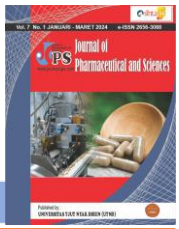
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Preliminary Evaluation of *Moringa oleifera* L. Leaf Ethanol Extract on Body-Weight Recovery in Protein-Energy Malnourished Mice

Evaluasi Awal Ekstrak Etanol Daun kelor (*Moringa oleifera* L.) terhadap Pemulihan Berat Badan pada Mencit dengan Malnutrisi Energi-Protein

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Abstract

Protein-energy malnutrition (PEM) is a condition caused by inadequate energy and protein intake that may result in body-weight loss and changes in physical condition. *Moringa oleifera* L. leaves contain nutrients and bioactive compounds that may support nutritional recovery. This study aimed to evaluate the preliminary effects of 96% ethanol extract of *Moringa oleifera* L. leaves on body-weight recovery in male Swiss Webster mice with experimentally induced PEM. This laboratory experimental study involved 20 mice divided into five groups: a negative control (K−), a positive control (K+), and three extract-treatment groups (P1, P2, and P3). PEM was induced by feeding a low-protein diet for 21 days, with a body-weight reduction of at least 20% used as the operational criterion. The treatment period lasted 30 days. The extract was administered orally at doses of 0.217, 0.272, and 0.326 mg/kg body weight, while the positive control received zinc sulfate monohydrate at 20 mg/kg body weight. Body weight was measured periodically, and physical condition and activity were observed descriptively. All mice met the induction criterion, with an average body-weight reduction of 20.57 ± 0.40%. The percentage of body-weight gain was 54.83 ± 0.42% in K−, 73.61 ± 0.32% in P1, 84.52 ± 0.57% in P2, 93.44 ± 0.20% in P3, and 95.81 ± 0.49% in K+. Levene's test indicated unequal variances ($p = 0.001$); therefore, the Kruskal–Wallis test was used and showed a statistically significant difference among the groups ($H = 18.286$, $df = 4$, $p = 0.001$). The extract-treatment groups showed progressively higher body-weight gains across the tested doses, although this pattern should be interpreted cautiously. These findings provide preliminary evidence that 96% ethanol extract of *Moringa oleifera* L. leaves may be associated with body-weight recovery in mice with experimentally induced PEM. Further studies with larger samples, controlled individual food-intake measurements, and additional nutritional and biochemical indicators are needed to confirm these findings.

***Keywords*: Moringa oleifera L.; ethanol extract; protein-energy malnutrition; nutritional recovery; body-weight recovery; mice**

Abstrak

Malnutrisi energi-protein (MEP) merupakan kondisi yang disebabkan oleh ketidakcukupan asupan energi dan protein yang dapat mengakibatkan penurunan berat badan serta perubahan kondisi fisik. Daun kelor (Moringa oleifera L.) mengandung berbagai zat gizi dan senyawa bioaktif yang berpotensi mendukung pemulihan status gizi. Penelitian ini bertujuan untuk mengevaluasi efek awal ekstrak etanol 96% daun kelor (Moringa oleifera L.) terhadap pemulihan berat badan mencit jantan galur Swiss Webster yang mengalami MEP akibat induksi eksperimental. Penelitian eksperimental laboratorium ini menggunakan 20 ekor mencit yang dibagi menjadi lima kelompok, yaitu kontrol negatif (K−), kontrol positif (K+), dan tiga kelompok perlakuan ekstrak (P1, P2, dan P3). Kondisi MEP diinduksi melalui pemberian pakan rendah protein selama 21 hari, dengan penurunan berat badan minimal 20% sebagai kriteria operasional keberhasilan induksi. Perlakuan diberikan selama 30 hari. Ekstrak diberikan secara oral dengan dosis masing-masing 0,217; 0,272; dan 0,326 mg/kg berat badan, sedangkan kelompok kontrol positif diberikan zink sulfat monohidrat dengan dosis 20 mg/kg berat badan. Pengukuran berat badan dilakukan secara berkala, sedangkan kondisi fisik dan aktivitas mencit diamati secara deskriptif. Seluruh mencit memenuhi kriteria keberhasilan induksi MEP dengan rerata penurunan berat badan sebesar 20,57 ± 0,40%. Persentase peningkatan berat badan pada kelompok K−, P1, P2, P3, dan K+ masing-masing sebesar 54,83 ± 0,42%; 73,61 ± 0,32%; 84,52 ± 0,57%; 93,44 ± 0,20%; dan 95,81 ± 0,49%. Hasil uji Levene menunjukkan bahwa varians antarkelompok tidak homogen ($p = 0,001$), sehingga analisis dilanjutkan menggunakan uji Kruskal–Wallis yang menunjukkan adanya perbedaan bermakna secara statistik antarkelompok ($H = 18,286$; $df = 4$; $p = 0,001$). Kelompok perlakuan ekstrak menunjukkan kecenderungan peningkatan berat badan yang semakin besar seiring dengan peningkatan dosis, meskipun pola tersebut perlu diinterpretasikan secara hati-hati. Hasil penelitian ini memberikan bukti awal bahwa pemberian ekstrak etanol 96% daun kelor (Moringa oleifera L.) berpotensi berhubungan dengan pemulihan berat badan pada mencit yang mengalami MEP akibat induksi eksperimental. Penelitian lebih lanjut dengan jumlah sampel yang lebih besar, pengukuran asupan pakan individual yang terkontrol, serta pemeriksaan parameter gizi dan biokimia tambahan diperlukan untuk mengonfirmasi temuan tersebut.

