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BLACK MULBERRY AS A FUNCTIONAL FOOD: A CHEMICAL AND NUTRITIONAL EVALUATION OF ITS PHYSIOLOGICAL EFFECTS IN OBESE RATS

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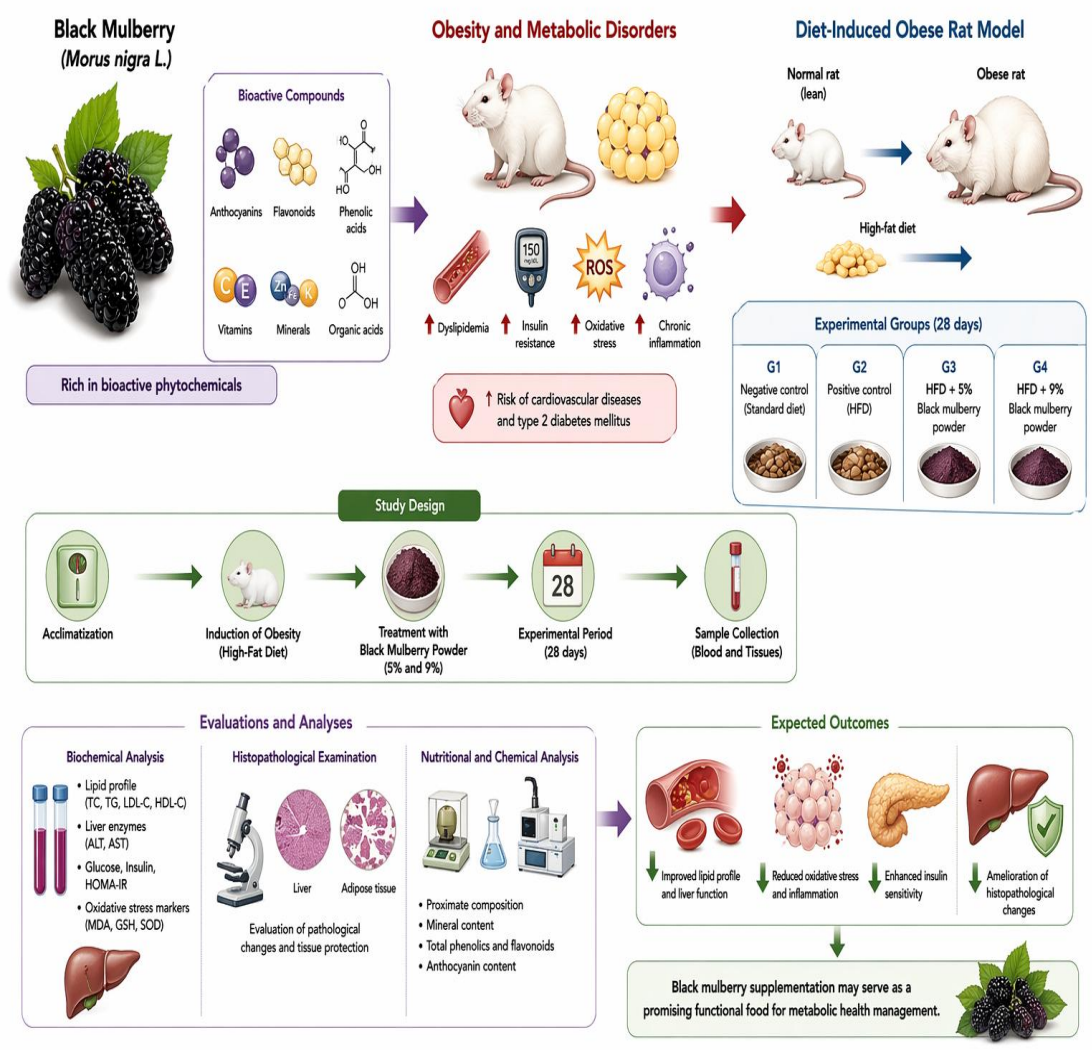
ABSTRACT

Black mulberry (*Morus nigra* L.) has gained considerable interest as a functional food owing to its high content of bioactive phytochemicals and its potential role in improving metabolic health, particularly in obesity-related disorders. This study aimed to characterize the nutritional composition of black mulberry and evaluate its physiological effects in an experimental model of diet-induced obesity. Twenty-four adult male albino rats (150 ± 10 g) were randomly divided into four equal groups (n = 6). The first group served as the normal control and received a standard basal diet. The second group represented the obese control and was fed the basal diet without supplementation. The third and fourth groups consisted of obese rats receiving the basal diet supplemented with black mulberry powder at concentrations of 5% and 9%, respectively. The experiment was conducted over a period of 28 days. At the end of the experimental period, blood samples were collected for biochemical analysis, and tissue specimens were obtained for histopathological examination to assess metabolic and structural changes associated with obesity and the potential protective effects of black mulberry supplementation. The findings demonstrate that black mulberry exerts significant beneficial effects on lipid profile, glycemic control, and hepatic and renal function parameters. These effects are primarily attributed to its richness in phenolic compounds, which enhance antioxidant defense, reduce oxidative stress, and modulate key metabolic pathways involved in energy and lipid metabolism. Overall, black mulberry shows promising potential as a functional food for the management of obesity-related metabolic disturbances.

KEYWORDS: Morus nigra, functional food, obesity, biochemical analysis, phytochemicals, metabolic disorders.



Graphical Abstract: Black Mulberry as a Functional Food Against Obesity-Induced Disorders"

INTRODUCTION

Black mulberry (*Morus nigra* L.) is a widely distributed perennial fruit species belonging to the Moraceae family, traditionally valued for both its nutritional and therapeutic properties. It is characterized by a rich composition of bioactive phytochemicals, including phenolic acids, flavonoids, anthocyanins, vitamins, minerals, and organic acids. These compounds are largely responsible for its strong antioxidant capacity and diverse biological activities, which have supported its recognition as a functional food (Polumackanycz et al.,2021). Functional foods are generally defined as foods that provide health benefits beyond basic nutrition and may contribute to the prevention or management of chronic diseases (Batiha et al.,2023). In recent years, increasing attention has been directed toward plant-derived functional foods due to their potential role in improving metabolic health and preventing lifestyle-related disorders. Obesity is a complex metabolic disease characterized by excessive fat accumulation resulting from an imbalance between energy intake and expenditure. It is strongly associated with insulin resistance, dyslipidemia, oxidative stress, and chronic low-grade inflammation, all of which contribute to the development of cardiovascular diseases and type 2 diabetes mellitus (Ferraz et al.,2024). Consequently, there is a growing interest in dietary strategies based on natural bioactive compounds as complementary approaches for obesity management.

Polyphenol-rich plant foods have been shown to modulate key metabolic pathways involved in lipid and glucose homeostasis. These compounds exert antioxidant and anti-inflammatory effects by regulating oxidative stress and inflammatory signaling pathways. In this context, black mulberry has gained scientific attention due to its high anthocyanin content, which is considered a major contributor to its biological activities (Alves et al., 2023). Anthocyanins and other phenolic compounds present in mulberry have been reported to improve lipid metabolism, enhance insulin sensitivity, and reduce adipogenesis in experimental studies **(Figure 1)**. Furthermore, previous investigations have demonstrated that mulberry extracts may influence the activity of enzymes involved in carbohydrate and lipid metabolism, thereby contributing to improved metabolic regulation. In addition, potential hypolipidemic and hepatoprotective effects have been reported, suggesting a protective role against obesity-related metabolic dysfunction and oxidative damage in vital organs such as the liver. These findings highlight the importance of further exploring its functional properties in experimental models.

