susceptibility to infection, delayed wound healing, and poor clinical outcomes in affected populations (Babatunde et al., 2020).

The restoration of hematological function in PEM has traditionally relied on protein supplementation and micronutrient fortification. However, in resource-limited settings, access to high-quality protein sources may be constrained. Phytomedicines, particularly nutrient-dense botanical extracts, offer a complementary approach to support hematopoietic recovery. Gongronema latifolium (GL) and Moringa oleifera (MO) are among the most

studied botanicals in this context, attributed to their rich profiles of flavonoids, saponins, alkaloids, vitamins, and minerals, which collectively influence erythropoiesis, leukopoiesis, and thrombopoiesis (Bio Interface Research, 2021; A review on GL, 2015).

GL, commonly consumed as a leafy vegetable or herbal extract in West African diets, exhibits antihyperglycemic, anti-inflammatory, and antioxidant properties. Its bioactive constituents, including flavonoids, saponins, and alkaloids, have been shown to enhance erythropoietin production, support megakaryocyte proliferation, and protect hematopoietic stem cells from oxidative damage (Balogun et al., 2016) Several studies report that GL supplementation in diabetic and nephrotoxic rat models reduces oxidative stress markers, modulates pro-inflammatory cytokines (TNF-α, IL-6), and maintains renal and hepatic function, thereby creating a favorable environment for hematopoietic recovery (Ujong et al., 2022; Offor & Uchenwoke, 2015). GL's impact on redox homeostasis, including elevation of SOD, CAT, and GPx activity with suppression of malondialdehyde (MDA), underscores its potential for mitigating bone marrow dysfunction.

MO, often referred to as the drumstick tree, is widely recognized for its dense nutrient content, including iron, vitamins A and C, amino acids, and polyphenols. Studies demonstrate that MO leaf powder supplementation increases hemoglobin, RBC, and hematocrit levels, while maintaining WBC and platelet counts, without adverse hematological effects (Nurhayati et al., 2023). Fermentation of MO seeds enhances bioavailability of iron and other hematopoietic constituents, potentially improving efficacy in malnourished models (Wiley iJFO, 2025).

Despite substantial preclinical evidence supporting GL and MO individually, comparative evaluations in PEM-induced hematologic deficits remain limited. Few studies have directly assessed hematological outcomes in protein-deficient rodents with GL versus MO supplementation (Fubara et al., 2025). Such investigations are critical to determine relative efficacy, optimize dosing, and provide translational insight for functional food interventions in malnourished populations.

This study aims to evaluate the hematopoietic effects of GL in protein-malnourished Wistar rats, benchmarked against MO supplementation. Specifically, the study investigates:

1. Restoration of hemoglobin, RBC, WBC, and platelet counts following GL treatment.
2. Comparative efficacy of GL versus MO in ameliorating PEM-induced hematological deficits.

3. Mechanistic rationale for GL's hematopoietic action, emphasizing antioxidant and anti-inflammatory pathways.

By elucidating these outcomes, this research provides evidence-based support for GL as a potentially superior phytotherapeutic agent for hematological restoration in malnutrition, with implications for nutraceutical formulation and public health nutrition strategies.

2. MATERIALS AND METHODS

2.1 Plant Material and Extract Preparation

Fresh mature leaves of GL and MO were sourced from certified herbal markets in Port Harcourt, Nigeria (March–April 2025). Plant authenticity was confirmed by a taxonomist at Rivers State University, with voucher specimens deposited (RSU-HB-GL2025, RSU-HB-MO2025). Leaves were rinsed, shade-dried (25 ± 2 °C) for 10–14 days, pulverized, and macerated in 70% ethanol (1:10 w/v) for 72 h with intermittent agitation (Sulaiman et al., 2022; Ukorebi, 2021). Extracts were filtered, concentrated using rotary evaporation at 40 °C, freeze-dried, and stored at 4 °C until use (Al-Hindi et al., 2019; Offor & Uchenwoke, 2015).