Kata kunci: Moringa oleifera L.; ekstrak etanol; malnutrisi energi-protein; pemulihan status gizi; pemulihan berat badan; mencit.

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Introduction

Stunting remains an important public health problem because chronic nutritional impairment during early life can affect physical growth, cognitive development, and long-term health. Globally, the prevalence of stunting among children under five years of age reached 22.3%, corresponding to approximately 148.1 million children in 2022 [1]. In Indonesia, the prevalence of stunting based on the 2022 Indonesian Nutrition Status Survey (SSGI) was 21.6% [2]. Although this prevalence has shown a declining trend, stunting remains an important challenge and requires sustainable nutritional interventions.

Stunting is not characterized solely by low body weight. It represents chronic impairment of linear growth and is generally assessed using length- or height-for-age indicators. In contrast, protein-energy malnutrition (PEM) refers specifically to inadequate protein and energy availability. The two conditions are therefore related but not interchangeable. In experimental animal research, body-weight loss and physical deterioration can be used as indicators of nutritional impairment and recovery, but they should not be interpreted as direct measures of stunting [3], [4], [5], [6], [7], [8], [9]

Nutritional deficiencies can interfere with protein synthesis, cell proliferation, energy metabolism, and growth-related signaling [4,6]. Severe nutritional deficiency may also be associated with weight loss, reduced physical activity, altered grooming behavior, deterioration of hair condition, and impaired physiological function [12], [13]. Accordingly, body-weight recovery and physical-condition indicators may provide useful preliminary evidence of nutritional recovery in an experimental PEM model, while direct assessment of stunting requires specific linear-growth endpoints.

Local food resources containing nutrients and bioactive compounds have attracted attention as potential components of nutritional interventions. *Moringa oleifera* L. is one such plant because its leaves contain proteins, vitamins, minerals, essential amino acids, and various bioactive compounds [14], [15]. Previous phytochemical and mineral studies have reported the presence of calcium, phosphorus, potassium, iron, and zinc in Moringa leaves [16], [17]. In addition, comprehensive reviews have described antioxidant, anti-inflammatory, immunomodulatory, pharmacological, and functional-food potential of *Moringa oleifera* [18], [19].

Previous intervention studies have also reported potential nutritional benefits of Moringa. Moringa-based nutritional intervention has been associated with improvement in protein and micronutrient intake among children with protein-energy deficiencies [15], while other studies have reported effects on hemoglobin levels and nutritional-related outcomes [16]. These findings provide a biological rationale for further investigation of Moringa in experimental nutritional deficiency models.

Importantly, however, the potential of Moringa for nutritional recovery is not a novel concept. Luthfiyah and Widjajanto [9] previously investigated Moringa leaf powder in a protein-energy malnutrition animal model and reported improvement in physical condition following Moringa administration. Therefore, improvement in body weight or physical condition following Moringa administration cannot be claimed as a novel finding by itself.

The present study was designed as an exploratory extension of this previous work. Rather than using whole-leaf powder, the present study evaluated a 96% ethanol extract of *Moringa oleifera* L. leaves. The experiment used male Swiss Webster mice with experimentally induced PEM and evaluated three graded extract doses of 0.217, 0.272, and 0.326 mg/kg body weight over a 30-day recovery period. In addition to body-weight recovery as the primary quantitative outcome, spontaneous movement, grooming behavior, and hair

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condition were observed descriptively. The extract was also characterized by qualitative phytochemical screening and FTIR spectroscopy.

Thus, the contribution of this study is not the first demonstration of a nutritional effect of Moringa in a PEM model. Instead, it provides a preliminary evaluation of an ethanol-extracted Moringa preparation using a different experimental animal model and graded extract doses, accompanied by preliminary chemical characterization. Because the phytochemical analysis was qualitative and the physical-condition observations were not based on validated numerical scoring, the study is positioned as an exploratory extension and preliminary validation of previous findings rather than as definitive evidence of a therapeutic or anti-stunting effect.

Based on this rationale, this study aimed to evaluate the preliminary effect of 96% ethanol extract of *Moringa oleifera* L. leaves on body-weight recovery in male Swiss Webster mice with experimentally induced PEM. Secondary exploratory observations included spontaneous movement, grooming behavior, and hair condition, while qualitative phytochemical screening and FTIR analysis were used to provide preliminary chemical characterization of the extract.

Research Methods

Research Design

This study was an exploratory quantitative laboratory experiment using an in vivo PEM model in male Swiss Webster mice. The research was conducted from August to December 2025 at the Biology Laboratory and Pharmacology Laboratory, Faculty of Medicine, Adiwangsa University, Jambi. All animal procedures received ethical approval under number LB.02.06/2/1148/2025.

Twenty mice were included, with four animals initially assigned to each of five experimental groups. No a priori sample-size or statistical power calculation was documented in the original study records. Therefore, the experiment was considered an exploratory pilot study, and the statistical findings and biological observations were interpreted as preliminary rather than confirmatory. The small sample size is acknowledged as an important limitation affecting the precision and generalizability of the findings.

Research Materials and Instruments

The main plant material was *Moringa oleifera* L. leaves obtained from Kerinci Regency, Jambi Province using purposive sampling. The leaves were selected based on uniform morphological characteristics, including fresh green appearance, appropriate maturity, and absence of visible pests, diseases, or physical damage.