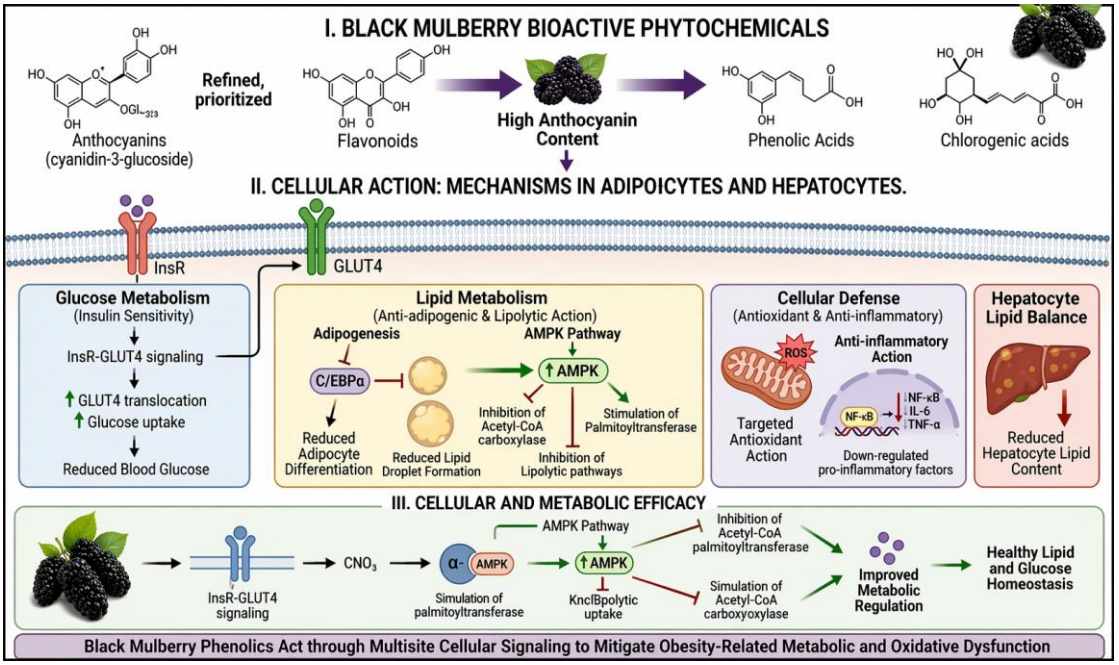


Figure 1: Proposed cellular and molecular mechanisms of Black Mulberry (*Morus nigra* L.) bioactive compounds in mitigating obesity-related metabolic dysfunction.

Animal models of diet-induced obesity are widely used in biomedical research to mimic human metabolic disorders under controlled conditions. These models allow comprehensive assessment of biochemical parameters such as lipid profiles, liver function enzymes, and oxidative stress markers, as well as histopathological alterations in target tissues (Reis et al.,2022). Such experimental approaches are essential for understanding the mechanistic effects of functional foods and plant-derived compounds. Despite the growing evidence supporting the biological activities of black mulberry, further research is still needed to clarify its dose-dependent effects and underlying mechanisms in obesity-related metabolic disturbances(Silva et al., 2022). Therefore, the present study was designed to evaluate the chemical and nutritional properties of black mulberry (*Morus nigra* L.) and to investigate its physiological effects in a diet-induced obese rat model (Al-Khalifah, et al .,2023). The study also aims to assess the impact of dietary supplementation with different concentrations of black mulberry powder on biochemical and histopathological parameters, providing deeper insight into its potential role as a functional food in metabolic health management

2. MATERIALS AND METHODS

2.1. Materials

Source of Plant Material: Specimens of black mulberry (*Morus nigra* L.) were obtained from local commercial outlets in Al-Baha City, Saudi Arabia (KSA). The black mulberry fruits were air-dried and ground to a fine powder according to the method described by **(Ammar et al., 2014)**. The botanical samples were prepared and processed for dietary incorporation as previously described.

Experimental Animals: Twenty-four mature male Sprague-Dawley albino rats, with an average body weight of 150 ± 10 g, were obtained from the animal colony at the Institute of Nutrition (Cairo, Egypt). The animals were selected based on their health status and uniform age to ensure the reliability of metabolic data.

Dietary Ingredients and Chemical Reagents: High-purity dietary components, including casein, cellulose, and standardized vitamin and mineral premixes, were obtained from Morgan Co. (Cairo, Egypt). For biochemical and histological procedures, analytical-grade chemicals and reagents specifically formalin (for tissue fixation), ethanol, and ethylenediaminetetraacetic acid (EDTA) were purchased from El-Nasr Pharmaceutical Chemicals (El-Amereia, Cairo, Egypt).

2.2. Preparation of Black Mulberry powder.

Black mulberry (*Morus nigra* L.) specimens were obtained from a local market in Al-Baha City, Saudi Arabia. To ensure purity, the samples were thoroughly washed with distilled water to remove any surface contaminants or debris. The cleaned fruits were then air-dried at a controlled ambient temperature (25 ± 2 °C) for 7–10 days until a constant weight was achieved. Subsequently, the dried material was ground into a fine powder using a laboratory-grade mechanical grinder and passed through a 40-mesh sieve to ensure uniform particle size. The homogenized powder was stored in light-resistant, airtight containers and kept at 4 °C to preserve its bioactive integrity for subsequent experimental and biochemical analyses, in accordance with established botanical preparation protocols (Ainsworth & Gillespie, 2007).

2.3. Experimental Animals and Housing

Twenty-four male Sprague Dawley rats were acclimatized for one week under controlled laboratory conditions: temperature 22 ± 2 °C, relative humidity 50–60%, and a 12-hour light/dark cycle. Animals had ad libitum access to standard basal diet and water during the acclimatization period. All procedures were conducted in accordance

with institutional guidelines for the care and use of laboratory animals.

2.4. Formulation and Administration of Experimental Diets.

The experimental regimens were formulated by incorporating powdered black mulberry (BM) into the basal diet at concentrations of 5% and 9% (w/w). To maintain nutritional quality and prevent oxidation of bioactive compounds, the diets were prepared fresh on a weekly basis and stored under refrigeration at 4 °C. The animals were provided with ad libitum access to their respective dietary treatments throughout the 28-day experimental period. Detailed proportions and the specific nutritional composition of each experimental diet are presented in Table 1.

Table (1): The basic and experimental diets' compositions.

Component (g)	Control (-)	Control (+)	5% (BM)	9% (BM)
Test ingredients	---	---	5	9
Casein	20	20	20	20
Corn oil	4.7	4.7	4.7	4.7
Mineral mix	3.5	3.5	3.5	3.5
Vitamin mix	1	1	1	1
Cellulose	5	5	5	5
Cholin chloride	2	2	2	2
Sucrose	10	10	10	10
Corn starch	**Up to 100**	**Up to 100**	**Up to 100**	**Up to 100**

2.5. Induction of Experimental Obesity:

To establish the obesity model, rats were maintained on a high-fat diet (HFD) for a duration of four weeks. The HFD was formulated using high-quality ingredients to achieve the desired metabolic induction. Each 100 g of the diet was composed of 30% total fat (a 1:1 ratio of tallow and corn oil), 12% casein as a protein source, 4% salt mixture, 1% vitamin mixture, and 5% dietary fiber. Additionally, the formulation included 0.3% D1-methionine, 2% choline chloride, and 0.2% bile acid, with corn starch added as the base to reach a total mass of 100 g, following the AIN-93 guidelines (AIN, 1993)**.**

2.6. Ethical Housing and Environmental Stabilization: Adult male Sprague-Dawley albino rats (weight: 150 ± 10 g; age: 14–16 weeks) were sourced from the Animal Laboratory and housed in sanitized polypropylene cages equipped with stainless steel lids. The experimental environment was strictly regulated under standard laboratory conditions, featuring a consistent 12-hour light/dark cycle. Throughout the study, the animals had unrestricted (ad libitum) access to a basal diet and fresh water, provided through specialized narrow-mouth bottles with metallic delivery tubes. To ensure physiological and metabolic stabilization, all rats underwent a seven-day acclimation period under these conditions before the experimental phase commenced.

2.7. Experimental Design and Animal Grouping

A total of **24 mature male Sprague-Dawley rats** were randomly allocated into four experimental groups, with six animals per group (n = 6). The experimental intervention was maintained for 28 consecutive days as follows:

- **Group 1 (Negative Control; G1):** Healthy rats received a standard basal diet and served as the baseline for normal physiological parameters.
- **Group 2 (Positive Control; G2):** Rats were fed a high-fat diet (HFD) to induce obesity and metabolic dysfunction, without any supplemental treatment.
- **Group 3 (BM 5%; G3):** Obese rats were fed a high-fat diet incorporated with 5% (w/w) black mulberry (*Morus nigra* L.) powder.
- **Group 4 (BM 9%; G4):** Obese rats were fed a high-fat diet incorporated with 9% (w/w) black mulberry (*Morus nigra* L.) powder.

