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Effectiveness of instant nano catfish–moringa liquid food on burn wound healing in Sprague Dawley rats

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ABSTRACT

Background: Burn injuries induce hypermetabolic and catabolic responses that impair tissue regeneration and increase nutrient demands. Nano-sized nutrients are expected to enhance bioavailability and accelerate wound recovery through improved cellular uptake and metabolic utilization.

Objective: This study evaluated the effectiveness of a nano-processed instant liquid food made from catfish and moringa flours on burn wound healing in Sprague Dawley rats.

Materials and Methods: Fifteen male rats were allocated into five groups: control (commercial formula), MCB15 and MCB30 (micro-size formulas providing 15% and 30% of caloric needs), and MCN15 and MCN30 (nano-processed formulas with particle sizes of approximately 692.2 nm for catfish flour and 1.8 µm for moringa flour). Data were analyzed using Friedman and one-way ANOVA tests with $p < 0.05$ considered statistically significant. Second-degree burns were induced by 5-second contact with a 2.5 × 5 cm heated plate on the dorsal area. The interventions lasted for 14 days. Wound area reduction, collagen deposition, and epithelial length were assessed macroscopically and histologically, while glucose, SGPT, SGOT, creatinine, urea, and hematological parameters were analyzed biochemically.

Results: All groups exhibited progressive wound contraction ($p < 0.05$). The MCN30 group demonstrated a trend toward greater (56.5%) wound closure and collagen deposition compared with other groups, although inter-group differences were not statistically significant. SGOT decreased significantly in MCN15 ($p < 0.05$), whereas creatinine showed a mild increase. Blood glucose, urea, and hematological indices remained within normal physiological ranges.

Conclusion: The nano-processed catfish-moringa liquid diet showed potential to support burn wound healing and collagen formation without adverse metabolic effects. These findings should be interpreted cautiously due to the exploratory nature and small sample size of the study.

Keywords: *Burn injury; catfish flour; liquid diet; moringa leaf; nano-formulation*

BACKGROUND

Burn injuries represent a major global public health problem, ranking among the leading causes of morbidity, disability, and mortality, particularly in low- and middle-income countries.[1] The pathophysiological response following burn trauma is characterized by a prolonged hypermetabolic and hypercatabolic state, leading to accelerated muscle protein breakdown, increased energy expenditure, impaired immune function, and delayed tissue repair.[2,3] These metabolic alterations significantly increase nutritional requirements, thereby making adequate nutritional support a critical component in post-burn management.[4]

Nutritional interventions for burn patients commonly emphasize high-protein, energy-dense diets delivered in liquid or semi-liquid forms to enhance tolerance and improve digestibility.[5] Protein is essential for collagen synthesis, immune cell proliferation, and re-epithelialization, all of which are vital to wound healing.[6] Among various protein sources, catfish (*Clarias gariepinus*) flour has demonstrated high digestibility and favorable amino acid profiles comparable to casein, making it a promising animal protein base for medical nutrition formulations.[7] Furthermore, *Moringa oleifera* leaf flour contains abundant phytochemicals flavonoids, polyphenols, vitamin C, zinc, and iron that contribute to antioxidant activity, epithelial regeneration, and immune modulation.[8,9]

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Recent developments in nanotechnology have introduced the concept of *nano-sized food ingredients*, which exhibit improved physicochemical properties, dispersion stability, and bioavailability compared to their conventional counterparts.[10,11] The reduction of particle size into the nanometer range increases surface area and enhances nutrient absorption and cellular utilization.[12,13] In food technology, nano-formulation has been shown to optimize the delivery of bioactive compounds, thereby amplifying their physiological efficacy.

Previous studies demonstrated that high-protein liquid food formulations containing catfish flour and Moringa oleifera leaf flour provide adequate macronutrient and micronutrient profiles for burn patients, including high protein, antioxidant, and mineral contents.[14,15] However, conventional formulations may still exhibit limited nutrient dispersion and bioavailability, particularly during hypermetabolic recovery conditions.

To improve nutrient delivery efficiency, the present study applied particle size reduction using planetary ball milling to produce nano-processed catfish flour and reduced-particle-size moringa flour. Reduction of particle size is known to increase surface area, dispersion stability, and nutrient absorption, thereby potentially enhancing tissue repair and metabolic recovery.[14] The integration of both ingredients in nano-processed form is therefore expected to provide synergistic benefits through improved cellular uptake of high-quality protein and antioxidant bioactive compounds. However, conventional liquid nutritional formulas often exhibit limited bioavailability and suboptimal dispersion of bioactive compounds, potentially reducing nutrient absorption efficiency during hypermetabolic conditions.

