**Comparative Analysis of the Hepatoprotective and Hematopoietic Effects of Moringa oleifera and Gongronema latifolium Extracts in Protein-Deficient Rats**

**Abstract**
*Protein malnutrition is a major contributor to hepatic and hematopoietic dysfunction, particularly in vulnerable populations. This study aimed to comparatively evaluate the hepatoprotective and hematopoietic effects of* Moringa oleifera *and* Gongronema latifolium *leaf extracts in Wistar rats maintained on a protein-deficient diet. Thirty male rats were randomized into five groups: Normal Control, Protein-Deficient Control, and three treatment groups receiving either* Moringa oleifera*,* Gongronema latifolium*, or their combination. Treatments lasted 28 days. Standard biochemical and hematological assays were performed to assess liver function and blood parameters, while liver histology confirmed biochemical findings. Protein deficiency significantly elevated ALT, AST, ALP, and bilirubin levels (p < 0.01), and reduced hemoglobin, RBC, and platelet counts, indicating liver damage and hematopoietic suppression.* Moringa oleifera *notably reduced liver enzymes and improved histoarchitecture, indicating strong hepatoprotective potential.* Gongronema latifolium *improved hematological indices more significantly, with moderate effects on hepatic markers. Interestingly, the combined extract group showed synergistic effects, simultaneously improving both hepatic and hematological parameters.These findings suggest that* Moringa oleifera *and* Gongronema latifolium *offer distinct but complementary benefits in managing protein-deficiency-induced organ dysfunction, and their combined use may provide a holistic therapeutic approach.*

***Keywords:*** *Protein malnutrition,* Moringa oleifera*,* Gongronema latifolium*, hepatoprotection, hematopoiesis*

**Introduction**

Protein-energy malnutrition (PEM) remains a global health challenge, especially in low- and middle-income countries, where it contributes significantly to childhood morbidity and mortality due to impaired organ development and compromised immunity (WHO, 2019). PEM is associated with oxidative stress, hepatocellular injury, and impaired hematopoiesis, culminating in anemia, immune dysfunction, and hepatic steatosis (Wokocha et al., 2024; Ezekwe et al., 2024; Amakiri el al., 2024). The liver, being central to metabolic homeostasis and detoxification, is particularly vulnerable to nutrient deficiency and reactive oxygen species (ROS) generation.

Phytotherapeutic interventions utilizing plant-based nutraceuticals are gaining attention for their ability to mitigate oxidative liver damage and restore hematopoietic balance (Kou et al., 2018). Among such botanicals, **Moringa oleifera** is extensively researched for its high nutritional content—rich in vitamins A, C, and E, essential amino acids, flavonoids, polyphenols, and bioactive isothiocyanates—which contribute to its potent antioxidant, hepatoprotective, anti-inflammatory, and hematopoietic activities (Anzano et al., 2022; Giacoppo et al., 2017; Masarkar et al., 2023; Kim et al., 2022; Kou et al., 2018;). Moringa’s efficacy is mediated through multiple pathways, including modulation of **Nrf2**, **NF-κB**, and **HO-1** signaling, all of which are essential in oxidative stress response and cytoprotection (Mundkar et al., 2022; Wright, 2020). Studies have also shown its ability to reverse neurodegeneration and memory impairment in oxidative stress models (Zhou et al., 2018), and its antimicrobial properties suggest a broader therapeutic potential (Sharma et al., 2020; Pagano et al., 2020).

In parallel, **Gongronema latifolium**, an indigenous African medicinal plant, has been traditionally used for its antioxidant, anti-inflammatory, and hematopoietic properties. Its flavonoids, saponins, and alkaloids are known to modulate immune responses and stimulate erythropoiesis (Antai et al., 2009). Despite the individual merits of Moringa and Gongronema, comparative investigations evaluating their therapeutic efficacy in a PEM model are limited.

This study aims to investigate and compare the hepatoprotective and hematopoietic effects of **Moringa oleifera** and **Gongronema latifolium** in protein-deficient Wistar rats. It also explores the potential synergistic interaction of both extracts in ameliorating malnutrition-induced liver dysfunction and hematological impairments, providing a basis for phytotherapeutic strategies in nutritional rehabilitation.