Figure 2. Experimental Design and Methodological Workflow for Evaluating the Effects of Black Mulberry (*Morus nigra* L.) Powder on High-Fat Diet-Induced Obesity in Sprague-Dawley Rats

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2.7. Chemical analysis

Moisture, ash, crude protein, and fat contents of the black mulberry powder were determined using the standard methods of **(AOAC, 2016)**.

2.8. Characterization of Phenolic Compounds via HPLC.

The phytochemical profile of black mulberry was analyzed using an Agilent Technologies 1260 Infinity II HPLC system, equipped with an autosampler and a diode-array detector (DAD), following the methodology described by Kim et al. (2006). Chromatographic separation was achieved using an Eclipse XDB-C18 analytical column (150 × 4.6 mm, 5 µm), protected by a C18 guard column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of a binary gradient system comprising acetonitrile (Solvent A) and 2% aqueous acetic acid (v/v) (Solvent B). A flow rate of 0.8 mL/min was maintained over a total run time of 60 minutes, using the following gradient program: 0–30 min, 100% to 85% B; 30–50 min, 85% to 50% B; 50–55 min, 50% to 0% B; and 55–60 min, returning to 100% B.

Prior to injection (50 µL), all extracts were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI, USA). Detection was performed by simultaneous monitoring at specific wavelengths: 280 nm for benzoic acid derivatives, 320 nm for cinnamic acid derivatives, and 360 nm for flavonoids. Phenolic constituents were identified by comparing their retention times and UV–Vis spectra with those of authentic commercial standards.

2.9. Biological Assessment and Growth Indices

Monitoring of nutritional and growth parameters was conducted throughout the 28-day experimental phase. Individual feed consumption was documented daily to determine total intake, while body weight fluctuations were recorded on a weekly basis. From these primary data, growth performance indicators including body weight gain percentage (BWG%) and feed efficiency ratio (FER) were determined. Furthermore, upon study completion, the relative weights of internal organs were calculated. All biological evaluations and growth metrics were derived in accordance with the standardized methodology described by Chapman, Castillo, and Campbell (1959).

2.10. Blood Sampling and Organ Preparation.

Upon completion of the 28-day experimental period, the rats were subjected to a 12-hour fasting period to ensure metabolic stability prior to sample collection. Blood samples were collected via the retro-orbital plexus using sterile glass capillary tubes. The collected samples were kept at 37 °C for 30 minutes to allow natural coagulation, followed by centrifugation at 3000 rpm for 10 minutes to separate the serum. The obtained serum was then aliquoted into sterile polypropylene microcentrifuge tubes and stored at −20 °C for subsequent biochemical analyses. Following blood collection, the animals were euthanized, and vital organs specifically the liver, kidneys, heart, and spleen were carefully excised. The organs were rinsed in physiological saline (0.9% NaCl), blotted dry, and weighed to determine relative organ weights. Finally, tissue specimens were fixed in 10% neutral buffered formalin for subsequent histopathological examination, according to the protocols established by Drury and Wallington (1980). The liver sections were stained with hematoxylin and eosin (H&E) for microscopic examination following the procedures outlined by (Bancroft & Layton, 2013).

2.11. Biochemical Analysis of Serum

The biochemical parameters were quantified using high-precision diagnostic kits and standardized

spectrophotometric techniques as follows:

1. Assessment of Liver Function Biomarkers:

- Aminotransferase Activities: The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using commercially available spectrophotometric kits (BioMerieux, France), in accordance with the colorimetric method established by (Reitman & Frankel, 1957).
- Alkaline Phosphatase (ALP): Serum ALP activity was measured colorimetrically following the protocol described by Roy (1970).
- Total Bilirubin: Quantitative determination of serum total bilirubin was performed calorimetrically at a wavelength of 578 nm following the methodology of Doumas et al. (1973).
- Total Protein (TP): The concentration of total protein in the serum was estimated according to the analytical procedures outlined by Varley et al. (1980).

2. Evaluation of Serum Lipid Profile:

The lipid components were analyzed to evaluate the metabolic impact of the treatments:

- Total Cholesterol (TC): Measured using the enzymatic approach described by Ratliff and Hall (1973).
- Triglycerides (TG) and High-Density Lipoprotein (HDL-c): These parameters were quantified employing enzymatic colorimetric methods as detailed by Jacobs and Van Denmark (1960).
- Lipoprotein Fractions: Very-low-density lipoprotein (VLDL-c) and low-density lipoprotein (LDL-c) concentrations were mathematically derived using the standard formulas provided by Lee and Nieman (1996).

3- Assessment of Renal Function Biomarkers. To evaluate the renal physiological status, the following biochemical indicators were quantified using standardized diagnostic kits:

- Serum Urea Nitrogen: The concentration of urea was determined using the enzymatic method established by Fawcett and Scott (1960), as further detailed and modified by Schultz (1984).
- Serum Uric Acid: Quantitative determination of uric acid was performed using an enzymatic colorimetric assay kit in accordance with the protocols described by Fossati and Prencipe (1982).

2.12. Statistical Analysis

The experimental data were expressed as mean ± standard deviation (SD). To assess the significance of differences among experimental groups, a one-way analysis of variance (ANOVA) was performed based on a completely randomized design, following the methodology of Armitage et al. (1987). In addition, Duncan's Multiple Range Test (DMRT) was applied for post-hoc comparisons to identify significant differences among group means. A probability value of less than 0.05 ($p < 0.05$) was considered the threshold for statistical significance across all analyses.

2.13. Ethical Statement and Institutional Approval

"The experimental protocols and animal handling procedures implemented in this study were meticulously reviewed and formally approved by the **Institutional Research Ethics Committee (REC) at Al-Baha University**, Saudi Arabia (Reference No. **46123022**; Approval date: **17 April 2025**). All interventions were conducted in strict adherence to the internationally recognized guidelines for the care and use of laboratory animals, ensuring the highest standards of animal welfare throughout the investigation."

3. RESULTS

3.1 Chemical Composition of Black Mulberry (*Morus nigra* L.)

The proximate chemical composition of black mulberry (*Morus nigra* L.) was determined on a wet weight basis (wwb), as presented in Table 2. The fruit exhibited a relatively high moisture content (13.12 ± 0.06%), indicating its fresh nature and susceptibility to post-harvest deterioration. Carbohydrates represented the predominant constituent, accounting for 83.68 ± 0.13%, highlighting their major contribution to the fruit's nutritional and energetic value. In contrast, the levels of macronutrients such as protein (0.38 ± 0.02%) and lipids (0.37 ± 0.01%) were relatively low, reflecting the fruit's low-fat and low-protein profile. The ash content was minimal (0.23 ± 0.03%), suggesting a low total mineral residue. Dietary fiber content was recorded at 2.22 ± 0.09%, indicating a modest contribution to the structural carbohydrate fraction.

Overall, the proximate analysis demonstrates that black mulberry is primarily a carbohydrate-rich fruit with low levels of protein, fat, and ash, along with a moderate fiber content, which supports its nutritional value in functional food applications.

Table 2. Chemical composition of black mulberry (*Morus nigra* L.) (wet weight basis, wwb).

Component	Component
Moisture	**13.12 ± 0.06**
Protein	**0.38 ± 0.02**
Total carbohydrates	**83.68 ± 0.13**
Lipids	**0.37 ± 0.01**
Ash	**0.23 ± 0.03**
Fiber	**2.22 ± 0.09**
Moisture	**13.12 ± 0.06**

Each value in the table represents the mean ± standard deviation of three independent replicates.