Botanical identification was performed at the Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Sciences, Universitas Padjadjaran, with determination number 40/HB/10/2025. The chemicals used included 96% ethanol as the extraction solvent, distilled water, hydrochloric acid (HCl), Mayer's reagent, Dragendorff's reagent, magnesium powder, amyl alcohol, and ferric chloride ($FeCl_3$) solution for qualitative phytochemical screening. Zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$) was used as the positive-control treatment.

Preparation and Characterization of Moringa Leaf Extract

A total of 4 kg of *Moringa oleifera* L. leaves was washed and mechanically reduced in size before extraction by maceration using 96% ethanol. The extraction was conducted at a plant-material-to-solvent ratio of 1:3 for three consecutive 24-hour maceration periods. The filtrate was separated from the plant residue and concentrated using a rotary evaporator at 55°C until a viscous extract was obtained. The resulting extract had an extraction yield of approximately 13%.

The original experimental records did not retain several additional preparation parameters, including the drying temperature and duration, final particle size or mesh number, and detailed agitation schedule during maceration. These parameters could therefore not be reconstructed retrospectively without introducing unsupported information. Their absence is acknowledged as a reproducibility limitation because drying and particle-size conditions may influence extraction yield and phytochemical composition.

Qualitative phytochemical screening was performed to determine the presence of alkaloids, flavonoids, tannins, and saponins in the 96% ethanol extract of *Moringa oleifera* L. leaves. Alkaloids were examined using Mayer's and Dragendorff's reagents following acidification of the extract with hydrochloric acid. Formation

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of a characteristic precipitate was interpreted as a positive reaction. Flavonoids were screened using the Shinoda reaction with magnesium powder and concentrated HCl followed by amyl alcohol, with characteristic color development interpreted as a positive reaction. Tannins were examined using ferric chloride ($FeCl_3$), with characteristic color development indicating a positive reaction. Saponins were assessed using the foam test by shaking the aqueous preparation and observing the persistence of foam.

The detailed qualitative procedures are presented in Supplementary Table S1. The original laboratory records did not retain a formal intensity scale for phytochemical reactions. Therefore, the results are reported as detected/not detected rather than retrospectively assigning intensity grades of +, ++, or +++. This approach avoids assigning unsupported semi-quantitative values to historical observations. Quantitative determination of total flavonoid content, total phenolic content, protein content, or mineral concentrations was not performed in the present study. Consequently, the extract was not quantitatively standardized, and batch-to-batch chemical consistency could not be established. These limitations are considered when interpreting the phytochemical and FTIR findings. FTIR analysis was conducted over the range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1} with 40 scans to characterize major functional groups present in the extract.

Animal Test and Treatment Procedures

The study used 20 male Swiss Webster mice (*Mus musculus*) with an initial body weight of approximately 20 g and a one-week acclimatization period. PEM was operationally defined as a reduction of at least 20% from the pre-induction body weight following low-protein feeding. The induction procedure was adapted from Luthfiyah and Widjajanto [9], whose reference model used a low-protein diet containing 5.6% protein and 67.8% carbohydrate until approximately 20% body-weight loss.

In the present study, the low-protein diet was used to induce the predefined nutritional-deficiency condition. The available study documentation identified the feed source as BioCalorix and recorded an induction period of 21 days; however, the complete ingredient-level and proximate nutrient composition of the feed used in the experiment was not retained. Therefore, the precise protein, carbohydrate, fat, fiber, mineral, and energy composition of the experimental feed cannot be reconstructed retrospectively.

All 20 mice reached the prespecified 20% body-weight-loss criterion, with individual losses ranging from 20.01% to 21.00% and a mean reduction of 20.57 ± 0.40%. After completion of PEM induction, the mice were divided into five groups, each initially consisting of four animals: a negative-control group (K−), a positive-control group (K+) receiving zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$), and three *Moringa* extract groups (P1, P2, and P3) receiving 0.217, 0.272, and 0.326 mg/kg body weight, respectively.

The positive-control group received zinc sulfate monohydrate at a dose of 20 mg/kg body weight by oral gavage. The administration volume was 0.5 mL per mouse. Zinc was selected as the positive control to provide a nutritional supplementation comparator in the PEM model. All treatments were administered orally once daily for 30 days.The three *Moringa* extract doses constituted the ascending exploratory dose series specified in the original protocol. The available records did not document a formal dose-finding study, human-equivalent dose conversion, or established therapeutic or toxicological thresholds for these doses. Therefore, the doses are interpreted as exploratory graded doses rather than validated therapeutic, subtherapeutic, or toxic doses. Future studies should establish the dose range through a priori dose-finding studies supported by pharmacological and toxicological evidence.

The exact vehicle and administration volume used for the negative-control group were not retained in the available experimental documentation. Therefore, these parameters were not retrospectively reconstructed. This limitation is acknowledged because vehicle matching and administration-volume standardization are important for rigorous interpretation of animal intervention studies.

A normal non-malnourished control group was not included in the original experimental design. Consequently, the present study compares recovery among animals subjected to the experimental PEM condition but does not provide a concurrent reference group representing normal nutritional status. One mouse in the negative-control group died between treatment days 13 and 16 and was unavailable for subsequent measurements.