Therefore, this study aimed to evaluate the effectiveness of a nano-processed instant liquid food made from catfish and moringa flours on burn wound healing, collagen formation, and systemic metabolic profiles in burn-induced Sprague Dawley rats. Although previous studies have explored high-protein diets and nano-processed nutrient delivery systems separately, limited studies have evaluated nano-processed combinations of animal and plant-based local food ingredients for burn wound recovery. Moreover, evidence regarding the application of nano-sized catfish–moringa formulations in burn models remains scarce.

MATERIALS AND METHODS

Experimental Design and Ethical Approval

This experimental study evaluated the effectiveness of nano- and micro-formulated instant liquid diets made from catfish (*Clarias gariepinus*) and moringa (*Moringa oleifera*) flours on burn wound healing in male Sprague Dawley rats. A total of fifteen healthy rats (3–4 months old, 200–300 g body weight) were used. They were acclimatized for 14 days under controlled temperature (25 ± 2°C), 12-hour light/dark cycle, and ad libitum access to water and standard chow.

Rats were randomly allocated into five experimental groups (n = 3 per group) using computer-generated random numbers. Allocation concealment was not applied due to the exploratory nature and small sample size of the study. No formal blinding procedure was applied during intervention administration; however, image analysis was conducted using ImageJ® software to minimize measurement bias. 1. Control – received a commercial enteral formula (Peptisol®); 2. MCB15 – micro-size formula providing 15% of burn caloric needs; 3. MCB30 – micro-size formula providing 30% of caloric needs; 4. MCN15 – nano formula providing 15% of caloric needs; 5. MCN30 – nano formula providing 30% of caloric needs.

All experimental procedures were conducted in accordance with ethical standards for animal experimentation and approved by the Animal Ethics Committee of the Tropical Biopharmaca Research Center, Institute of Research and Community Service, IPB University (Approval No. 049-2020 KEH TROP BRC). This study was designed as a pilot exploratory animal study to evaluate preliminary trends in wound healing responses following administration of nano- and micro-formulated catfish–moringa liquid diets.

Dietary Formulation and Dosage Calculation

The instant liquid formulas were developed using locally processed catfish and moringa flours produced in two particle-size variants: micro-size (MCB) and nano-milled (MCN) using a planetary ball mill. Particle size was confirmed through Scanning Electron Microscopy (SEM). Based on SEM characterization, the particle size of milled catfish flour reached approximately 692.2 nm, whereas moringa flour particles reached approximately 1,890 nm (1.8 µm), indicating nano-sized and micro-size characteristics, respectively.

The daily human caloric requirement for burn recovery (2,500 kcal/day) was converted to the rat equivalent using the conversion factor 0.018. Accordingly, the caloric contributions were set at 15% and 30% of this value, corresponding to 375 kcal and 750 kcal human-equivalent doses. These were translated to 2.25 mL/day and 3.38 mL/day for rats, respectively. Dose conversion from human to rat equivalent was adapted using body surface area normalization methods described by Nair and Jacob.[16]

All experimental groups received oral gavage once daily for 14 consecutive days, while maintaining standard chow *ad libitum*. Nutrient composition per dose is shown in Table 1.

Table 1. Nutrient Composition of Instant Liquid Formula per Dose

Group	Formula (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)
Control	3.40	13.5	0.76	0.16	2.32
MCB15	1.54	6.75	0.44	0.22	0.71
MCB30	3.09	13.5	0.87	0.43	1.43
MCN15	1.59	6.75	0.43	0.19	0.84
MCN30	3.18	13.5	0.86	0.37	1.68

Burn wound area was documented using digital photography and quantified using ImageJ® software under standardized measurement conditions. Histopathological analysis of skin tissue was conducted at the Primate Research Center (PSSP), IPB University, while animal handling and intervention procedures were performed at the Tropical Biopharmaca Research Center (TropBRC), IPB University.

Blood samples were collected from the tail vein for serial glucose and hematological measurements, while terminal blood collection on day 15 was performed under anesthesia for biochemical analysis (SGOT, SGPT, urea, and creatinine). Biochemical parameters were analyzed using standard enzymatic colorimetric methods according to the laboratory protocols of the Tropical Biopharmaca Research Center, IPB University.