3.2 Phenolic Profile of Black Mulberry (*Morus nigra* L.)

The phenolic composition of black mulberry (*Morus nigra* L.) was characterized using HPLC analysis, and the identified compounds along with their retention times (RT) and concentrations (µg/g) are presented in figre 3. The results revealed a diverse profile of phenolic acids and flavonoids, with notable variation in their abundance. Among the detected compounds, gallic acid was the predominant phenolic constituent, exhibiting the highest concentration (410.42 µg/g) at a retention time of 4.5 min. This was followed by protocatechuic acid (152.15 µg/g) and ferulic acid (79.79 µg/g), indicating their significant contribution to the overall phenolic content. Moderate levels were observed for p-hydroxybenzoic acid (56.05 µg/g), catechin (54.49 µg/g), rutin (32.40 µg/g), and sinapic acid (28.54 µg/g).

In contrast, several compounds were detected at relatively low concentrations, including caffeic acid (13.73 µg/g), vanillic acid (9.17 µg/g), cinnamic acid (7.82 µg/g), chlorogenic acid (6.58 µg/g), and p-coumaric acid (4.47 µg/g). Quercetin was present in trace amounts (1.65 µg/g), suggesting a minor contribution to the flavonoid fraction. Notably, a number of phenolic compounds, including gentisic acid, syringic acid, rosmarinic acid, apigenin-7-glucoside, apigenin, kaempferol, and chrysin, were not detected under the applied analytical conditions. Overall, the results demonstrate that black mulberry is particularly rich in phenolic acids, especially gallic and protocatechuic acids, which may contribute to its well-documented antioxidant and biological properties.

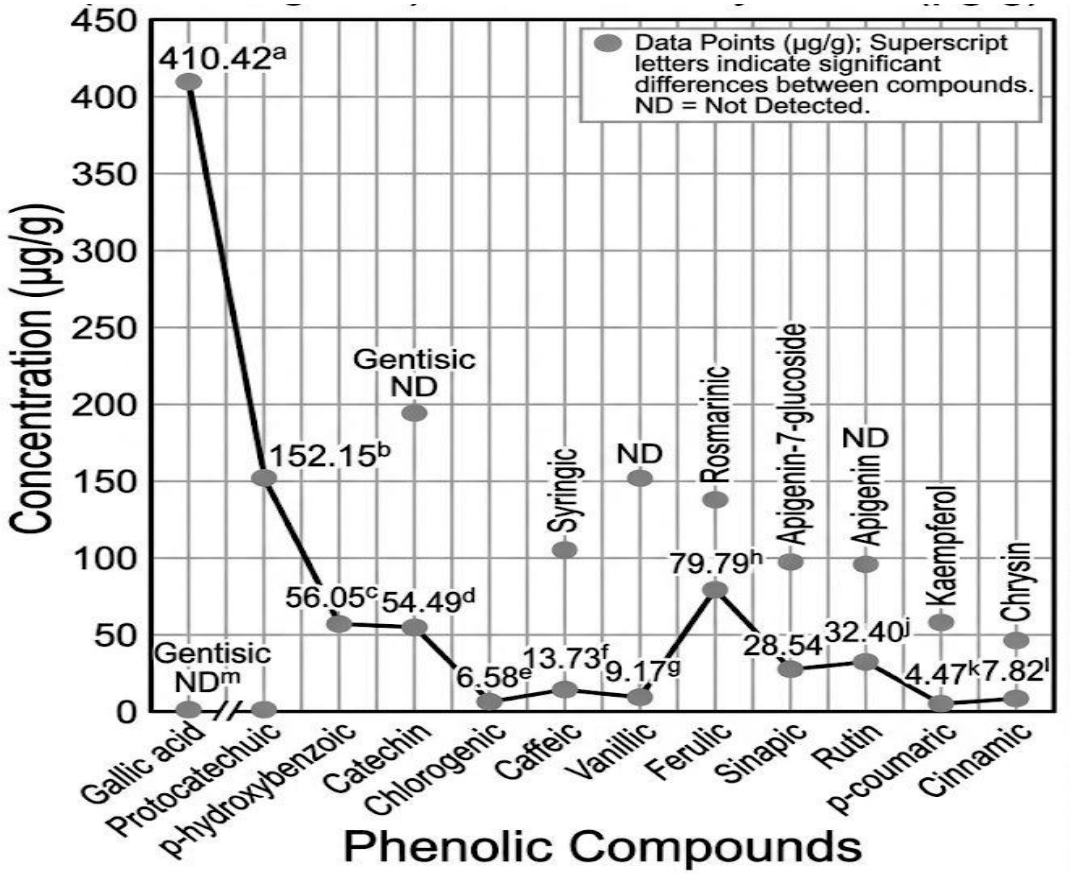


Figure 3: Phenolic profile of black mulberry (*Morus nigra* L.) determined by HPLC (µg/g)

3.3. Biological evaluation

The effects of the experimental diets on feed intake, feed efficiency ratio (FER), and body weight gain (BWG%) are illustrated in Figure 4. Feed intake did not show marked variation among most experimental groups, although the positive control group (G2) exhibited a slightly higher intake compared to the negative control (G1). Rats fed diets supplemented with black mulberry at 5% (G3) and 9% (G4) demonstrated comparable feed intake values to the control groups, indicating that mulberry incorporation did not adversely affect diet palatability.

In contrast, substantial differences were observed in feed efficiency ratio and body weight gain. The positive control group (G2) recorded the highest FER (0.185 ± 0.012) and BWG% (115.42 ± 9.32), reflecting the expected metabolic impact of the obesogenic condition. Supplementation with black mulberry at 5% (G3) resulted in a moderate reduction in both FER (0.162 ± 0.025) and BWG% (84.12 ± 7.65) compared to the positive control, suggesting a partial ameliorative effect.

Notably, the 9% black mulberry group (G4) exhibited the lowest FER (0.085 ± 0.009) and one of the lowest BWG% values (40.22 ± 6.45), closely resembling the negative control group (G1). This finding indicates a dose-dependent effect of black mulberry in reducing weight gain and improving feed efficiency.

Overall, the results suggest that dietary inclusion of black mulberry, particularly at the higher level (9%), may

effectively modulate body weight gain and feed utilization, potentially contributing to the management of obesity-related metabolic alterations, as clearly demonstrated in Figure 3.

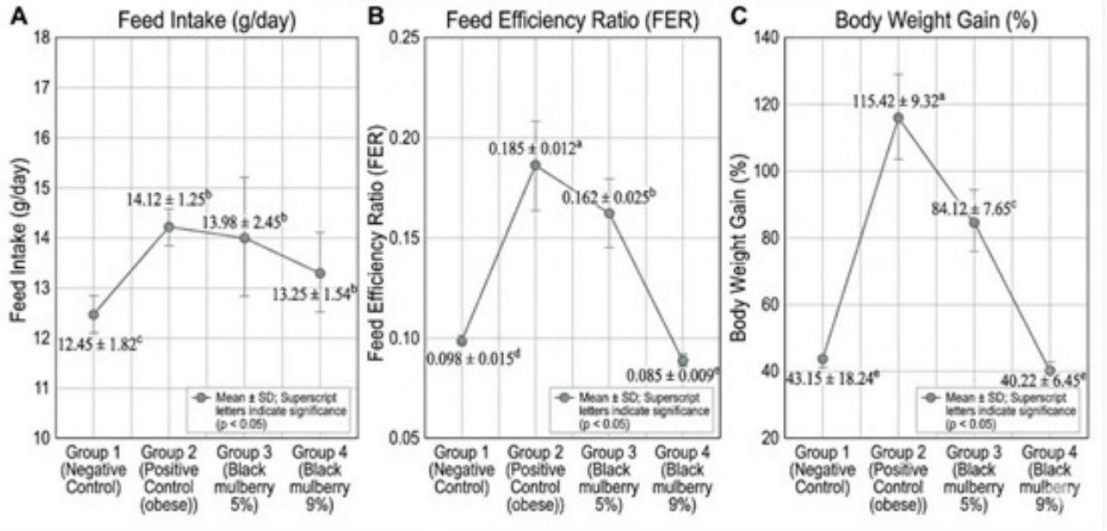


Figure 4: Impact of varying levels of black mulberry (*Morus nigra* L.) seed supplementation on nutritional parameters (feed intake, feed efficiency ratio, and body weight gain %) of obese rats.

