

# Efficiency of Extracts from Black and White Mulberry Fruits and Leaves Against a High-Cholesterol Diet

Fatma El-Zhraa A. El-Sherif <sup>1</sup> , Eslam A. Header <sup>1</sup> , Aya Sayed Mohammed Mohammed <sup>1,\*</sup> 

<sup>1</sup> Department of Nutrition and Food Sci., Faculty of Home Economics, Menoufia Univ, Shebin EL-Kom, Egypt; drfatmazhraa1949@gmail.com (F.E.-Z.A.E.-S.); islam.hyder@hec.menofia.edu.eg (E.A.H.); elhalawanyaya22@gmail.com (A.S.M.M.);

\* Correspondence: elhalawanyaya22@gmail.com;

Received: 3.10.2024; Accepted: 11.04.2025; Published: 25.11.2025

**Abstract:** The present study aimed to investigate the cholesterol-lowering effect of black and white mulberry (fruits and leaves) in rats fed a high-cholesterol diet. Sixty white male albino rats, weighing (170-180 g) were divided into two main groups; one group served as the negative control, while the second main group (54 rats) was fed for two weeks on a basal diet plus cholesterol of 1.5% to induce hypercholesterolemia. After 2 weeks of feeding, the second main group was divided into 9 groups of rats, and one of these groups was fed only a basal diet. The remainder group was treated with black and white mulberry (fruits and leaves) extract (150 mg/kg and 300 mg/kg). After taking black and white extract supplements for four weeks, there were minor changes in body weight gain (BWG) and food efficiency ratio (FER), but there was a significant decrease in serum low-density lipoprotein cholesterol (LDL-C) and triglycerides, a decrease in serum cholesterol (TC), and an improvement in kidney and liver function ( $p < 0.05$ ). Mulberry fruit and leaf extract improves the histopathological changes in the liver. Black mulberry fruit extract BMFE and WMFE at a dosage of 300 mg/kg enhanced hepatic function and improved the structural integrity of liver tissues with an elevation in antioxidant concentrations.

**Keywords:** mulberry; bioactive compound; fruits and leaves; cholesterol; extract; liver function.

© 2025 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The authors retain copyright of their work, and no permission is required from the authors or the publisher to reuse or distribute this article, as long as proper attribution is given to the original source.

## 1. Introduction

Hypercholesterolemia is a genetically inherited disorder characterized predominantly by autosomal dominant inheritance. This condition manifests through exceedingly high cholesterol levels within the bloodstream [1, 2]. Globally, cardiovascular disease (CVD) continues to be the primary cause of death [3]. The World Health Organization (WHO) predicts that by 2030, there will be 22.3 million yearly deaths from CVD, an increase of almost 27% from 2012 [4]. One of the main risk factors for CVD is dyslipidemia, characterized by reduced HDL-C levels, elevated TG levels, and low-density lipoprotein cholesterol (LDL-C) [5]. HDL-C levels and CVD risk are negatively correlated. HDL-C is a powerful CVD predictor [6,7]. Reverse cholesterol transport from aortic foam cells to the liver and anti-inflammatory, anti-oxidative, and anti-apoptotic activities are among HDL-C's cardioprotective qualities [8,9]. Therefore, enhancing HDL synthesis and function is essential for the prevention and/or treatment of CVD [10].

Egypt's pivotal role in the Middle East is partly attributed to its longstanding mulberry cultivation tradition. Among the various governorates, Sohag is distinguished by its remarkable proficiency, extending beyond cultivation to expertise in utilizing mulberry leaves for sericulture, which subsequently leads to silk production [11]. The mulberry plant, assigned to the genus *Morus* and situated taxonomically within the Moraceae family, is hypothesized to have emanated from the liminal zone adjacent to the Indo-Chinese region [12]. Mulberries have been shown in numerous studies to possess a variety of biological activities, such as antioxidant [13], anti-diabetic [14], and anti-inflammatory [15]. Furthermore, a variety of biologically active substances, such as flavonols and anthocyanins, have been documented [16,17]. Therefore, the purpose of the present study was to investigate the biological effects of black and white mulberry leaf extract (BMLE and WMLE) and black and white mulberry fruit extract (BMFE and WMFE) on hypercholesterolemic white albino rats.

## 2. Materials and Methods

### 2.1. Materials.

#### 2.1.1. Plants.

Samples of Black mulberry (*Morus nigra* L.) and White mulberry (*Morus alba* L.) were obtained from El-Arish City, North Sinai, Governorate. These samples were harvested in mid-April to mid-May 2023 as leaves and fruit and dried for the preparation of ethanol extracts.

#### 2.1.2. Rats.

Sixty white male albino rats (Sprague Dawley strain) were purchased from the Medical Insect Research Institute in Doki, Cairo, Egypt.

#### 2.1.3. Cholesterol.

The source of the pure white crystalline powder cholesterol is Elgomhoriya Company for Medical Preparations, Chemicals, and Medical Equipment, located in Cairo, Egypt.

#### 2.1.4. Diet.

The rats were fed a ration consisting of wheat bran, fish meal, molasses, 3.3% fiber, calcium carbonate, calcium phosphate, sodium chloride, methionine, ash (net protein 22% and lipids 4.7%), and soybean powder (44%). It was produced by Cairo Agricultural Development Company, 6 October City, Giza, Egypt.