Burn Injury Induction

After acclimatization, rats were anesthetized with ketamine–xylazine (80 mg/kg + 10 mg/kg BW, intraperitoneal). The dorsal region was shaved and disinfected using 70% alcohol. Second-degree burns (grade IIB) were induced by applying a pre-heated (15 minutes) metal plate (2.5 cm × 5 cm) to the dorsal area for 5 seconds. Rats were monitored until full recovery before intervention began. The burn area was visually confirmed by blistering and erythema without full dermal destruction.

Sample Inclusion and Exclusion Criteria

Inclusion criteria: healthy male rats (Sprague Dawley strain), age 3–4 months, weight 200–300 g, physically active with clean fur and clear eyes. Exclusion criteria: previous involvement in studies or >10% weight loss during acclimatization. Minimum sample size was determined using Arifin and Zahiruddin's formula ($n = 10/k + 1$), resulting in 3 animals per group. Due to the exploratory nature of the study and ethical considerations in animal experimentation, a minimum sample size approach based on Federer/Arifin and Zahiruddin formula was applied.

Data collection and outcome

Table 2. Data were collected at various time points

Variable	Method	Frequency	Timepoints
Skin histopathology	Tissue excision	2×	Days 7 and 15
SGOT, SGPT, urea, creatinine	Blood sampling	1×	Day 15
Complete blood count	Blood sampling	2×	Days 3 and 15
Body weight	Weighing	5×	D0, D3, D7, D15
Burn surface area	Wound area measurement	4×	Days 1, 3, 7, 15
Blood glucose	Blood sampling	6x	Pre-burn, 30 min post-burn, D1, D3, D7, D15

Note: Histological analysis used one rat per group per time point. Collagen deposition and epithelial length were quantified using ImageJ® software.

Statistical Analysis

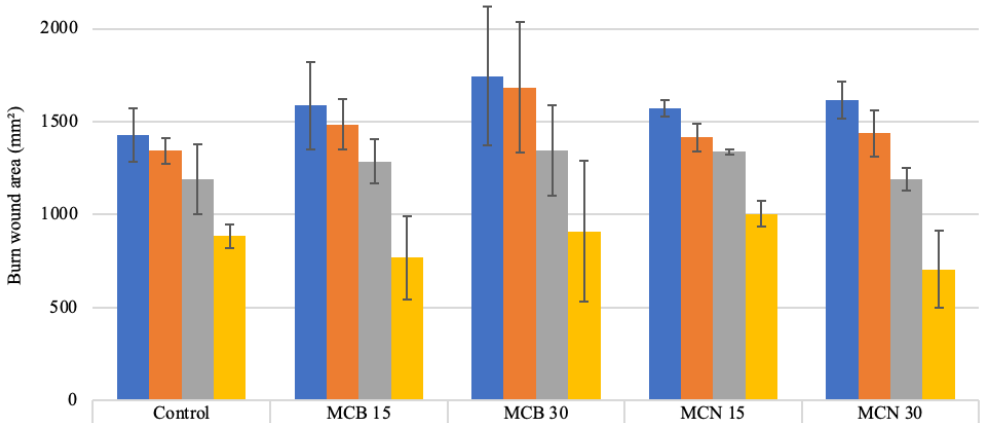
All data were expressed as mean ± standard deviation (SD). Macronutrient and physicochemical comparisons between nano and micro-size flours were analyzed using paired *t*-tests. Total phenolic content and dispersion capacity were evaluated using independent *t*-tests.

For biological parameters: 1) Repeated wound area and blood glucose were analyzed using Friedman tests; 2) SGOT, SGPT, urea, and creatinine by one-way ANOVA followed by Duncan's post hoc; 3) Within-group hematological differences (Day 3 vs Day 15) by paired *t*-tests. A p-value < 0.05 was considered

statistically significant. Statistical analyses were conducted using SPSS Version 22. Due to the small sample size (n = 3/group), data distribution assumptions were assessed descriptively and non-parametric approaches were prioritized for repeated measurements. Statistical analyses were interpreted exploratorily, and non-significant findings were not considered evidence of equivalence between groups.

RESULTS

All treatment groups exhibited a progressive reduction in burn wound area over the 14-day intervention period. Wound size was measured on days 1, 3, 7, and 15 using digital planimetry. Within-group reductions were statistically significant ($p < 0.05$), although inter-group differences were not significant. Among all treatments, the MCN30 group showed the greatest wound area contraction, with a decrease of 56.5% compared to 38.1% in the control group (Figure 1).



	Control	MCB 15	MCB 30	MCN 15	MCN 30
Day-1	1,427.00	1,587.00	1,746.00	1,572.67	1,616.00
Day-3	1,342.67	1,484.67	1,684.67	1,414.67	1,437.00
Day-7	1,191.33	1,286.67	1,345.33	1,337.00	1,191.33
Day-15	883.33	767.33	910.67	1,004.00	705.00

Data are presented as mean (n = 3/group). Statistical analysis was performed using Friedman test; *p < 0.05 compared to baseline within the same group. Error bars indicate standard deviation.