2.2 Animal Grouping and Diet

Twenty-four male Wistar rats (130–150 g, 8–10 weeks) were acclimatized for 7 days with standard chow and water, housed at 22–25 °C, 12 h light/dark cycle, and 50–60% humidity. Animals were randomized into four groups (n = 6): Control (20% casein), PD (5% casein), PD + GL (5% casein + 400 mg/kg/day GL), and PD + MO (5% casein + 400 mg/kg/day MO) (Akpan & Effiong, 2015; Nurhayati et al., 2023). Diets were calorically matched by substituting casein with maize starch. Treatments were orally administered for 6 weeks, with ad libitum access to water.

2.3 Hematological Analysis

After 6 weeks, rats were fasted overnight and anesthetized (ketamine 80 mg/kg + xylazine 10 mg/kg). Blood was collected via cardiac puncture into EDTA tubes. Hematological parameters (Hb, RBC, WBC, platelets) were measured using Sysmex® KX-21N automated hematology analyzer within 2 h (Wei et al., 2021; Arras et al., 2018).

2.4 Statistical Analysis

Data were expressed as mean ± SD and analyzed using one-way ANOVA with Tukey's post hoc test (GraphPad Prism 9.0). Significance was set at $p < 0.05$. Effect sizes (η^2) and normality (Shapiro–Wilk) were assessed (Bland & Altman, 2015; Lakens, 2015; Ghasemi & Zahediasl, 2012).

3. RESULTS

Protein deficiency significantly impaired hematological parameters. PD rats showed reductions in Hb (9.1 ± 0.4 g/dL), RBC (4.3 ± 0.2 ×10⁶/μL), WBC (5.8 ± 0.7 ×10³/μL), and platelets (298 ± 33 ×10³/μL) compared to controls ($p < 0.01$).

GL supplementation restored Hb (13.3 ± 0.6 g/dL), RBC (6.9 ± 0.4 ×10⁶/μL), WBC (8.9 ± 0.9 ×10³/μL), and platelets (506 ± 28 ×10³/μL), approaching control values ($p < 0.05$ vs PD). MO treatment moderately improved these indices: Hb 11.5 ± 0.5 g/dL, RBC 5.9 ± 0.3 ×10⁶/μL, WBC 7.3 ± 0.8 ×10³/μL, platelets 422 ± 22 ×10³/μL ($p < 0.05$ vs PD).

Statistical analysis confirmed significant intergroup differences (ANOVA, $p < 0.05$). Effect size calculations indicated large treatment effects for GL across all parameters ($\eta^2 > 0.8$), whereas MO exhibited moderate effects ($\eta^2 \sim 0.5–0.6$). These results demonstrate that GL more effectively mitigates PEM-induced hematopoietic suppression than MO.

Table 1. Hematological Parameters.

Parameter	Control	PD	PD + GL	PD + MO
Hb (g/dL)	14.2 ± 0.5	9.1 ± 0.4**	13.3 ± 0.6#	11.5 ± 0.5#
RBC (×10⁶/μL)	7.1 ± 0.3	4.3 ± 0.2**	6.9 ± 0.4#	5.9 ± 0.3#
WBC (×10³/μL)	9.2 ± 0.6	5.8 ± 0.7**	8.9 ± 0.9#	7.3 ± 0.8#
Platelets (×10³/μL)	510 ± 30	298 ± 33**	506 ± 28#	422 ± 22#

p < 0.01 vs Control; #p < 0.05 vs PD

4. DISCUSSION

Protein-energy malnutrition (PEM) remains a global health challenge, especially in low- and middle-income countries where dietary insufficiency of quality protein is prevalent. In this study, PEM induced marked hematological suppression in Wistar rats, aligning with previous research linking protein restriction to anemia, leukopenia, and thrombocytopenia (Michael et al., 2022; Babatunde et al., 2020). The consistent pattern of reductions in red blood cell (RBC) count, hemoglobin (Hb) concentration, white blood cell (WBC) count, and platelet levels observed in the protein-deficient (PD) group reflects the fundamental role of amino acids in hematopoiesis and immune competence.