Operational Definitions and Outcome Assessment

Body-weight recovery was defined as:

$$\frac{\text{(day-30 body weight – treatment day-0 body weight)} \times 100\%}{\text{Treatment day-0 body weight}}$$

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Movement activity was observed descriptively based on spontaneous walking, running, cage exploration, persistent inactivity, and crouched or prostrate posture. Grooming behavior was assessed descriptively based on the apparent return of self-grooming behavior. Hair condition was assessed visually based on texture, dullness, and apparent hair loss.

The original observation records did not contain a predefined numerical scoring system, validated behavioral assessment instrument, documentation of observer blinding, or inter-rater reliability measurements. Therefore, no retrospective behavioral scores were assigned, and these exploratory observations were not subjected to inferential statistical analysis.

The absence of standardized behavioral scoring is considered a methodological limitation. Future confirmatory studies should employ predefined scoring criteria, blinded observers, video-based behavioral assessment, and independent raters with formal inter-rater reliability analysis. Standardized behavioral paradigms such as the open-field test may also be considered where appropriate to the research question.

Data Collection

Body weight was measured using a digital balance during the treatment period on days 0, 4, 7, 10, 13, 16, 19, 23, 27, and 30. Physical condition was observed at corresponding intervals, focusing on spontaneous movement, inactivity or crouched posture, grooming behavior, and hair appearance. Hair observations considered texture, dullness, and apparent hair loss. Because the original physical-condition records did not contain validated numerical scores, these observations were retained as qualitative descriptive outcomes rather than quantitative endpoints.

Data Analysis

Raw individual body-weight data were analyzed descriptively. Continuous data are presented as mean ± standard deviation (SD). The primary quantitative endpoint was the percentage of body-weight gain from the beginning to the end of the 30-day treatment period. Homogeneity of variance was assessed using Levene's test. The test indicated unequal variances (Levene statistic = 8.730, $p = 0.001$).

Therefore, the Kruskal–Wallis test was used to evaluate overall differences in percentage body-weight gain among the five experimental groups. Statistical significance was set at $\alpha = 0.05$. Repeated-measures ANOVA was not performed because one negative-control animal died between treatment days 13 and 16, resulting in incomplete longitudinal observations. Longitudinal body-weight trajectories were therefore presented descriptively as mean ± SD. Qualitative phytochemical findings, FTIR results, movement activity, grooming behavior, and hair condition were interpreted descriptively.

Results and Discussion

Characterization of Moringa Leaf Extract *(Moringa oleifera L.)*

Botanical determination at the Jatinangor Herbarium confirmed that the plant material used in this study was *Moringa oleifera* L., with determination number 40/HB/10/2025. This identification was performed before extraction and provided taxonomic confirmation of the plant material.

Table 1. Qualitative Phytochemical Screening of *Moringa oleifera L.* Leaf Ethanol Extract

Phytochemical	Qualitative procedure or indicator	Result
Alkaloids	Mayer's and Dragendorff's precipitation reactions	Detected
Tannins	Ferric chloride ($FeCl_3$) reaction	Detected
Flavonoids	Shinoda (Mg-HCl) reaction followed by amyl alcohol	Detected
Saponins	Foam test	Detected

Note: Detected indicates a positive qualitative reaction only and does not represent concentration or intensity. Intensity grades (+, ++, +++) and quantitative phytochemical concentrations were not recorded during the original experiment. Therefore, total flavonoid, total phenolic, protein, and mineral contents remain unmeasured.

Extraction of 4 kg of Moringa leaves using 96% ethanol produced a 13% extract yield. This yield was lower than the 18.1% ethanol-extract yield reported in a previous study [13]. Differences in plant material, environmental conditions, and extraction procedures may contribute to variation in extraction yield. However, because several extraction parameters were not retained in the present study, including drying conditions, particle size, solvent-to-material ratio, and maceration duration, the observed 13% yield should be

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interpreted as a batch-specific result rather than a standardized extraction benchmark. Qualitative phytochemical screening detected alkaloids, tannins, flavonoids, and saponins in the 96% ethanol extract (Table 1). These findings indicate the qualitative presence of the corresponding phytochemical classes but do not provide information about their concentrations or relative abundance.

FTIR analysis provided complementary information on the functional groups present in the extract. Prominent absorption bands occurred at approximately 3397.76, 3295.84, 2957.07, 2853.72, 1791.47, 1683.81, 1560.36, and 1498.63 cm^{-1}. These bands were broadly compatible with O-H, C-H, C=O, and C=C functionalities commonly observed in plant extracts. The FTIR findings provide preliminary functional-group characterization but do not identify or quantify individual phytochemical compounds.

The qualitative phytochemical findings are generally consistent with previous reports describing multiple secondary-metabolite classes in *Moringa oleifera* [14,15]. Flavonoids such as quercetin and kaempferol have been reported in Moringa leaves and are associated with antioxidant activity [14,15]. Saponins have also been described among Moringa leaf constituents [14]. Nevertheless, the detection of these phytochemical classes in the present study should not be interpreted as evidence that individual compounds were present at specific concentrations.

Similarly, the nutritional literature reports that Moringa leaves contain proteins, vitamins, minerals, and micronutrients such as iron, zinc, calcium, magnesium, and potassium [15]. These constituents provide biological plausibility for studying Moringa in nutritional deficiency models. However, because the present experiment did not quantify these nutrients in the administered extract, the observed biological response cannot be attributed quantitatively to a specific nutrient or phytochemical.