**MATERIALS AND METHODS**

**Diet Formulation and Grouping**

Rats were acclimatized for 7 days and randomly divided into five groups (n = 6 per group):

* **Group 1 (Normal Control):** Standard protein diet (20% casein).
* **Group 2 (Protein-Deficient Control):** Low-protein diet (5% casein).
* **Group 3 (PD + Moringa):** Low-protein diet + *Moringa oleifera* extract (400 mg/kg/day).
* **Group 4 (PD + Gongronema):** Low-protein diet + *Gongronema latifolium* extract (400 mg/kg/day).
* **Group 5 (PD + Combined Extract):** Low-protein diet + *Moringa oleifera* and *Gongronema latifolium* (200 mg/kg each/day).

Treatment lasted for 28 consecutive days via oral gavage using a sterile cannula.

**Preparation of Plant Extracts**

Fresh leaves of Moringa oleifera and Gongronema latifolium were authenticated at the Department of Botany, University of Port Harcourt and voucher specimens (MO-002 and GL-007) were deposited. The leaves were washed, air-dried at room temperature, and ground into fine powder. Aqueous extracts were prepared by cold maceration in distilled water (1:10 w/v) for 48 hours with occasional stirring. The mixtures were filtered using Whatman No.1 filter paper, and filtrates were concentrated using a rotary evaporator at 40°C and stored at 4°C until use.

**Biochemical Assays**

At the end of the treatment, rats were fasted overnight and anesthetized using intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood samples were collected via cardiac puncture. Serum was separated by centrifugation (3000 rpm, 15 min) and analyzed for:

* **Alanine aminotransferase (ALT)**
* **Aspartate aminotransferase (AST)**
* **Alkaline phosphatase (ALP)**
* **Total bilirubin**

Assays were conducted using diagnostic enzymatic kits (Randox Laboratories, UK) according to the manufacturer’s instructions, based on the Reitman and Frankel method (1957).

**Hematological Analysis**

Whole blood samples collected in EDTA tubes were analyzed using an automated hematology analyzer (Sysmex XT-1800i, Sysmex Corporation, Japan) for:

* Hemoglobin concentration (Hb)
* Red blood cell count (RBC)
* White blood cell count (WBC)
* Platelet count

**Histopathological Examination**

Liver tissues were harvested and fixed in 10% neutral buffered formalin, processed using routine paraffin embedding techniques, sectioned at 5 μm thickness, and stained with hematoxylin and eosin (H&E). Microscopic evaluation was performed under a light microscope (×400 magnification), and images were captured using a digital photomicroscope (Leica Microsystems, Germany). Histological scoring of inflammation and necrosis followed a semi-quantitative grading scale adapted from Klaunig et al. (2011).

**Statistical Analysis**

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 9.0. One-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison post hoc test was used to assess intergroup differences. Significance was considered at *p* < 0.05.

**RESULTS**

**Liver Function Biomarkers**

The protein-deficient control group exhibited significant elevations in serum ALT, AST, ALP, and total bilirubin compared to the normal control (*p* < 0.05), indicating hepatic dysfunction (Table 1). Treatment with *Moringa oleifera* significantly reduced these enzyme levels compared to the protein-deficient group (*p* < 0.05). *Gongronema latifolium* produced moderate but significant reductions, while the combined extract group demonstrated the most substantial normalization of liver function markers, nearly restoring values to control levels.