### 2.2. Methods.

#### 2.2.1. Preparation of ethanol extracts for black and white mulberry (leaves - fruit) extract.

Leaves were collected, cleaned, washed with deionized water, and left to dry for 4 days. After complete drying, the leaves were ground and sieved through sieve number 40 to obtain a coarse powder. About (1 kg) of leaf powder was macerated in absolute ethanol (99% - 50 ml) for 48 hrs. The supernatants were collected and rotary evaporated at 40°C; the dried extract was resuspended in deionized water, filtered through a 25 µm filter, and lyophilized to obtain the dried active ingredient, which was then redissolved in deionized water (400 mg/25 ml) and

stored at 4°C for further characterization. The resultant solution of the blackberry leaves and white berry leaves extracts will be labeled as BMLE and WMLE, respectively [18]. 1 kg of fresh fruit was processed at room temperature for 10 minutes using four filters with 80% ethanol. The IKA T-45 ultra-Turrax homogenizer operating at 4000rpm was used. The resulting combined solution will then undergo rotary evaporation at 40°C, ultimately producing approximately 160 g of black mulberry extract and 160 g of white mulberry extract [19].

### 2.2.2. Chemical analysis.

Chemical analysis of samples, including moisture, crude protein, crude fat, and ash, was performed on a dry-weight basis [20]. Moisture and ash contents were determined according to methods described by the AOAC [21]. The total ash content of the resultant powder will be estimated by direct incorporation into a Muffle Furnace Box (Model: COMEC:2200851, SNOL) at 55°C, following the protocol [21, 22]. To calculate the crude protein content, total nitrogen was determined using the micro-Kjeldahl method [21, 23]. Fat content was estimated according to the AOAC method [21] using the Soxhlet apparatus and hexane as the solvent. (model; J.P SELECATA:63008, Spain) [21, 24]. Total carbohydrates were determined by Equation (1) as follows [20].

$$\text{Total carbohydrates} = 100 - (\% \text{protein} + \% \text{fat} + \% \text{moisture} + \% \text{ash}) \quad (1)$$

The caloric value of the formulas was calculated by using the factors as described by FAO/WHO/UNU [25] according to the following Equation (2) as follow:

$$\text{The caloric value} = 4 \times (\text{Protein \%} + \text{Carb. \%}) + 9 \times (\text{Fat \%}) \quad (2)$$

The extracted crude fiber was used to determine fiber content by digesting fat-free samples (1.25%) with sulfuric acid and filtering through ceramic fiber filters, following the AOAC protocol [21, 26]. The nitrogen-free extract (NFE) was quantified by subtracting the % ages of total ash, crude protein, crude fat, crude fiber, and moisture from 100 [27].

### 2.2.3. The experimental design.

#### 2.2.1.1. Experimental animals.

The male albino rats (Sprague-Dawley strain) weighing between 170 and 180 grams were kept in a laboratory for a week before the commencement of the study to acclimate them to their new environment. The biological investigation was carried out in the animal house facility of the faculty of Home Economics, Menoufia University, where animals were housed in clean wire cages with no more than 4 animals per cage and maintained under standard laboratory conditions (temperature  $25 \pm 2$  °C with a 12/12 h dark/light cycle). They were fed a standard ration, according to [28], and water was provided *ad libitum* for the experimental period. The rats' feed and water intake were monitored daily in line with the American Institute of Nutrition AIN-93 criteria [29]. Daily body weight measurements were taken, and care was taken to comply with Egyptian regulations on animal protection. All procedures described were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Menoufia University, Egypt, Approval No. MUFHE/S/NFS/16/23.

#### 2.2.1.2. High-cholesterol diet preparation.

With minor modifications, a high-fat diet was created using the previously published procedure [30]. The high-fat diet was prepared from a combination of a 78.4% standard ration

(normal diet), 1.5 % cholesterol powder, 15% corn starch, 0.1% cholic acid, and 5% corn oil [31].

#### 2.2.1.3. Groups of rats.

The study was divided into two main groups. The first group (6 rats) was designated the negative control group and fed a standard ration throughout the experimental period. The second main group (54 rats) was fed a high-fat diet for 2 weeks to induce hypercholesterolemia before the experiment began. After 2 weeks of feeding, the second main group of rats was divided into 9 groups (6 rats each), and one of these hypercholesterolemic rat groups was fed a standard diet, representing the control positive. The remaining eight hypercholesterolemic groups received a single daily dose of BMLE and WMLE (150 mg/kg body weight, b.wt. and 300 mg/kg b.wt.) and BMFE and WMFE (150 mg/kg b.wt. and 300 mg/kg b.wt.) for six weeks, each group consisted of six rats as follows:

Group (1): Negative control group (-) normal rats were fed on a ration only.

Group (2): Positive control group (+) hypercholesterolemic rats were fed only on a ratio.

Group (3): The hypercholesterolemic rats were fed on a ration + oral injection of BMLE once daily at a dose of 150 mg/kg b.wt.

Group (4): The hypercholesterolemic rats fed on a ration + oral injection of BMLE once daily at a dose of 300 mg/kg b.wt.

Group (5): The hypercholesterolemic rats were fed on a ration + oral injection of WMLE once daily at a dose of 150 mg/kg b.wt.

Group (6): The hypercholesterolemic rats were fed on a ration + oral injection of WMLE once daily at a dose of 300 mg/kg b.wt.

Group (7): The hypercholesterolemic rats were fed on a ration + oral injection of BMFE once daily at a dose of 150 mg/kg b.wt.

Group (8): The hypercholesterolemic rats were fed on a ration + oral injection of BMFE once daily at a dose of 300 mg/kg b.wt.

Group (9): The hypercholesterolemic rats were fed on a ration + oral injection of WMFE once daily at a dose of 150 mg/kg b.wt.

Group (10): The hypercholesterolemic rats were fed on a ration + oral injection of WMFE once daily at a dose of 300 mg/kg b.wt.

#### 2.2.4. Blood collection.

6 weeks after the last meal, blood samples were collected by sacrificing each hypercholesterolemic rat and a control rat. The rats were anesthetized in a chamber containing diethyl ether. Blood samples were collected from the hepatic portal vein. Two blood samples were collected from each animal into a heparin-containing tube and a plain tube, respectively. The blood samples were collected into sterile centrifuge tubes and allowed to coagulate in water at 37 °C for 30 minutes. In the next step, the tubes were centrifuged at a speed of 4000 revolutions per minute for 10 minutes to separate the serum. The serum was then carefully removed and put into clean cuvette tubes. Finally, the serum samples were collected until the subjects were at ease with the analysis [32].