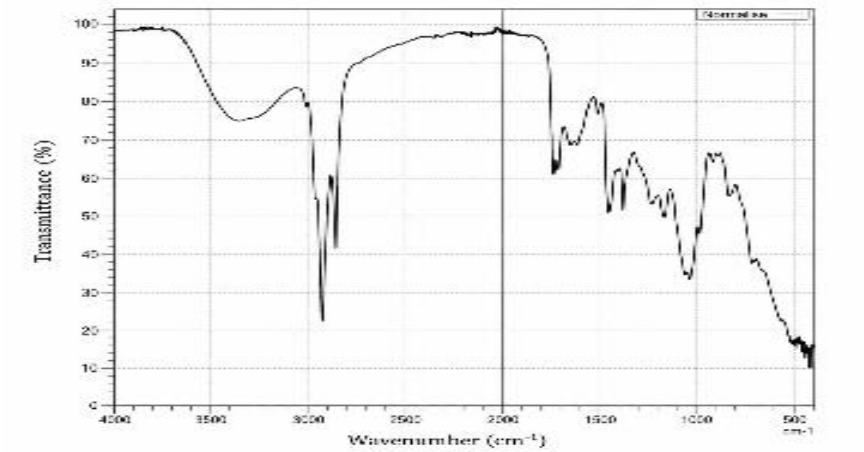


Figure 1. FTIR Moringa Leaf Extract.

Mice Weight Changes

All 20 mice met the operational PEM criterion of at least 20% body-weight loss during the induction period. The mean reduction was 20.57 ± 0.40%, with individual reductions ranging from 20.01% to 21.00%. During the 30-day recovery period, one mouse in the negative-control group died between days 13 and 16; therefore, three surviving animals contributed to the day-30 negative-control analysis.

Mean body-weight recovery differed among the experimental groups. The positive-control group showed a mean gain of 95.81 ± 0.49%, whereas the negative-control group showed 54.83 ± 0.42%. The three extract groups showed mean gains of 73.61 ± 0.32%, 84.52 ± 0.57%, and 93.44 ± 0.20% for P1, P2, and P3, respectively.

Table 2. Body Weight Recovery and Between-Group Statistical Comparison

Group	Day 0 weight (g)	Day 30 weight (g)	Weight gain (%)
Positive control (n=4)	16.93 ± 0.37	33.16 ± 0.68	95.81 ± 0.49
Negative control (n=4; day 30 n=3)	20.15 ± 0.45	31.00 ± 0.64	54.83 ± 0.42
P1 (n=4)	17.89 ± 0.35	31.05 ± 0.60	73.61 ± 0.32
P2 (n=4)	17.88 ± 0.35	32.98 ± 0.69	84.52 ± 0.57
P3 (n=4)	16.31 ± 0.38	31.55 ± 0.73	93.44 ± 0.20

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The percentage of body-weight gain varied among the five experimental groups. The negative control group (K−) showed a body-weight gain of 54.83 ± 0.42%, while the positive control group (K+) showed a gain of 95.81 ± 0.49%. Among the extract-treated groups, body-weight gain was 73.61 ± 0.32% in P1, 84.52 ± 0.57% in P2, and 93.44 ± 0.20% in P3. The Kruskal–Wallis test indicated an overall statistically significant difference among the five groups (H = 18.286, df = 4, p = 0.001). The table presents descriptive results for each group, while the statistical test evaluates the overall difference among groups. Longitudinal body-weight trajectories are presented in Figure 2. The negative-control group had four animals through day 13 and three animals thereafter because of the death of one animal.

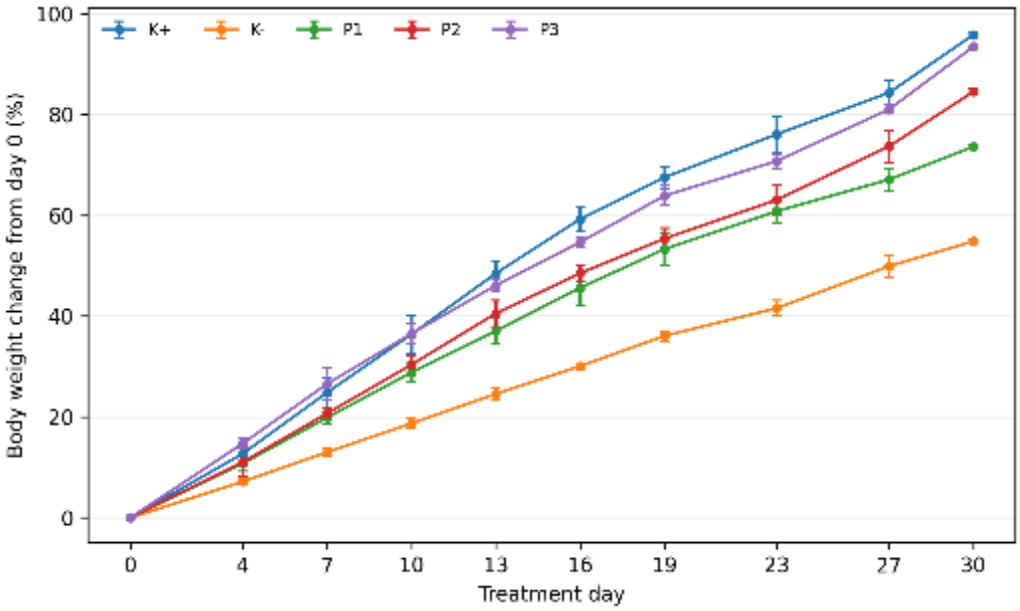


Figure 2. Mean percentage change in body weight from treatment day 1 across the 30-day recovery period. Error bars represent SD. K- had n = 4 through day 13 and n = 3 thereafter because one mouse died before day 16.