Figure 1. Burn Wound Area Measured on Days 1, 3, 7, and 15 in Sprague Dawley Rats Following Dietary Intervention

Macroscopic observation of wound surfaces (Figure 2) showed that by day 15, scab detachment and complete wound closure were most apparent in the MCN30 group. This indicated enhanced epithelialization and tissue contraction, supported by visible granulation and remodelling processes.

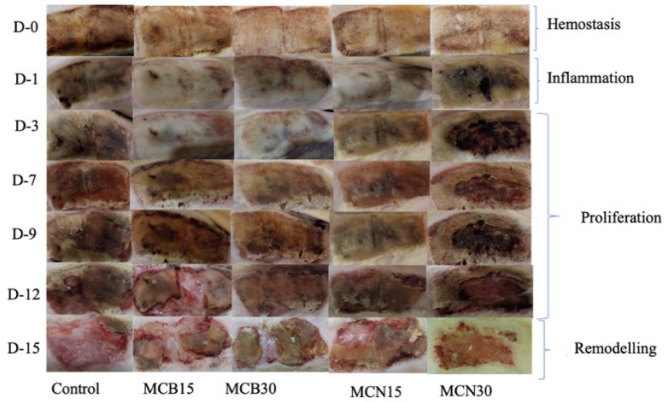
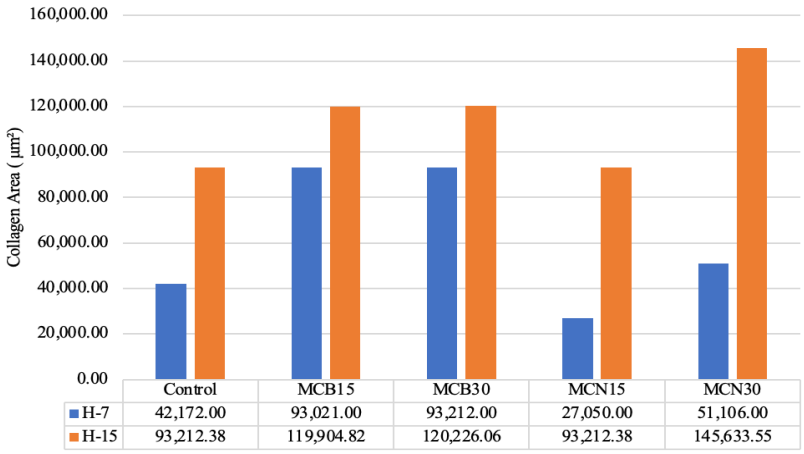


Figure 2. Macroscopic Appearance of Rat Skin from Day 0 to Day 15 in Each Group Post-Burn Induction

Representative histological observations suggested increased collagen deposition in all groups, particularly in MCN30, where the collagen area increased from 51,106 µm² (day 7) to

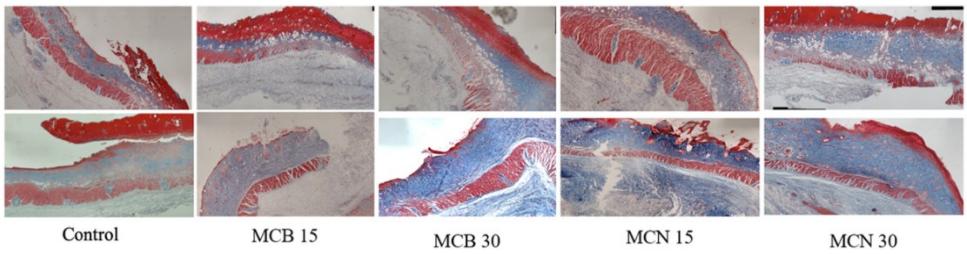
145,633.55 µm² (day 15). This finding corresponded with macroscopic wound contraction (Figure 3).