### 2.2.5. Biological evaluation.

Throughout the experimental period, daily amounts of feed ingested and/or wasted were recorded, and the total feed intake (FI) was computed. Furthermore, the weight of the rat's body was recorded. Biological evaluation of the different diets was carried out by determination of body weight gain (BWG%) and Feed efficiency ratio (FER) according to [33]. Using Equation (3) and Equation (4), the relative body weight gain (rBWG) and survival rate were calculated using Equation (5) as follows according to [34]:

$$\text{BWG rate (\%)} = \frac{(\text{final body weight} - \text{initial body weight})}{\text{initial body weight}} \times 100 \quad (3)$$

$$\text{FER} = \frac{\text{Weight Gain (g)}}{\text{Feed intake (g)}} \quad (4)$$

$$\text{rBWG (\%)} = \frac{(\text{BWG rate of the infected/unmedicated control or drug-treated group} - \text{BWG rate of the healthy control group})}{\text{BWG rate of the healthy control group}} \times 100 \quad (5)$$

### 2.2.6. Biochemical analysis.

#### 2.2.6.1. Lipid profile.

Triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-c) were measured using an automated method. The diagnostic kit used in the study's findings was based on the methods described [35-37] for TG, TC, and HDL-c, respectively.

Determination of low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) was calculated using the method [38,39]. In clinical laboratories, LDL-C is most often determined using the "Friedewald formula" [40] using measured values for TC, HDL-C, and TG as shown below in Equations (6), Equations (7), or Equations (8):

$$\text{LDL (mg/dl)} = \text{TC} - (\text{HDL} + \text{VLDL}) \quad (6)$$

$$\text{VLDL - C (mg/dL)} = \frac{\text{TG (mg/dL)}}{5} \quad (7)$$

$$\text{VLDL - C (mmol/L)} = \frac{\text{TG (mmol/L)}}{1.181} \quad (8)$$

#### 2.2.6.2. Atherogenic index (AI).

The principal use of the atherogenic index was calculated as shown in Equation (9), Equation (10), and Equation (11) according to equation[41,42].

$$\text{Atherogenic Index} = \frac{\text{LDLC}}{\text{HDLc}} \quad (9)$$

$$\text{Atherogenic Index} = \frac{\text{TC}}{\text{LDL}} \quad (10)$$

$$\text{Atherogenic Index} = \frac{\text{TC}}{\text{HDLc}} \quad (11)$$

#### 2.2.6.3. Determination of antioxidant enzyme.

The enzymatic perspective proposed has been deployed to assess the activity of antioxidant enzymes[43]. The RBCs were lysed by mixing chilled water with the RBC. Lysate was used to estimate antioxidant enzymes, namely catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) [43] (pp. 2). CAT activity was determined spectrophotometrically by the method [44], and SOD activity was determined spectrophotometrically according to the method [45]. Malondialdehyde (MDA) was determined according to the method [46].

### 2.2.7. Statistical analysis.

The results were presented in the form of mean  $\pm$  standard errors (SE). Statistical analysis of the results was performed using the Statistical Package for the Social Sciences (SPSS) version 24 for Windows (SPSS, 24). A paired-samples t-test was used to compare the parameters between the control-positive group and the hypercholesterolemic rat groups. A P-value less than 0.05 was reflected as statistically significant.

### 2.2.8. Histological examination.

Tissue samples were collected from all experimental groups and preserved in 10% neutral buffered formalin. Following this, the samples were dehydrated using increasing concentrations of ethanol (70%, 80%, 90%), cleared in xylene, and finally embedded in paraffin. Thin sections (4–4 micrometers thick) were then prepared and stained with hematoxylin and eosin, following the method outlined [47].

## 3. Results and Discussion

### 3.1. Chemical composition.

In many nations, mulberry leaves and fruits have been used as ingredients in medications, beverages, and functional foods; however, it remains unclear how well-suited these materials are for these uses. The nutritional and chemical compositions were examined in this study. Analysis has been performed to determine the chemical structure's constituents and their corresponding percentages. The manuscript explains the multifaceted applications of mulberry leaves and fruits across various medicinal and functional foods. Mulberries have numerous health advantages; they have a positive impact on the human body. Recognized for their prolific health advantages and beneficial effects on human well-being, mulberries are the subject of thorough investigation within this study. Through detailed analysis, the research describes the chemical compounds in mulberries and quantifies their concentrations, thus providing valuable insights into their potential uses.

Table 1 shows the chemical composition of black mulberry leaves (BML), black mulberry fruit (BMF), white mulberry leaves (WML), and white mulberry fruit (WMF) (g/100g w/w). The data proved that protein, fat, ash, moisture, carbohydrate (CHO) fiber, energy, PH%, and total nitrogen% of BML were (1.225%, 2.5%, 2.76%, 73.635%, 8.98%, 10.9%, 63.32 kcal, 6.69%, and 0.196%) respectively, which for BMF were (1.25%, 0.9%, 0.74%, 72.71%, 17.9%, 6.5% 84.7kcal, 5.56%, and 0.2%,) respectively; however, it was (1.75%, 2.3%, 2.81%, 74.45%, 8.79%, 9.9% 62.86kcal, 6.69%, and 0.28%,) respectively of WML, in addition for WMF was (1.63%, 1.1%, 0.85%, 73.02%, 15.8%, 7.6% 79.62kcal, 4.82%, and 0.261%,) respectively. Besides, the leaves also contain substantial moisture and numerous other nutrients, including nitrogen, copper, iron, and manganese [48]. The present results agree with Author [27] (p. 3), who reported that the moisture content of fresh mulberry (*Morus alba*) leaves fluctuates between 71.13% and 76.68%. Some contain 4.72% to 9.96% crude protein, 4.26% to 5.32% total ash, 8.15% in natural detergent fibers (NFD), and 0.64% to 11.3% crude fat. Also, observed that mulberries are highly nutritious, including roughly 0.5-1.4% protein and about 7.8-9% carbs [49]. On the other hand, the results of Author [50] revealed that the proximate analysis of mulberry leaves included moisture, protein, fat, carbohydrate, fiber, and ash, with contents of 5.2%, 18.40%, 6.46%, 28.37%, 25.12%, and