Table 3. Post-hoc Pairwise Comparisons (Tukey HSD)

No.	Group Comparison	Mean Difference	Sig. (p)	Interpretation
1	Positive control vs. Negative control	54.683	0.000	Significant
2	Positive control vs. P1	22.203	0.129	Not significant
3	Positive control vs. P2	11.293	0.694	Not significant
4	Positive control vs. P3	2.368	0.999	Not significant
5	Negative control vs. P1	−32.480	0.014	Significant
6	Negative control vs. P2	−43.390	0.001	Significant
7	Negative control vs. P3	−52.315	0.000	Significant
8	P1 vs. P2	−10.910	0.719	Not significant
9	P1 vs. P3	−19.835	0.203	Not significant
10	P2 vs. P3	−8.925	0.838	Not significant

The Kruskal–Wallis test revealed a statistically significant overall difference in body-weight gain among the five experimental groups (H = 18.286, df = 4, p = 0.001). The negative control group (K−) showed the lowest percentage of body-weight gain (54.83 ± 0.42%), whereas the positive control group (K+) showed the highest (95.81 ± 0.49%). Among the extract-treated groups, body-weight gain was 73.61 ± 0.32% in P1, 84.52 ± 0.57% in P2, and 93.44 ± 0.20% in P3.

According to the reported post-hoc comparisons, significant differences were observed between the negative control group and P1, P2, and P3, as well as between the positive and negative control groups. No statistically significant differences were reported between the positive control and the extract-treated groups or among P1, P2, and P3. These findings suggest a tendency toward greater body-weight gain in the extract-treated groups compared with the negative control, although the differences among extract doses were not statistically significant in the reported pairwise comparisons. The findings should be interpreted as preliminary evidence rather than definitive proof of the extract's effectiveness in restoring nutritional status.

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Discussion

Interpretation of Nutritional Recovery

The present study provides preliminary evidence of an association between the administration of 96% ethanol extract of *Moringa oleifera* L. leaves and body-weight recovery in male Swiss Webster mice subjected to an experimentally induced protein-energy malnutrition (PEM) condition. The Kruskal–Wallis test showed an overall statistically significant difference in percentage body-weight gain among the five experimental groups ($H = 18.286$, $df = 4$, $p = 0.001$). Descriptively, body-weight gain increased across the three extract groups, from 73.61 ± 0.32% in P1 to 84.52 ± 0.57% in P2 and 93.44 ± 0.20% in P3. The P3 mean was close to that of the positive-control group (95.81 ± 0.49%). However, the overall statistical result does not demonstrate that every pair of groups differed significantly, and the descriptive pattern alone does not establish a definitive dose–response relationship. Given the small sample size and the exploratory nature of the dose series, these findings should be interpreted as preliminary rather than confirmatory.

The findings should also be interpreted in the context of previous research. Luthfiyah and Widjajanto [9] previously demonstrated improvement in physical condition in a protein-energy malnutrition animal model treated with *Moringa* leaf powder. Therefore, the present study does not establish for the first time that *Moringa* can improve nutritional or physical recovery in a PEM model.

Instead, the present study extends the previous observation by evaluating an ethanol-extracted preparation in male Swiss Webster mice using three graded extract doses and providing preliminary phytochemical and FTIR characterization. The use of an ethanol extract represents a different preparation from whole-leaf powder because extraction may selectively transfer compounds according to their physicochemical properties. However, because the present study did not quantitatively characterize the extract, it cannot establish that the ethanol extract has a superior or distinct phytochemical profile compared with the powder preparation used previously.

The observed ordered increase in body-weight recovery from P1 to P3 may indicate a potential dose-associated pattern within the range examined. Nevertheless, the three doses were derived from an exploratory series in the original protocol, and no formal dose-finding study or human-equivalent dose conversion was documented. The pattern should therefore not be interpreted as definitive evidence of a pharmacological dose-response relationship.

Phytochemical Findings

Qualitative screening detected alkaloids, flavonoids, tannins, and saponins, consistent with previous descriptions of the phytochemical diversity of *Moringa oleifera* leaves [14,17,18]. Flavonoids such as quercetin and kaempferol have been reported as important constituents of *Moringa* and may contribute to antioxidant activity [18,19]. Saponins and other secondary metabolites may also contribute to biological activity.

However, qualitative detection alone cannot establish the concentration or biological contribution of these compounds. The present study therefore does not support attribution of the observed body-weight recovery to a specific phytochemical. Similarly, the FTIR spectrum provides evidence of several functional groups but cannot be used to quantify individual compounds.

Quantitative chemical characterization would strengthen future investigations. Total flavonoid content could be expressed as quercetin equivalents, total phenolic content as gallic acid equivalents, while protein and mineral concentrations could be quantified using validated analytical methods. Such measurements would enable chemical standardization and improve comparison between extraction batches and experimental studies.