**Table 1.** Liver Function Biomarkers (Mean ± SD, *n* = 6)

| **Parameter** | **Control** | **Protein-Deficient** | **+Moringa** | **+Gongronema** | **Combined Extract** |
| --- | --- | --- | --- | --- | --- |
| ALT (U/L) | 35.2 ± 3.1 | 78.5 ± 5.6\* | 40.3 ± 4.2# | 55.7 ± 5.0# | 38.9 ± 3.8# |
| AST (U/L) | 42.7 ± 4.0 | 90.2 ± 7.4\* | 44.9 ± 3.9# | 62.1 ± 5.5# | 41.5 ± 3.6# |
| ALP (U/L) | 90.3 ± 6.8 | 145.6 ± 9.8\* | 98.5 ± 7.1# | 120.3 ± 8.4# | 94.7 ± 6.7# |
| Total Bilirubin (mg/dL) | 0.9 ± 0.1 | 2.2 ± 0.3\* | 1.1 ± 0.2# | 1.6 ± 0.2# | 1.0 ± 0.1# |

\*Significantly different from Control, *p* < 0.05
#Significantly different from Protein-Deficient, *p* < 0.05

‘+’ denotes treatment with the indicated plant extract in rats fed a protein-deficient diet.”

**Hematological Parameters**

Protein deficiency significantly reduced Hb, RBC, WBC, and platelet counts compared to control (*p* < 0.05). While *Moringa oleifera* treatment yielded moderate improvements, *Gongronema latifolium* significantly improved all hematological indices (*p* < 0.05). The combined extract group showed the greatest enhancement across all parameters, approaching normal control values (Table 2).

**Table 2.** Hematological Indices (Mean ± SD, *n* = 6)

| **Parameter** | **Control** | **Protein-Deficient** | **+Moringa** | **+Gongronema** | **Combined Extract** |
| --- | --- | --- | --- | --- | --- |
| Hemoglobin (g/dL) | 14.6 ± 0.7 | 9.0 ± 0.6\* | 11.2 ± 0.7# | 12.9 ± 0.8# | 13.5 ± 0.6# |
| RBC (×10⁶/µL) | 7.9 ± 0.5 | 5.0 ± 0.4\* | 6.0 ± 0.5# | 7.1 ± 0.4# | 7.4 ± 0.4# |
| WBC (×10³/µL) | 8.4 ± 0.6 | 5.2 ± 0.5\* | 6.4 ± 0.6# | 7.8 ± 0.5# | 8.1 ± 0.5# |
| Platelet (×10³/µL) | 312 ± 21 | 208 ± 17\* | 250 ± 19# | 290 ± 20# | 300 ± 22# |

\*Significantly different from Control, *p* < 0.05
#Significantly different from Protein-Deficient, *p* < 0.05

‘+’ denotes treatment with the indicated plant extract in rats fed a protein-deficient diet.”

**Histopathological Observations**

Histological analysis of liver sections revealed preserved hepatic lobular structure with normal hepatocytes in the control group(A). Protein-deficient rats showed hepatocyte degeneration, vacuolation, sinusoidal congestion, and moderate portal inflammation(B). *Moringa oleifera* mitigated these changes with marked architectural restoration and minimal necrosis (C). *Gongronema latifolium* treatment showed moderate protection with some residual inflammatory features (D). The combined extract group exhibited near-normal hepatic morphology, indicating synergistic hepatoprotective efficacy (E)

Fig 1 **Histopathological Observation 1**



Fig 2 **Histopathological Observation 2**



Fig 3 **Histopathological Observation 3**



Fig 4 **Histopathological Observation 4**



Fig 5 **Histopathological Observation 5**



**Discussion**

This study corroborates earlier findings that protein-energy malnutrition induces significant hepatic injury and hematological disturbances, likely through mechanisms involving oxidative stress, impaired redox signaling, and inflammatory responses (Klaunig et al., 2011;). Elevated serum levels of liver enzymes and bilirubin in PEM-exposed rats indicate hepatocellular necrosis and intrahepatic cholestasis, affirming the sensitivity of the liver to nutritional insults.

Treatment with **Moringa oleifera** significantly mitigated hepatic damage, as evidenced by normalized liver enzymes and preserved hepatic architecture. This aligns with previous reports attributing Moringa's hepatoprotective effects to its polyphenol-rich profile and ability to modulate antioxidant enzymes (Anzano et al., 2022; Kim et al., 2022; Kou et al., 2018). Notably, the activation of Nrf2-mediated antioxidant defense pathways, as described by Kim et al. (2022) and Mundkar et al. (2022), may underpin the observed hepatocellular protection. Furthermore, Moringa's isothiocyanates, as shown by Giacoppo et al. (2017), may contribute to its anti-inflammatory properties, suppressing neuroinflammation and systemic oxidative stress—mechanisms also relevant to hepatic recovery.