8.7%, respectively. Likewise, discovered in another study the contents of ash, moisture, lipid, fiber, and protein, in *Morus alba* L. leaves were 8.91%, 5.3%, 6.57%, 10.11%, and 18.41%, respectively, while in *Morus nigra* L. leaves they were 9.12, 6.7, 5.13, 12.32, and 19.76, respectively [22] (pp. 6).

**Table 1.** Chemical composition of black mulberry leaves (BML), black mulberry fruit (BMF), white mulberry leaves (WML), and white mulberry fruit (WMF) (g/100g w/w).

Nutrient contents	Protein	Fat	Ash	Moisture	CHO	Fiber	Energy (Kcal)	Total PH%	Total Nitrogen%
BML	1.225	2.5	2.76	73.635	8.98	10.9	63.32	6.69	0.196
BMF	1.25	0.9	0.74	72.71	17.9	6.5	84.7	5.56	0.2
WML	1.75	2.3	2.81	74.45	8.79	9.9	62.86	6.69	0.28
WMF	1.63	1.1	0.85	73.02	15.8	7.6	79.62	4.82	0.261

**Kcal:** Kilocalories

Correspondingly, showed that the contents of mulberry fruits and leaves are 9.0 and 8.48% of carbs, 1.28 and 3.16% protein, 0.49 and 0.33% fat, 86.63 and 81.14% moisture, 1.03 and 4.25% ash, and 1.57 and 2.64% fiber, respectively [51]. According to [52], mulberries contain 5.31% protein, 2.09% fat, 9.9% crude fiber, 27.6% dietary fiber, and 11.3% ash by dry weight. According to earlier research, the highest concentrations of beneficial chemicals have been found in leaves and dark fruits [53,54]. Author [55] found the moisture contents of white (*Morus alba*) and black (*Morus nigra*) mulberry fruits were in the range of (82.40 to 81.72) g/100 g fresh weight (FW). Also, the Turkish-origin mulberry fruit species had moisture levels ranging from 71.5% to 74.6% [56]. According to Author [55] (pp. 6), the ash, lipids, protein, and fiber contents of white (*Morus alba*) and black (*Morus nigra*) mulberry fruits were in the range of (0.57 to 0.5), (0.48 to 0.55), (1.55 to 0.96), and (1.47 to 11.75) g/100 g dry weight (DW), respectively.

Overall, the findings indicated that the fruit samples might provide lipids, proteins, fibers, carbohydrates, and, ultimately, energy. Nevertheless, differences were observed in the parameters under investigation; as a result, fruits should be selected based on consumer demand when used for food. According to the author's published literature [27, 50, 51, 55] (pp. 3–6), the results are in good agreement.

### 3.2. Biological characteristics.

The effect of orally administered BML, BMF, WML, and WMF extracts at 150 and 300mg/kg on the biological characteristics of hypercholesterolemic rats was summarized in Table 2. At the end of the experimental period, the feed efficiency ratio (FER) was significantly lower in the groups fed BMLE300mg/kg, WMFE300mg/kg, WMLE300mg/kg, WMLE150mg/kg, and BMLE150mg/kg, in descending order, than in the positive control group. Body weight gain (BWG) significantly decreased in all high-cholesterol diet-treated groups compared to the positive control group. The BWG and relative body weight gain (rBWG) for the negative control group (22.17±1.05;  $P<0.001$  and 100%), BMLE150mg/kg (20.3±1.01,  $P<0.01$  and 91.6%), WMLE150mg/kg (22.1±2.35,  $P<0.001$  and 99.7%), BMLE300mg/kg (24.8±0.58,  $P<0.05$  and 111.9%), WMLE300mg/kg (20.6±1.42,  $P<0.001$  and 92.9%), BMFE150mg/kg (26.6±1.03,  $P<0.05$  and 120%), WMFE150mg/kg (25.6±1.62,  $P<0.01$  and 115.5%), BMFE300mg/kg (24.8±0.72,  $P<0.01$  and 111.9%), and WMFE300mg/kg (24.9±1.92,  $P<0.01$  and 112.3%).

**Table 2.** Effect of BMLE, BMFE, WMLE, WMFE on body weight gains (BWG), relative body weight gain (rBWG), and feed efficiency ratio (FER) of Hypercholesterolemic rats.