Relationship to PEM

The induction phase resulted in a mean body-weight reduction of 20.57 ± 0.40%, and all animals achieved the prespecified 20% reduction criterion. This approach was adapted from the previously reported low-protein nutritional-deficiency model [9]. Protein restriction can reduce amino-acid availability, alter protein synthesis and energy metabolism, and promote mobilization of body-energy reserves [8,19]. These mechanisms support interpretation of the experimental condition as PEM.

During the induction phase, mice were also described as less active, with persistent inactivity, crouched or prostrate posture, and poorer hair appearance. Similar physical deterioration has been described in protein-energy-deficient animal models [9]. However, because these observations were not collected using validated

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numerical scoring, blinded assessment, or inter-rater reliability procedures, they should be interpreted only as qualitative supportive observations.

Behavioral and Hair Observations

During the recovery period, qualitative observations suggested improvement in spontaneous activity and grooming behavior in the treatment groups. Hair appearance also appeared to improve descriptively. However, these observations cannot be given the same evidentiary weight as the measured body-weight endpoint because no validated numerical scoring system was used.

The absence of standardized behavioral assessment limits reproducibility and makes quantitative comparison between groups inappropriate. Future studies should employ predefined behavioral scales or validated behavioral paradigms, together with blinded observers, video recording, and independent scoring. Inter-rater reliability should also be formally assessed. Where locomotor activity is a specific outcome of interest, an open-field test or another validated behavioral paradigm may provide more objective measurements.

Stunting Interpretation

The findings of this study should not be interpreted as direct evidence of stunting prevention. Stunting represents chronic linear-growth impairment, whereas the present study primarily measured body-weight recovery and descriptively observed physical condition in a mouse model of PEM [3–6].

Direct investigation of growth impairment would require additional endpoints, such as body length, tibial length, growth-plate histology, and growth-related biomarkers such as IGF-1. The absence of these measurements means that the present study cannot establish whether *Moringa* extract prevents or reverses a stunting-like linear-growth phenotype.

Therefore, the present findings suggest potential relevance to nutritional recovery but do not confirm an anti-stunting effect in animals or humans. Any potential application to human stunting prevention should be investigated through appropriately designed nutritional and clinical studies rather than extrapolated directly from the current animal model.

Study Limitations and Future Directions

This study has several important limitations. First, it was an exploratory pilot experiment with only four animals initially assigned to each group, and no a priori sample-size or statistical power calculation was documented. Although statistically significant differences were observed, the small group sizes limit the precision and generalizability of the findings. The statistical results should therefore be interpreted as preliminary rather than confirmatory.

Second, one negative-control mouse died between treatment days 13 and 16, resulting in incomplete longitudinal observations. In addition, no normal non-malnourished control group was included. Consequently, the study does not provide a concurrent reference for normal nutritional recovery. Third, several methodological parameters were not retained in the original experimental records. These included drying temperature and duration, final particle size or mesh number, detailed agitation conditions, complete low-protein feed composition, and the exact vehicle and administration volume of the negative-control treatment. Although the extraction ratio and maceration duration were documented as 1:3 and 3 × 24 hours, respectively, the absence of several preparation parameters limits complete reproducibility.

Fourth, the rationale for the specific *Moringa* extract doses was not documented through a formal dose-finding study, human-equivalent dose conversion, or established therapeutic or toxicological thresholds. The doses should therefore be regarded as an exploratory graded series rather than validated therapeutic doses.

Fifth, phytochemical characterization was limited to qualitative screening and FTIR analysis. Although alkaloids, flavonoids, tannins, and saponins were detected, quantitative measurements of total flavonoids, total phenolics, protein, zinc, iron, calcium, or other relevant nutrients were not performed. Consequently, the extract could not be quantitatively standardized, and the contribution of individual bioactive constituents to the observed biological response could not be established. Sixth, movement activity, grooming behavior, and hair condition were assessed descriptively without a predefined numerical scoring system, validated behavioral instrument, documented observer blinding, or inter-rater reliability assessment. These observations should therefore be regarded as qualitative supportive findings rather than reproducible quantitative behavioral endpoints. Finally, the study did not directly measure linear growth, growth-plate development, or growth-related biomarkers. Therefore, the results cannot establish prevention of stunting.

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Conclusions

In this preliminary study using a mouse model of protein-energy malnutrition (PEM), administration of 96% ethanol extract of *Moringa oleifera* L. leaves was associated with an increasing descriptive pattern of body-weight gain across the three extract-treatment groups. Percentage body-weight gain was 73.61 ± 0.32% in P1, 84.52 ± 0.57% in P2, and 93.44 ± 0.20% in P3, compared with 54.83 ± 0.42% in the negative-control group and 95.81 ± 0.49% in the positive-control group. The Kruskal–Wallis test indicated an overall statistically significant difference among the five groups ($H = 18.286$, $df = 4$, $p = 0.001$). However, these findings do not establish that all groups differed significantly or confirm a definitive dose–response relationship. Qualitative phytochemical screening detected alkaloids, flavonoids, tannins, and saponins, while FTIR analysis indicated the presence of several organic functional groups. Because quantitative chemical characterization was not performed, the contribution of individual phytochemicals or nutrients to body-weight recovery could not be determined. Changes in movement activity, grooming behavior, and hair appearance were observed descriptively and were not assessed using validated numerical scores. Overall, the findings provide preliminary evidence of a potential association between Moringa leaf ethanol extract administration and body-weight recovery in the experimental PEM model. However, actual feed intake was not measured quantitatively, the sample size was small, and the study did not directly assess linear growth or growth-related biomarkers. Therefore, the findings cannot establish that the extract alone caused the observed recovery and should not be interpreted as evidence of stunting prevention. Further studies with adequate sample sizes, standardized extract characterization, appropriate statistical procedures, and quantitative nutritional and growth-related measurements are needed.