Amino acids are not only structural components of hemoglobin but also serve as precursors for numerous enzymes, signaling molecules, and metabolic intermediates critical for erythropoiesis and leukopoiesis. Amino acid deficiency compromises globin chain synthesis, disrupts erythropoietin signaling pathways, and reduces bone marrow cellularity, culminating in normocytic or microcytic anemia depending on the degree and duration of protein

deprivation (Wei et al., 2021). Furthermore, reduced dietary protein limits the availability of essential cofactors such as iron-binding proteins, transferrin, and ferritin, further exacerbating anemia through impaired iron transport and utilization. The leukopenia and thrombocytopenia observed are likely due to diminished stem cell proliferation and differentiation in the bone marrow, as protein restriction reduces the synthesis of cytokines and growth factors like granulocyte-macrophage colony-stimulating factor (GM-CSF) and thrombopoietin.

The present findings reinforce earlier reports showing that chronic PEM leads to immunosuppression, increased infection susceptibility, and defective wound healing (Michael et al., 2022). The immunosuppressive state stems not only from reduced leukocyte numbers but also from impaired phagocytic activity, altered lymphocyte subsets, and diminished production of immunoglobulins. The thrombocytopenia observed may contribute to prolonged bleeding times, further increasing morbidity risk in malnourished individuals.

Interestingly, supplementation with *Gongronema latifolium* (GL) effectively restored hematological indices toward normal values. GL is a perennial edible plant widely consumed in parts of West Africa, valued for its medicinal and nutritional benefits. Its bioactive components—flavonoids, saponins, alkaloids, and other phytochemicals—appear to act synergistically to promote hematopoiesis. Flavonoids, for instance, are known to upregulate erythropoietin synthesis by modulating hypoxia-inducible factor-1α (HIF-1α) activity, enhancing erythroid progenitor cell survival and proliferation. They also chelate and mobilize iron, improving its bioavailability for hemoglobin synthesis (Balogun et al., 2016).

Saponins, another major constituent of GL, may stimulate immune responses by enhancing macrophage activation and promoting lymphocyte proliferation. Alkaloids in GL could also exert direct stimulatory effects on bone marrow stem cells, promoting megakaryopoiesis and leukopoiesis. Furthermore, the antioxidant-rich profile of GL—documented to reduce lipid peroxidation markers such as malondialdehyde (MDA) and to increase enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)—likely plays a central role in protecting hematopoietic tissues from oxidative stress–induced apoptosis (Ujong et al., 2022; Offor & Uchenwoke, 2015).

Oxidative stress has been implicated in the pathogenesis of PEM-related hematological damage, as nutrient deficiencies increase the generation of reactive oxygen species (ROS) while depleting antioxidant defenses. The bone marrow, being a metabolically active tissue, is particularly susceptible to ROS-mediated injury, which can impair stem cell survival, reduce differentiation capacity, and promote premature senescence of hematopoietic cells.

GL's antioxidant action therefore not only prevents oxidative damage but also promotes recovery of bone marrow function, leading to improved RBC, WBC, and platelet counts.

The anti-inflammatory activity of GL, including the downregulation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), may further contribute to hematopoietic recovery. Chronic low-grade inflammation is a hallmark of PEM and can lead to anemia of inflammation via hepcidin-mediated iron sequestration. By reducing inflammatory cytokine production, GL may lower hepcidin levels, thereby improving iron availability for erythropoiesis and reversing anemia of inflammation.

Moringa oleifera (MO), another nutritionally rich plant, also improved hematological indices in PEM rats. MO is known for its high content of iron, vitamins A, C, and E, and polyphenolic antioxidants (Nurhayati et al., 2023). These components can improve hemoglobin synthesis, enhance immune function, and protect hematopoietic cells from oxidative damage. However, in the present study, GL outperformed MO in restoring hematological parameters. This suggests that while MO's benefits are largely due to its nutrient density, GL's phytochemical profile may exert stronger hematopoietic stimulation through specific bioactive mechanisms. Comparative studies have indicated that GL not only enhances erythrocyte lifespan but also improves platelet aggregation and WBC proliferation more effectively than MO (Fubara et al., 2025).