Parameters groups	IBW(g)	FBW(g)	FI (g/day)	FER (g)	BWG (%)	rBWG (%)
Negative(-ve)	230±2.71	281±3.77	21.7±1.01***	0.0102±0.0003*	22.17±1.05***	100.0
Positive(+ve)	177±1.60	240±1.93	31.4±0.73	0.0113±0.0009	35.95±1.81	162.2
BMLE150mg/kg	214±2.15	258±2.21	26.6±1.90*	0.007±0.0006**	20.3±1.01**	91.6
BMLE300mg/kg	194±1.58	242±1.73	22.7±0.53**	0.011±0.0003*	24.8±0.58*	111.9
WMLE150mg/kg	219±1.81	267±3.39	30.9±0.54	0.007±0.0007**	22.1±2.35***	99.7
WMLE300mg/kg	215±0.83	259±2.31	29.1±0.60*	0.007±0.0006**	20.6±1.42***	92.9
BMFE150mg/kg	194±1.49	245±1.89	21.7±1.01**	0.012±0.0006	26.6±1.03*	120.0
BMFE300mg/kg	181±1.14	226±2.09	19.9±0.57***	0.013±0.0007	24.8±0.72**	111.9
WMFE150mg/kg	201±1.70	252±1.72	25.1±0.80*	0.0102±0.0006*	25.6±1.62**	115.5
WMFE300mg/kg	186±1.78	232±1.65	23.9±1.59**	0.0108±0.0015*	24.9±1.92**	112.3

**IBW:** initial body weight - **FBW:** Final body weight - **FI:** Food intake – **BWG:** Body weight gains, **rBWG:** Relative body weight gains, and **FER:** Feed efficiency ratio. Data are expressed as the Mean±SE of six experimental rats. A P-value less than 0.05 was considered statistically significant. The parameters of the Negative and treatment groups were compared to the positive groups. \*(P≤0.05) significant change, \*\*(P≤0.01) high significant change; \*\*\*(P≤0.001) very high significant change.

The lowest FER, BWG, and rBWG were observed in the BMLE150mg/kg group, followed by the WMLE300mg/kg and WMLE150mg/kg groups; FER in the BMFE150mg/kg and BMFE300mg/kg groups did not significantly differ from the positive control group. This suggests that daily intake of BMLE is effective in reducing weight gain. Our results agree with those reported that BWG in negative controls decreased significantly compared with high-fat diet-induced obese controls [57]. Mice treated with mulberry leaf and fruit extracts showed a decrease in BWG compared with high-fat obese controls, despite no differences in food intake [55] (pp. 2). Nevertheless, others reported that BWG in the negative control group was a significant increase ( $p \leq 0.001$ ) compared with the hyperlipidemic control group [51] (pp. 6). Also, it has been demonstrated that mulberry leaf (ML) and mulberry leaf extract (MLE) have numerous biological properties, including regulating sugar and lipid metabolism, reducing blood glucose levels, and increasing insulin secretion [58].

### 3.3. Lipid profile.

Table 3 shows the impact of supplementing BMLE, BMFE, WMLE, and WMFE on the lipid profile of hypercholesterolemic rats. Low-density lipoprotein cholesterol(LDL-c), high-density lipoprotein cholesterol(HDL-c), very low-density lipoprotein cholesterol(VLDL-c), and total cholesterol (TC) are all evaluated as part of the lipid profile. According to the study's results, the cohort of rats, including the positive control group (+), showed elevated baseline cholesterol levels. The study revealed that rats fed a high-fat diet (HFD) showed a substantial increase in cholesterol levels compared to the negative control group. Moreover, upon completing the 42-day trial period, the total cholesterol concentration for treatment groups exhibited a noteworthy reduction compared to the positive control group. So the rats were administered injections of BMLE, BMFE, WMLE, and WMFE at the dosages of 150 and 300mg/kg exhibited positive responses, with a gradual reduction in their cholesterol levels throughout the treatment until they recovered. Remarkably, the group that received injections of BMFE at a dosage of 300 mg/kg demonstrated the greatest significant ( $P < 0.001$ ) effects (187.8±2.04 and 282.9±1.38 mg/dl, respectively).

The statistical data indicate that feeding rats on HFD or the positive control group (+) resulted in a rise in TC, TG, LDL-c, and VLDL-c. At the same time, levels of HDL-c were

significantly decreased ( $P<0.001$ ) compared to the negative control group (-) being ( $82.7\pm0.59$  and  $53.1\pm2.86$  mg/dl), respectively. HDL-c decreased, and LDL-c and VLDL-c increased in response to an HFD in the positive control group.

**Table 3.** Effect of BMLE, BMFE, WMLE, WMFE on Lipid profile, {Total cholesterol (TC), Triglycerides (TG), High-density lipoprotein (HDL-c), Low-density lipoprotein(LDL-c), and Very low-density lipoprotein (VLDL-c)} of Hypercholesterolemic rats.

Parameters groups	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Negative (- V)	181.1±1.10**	150.7±1.578***	82.7±0.59 ***	68.2±0.716***	30.2±0.158***
Positive (+V)	282.9±1.38	267.8±3.024	53.1±2.86	176.1±4.53	53.7±0.987
BMLE150mg/kg	219.8±1.97**	213.7±0.584**	65.2±0.19**	111.9±1.491**	42.7±1.116**
BMLE300mg/kg	203.1±1.59**	177.3±2.498***	76.2±1.41 ***	91.3±1.158***	35.5±0.074***
WMLE150mg/kg	253.9±3.59**	236.8±1.712*	58.3±3.40 *	148.2±2.610*	47.4±2.140*
WMLE300mg/kg	216.3±1.99**	207.5±3.569**	68.6±1.58 **	106.2±4.726**	41.5±4.085**
BMFE150mg/kg	206.2±2.03**	186.3±1.047***	74.7±0.66 ***	94.2±0.583***	37.3±0.243***
BMFE300mg/kg	187.8±2.04***	161.7±0.736***	80.9±3.58 ***	74.6±1.020***	32.3±1.147***
WMFE150mg/kg	213.7±3.09**	198.5±2.242***	70.1±0.582 ***	103.9±1.137***	39.7±0.254***
WMFE300mg/kg	198.1±2.04***	170.5±1.406***	78.5±1.582***	85.5±3.289***	34.1±2.080***

Data are expressed as the Mean±SE of six experimental rats. A P-value less than 0.05 was considered statistically significant. The parameters of the Negative and treatment groups were compared to the positive groups. \*( $P\leq0.05$ ) significant change, \*\*( $P\leq0.01$ ) high significant change; \*\*\*( $P\leq0.001$ ) very high significant change.