Histologically, Moringa-treated rats demonstrated hepatocyte regeneration with minimal steatosis, possibly due to the bioactive compounds such as quercetin and chlorogenic acid that improve lipid metabolism and ROS scavenging (Masarkar et al., 2023). Its application in wound healing (Pagano et al., 2020) and antimicrobial food preservation (Sharma et al., 2020) further reflects its systemic therapeutic value.

**Gongronema latifolium**, on the other hand, exhibited superior enhancement in hematological indices. This may be attributed to its flavonoids and saponins, which have been implicated in bone marrow stimulation, erythropoiesis, and leukopoiesis (Antai et al., 2009;). These phytochemicals likely counteract oxidative stress-induced bone marrow suppression, thereby promoting hematopoietic recovery. While Moringa has shown some hematopoietic activity (Tuorkey, 2020; Omodanisi et al., 2017), the greater effect of Gongronema in this regard suggests plant-specific differences in bioactivity.

Interestingly, the **combined extract treatment** yielded the most favorable outcomes in both liver and blood parameters. This synergism may arise from the complementary mechanisms of Moringa’s antioxidative and anti-inflammatory actions and Gongronema’s marrow-stimulating properties (Omodanisi et al., 2017; Masarkar et al., 2023). Such botanical combinations may offer holistic strategies for addressing multifaceted consequences of malnutrition.

While these results are promising, this study is limited by its short duration and lack of detailed molecular analyses. Future studies should include gene expression profiling of antioxidant and inflammatory markers, as well as explore the clinical translational potential in human populations suffering from PEM. Additionally, exploration of neuroprotective effects—such as those reported by Zhou et al. (2018) and Wright (2020)—may broaden the therapeutic applicability of these extracts.

This manuscript provides valuable insights into the potential of Moringa oleifera and Gongronema latifolium plant extracts in addressing the global health burden of Protein-Energy Malnutrition (PEM). It highlights the hepatoprotective and hematopoietic effects of these extracts in PEM-induced models, reinforcing their therapeutic potential. Furthermore, the study lays a foundation for investigating critical mechanistic pathways such as Nrf2, NF-κB, and HO-1 signaling, paving the way for future translational and clinical research. The findings contribute meaningfully to the growing scientific interest in plant-based interventions as alternatives or adjuncts to conventional therapies.

**Conclusion**

This study demonstrates that Moringa oleifera and Gongronema latifolium exert distinct but complementary protective effects against protein-deficiency-induced hepatic and hematological dysfunctions in Wistar rats. Protein malnutrition significantly impaired liver function and hematopoiesis, as evidenced by elevated liver enzymes, hyperbilirubinemia, and reductions in hemoglobin, red blood cell, white blood cell, and platelet counts. Treatment with Moringa oleifera notably ameliorated hepatic injury and preserved liver histoarchitecture, highlighting its potent hepatoprotective potential. Conversely, Gongronema latifolium markedly restored hematological indices, suggesting its stimulatory effect on hematopoietic recovery. Importantly, the combined administration of both extracts yielded synergistic benefits, offering near-complete restoration of biochemical and histological parameters to normal levels. These findings support the therapeutic promise of these botanicals—individually and in combination—as a natural, cost-effective intervention for mitigating the systemic complications of protein malnutrition. Further studies are recommended to explore the mechanistic pathways and clinical relevance of these effects in humans.

Animals and Ethical Approval

Thirty adult male Wistar rats (weighing 150–180 g) were obtained from the Animal House of the Department of biochemistry, Faculty of Basic Medical Sciences, Rivers State University.”Animals were housed under standard laboratory conditions (12 h light/12 h dark cycle, temperature 22 ± 2°C, relative humidity 50–60%) with access to food and water ad libitum. The experimental protocol adhered to the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 2011) and was approved by the Institutional Animal Care and Use Committee (IACUC) of [Rivers State University, Approval number: 1233280.

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