3.4. Biochemical Analysis

- **Results (Serum Lipid Profile)**

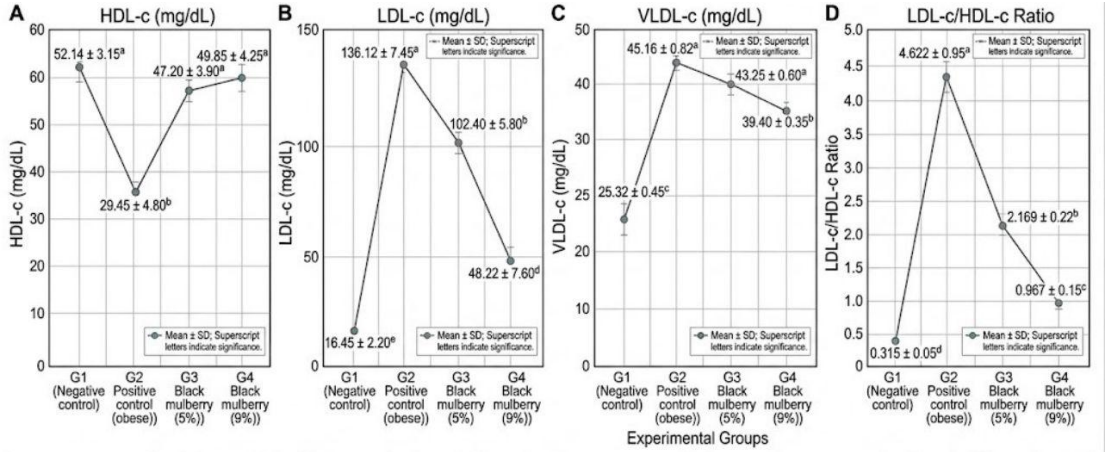
The impact of the experimental dietary treatments on serum lipid biomarkers is summarized in Figure 5, revealing clear differences among groups in response to black mulberry supplementation. The negative control group (G1) maintained a physiologically balanced lipid profile, as evidenced by elevated HDL-c levels (52.14 ± 3.15 mg/dL) and markedly low LDL-c (16.45 ± 2.20 mg/dL) and VLDL-c (25.32 ± 0.45 mg/dL) concentrations. Consequently, the LDL-c/HDL-c ratio remained minimal (0.315 ± 0.05), reflecting a low atherogenic risk under normal metabolic conditions.

In contrast, the positive control group (G2) exhibited a pronounced dyslipidemic profile, characterized by a significant depletion in HDL-c (29.45 ± 4.80 mg/dL) alongside substantial elevations in LDL-c (136.12 ± 7.45 mg/dL) and VLDL-c (45.16 ± 0.82 mg/dL). This imbalance resulted in a sharply increased LDL-c/HDL-c ratio (4.622 ± 0.95), indicating severe disruption of lipid homeostasis and a markedly elevated cardiovascular risk. These findings confirm the effectiveness of the experimental model in inducing obesity-associated lipid abnormalities.

Dietary intervention with black mulberry produced a dose-dependent improvement in lipid parameters. In the group receiving 5% supplementation (G3), HDL-c levels were considerably restored (47.20 ± 3.90 mg/dL), while LDL-c and VLDL-c concentrations were reduced relative to the positive control, although they remained higher than those observed in the negative control group. The LDL-c/HDL-c ratio (2.169 ± 0.22) was notably decreased, indicating partial correction of lipid imbalance. More pronounced effects were observed in the 9% supplementation group (G4), where lipid parameters approached near-normal levels. HDL-c concentrations increased to 49.85 ± 4.25 mg/dL, closely resembling those of the negative control, while LDL-c levels were substantially reduced to 48.22 ± 7.60 mg/dL. Additionally, VLDL-c levels declined to 39.40 ± 0.35 mg/dL, accompanied by a marked reduction in the LDL-c/HDL-c ratio (0.967 ± 0.15). This substantial improvement suggests enhanced lipid metabolism and reduced atherogenic potential.

The observed hypolipidemic effect of black mulberry may be attributed to its rich phenolic composition, particularly compounds such as gallic acid, protocatechuic acid, and flavonoids, which are known to modulate lipid metabolism. These bioactive constituents may contribute to reducing cholesterol synthesis, enhancing lipid clearance, and improving antioxidant defense mechanisms, thereby mitigating oxidative stress associated with dyslipidemia.

Overall, the data demonstrate that black mulberry supplementation exerts a significant protective effect against diet-induced lipid disturbances, with the higher inclusion level (9%) showing superior efficacy. This supports its potential application as a functional dietary component for improving lipid homeostasis and reducing cardiovascular risk



*Data are expressed as Mean ± SD (n=6). Superscript letters indicate significance, means with the same letters are not significantly different (p > 0.05).

Figure 5. Graphical representation illustrating the therapeutic effects of dietary supplementation with black mulberry (BM) at concentrations of 5% and 9% on serum lipid profile parameters. (A) High-density lipoprotein cholesterol (HDL-c), (B) Low-density lipoprotein cholesterol (LDL-c), (C) Very-low-density lipoprotein cholesterol (VLDL-c), and (D) LDL-c/HDL-c ratio as an indicator of cardiovascular risk. Data are expressed as mean ± standard deviation (SD) (n = 6 per group). Different superscript letters (a–e) above the bars indicate statistically significant differences among groups at p < 0.05, according to Duncan's Multiple Range Test (DMRT).

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- **Results (Liver Function Biomarkers: (AST and ALT)**

The effects of black mulberry supplementation on serum liver enzymes are presented in figure 6 . The negative control group exhibited normal hepatic enzyme activities, with AST and ALT levels of 29.15 ± 1.25 U/L and 27.85 ± 1.10 U/L, respectively, indicating intact liver function under physiological conditions. In contrast, the positive control group (obese rats) showed a significant elevation in both AST (58.40 ± 4.95 U/L) and ALT (55.22 ± 1.35 U/L), reflecting pronounced hepatic stress and metabolic impairment associated with obesity. Dietary supplementation with black mulberry resulted in a clear improvement in liver function markers. The 5% black mulberry group showed moderate reductions in AST (37.92 ± 1.50 U/L) and ALT (40.15 ± 0.40 U/L) compared with the positive control group. A more pronounced improvement was observed in the 9% group, where AST and ALT levels decreased further to 33.45 ± 0.65 U/L and 30.90 ± 1.80 U/L, respectively, approaching values observed in the negative control group.

Overall, these findings suggest that black mulberry supplementation exerts a hepatoprotective effect in a dose-dependent manner, likely due to its rich phenolic content and antioxidant properties, which help mitigate obesity-induced liver damage.

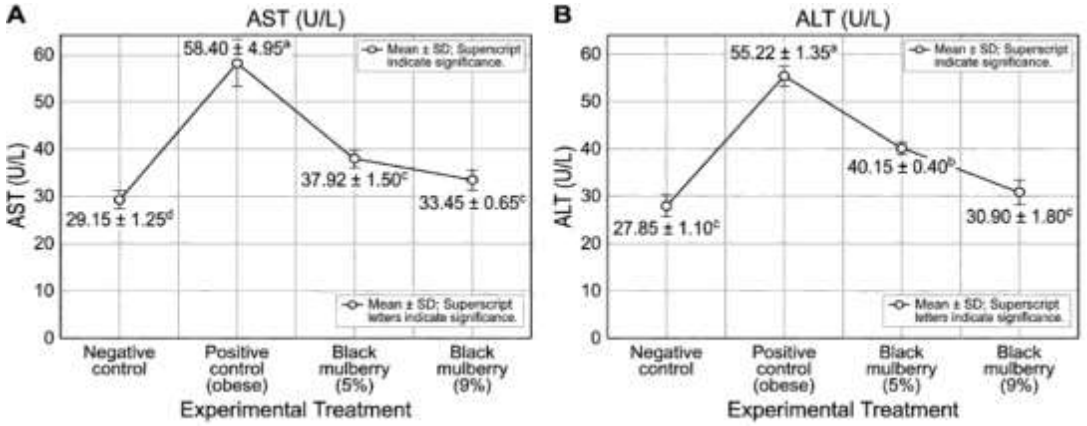

Figure 6. Comparison of serum liver biomarkers among experimental groups. (A) AST and (B) ALT activities. Data are expressed as mean ± SD (n = 6). The positive control group showed elevated AST and ALT levels, indicating hepatic stress, whereas black mulberry supplementation (5% and 9%) significantly reduced both enzymes, suggesting a hepatoprotective effect. Different superscript letters (a–d) indicate significant differences at p < 0.05 according to Duncan's Multiple Range Test (DMRT).