In addition, injecting rats once daily with 150 and 300mg/kg of BMLE, BMFE, WMLE, and WMFE for 6 weeks resulted in a significant decrease in all parameters, except HDL-c, which showed a significant increase compared to the positive control group. The highest significant ( $P<0.001$ ) decrease of TG and VLDL-c were noticed in the group that received BMFE300mg/kg compared with the control positive group ( $161.7\pm0.736$  and  $267.8\pm3.024$ mg/dl) and ( $32.3\pm1.147$  and  $53.7\pm0.987$  mg/dl) respectively, also the highest significant ( $P<0.001$ ) increase of HDL-c was noticed in the group that received BMFE300mg/kg compared with the control positive group ( $85.5\pm3.289$  and  $53.1\pm2.86$ mg/dl) respectively.

In the current investigation, the lipid profile was disrupted in HFD rats. The HFD group exhibited significantly higher plasma cholesterol and LDL-C levels. Additionally, comparing the HFD groups to the negative control group, there was a significant decrease in the HDL-C level. These results agree with [59,10] (pp.1), who found that rats fed a high-cholesterol (HC) diet showed higher serum TG, TC, and LDL-c contents than animals fed a normal diet. Another study showed that TC, TG, LDL, and VLDL in the negative control group decreased significantly ( $P<0.001$ ) compared to the group that received HFD, control (+) [51] (pp. 6). However, it was found that mulberry polysaccharide effectively reduces low-density lipoprotein (LDL-c), triglycerides, and total cholesterol in rats' serum and liver on a high-fat diet [60]. Additionally, compared with the HC group, mulberry fruit extract supplementation significantly reduced serum TG, TC, and LDL-c concentrations by 54.3%, 26.8%, and 24.1%, respectively ( $P<0.05$ )[10]. Interestingly, rats in the high mulberry fruit extract group had higher serum HDL-c levels than those in the HC group ( $P<0.05$ ). In a separate study, examined the effect of mulberry leaf extract (MLE) on atherosclerosis development was examined using aortic vascular smooth muscle cells (VSMCs) and high-cholesterol-fed New Zealand white rabbits [61]. The results demonstrate that after MLE treatment, there is a significant reduction in atheroma load, serum cholesterol, triglycerides, and low-density lipoprotein (LDL) levels, as well as an improvement in liver function. Rats fed a high-fat diet gained weight and

increased adipose tissue compared with the control group, and also exhibited insulin resistance and hyperlipidemia, conditions linked to obesity [59]. Increased flow of free fatty acid (FFA) from adipose tissue to the liver via the portal vein when visceral TG levels rise is one of the main causes of dyslipidemia. FFA is released by adipocytes in obese individuals due to increased lipolysis [62]. The uncontrolled release of free fatty acids (FFA) from visceral adipose tissue increases the flow of FFA to the liver. It promotes the production and secretion of very low-density lipoprotein (VLDL). It also suppresses lipoprotein lipase in skeletal muscle and adipose tissue, leading to hypertriglyceridemia [63, 64]. Hypertriglyceridemia encourages the production of small-dense lipoprotein LDL particles, cholesteryl ester transport protein-induced HDL decrease, and consequent hepatic lipase production [61].

Correspondingly, found the consumption of mulberry leaves and fruits at the dose of 7.5 % of the basal diet showed a significant decrease in mean values of TC, TG, LDL, and VLDL compared positive control group, the value of HDL-c of control (-) showed a highly significant increase ( $P<0.001$ ) as compared with the control (+) group, plus the consumption of mulberry fruits at 7.5% significant increase ( $P<0.01$ ) when compared to the control (+) group while the consumption of mulberry leaves at 7.5 % [51] (pp. 6).

Nevertheless, New Zealand white rabbits fed a high-cholesterol diet (HCD) for 10 weeks that included 0.5% or 1.0% water extract of mulberry fruits in addition to 95.7% standard Purina chow, 3% lard oil, and 1.3% cholesterol had lower levels of triglycerides, LDL cholesterol, and total cholesterol than the rabbits fed a diet that only contained lard oil. The same authors also demonstrated that feeding HCD with 0.5% or 1.0% water extract of mulberry fruits dramatically reduced the incidence of severe aortic atherosclerosis by 42–63%. Histopathological analysis of the rabbits' blood vessels also supported these results. It has been reported that there is a dose-dependent relationship between the levels of total and low-density lipoprotein cholesterol and the water extract from mulberry fruits [65].

Our results also agree that rats fed an HF diet supplemented with 5% or 10% mulberry fruit significantly reduced serum and liver TG, TC, and LDL-c concentrations and increased HDL-c [66]. Mulberry fruits include dietary fiber, which promotes LDL-receptor activation and suppresses hepatic lipogenesis [67]. In addition, reported that mulberry fruits are rich in dietary fiber and linoleic acid, suggesting they may have a hypolipidemic effect [66]. However, the beneficial effect of the mulberry extract on HC-decreased HDL-C levels, a powerful predictor of CVD [10].

### 3.4. Atherogenic indices.

Table (4) shows the atherogenic indices of Hyperlipidemic Rats Fed on BMLE, BMFE, WMLE, and WMFE. A decreased risk of coronary heart disease (CHO) is associated with lower ratios of total cholesterol to HDL and LDL-c to HDL-c. Conversely, the risk of (CHD) decreases with increasing total cholesterol/LDL-c ratio. Our results revealed that the treatments with BMFE300 mg/kg and WMFE300 mg/kg had the lowest ratios of total cholesterol to HDL-c and LDL-c to HDL-c compared with the HFD group. Additionally, compared with the positive control group (HFD), the same treatment showed the highest total cholesterol-to-LDL-c ratio. Our results agree that when comparing the mulberry fruit ethanol extract (MBEE)-treated groups to the high-fat diet group (HFD group, significant reductions in the cholesterol, LDL-C, and TC/HDL-C ratio were observed, particularly for the group that received 150 mg/kg/day (LMB) of MBEE. Nevertheless, the LMB group showed greater effects on LDL-C and TC/HDL-C than the high-dose HMB group [59].