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References

[1] World Health Organization, UNICEF, and World Bank, *Levels and trends in child malnutrition: Key findings of the 2023 edition*. World Health Organization, 2023.

[2] Kementerian Kesehatan Republik Indonesia, "Peraturan Menteri Kesehatan Republik Indonesia Nomor 30 Tahun 2022," JDIH Kemkes, Jakarta, 2022.

[3] L. A. Laily and S. Indarjo, "Dampak stunting terhadap pertumbuhan dan perkembangan anak," *Higeia*, vol. 7, no. 3, pp. 354–364, 2023.

[4] A. R. Ananda, S. T. Messakh, and Darry, "Gambaran status gizi dan perkembangan motorik anak usia 3 - 5 tahun di Kelurahan Pulutan, Salatiga.," *Jurnal Sains dan Kesehatan*, vol. 3, no. 1, pp. 242–247, 2020.

[5] A. P. Dewi, A. Rahmadini, J. Setiawati, and et al, "Analisis dampak, solusi serta pencegahan stunting: Literature review impact," *Jurnal Riset Gizi*, vol. 12, no. 1, pp. 64–71, 2024.

[6] M. H. Ramadhan, L. Salawati, and S. Y. Fitri, "Hubungan tinggi badan ibu, sosial ekonomi dan asupan sumber zinc dengan kejadian stunting pada anak usia 3-5 tahun di Puskesmas Kopelma," *Averrous: Jurnal Kedokteran dan Kesehatan Malikussaleh*, vol. 6, no. 1, pp. 55–65, 2020.

[7] Babirekere-Iriso E., Johanne M., Rytter H., and et al., "Physical activity level among children recovering from severe acute malnutrition," *Tropical Medicine and International Health*, vol. 3, no. 1, pp. 1–18, 2017.

[8] Bhutta Z.A., Berkley J.A., Bandsma R.H.J., and et al., "Severe childhood malnutrition," *Nature Reviews Disease Primers*, vol. 3, no. 1, pp. 1–18, 2017.

[9] W. Luthfiyah, "Serbuk daun kelor memulihkan kondisi fisik gizi buruk pada tikus model kurang energi protein," *Jurnal Kedokteran Brawijaya*, vol. 26, no. 3, pp. 131–135, 2011.

[10] A. R. Ananda, S. T. Messakh, and Dary, "Gambaran status gizi dan perkembangan motorik anak usia 3 - 5 tahun di Kelurahan Pulutan, Salatiga," *Jurnal Sains dan Kesehatan*, vol. 3, no. 1, pp. 242–247, 2020.

[11] M. H. Ramadhan, L. Salawati, and S. Y. Fitri, "Hubungan tinggi badan ibu, sosial ekonomi dan asupan sumber zinc dengan kejadian stunting pada anak usia 3-5 tahun di Puskesmas Kopelma," *Averrous: Jurnal Kedokteran dan Kesehatan Malikussaleh*, vol. 6, no. 1, pp. 55–65, 2020.

Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com

[12] E. Babirekere-Iriso *et al.*, "Physical activity level among children recovering from severe acute malnutrition," *Tropical Medicine and International Health*, vol. 3, no. 1, pp. 1–18, 2017, doi: 10.1111/tmi.13022.

[13] Z. A. Bhutta, J. A. Berkley, R. H. J. Bandsma, and M. Kerac, "Severe childhood malnutrition," *Nature Reviews Disease Primers*, vol. 3, no. 1, pp. 1–18, 2017, doi: 10.1038/nrdp.2017.67.

[14] Z. Irwan, "Kandungan zat gizi daun kelor (*Moringa oleifera*) berdasarkan metode pengeringan," *Jurnal Kesehatan Manarang*, vol. 6, no. 1, pp. 69–77, 2020.

[15] V. S. Srikanth, S. Mangala, and Subrahmanyam, "Improvement of protein energy malnutrition by nutritional intervention with *Moringa oleifera* among Anganwadi children in rural area in Bangalore, India," *Journal of Scientific Study*, vol. 2, no. 1, pp. 1–2, 2014.

[16] A. B. Manggara and M. Shofi, "Analisis kandungan mineral daun kelor (*Moringa oleifera* Lamk.) menggunakan spektrometer XRF," *Akta Kimia Indonesia*, vol. 3, no. 1, pp. 104–111, 2018.

[17] Z. Anaswa, V. Arianti, D. Adrianto, and D. Maulina, "Skrining fitokimia dan standarisasi ekstrak etanol pada daun kelor (*Moringa oleifera* L.) dengan parameter," *Jurnal Akademika Kimia*, vol. 5, no. 6, pp. 1457–1467, 2025.

[18] A. Pareek, M. Pant, M. M. Gupta, P. Kashania, and Y. Ratan, "*Moringa oleifera*: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects," *Frontiers in Pharmacology*, 2023.

[19] R. Kumar, S. Khatak, V. Adarsh, K. Shukla, S. Panwar, and A. Kumar, "Comprehensive review," *Food Science and Nutrition*, pp. 271–287, 2025, doi: 10.1002/fob2.70013.

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