- **Results (Renal Function Biomarkers)**

The effects of dietary supplementation with black mulberry (*Morus nigra* L.) on renal function biomarkers are presented in figure 7. The negative control group (G1) exhibited normal renal function, as indicated by low uric acid (0.92 ± 0.10 mg/dL) and urea nitrogen (15.10 ± 0.85 mg/dL) levels. In contrast, the positive control group (G2) showed a significant elevation in both uric acid (3.96 ± 0.15 mg/dL) and urea nitrogen (27.20 ± 2.10 mg/dL), reflecting impaired renal function associated with obesity-induced metabolic stress. Dietary supplementation with

black mulberry resulted in a clear improvement in renal biomarkers. The 5% black mulberry group (G3) demonstrated a reduction in uric acid (2.85 ± 0.08 mg/dL) and urea nitrogen (25.10 ± 1.30 mg/dL) compared with the positive control group, indicating a partial ameliorative effect. A more pronounced improvement was observed in the 9% group (G4), where uric acid (1.62 ± 0.12 mg/dL) and urea nitrogen (21.40 ± 0.55 mg/dL) levels were further reduced, approaching values observed in the negative control group. Overall, these findings suggest that black mulberry supplementation exerts a dose-dependent protective effect on renal function in obese rats, likely through its bioactive and antioxidant constituents that help mitigate obesity-induced renal dysfunction

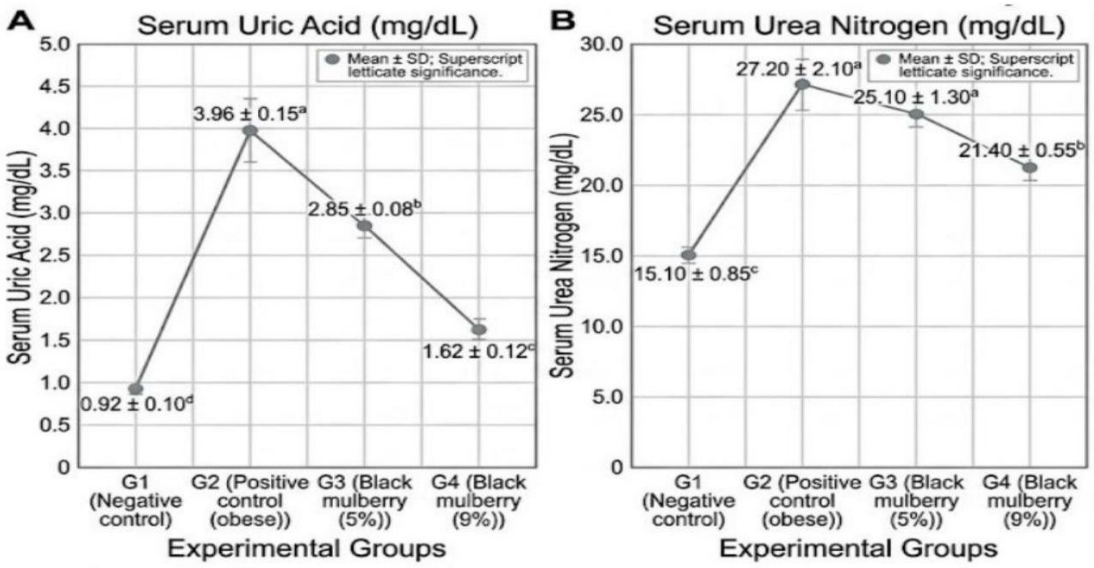

Figure 7. Renal function parameters across experimental groups. (A) Uric acid and (B) urea nitrogen levels. Data are expressed as mean ± SD (n = 6). The positive control group showed elevated values indicating renal impairment, while black mulberry, S. officinalis, and their mixture significantly reduced both biomarkers in a dose-dependent manner, suggesting nephroprotective effects. Different letters (a–d) indicate significant differences at p < 0.05 (DMRT).

- **Results (Serum Glucose Levels)**

The effect of dietary supplementation with black mulberry (*Morus nigra* L.) on serum glucose levels in obese rats is presented in figure 8. The negative control group (G1) maintained normal glucose homeostasis, with a serum glucose level of 112.45 ± 1.85 mg/dL.

In contrast, the positive control group (G2) exhibited a marked elevation in serum glucose (188.60 ± 2.75 mg/dL), confirming the development of hyperglycemia associated with obesity.

Supplementation with black mulberry resulted in a significant reduction in serum glucose levels. The group receiving 5% black mulberry (G3) showed a moderate decrease to 145.30 ± 1.40 mg/dL compared to the positive control. A more pronounced improvement was observed in the 9% group (G4), where serum glucose levels declined further to 124.15 ± 1.35 mg/dL, approaching values observed in the negative control group.

Overall, these findings indicate that black mulberry supplementation exerts a beneficial effect on glycemic control in obese rats in a dose-dependent manner, suggesting its potential role in mitigating obesity-related hyperglycemia.

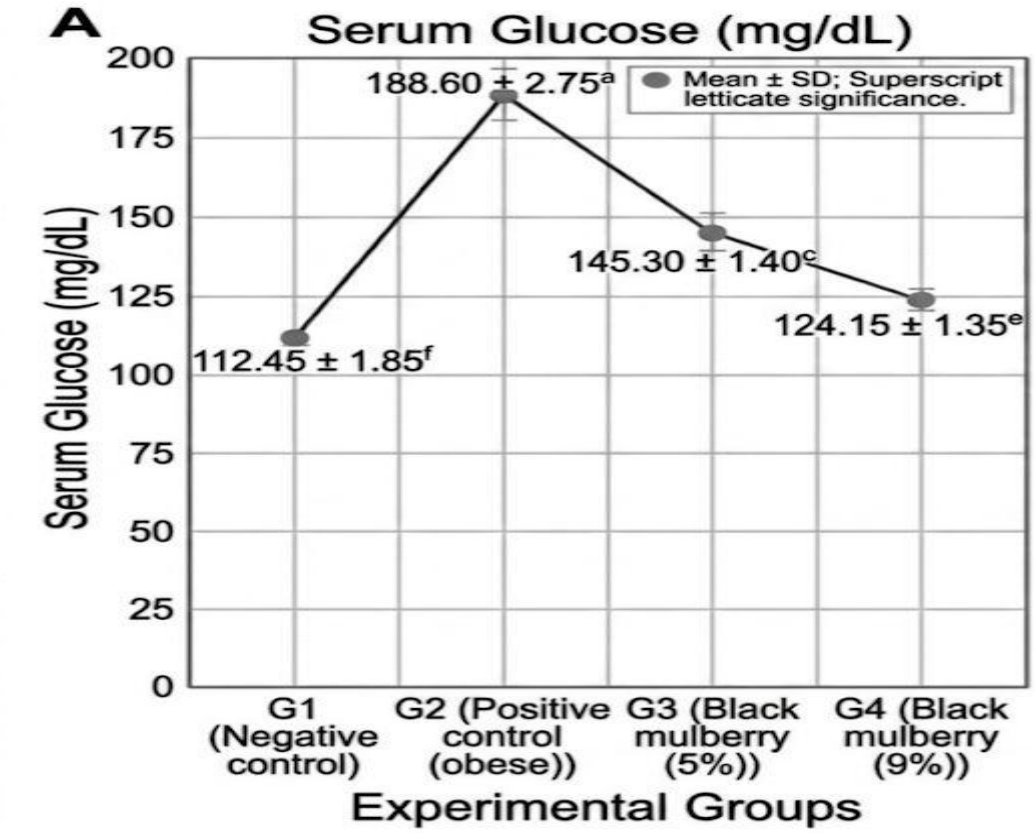

Figure 8: Effect of black mulberry at different concentrations (5% and 9%) on Serum Glucose levels (mg/dL) in obese experimental groups.