**Table 4.** Atherogenic indices of Hyperlipidemic Rats Fed on BMLE, BMFE, WMLE, and WMFE.

Parameters groups	TC/HDL Ratio	TC/LDL ratio	LDL/HDL ratio
Negative (- V)	2.19±0.21	2.66±0.68	0.82±0.09
Positive (+V)	5.33±1.04	1.61±0.37	3.32±0.98
BMLE150mg/kg	3.37±0.98	1.96±0.81	1.72±0.57
BMLE300mg/kg	2.66±0.85	2.22±0.76	1.20±0.61
WMLE150mg/kg	4.36±1.01	1.71±0.38	2.54±0.52
WMLE300mg/kg	3.15±0.96	2.04±0.94	1.55±0.57
BMFE150mg/kg	2.76±0.99	2.19±0.87	1.26±0.38
BMFE300mg/kg	2.32±0.87	2.52±0.59	0.92±0.08
WMFE150mg/kg	3.05±0.93	2.06±0.81	1.48±0.36
WMFE300mg/kg	2.52±0.87	2.32±0.76	1.09±0.53

Data are expressed as the Mean±SE of six experimental rats. A P-value less than 0.05 was considered statistically significant. The parameters of the Negative and treatment groups were compared with those of the positive groups. \*(P≤0.05) significant change, \*\*(P≤0.01) high significant change; \*\*\*(P≤0.001) very high significant change.

Using the LDL-c/HDL-c ratio rather than the non-HDL-c/LDL-c ratio did not reveal an association [68]. There is increasing evidence that HDL-C's functional characteristics, rather than its levels in the blood, may be more significant in predicting coronary heart disease risk [69].

Contemporary studies have shown that elevated HDL-c does not necessarily reduce the risk of CHD [68]. According to the Framingham study, about 40% of coronary heart disease (CHD) events occurred in persons with normal or raised HDL levels [70]. Additionally, very high HDL-C levels have been shown to be unrelated to the risk of vascular events [71]. According to the data, there may be a clinical benefit to enhancing HDL-C function rather than raising its levels [72]. While a decreased LDL-c/HDL-c ratio, achieved with pharmacological interventions, may be associated with coronary plaque regression, a high LDL-c/HDL-c ratio is associated with coronary plaque development [68]. Certain researchers have suggested that individuals with abnormal cholesterol levels and a high LDL-c/HDL-c ratio should start treatment [73]. An index of the LDL-c/HDL-c ratio and alterations in coronary plaque volume showed a positive linear relationship, according to a pooled analysis of four prospective randomized trials [74].

In addition, a high LDL-c/HDL-c ratio predicts the presence of coronary lipid-rich plaques and plaque vulnerability, which increases the risk of sudden cardiac death (SCD) [75,76]. It is thought that the primary route for the development of coronary thrombosis, which ultimately results in acute myocardial infarction (MI) and SCD, is the rupture of high-risk susceptible plaques [77]. Coronary heart disease (CHD) is the most common pathology underlying SCD [78,79]. Nevertheless, a study found that when the analysis was limited to men without a history of CHD, the correlation between the LDL-c/HDL-c ratio and the risk of SCD persisted [68] (pp. 9). The same outcome demonstrates a clear, independent association between the LDL-c/HDL-c ratio and SCD risk, with possible clinical implications. Patients' CVD risk is already predicted in clinical practice using assays for these lipoproteins. Lowered plasma cholesterol, LDL-C, TC/HDL-C, and hepatic TG could be protected against MBEE, which also lowers hepatic TG and aberrant lipid metabolism brought on by a high-fat diet [59]. According to a study, MLE and MLPE can effectively suppress the proliferation and migration of aortic VSMCs, enhance vascular endothelial function, and lower atheroma load, all of which can prevent atherosclerosis in addition to their hypolipidemic effects [61]. Moreover, it was found that mulberry polysaccharide effectively decreases the atherogenic index and increase high-density lipoprotein (HDL) levels in serum [60]. Suggested that different kinds of mulberry fruit are useful for hypercholesterolemic patients [80]. Because mulberry fruits have

antioxidant and anti-hyperlipidemic properties that prevent LDL oxidation, eating them may lower the risk of atherosclerosis [81].

One of the main risk factors for Cardiovascular disease (CVD) is hyperlipidemia [82]. One of the leading causes of death worldwide, CVD accounts for around 17 million deaths annually. This includes deaths from coronary heart disease and stroke [83,84]. In the future, CVD will still account for the greatest portion of mortality worldwide [85].

### 3.5. Antioxidant markers.

Table (5) the effect of BMLE, BMFE, WMLE, and WMFE on serum levels of the antioxidant markers {glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), catalase activities (CAT)} of hypercholesterolemic rats. Levels of the antioxidant markers (GSH, SOD, and CAT) have significantly decreased ( $P < 0.001$ ) in the Positive (+V) group, while MDA has significantly increased ( $P < 0.01$ ) compared to the Negative(-V) control ( $65.7 \pm 1.16$  and  $98.8 \pm 3.02$  nmol/ml), respectively. On the other hand, treatment of hypercholesterolemic rats with orally administered BMFE 300mg/kg caused a significant increase ( $P < 0.05$ ) in GSH, SOD, and CAT compared with the positive control group. Furthermore, the treatment showed significant decreases ( $P < 0.001$ ) in MDA in the same group, compared with the hypercholesterolemic group ( $68.8 \pm 0.39$  and  $98.8 \pm 3.02$  nmol/g, respectively).