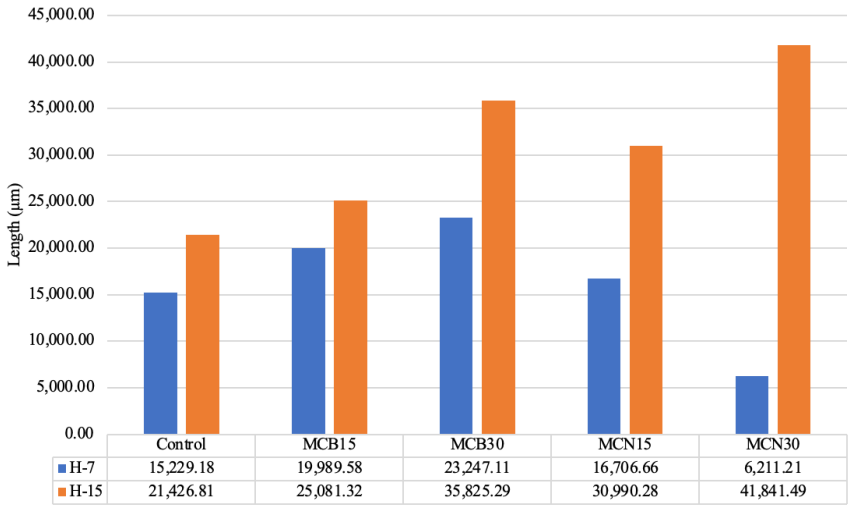
	Control	MCB15	MCB30	MCN15	MCN30
H-7	42,172.00	93,021.00	93,212.00	27,050.00	51,106.00
H-15	93,212.38	119,904.82	120,226.06	93,212.38	145,633.55

Figure 3. Collagen Area in Burn Wound Tissue on Days 7 and 15. Collagen stained in blue using Masson's trichrome

Epithelial regeneration also improved across groups. The MCN30 group exhibited the greatest epithelial coverage (41,841.49 µm) at day 15 compared with the control (21,426.81 µm), reflecting faster epidermal closure (Figure 4).



(a)

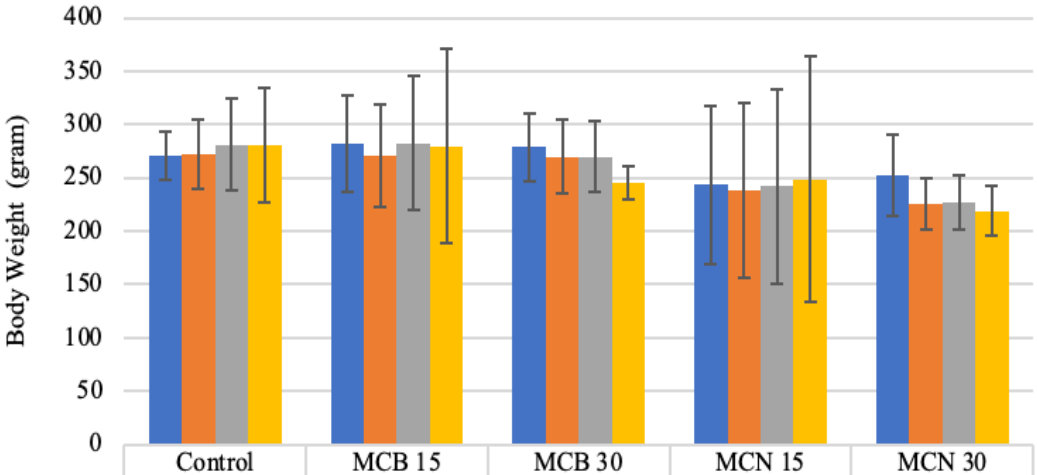


	Control	MCB15	MCB30	MCN15	MCN30
H-7	15,229.18	19,989.58	23,247.11	16,706.66	6,211.21
H-15	21,426.81	25,081.32	35,825.29	30,990.28	41,841.49

(b)

Figure 4. Length of Newly Formed Epithelium on Days 7 and 15. (a) histological appearance, (b) diagram

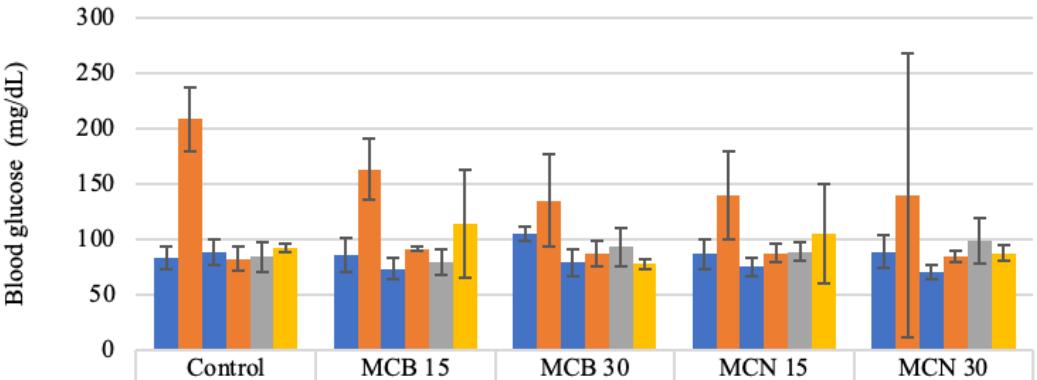
Body weight decreased consistently in all groups following burn induction, reflecting the hypermetabolic response. The most marked reduction occurred in the MCN30 group (12.94% from baseline) (Figure 5).