- **Inflammatory Markers Analysis**

The data presented in the figure 9 illustrate the levels of inflammatory biomarkers (TNF-α, IL-6, and hs-CRP) across all experimental groups. The negative control group (G1) showed the lowest concentrations of TNF-α, IL-6, and hs-CRP, with values of 11.90±1.05 pg/mL, 43.10±3.00 pg/mL, and 0.80±0.09 mg/L, respectively. In contrast, the positive control group (G2), representing the obese condition, exhibited a marked elevation in all measured inflammatory markers, recording 50.10±4.60 pg/mL for TNF-α, 118.20±9.10 pg/mL for IL-6, and 3.60±0.30 mg/L for hs-CRP. Regarding the treated groups, supplementation with black mulberry resulted in a notable reduction in inflammatory parameters. The G3 group (5% black mulberry) showed intermediate decreases, with TNF-α, IL-6, and hs-CRP levels of 30.25±2.70 pg/mL, 78.90±5.90 pg/mL, and 2.05±0.16 mg/L, respectively. A more pronounced improvement was observed in the G4 group (9% black mulberry), which demonstrated further reductions in TNF-α (19.80±1.70 pg/mL), IL-6 (60.10±4.40 pg/mL), and hs-CRP (1.30±0.11 mg/L), approaching values closer to those of the negative control group. Overall, these findings suggest a dose-dependent anti-inflammatory effect of black mulberry supplementation in the experimental model.

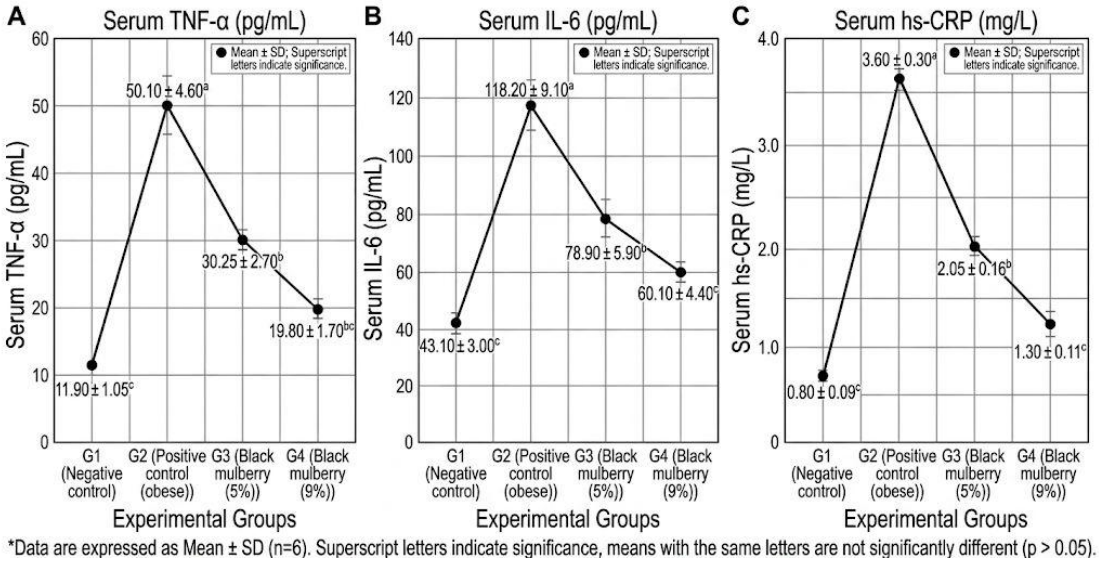

Figure 9: Effect of Black Mulberry on Serum Pro-inflammatory Markers (TNF-α, IL-6, and hs-CRP) in Different Experimental Groups.

3.5. Histopathological Examination of Liver Tissue

The histopathological features of liver sections from different experimental groups are illustrated in Figure 10, where each numbered panel corresponds to a specific group.

Group 1 (Negative Control): Liver tissue exhibits normal histological architecture, with well-arranged hepatic cords radiating from the central vein. Hepatocytes appear intact, with homogeneous cytoplasm and centrally located nuclei. No evidence of fatty changes, necrosis, or inflammatory infiltration is observed.

Group 2 (Positive Control – Obese): Severe histopathological alterations are observed, including extensive hepatic steatosis with large lipid vacuoles occupying hepatocytes, along with cellular ballooning and distortion of hepatic architecture. These changes indicate significant liver damage associated with obesity-induced metabolic stress.

Group 3 (Black Mulberry 5%): Liver sections from rats treated with 5% black mulberry (*Morus nigra* L.) show moderate improvement compared to the positive control group. A reduction in lipid accumulation is evident, although some hepatocytes still exhibit mild vacuolar degeneration. The hepatic architecture appears partially restored, indicating a protective effect at this concentration.

Group 4 (Black Mulberry 9%): A more pronounced improvement is observed in the group treated with 9% black mulberry. Liver tissue shows minimal fat deposition, with hepatocytes regaining near-normal morphology and arrangement. The hepatic cords appear well-organized, and the overall architecture is largely preserved, reflecting a strong hepatoprotective effect at the higher dose. Overall Interpretation: The histopathological findings demonstrate that dietary supplementation with black mulberry exerts a dose-dependent protective effect against obesity-induced liver damage, with the 9% level showing superior efficacy in restoring normal hepatic structure.

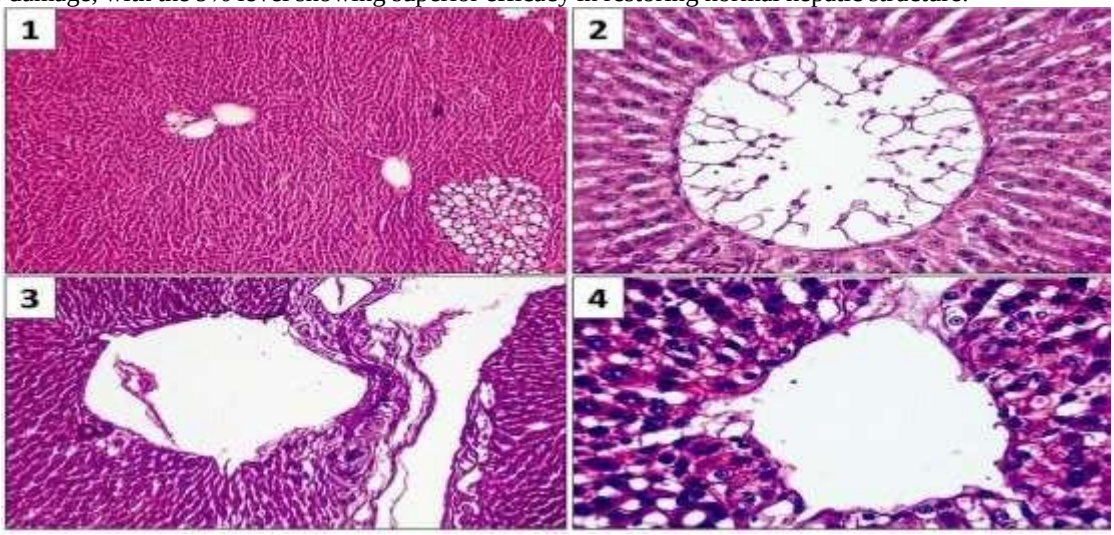

Figure 10. Histopathological evaluation of liver tissue in experimental groups showing the protective effect of black mulberry (*Morus nigra* L.) supplementation at 5% and 9% against obesity-induced hepatic alterations.

4. DISCUSSION

The present study provides comprehensive evidence supporting the potential of black mulberry (*Morus nigra* L.) as a functional food with significant metabolic, biochemical, and histological benefits in obesity-induced experimental models. The findings demonstrate that dietary supplementation with black mulberry, particularly at higher inclusion levels (9%), exerts pronounced protective effects against obesity-associated metabolic disturbances. These results are strongly consistent with recent scientific literature highlighting the multi-target therapeutic properties of Morus nigra and its bioactive compounds.