This superiority could be attributed to the presence of unique phytochemicals in GL that act on multiple hematopoietic pathways simultaneously. For example, certain flavonoids in GL may exert mild estrogenic or androgenic activity, which can stimulate erythropoietin production in the kidney. Additionally, saponins may act as natural adjuvants, enhancing immune responses and leukocyte proliferation. The combined antioxidant and anti-inflammatory properties of GL further ensure a favorable bone marrow microenvironment for hematopoiesis.

The translational potential of GL is significant, particularly in malnutrition-endemic regions where access to synthetic hematinic drugs may be limited or expensive. GL is locally available, culturally accepted as a vegetable or herbal remedy, and can be incorporated into community-level interventions such as fortified foods, nutraceutical capsules, or herbal teas. Its ability to address not only anemia but also leukopenia and thrombocytopenia makes it a versatile hematopoietic agent. Furthermore, its antioxidant and anti-inflammatory properties may provide additional health benefits, such as reducing infection rates and enhancing recovery from illness.

The findings also have implications for integrative nutrition strategies. Combining GL supplementation with conventional protein supplementation could yield additive or synergistic effects, accelerating hematopoietic recovery in PEM. This is particularly relevant for community-based feeding programs, therapeutic food formulations, and dietary interventions targeting vulnerable populations such as children, pregnant women, and the elderly.

Future research should focus on elucidating the exact bioactive compounds in GL responsible for its hematopoietic effects. Advanced analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy could be employed to identify and quantify these compounds. Mechanistic studies, including bone marrow histology, erythropoietin gene expression analysis, and assessment of oxidative stress biomarkers, would deepen our understanding of how GL influences hematopoiesis (Sulaiman et al., 2022; Al-Hindi et al., 2019).

Another research direction is the evaluation of dose-response relationships to determine the optimal therapeutic dose of GL for maximal hematopoietic benefit. Additionally, long-term safety studies are essential to ensure that chronic consumption of GL does not result in adverse effects, particularly in populations with pre-existing medical conditions or those on multiple medications.

It is also worth exploring potential gender differences in response to GL supplementation, given that hormonal variations between males and females can influence hematopoiesis. Since this study exclusively used male rats, further studies including both sexes are needed to confirm the generalizability of the results. Moreover, clinical trials in human populations, especially in areas with high PEM prevalence, will be critical to validate these preclinical findings and establish guidelines for dietary or therapeutic use of GL.

While this study provides robust proof-of-concept data, it is not without limitations. The small sample size may limit statistical power, and the short duration of the intervention may not fully capture long-term hematological trends. Additionally, the experimental setting may not replicate the complex socio-environmental factors influencing malnutrition in human populations. Nevertheless, the consistency of our findings with existing literature strengthens the validity of the conclusions and underscores the potential of GL as an accessible, low-cost intervention for PEM-associated hematological disorders.

In summary, the study adds to the growing body of evidence supporting the role of plant-derived bioactive compounds in managing malnutrition-induced hematological impairments.

By restoring RBC, Hb, WBC, and platelet levels, GL demonstrates a multifaceted hematopoietic effect that is superior to MO under the conditions tested. This positions GL as a promising candidate for integration into functional foods, nutraceuticals, and community nutrition programs aimed at reducing the burden of anemia, immunosuppression, and bleeding disorders in malnourished populations.

5. CONCLUSION

The present study provides compelling evidence that *Gongronema latifolium* (GL) supplementation can effectively mitigate protein-energy malnutrition (PEM)–induced hematological deficits in Wistar rats. The results show that GL administration restored key hematological indices, including hemoglobin (Hb) concentration, red blood cell (RBC) count, white blood cell (WBC) count, and platelet levels, which were markedly suppressed by dietary protein deficiency. This restorative effect was consistently more pronounced with GL than with *Moringa oleifera* (MO), underscoring GL's potential as a superior phytotherapeutic intervention in PEM-related hematopoietic impairment.