	Control	MCB 15	MCB 30	MCN 15	MCN 30
Day-0	271.00	282.00	278.67	243.33	252.33
Day-3	272.00	270.67	269.67	238.67	225.67
Day-7	281.00	282.67	269.67	242.00	226.67
Day-15	280.00	279.67	245.00	248.67	219.00

Figure 5. Body Weight Changes in Rats Before and After Burn Induction Over the 14-Day Intervention Period

Blood glucose levels rose sharply 30 minutes post-burn, indicating transient stress-induced hyperglycemia. Glucose normalized by day 1 and remained within the physiological range until day 15, with no significant inter-group differences (Figure 6).

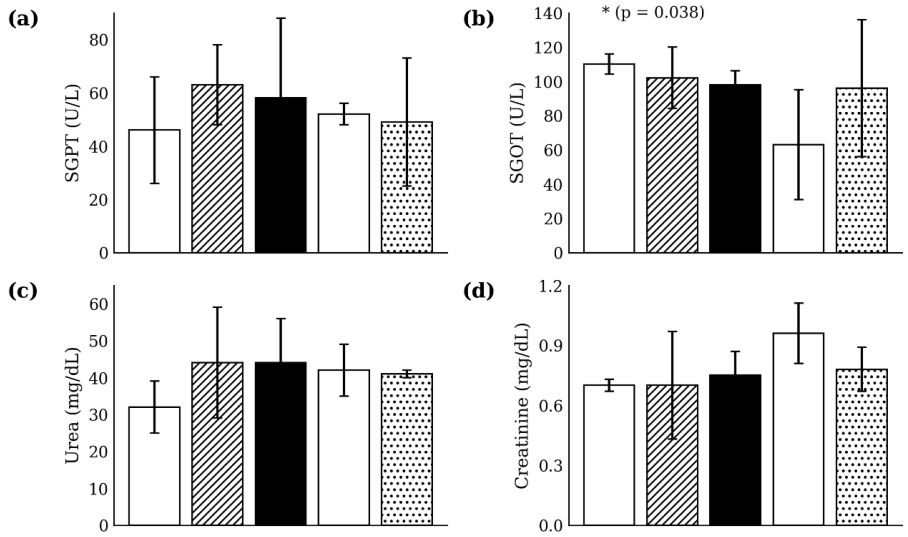


	Control	MCB 15	MCB 30	MCN 15	MCN 30
Before	84.00	86.00	105.33	87.33	89.33
30 menit	208.67	163.33	135.33	140.00	139.67
Day-1	88.67	73.67	79.33	75.67	70.67
Day-3	83.00	91.67	87.67	88.00	85.00
Day-7	84.67	80.00	93.33	89.33	99.00
Day-15	92.33	114.00	78.00	105.67	88.00

Note: Data are presented as mean (n = 3/group). Statistical analysis was performed using Friedman test; *p < 0.05 compared to baseline within the same group

Figure 6. Blood Glucose Levels at Multiple Time Points Post Burn Induction.

Liver (SGPT, SGOT) and kidney (urea, creatinine) function tests remained within normal ranges across groups. A significant decrease in SGOT levels was observed in MCN15 ($p < 0.05$), suggesting a potential hepatoprotective effect of the nano-formulation (Figure 7).



Note: Data are presented as mean ± SD (n = 3/group). Statistical analysis was performed using one-way ANOVA followed by Duncan's post hoc test; $p < 0.05$.

Figure 7. Liver Enzyme (a) SGPT, (b) SGOT and Kidney Biomarker (c) Urea, (d) Creatinine Levels on Day 14.

Haematological parameters (WBC, RBC, Hb, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW) exhibited minor fluctuations but remained within physiological reference ranges. No clinically significant deviations were detected across groups (Figure 8).