The proximate analysis revealed that black mulberry is predominantly composed of carbohydrates with relatively low levels of protein and lipids. This nutritional profile is consistent with previous reports describing mulberry fruits as energy-rich but low-fat foods suitable for metabolic health interventions. Recent nutritional studies also emphasize that, despite their carbohydrate content, mulberries possess a low glycemic impact due to their fiber and polyphenol content, which modulate glucose absorption and improve postprandial glycemic control. The moderate fiber content may further enhance satiety, delay gastric emptying, and contribute to improved metabolic regulation in obesity models (Saeed et al.,2023).

The HPLC analysis highlighted a rich phenolic profile dominated by gallic acid, protocatechuic acid, and ferulic acid. These compounds are well-documented for their potent antioxidant and anti-inflammatory activities. Recent phytochemical studies confirm that Morus nigra contains high concentrations of phenolic acids and flavonoids capable of scavenging reactive oxygen species and enhancing endogenous antioxidant defense systems. Importantly, recent mechanistic evidence shows that these compounds activate the Nrf2 signaling pathway, a key regulator of cellular antioxidant responses, while simultaneously suppressing NF-κB-mediated inflammatory signaling. This dual regulatory effect provides a strong mechanistic explanation for the physiological improvements observed in the present study (Zhang et al.,2022). In terms of biological performance, black mulberry supplementation did not significantly affect feed intake, indicating good palatability and excluding appetite suppression as a cause of weight reduction. However, it markedly reduced body weight gain and improved

feed efficiency in a dose-dependent manner. These findings are consistent with recent animal studies demonstrating that mulberry polyphenols regulate energy metabolism by enhancing AMP-activated protein kinase (AMPK) activity, which promotes fatty acid oxidation and inhibits lipogenesis. Activation of AMPK has been widely recognized as a central mechanism in the prevention of diet-induced obesity, supporting the metabolic effects observed in this study (**Figure 11**).

The lipid profile results further support the hypolipidemic potential of black mulberry. The significant increase in HDL-c and reduction in LDL-c, VLDL-c, and LDL/HDL ratio indicate improved lipid metabolism and reduced atherogenic risk (Li et al.,2023). Recent experimental studies confirm that Morus nigra extracts improve dyslipidemia by suppressing hepatic cholesterol synthesis, enhancing bile acid excretion, and improving adipokine balance (Chen et al., 2022). Additionally, polyphenols have been shown to modulate lipid metabolism through regulation of PPARs (peroxisome proliferator-activated receptors), which play a central role in fatty acid oxidation and cholesterol homeostasis (**Figure 11**).

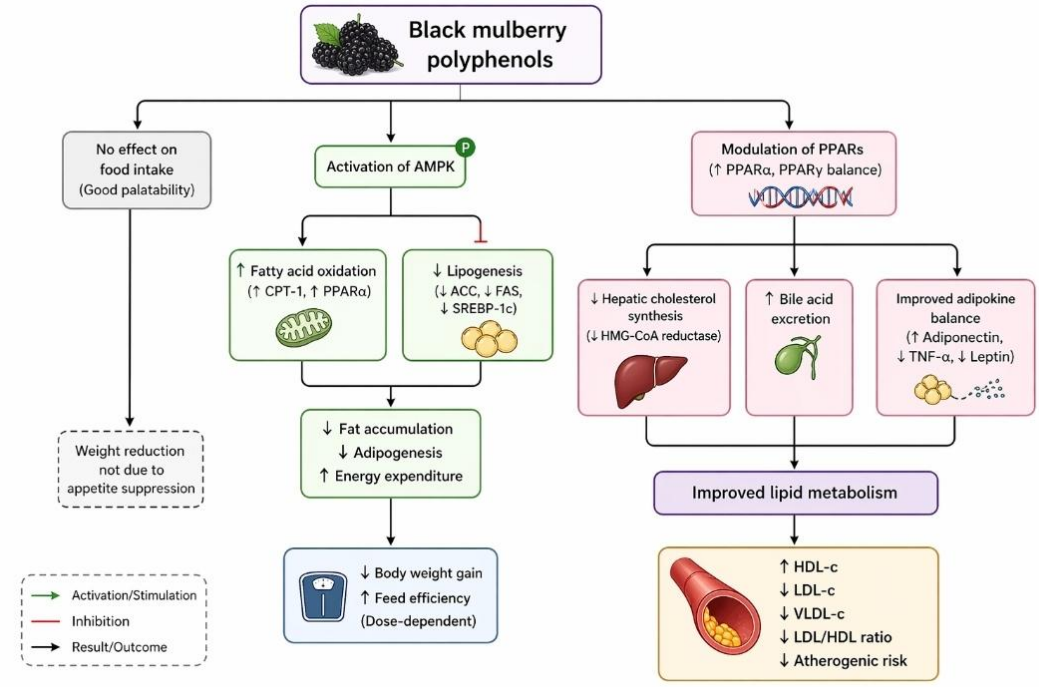

Figure 11: Proposed mechanism of Black mulberry (*Morus nigra* L.) polyphenols in modulating serum glucose levels and lipid metabolism.

Liver function biomarkers (AST and ALT) were significantly elevated in obese rats, reflecting hepatic injury and metabolic stress. However, black mulberry supplementation significantly reduced these enzyme levels, indicating restoration of hepatocellular integrity. These findings are strongly supported by recent studies showing that mulberry polysaccharides and phenolic compounds activate antioxidant defense pathways and reduce hepatic lipid accumulation. Histopathological findings in the present study, showing reduced steatosis and improved hepatic architecture, are consistent with experimental evidence demonstrating that Morus nigra alleviates non-alcoholic fatty liver disease (NAFLD) through oxidative stress reduction and mitochondrial protection (Aljahdali et a;., 2025). Renal function markers also showed significant improvement following treatment. Elevated urea and uric acid levels in obese animals reflect impaired renal clearance and increased metabolic stress. The reduction of these biomarkers in treated groups aligns with recent studies reporting that mulberry polyphenols protect renal tissues by reducing oxidative damage, suppressing inflammatory cytokines, and improving glomerular filtration efficiency (Abd Elmeged, & Albaggar 2026). These nephroprotective effects are increasingly attributed to the systemic antioxidant activity of phenolic compounds and their ability to regulate nitric oxide balance and vascular function.

Furthermore, the observed reduction in serum glucose levels highlights the antidiabetic potential of black mulberry. Recent pharmacological studies demonstrate that mulberry flavonoids inhibit carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, thereby delaying glucose absorption in the intestine. In addition, these compounds enhance insulin sensitivity and promote GLUT4 translocation in peripheral tissues, leading to improved glucose uptake (Mashnafi et al., 2025). These mechanisms collectively support the hypoglycemic effects observed in this study and align with current evidence on plant-derived polyphenols in glycemic regulation (Abbas et al .,2025).

Overall, the integration of biochemical, physiological, and histological findings demonstrates that black mulberry acts through multiple complementary mechanisms. These include antioxidant activity via Nrf2 activation, anti-inflammatory effects through NF-κB suppression, and metabolic regulation via AMPK signaling. Recent studies strongly support this multi-target action model, highlighting that the therapeutic effects of Morus nigra are not the result of a single compound but rather the synergistic interaction of its bioactive constituents.

5. CONCLUSION

In conclusion, accumulating scientific evidence suggests that black mulberry (*Morus nigra* L.) has strong potential as a functional food with notable effects against obesity, as well as protective roles for the liver and kidneys. It also demonstrates lipid-lowering and blood glucose–regulating activities, making it relevant in the context of metabolic and endocrine health. These beneficial effects are largely attributed to its high content of phenolic compounds and its capacity to influence key metabolic, antioxidant, and inflammatory mechanisms. In addition, incorporating black mulberry into the diet may support overall metabolic balance and help reduce the risk of developing chronic metabolic disorders. Nevertheless, further well-designed clinical trials are necessary to confirm these effects in humans and to determine appropriate therapeutic intake levels and long-term safety

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