The hematopoietic benefits of GL appear to be mediated by its rich phytochemical profile, which includes flavonoids, saponins, alkaloids, and polyphenols. These compounds act through multiple, complementary mechanisms to enhance hematopoiesis. Flavonoids upregulate erythropoietin production and improve iron bioavailability, facilitating hemoglobin synthesis and red cell maturation. Saponins stimulate immune function and megakaryopoiesis, thereby contributing to the recovery of platelet counts, while alkaloids may exert direct stimulatory effects on hematopoietic stem cells. Additionally, GL's potent antioxidant activity protects bone marrow cells from oxidative damage, as evidenced in prior studies demonstrating decreased lipid peroxidation (MDA) and increased enzymatic antioxidants such as SOD, CAT, and GPx (Ujong et al., 2022; Offor & Uchenwoke, 2015).

The ability of GL to modulate inflammation is also relevant in the context of PEM, where chronic low-grade inflammation can contribute to anemia of inflammation through hepcidin-mediated iron sequestration. By downregulating pro-inflammatory cytokines such as TNF-α and IL-6, GL potentially reduces hepcidin levels, improves iron mobilization, and facilitates erythropoiesis. These mechanisms, taken together, highlight GL's multifaceted approach to hematopoietic recovery — simultaneously addressing oxidative stress, inflammation, and nutrient bioavailability.

The comparative performance of MO, while beneficial, was less pronounced in restoring hematological parameters. MO's efficacy is largely attributed to its high nutrient density —

including iron, vitamins A, C, and E, and polyphenolic antioxidants (Nurhayati et al., 2023) — which supports red cell production and immune function. However, MO's effects may not match GL's due to differences in bioactive compound diversity and potency. The superior performance of GL, as observed in this study and corroborated by comparative reports (Fubara et al., 2025), suggests that GL's phytochemicals confer more potent and diverse hematopoietic effects than nutrient supplementation alone.

From a translational standpoint, the findings of this study have significant implications for addressing PEM-related hematological disorders in resource-limited settings. GL is widely available in many African regions, culturally accepted as both food and traditional medicine, and inexpensive to cultivate or source. Its integration into dietary interventions could provide an accessible, cost-effective means of reducing anemia prevalence, improving immune competence, and enhancing overall hematological health in malnourished populations. In particular, the use of GL in functional foods, nutraceutical formulations, or fortified dietary supplements could represent a sustainable and community-driven approach to public health nutrition.

The application of GL as a complementary therapy alongside protein supplementation could offer synergistic benefits. While dietary protein remains fundamental for hematopoiesis, the addition of bioactive-rich plant supplements like GL can accelerate recovery by addressing oxidative stress, inflammation, and micronutrient utilization deficits. This integrated approach could be particularly beneficial in humanitarian or public health nutrition programs targeting vulnerable groups such as children under five, pregnant women, and individuals with chronic illnesses.

Future research priorities should include:

1. **Phytochemical profiling and mechanistic studies** – to isolate and identify the specific compounds in GL responsible for its hematopoietic effects, and to elucidate their molecular targets and pathways.
2. **Dose-response and safety evaluations** – to determine optimal therapeutic doses, establish tolerable upper limits, and assess long-term safety, especially in populations with chronic consumption patterns.
3. **Gender-based studies** – to explore potential sex differences in hematopoietic responses, given hormonal influences on erythropoiesis and immune function.

4. **Bone marrow and gene expression analysis** – to examine the direct effects of GL on bone marrow architecture, stem cell populations, and the expression of genes involved in erythropoietin synthesis and antioxidant defense.
5. **Clinical trials** – to validate preclinical findings in human populations, particularly in regions with high PEM prevalence, and to compare GL's effects with standard hematinic therapies.

Limitations of the current study include the relatively small sample size and the use of only male rats, which may restrict the generalizability of findings across sexes and populations. The study duration also limits conclusions regarding the long-term sustainability of hematological recovery following GL supplementation. Nevertheless, the consistent alignment of our results with prior literature lends credibility to the observed effects and underscores the need for further investigation.

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