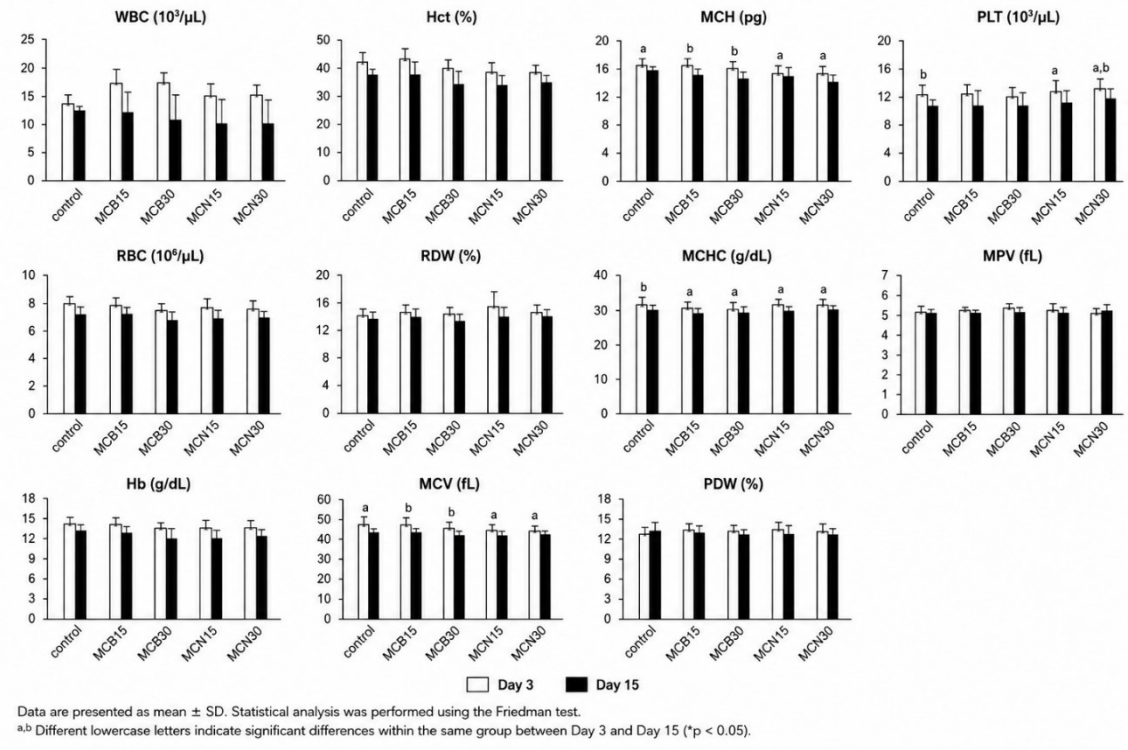


Figure 8. Haematological Profiles of Rats on Days 3 and 14 Post Burn Injury and Intervention.

Overall, the MCN30 intervention resulted in the most favorable wound healing outcomes—evidenced by faster wound contraction, greater collagen deposition, and longer epithelial coverage. These findings indicate that nano-processed liquid food may support tissue repair without causing any adverse effects on metabolic or organ function markers.

DISCUSSION

Burn injuries induce complex metabolic and physiological responses characterized by hypermetabolism, inflammation, and oxidative stress that significantly affect wound healing outcomes.[1] The results of this study demonstrate that all intervention groups experienced progressive wound area reduction, with the nano-processed group receiving 30% of caloric needs (MCN30) demonstrating trends toward improved outcome. These findings indicate that a nano-processed catfish moringa liquid diet may support tissue repair without causing metabolic disturbance.

The progressive wound contraction observed in this study aligns with the essential role of protein in collagen synthesis and matrix remodeling.[2] Catfish flour provides high-quality protein with balanced amino acids that contribute to fibroblast proliferation and granulation tissue formation.[3] The addition of *Moringa oleifera* leaf flour enhances the formulation with antioxidants, vitamins, and minerals particularly vitamin C, zinc, and polyphenols that protect cells from oxidative damage and promote epithelial regeneration.[4,5] This synergistic combination explains the improvement in wound closure and collagen deposition seen in the MCN30 group.

The use of reduced particle-size ingredients further strengthens the biological effect through enhanced nutrient bioavailability. Particle size reduction to the nanometer scale increases surface area, dispersion uniformity, and absorption efficiency at the intestinal level.[6,7] Nano-processed proteins and phytochemicals may be more readily transported across biological membranes, allowing faster cellular uptake and utilization for tissue synthesis.[8] These findings support prior reports that nano-processed food components can improve tissue regeneration and healing responses compared with conventional formulations.[9]

Histological findings should be interpreted descriptively because only one animal per group was evaluated at each time point. Histological results in this study demonstrated a trend toward increased collagen deposition and longer epithelial coverage in the MCN30 group. Collagen is a key structural component of granulation tissue and serves as a marker of wound maturation.[10] Enhanced collagen formation implies better fibroblast activity and angiogenesis. The visible granulation and re-epithelialization observed macroscopically suggest an accelerated healing phase, consistent with the mechanism of protein-driven fibroblast proliferation and antioxidant-mediated modulation of inflammation.[11]

The biochemical parameters indicated that the nano-processed liquid diet appeared metabolically safe. Liver enzymes (SGOT, SGPT) and kidney biomarkers (urea, creatinine) remained within physiological limits, implying no hepatic or renal toxicity during intervention. Interestingly, SGOT levels decreased significantly in the MCN15 group, suggesting potential hepatoprotective effects of moringa bioactives, as reported in previous studies.[12,13] The significant SGOT reduction observed only in MCN15 may indicate variability associated with small sample size rather than a dose-dependent response. Therefore, these findings should be interpreted cautiously. Blood glucose levels exhibited a transient post-burn increase, reflecting acute stress response, but normalized by day one. These findings correspond with physiological recovery patterns seen in burn models receiving adequate nutritional support.[14]

Hematological stability across groups suggests that the liquid diet maintained normal erythropoietic and immune functions. Adequate protein intake and antioxidant supply may help sustain hemoglobin synthesis and mitigate systemic inflammation.[15] Body weight reduction observed in all rats was expected due to burn-induced hypermetabolism; however, it did not result in critical catabolism or organ failure, implying sufficient energy compensation from the formulated diets.

The greater body weight reduction observed in the MCN30 group, despite higher caloric supplementation, may reflect the hypermetabolic response following burn injury and increased metabolic utilization during accelerated tissue repair. However, interpretation should be cautious due to the limited sample size.

The overall outcomes indicate that nano-processed catfish–moringa liquid food supports burn wound healing through multiple mechanisms: enhanced nutrient absorption, protein-mediated collagen synthesis, and antioxidant-driven epithelial regeneration. These results are consistent with earlier studies that emphasized the importance of high-protein and antioxidant-rich diets in accelerating wound repair.[16,17]

This study provides one of the first in vivo evaluations of nano-processed catfish moringa liquid diets for burn recovery. Its strengths include a well-defined experimental design, detailed biochemical monitoring, and histological validation of healing markers. However, limitations include a small sample size, short intervention duration, and absence of inflammatory cytokine measurements. Future research should focus on longer-term effects, molecular pathways, and translational studies in human subjects to validate the clinical potential of this formulation.

The findings highlight the potential of locally sourced ingredients catfish and moringa—combined with nanotechnology, to develop effective, affordable, and safe therapeutic foods for burn patients. The application of nano-processing in medical nutrition could represent a novel approach in clinical dietetics, especially for individuals requiring high nutrient density and bioavailability during recovery.

This study has several limitations. First, the small sample size limited statistical power and reduced the ability to detect significant inter-group differences. Second, the intervention duration was relatively short and may not fully represent long-term wound healing responses. Third, the use of an animal model limits direct extrapolation to human burn patients. In addition, the study did not evaluate molecular biomarkers or direct mechanistic evidence of nano-bioavailability. Therefore, the findings should be considered preliminary and exploratory, requiring further validation through larger and longer-term studies.

CONCLUSION

The nano-processed instant liquid food made from catfish (*Clarias gariepinus*) and moringa (*Moringa oleifera*) flours showed promising trends in supporting burn wound healing in Sprague Dawley rats. This effect was evidenced by faster wound area reduction, increased collagen deposition, and enhanced epithelial regeneration compared to micro-size formulations.

No adverse changes were observed in biochemical or hematological parameters, indicating that the nano-processed formulation appeared metabolically safe. The tendency to demonstrate improved wound healing indicators in the MCN30 group suggests that nanotechnology may enhance nutrient bioavailability and utilization in tissue repair processes. However, inter-group differences were not statistically significant, and the findings should be interpreted cautiously.

These findings highlight the potential application of nano-processed local food ingredients as a promising nutritional support for patients with burn injuries or high metabolic stress conditions. Further studies involving larger sample sizes and longer intervention periods are recommended to confirm these preliminary findings.

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CONFLICT OF INTEREST

The authors declare no conflict of interest to disclose.

DECLARATION USE AI

During the preparation of this work, the authors used ChatGPT (OpenAI) in order to improve language clarity, grammar, and manuscript readability. The authors have reviewed and edited the content as needed. The authors take full responsibility for the content of the published article.

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