Nevertheless, significant decreases were recorded in the levels of (antioxidant parameters) in treatment groups (BMFE300mg/kg ( $P < 0.001$ ), WMFE300mg/kg ( $P < 0.01$ ), BMLE300mg/kg ( $P < 0.05$ ), and WMLE300mg/kg ( $P < 0.001$ ) respectively) as compared to the non-treatment group of Hypercholesterolemic rats. According to our findings, injecting 150 and 300 mg/kg of BMLE, BMFE, WMLE, and WMFE extracts enhances antioxidant markers in rats with high cholesterol. When compared to the control (+) group, the rats in the BMFE300 mg/kg group received improved treatment. Our findings supported the study's main objective of differentiating the biological effects of BMLE, BMFE, WMLE, and WMFE extracts on hypercholesterolemic rats with elevated MDA levels. We also found that extracts improve antioxidant marker outcomes.

**Table 5.** Effect of BMLE, BMFE, WMLE, WMFE on serum antioxidant, glutathione (GSH), Malondialdehyde (MDA), superoxide dismutase (SOD), catalase activities (CAT) of Hypercholesterolemic rats.

Parameters groups	MDA (nmol/ml)	CAT (ng/ml)	SOD (ul/ml)	GSH (ng/ml)
Negative(-ve)	$9.26 \pm 0.035^{***}$	$28.85 \pm 0.049^*$	$2.89 \pm 0.0135^{***}$	$13.37 \pm 0.017^{**}$
Positive(+ve)	$15.36 \pm 0.032$	$13.75 \pm 0.036$	$1.33 \pm 0.022$	$7.44 \pm 0.030$
BMLE150mg/kg	$13.43 \pm 0.029^{**}$	$17.31 \pm 0.239^{**}$	$1.60 \pm 0.015^{**}$	$9.66 \pm 0.043^*$
BMLE300mg/kg	$10.78 \pm 0.020^*$	$25.60 \pm 0.055^{***}$	$2.31 \pm 0.029^*$	$12.39 \pm 0.016^{**}$
WMLE150mg/kg	$14.88 \pm 0.024^{**}$	$14.38 \pm 0.055^{**}$	$1.42 \pm 0.017^{**}$	$7.78 \pm 0.019^{***}$
WMLE300mg/kg	$12.80 \pm 0.007^{**}$	$16.79 \pm 0.538^{**}$	$1.73 \pm 0.026^{**}$	$10.22 \pm 0.028^*$
BMFE150mg/kg	$11.28 \pm 0.012^*$	$23.78 \pm 0.043^{***}$	$2.03 \pm 0.008^*$	$11.90 \pm 0.020^*$
BMFE300mg/kg	$9.70 \pm 0.017^{***}$	$28.39 \pm 0.033^*$	$2.69 \pm 0.035^{***}$	$13.05 \pm 0.015^{**}$
WMFE150mg/kg	$12.28 \pm 0.025^{**}$	$21.15 \pm 0.157^{***}$	$1.82 \pm 0.027^{**}$	$10.83 \pm 0.003^{**}$
WMFE150mg/kg	$10.19 \pm 0.007^*$	$27.81 \pm 0.069^*$	$2.47 \pm 0.013^*$	$12.77 \pm 0.020^{**}$

Data are expressed as the Mean $\pm$ SE of six experimental rats. A P-value less than 0.05 was considered statistically significant. The parameters of the Negative and treatment groups were compared to the positive groups. \*( $P \leq 0.05$ ) significant change, \*\*( $P \leq 0.01$ ) high significant change; \*\*\*( $P \leq 0.001$ ) very high significant change.

The results revealed a noteworthy increase in blood MDA levels and an overall reduction in enzyme activity (GSH, SOD, and CAT) compared with the uninfected group consuming a basic diet. Rats were assigned to groups BMLE, BMFE, WMLE, and WMFE,

and doses of 150 and 300 mg/kg were administered. Furthermore, the results showed that rats administered BMFE at 300 mg/kg demonstrated a marked enhancement in oxidative stress markers.

Our results agree with those reported by others, who found that the presence of anthocyanins in mulberry fruits might act as antioxidants, reducing exercise-induced oxidative stress and physical fatigue. Regarding the changes in antioxidant activities during lactic fermentation, it is noteworthy that the antioxidant activities of mulberry at any stage of fermentation were higher in mulberry fruits (MF) than those in mulberry leaves (ML) [86]. Since antioxidant activity has been regarded as a beneficial indicator of fruit products, it has a positive effect on the treatment of consumption [87,88].

Another study examined the protective effect of mulberry fruit (MF) extract against ethyl carbamate (EC)-induced cytotoxicity in human hepatoma (HepG2) cells [86] (pp. 11). Reported no reduction in cell viability with the treatments of MF extract (0.5 mg/mL, 1.0 mg/mL, and 2.0 mg/mL) [13] (pp. 2). Therefore, it is suggested that MF can be used to protect against EC-induced cytotoxicity and oxidative stress. Also, in a study investigating the effect of MF consumption on anti-fatigue activity in mice using a weight-loaded swimming test, it was reported that mice fed mulberry juice purification or mulberry marc purification had increased endurance capacity compared to the control group [89]. The same authors suggested that the presence of anthocyanins in MF might act as antioxidants, reducing physical fatigue and exercise-induced oxidative stress. Therefore, a relationship between the polysaccharides found in three fractions of black mulberry fruit and their ability to prevent parasitic and lipotoxic damage (BP1) [90]. One of three polysaccharide fractions had a significant impact on cell viability and survival. Additionally, BP1 treatment demonstrated a remarkable ability to reduce reactive oxygen species accumulation in HepG2 cells, a major cause of plasmatic acid-induced lipotoxicity. Furthermore, BP1 treatment increased the levels of antioxidant enzymes, such as glutathione peroxidase and catalase, which are essential for maintaining a balanced level of oxidative stress.

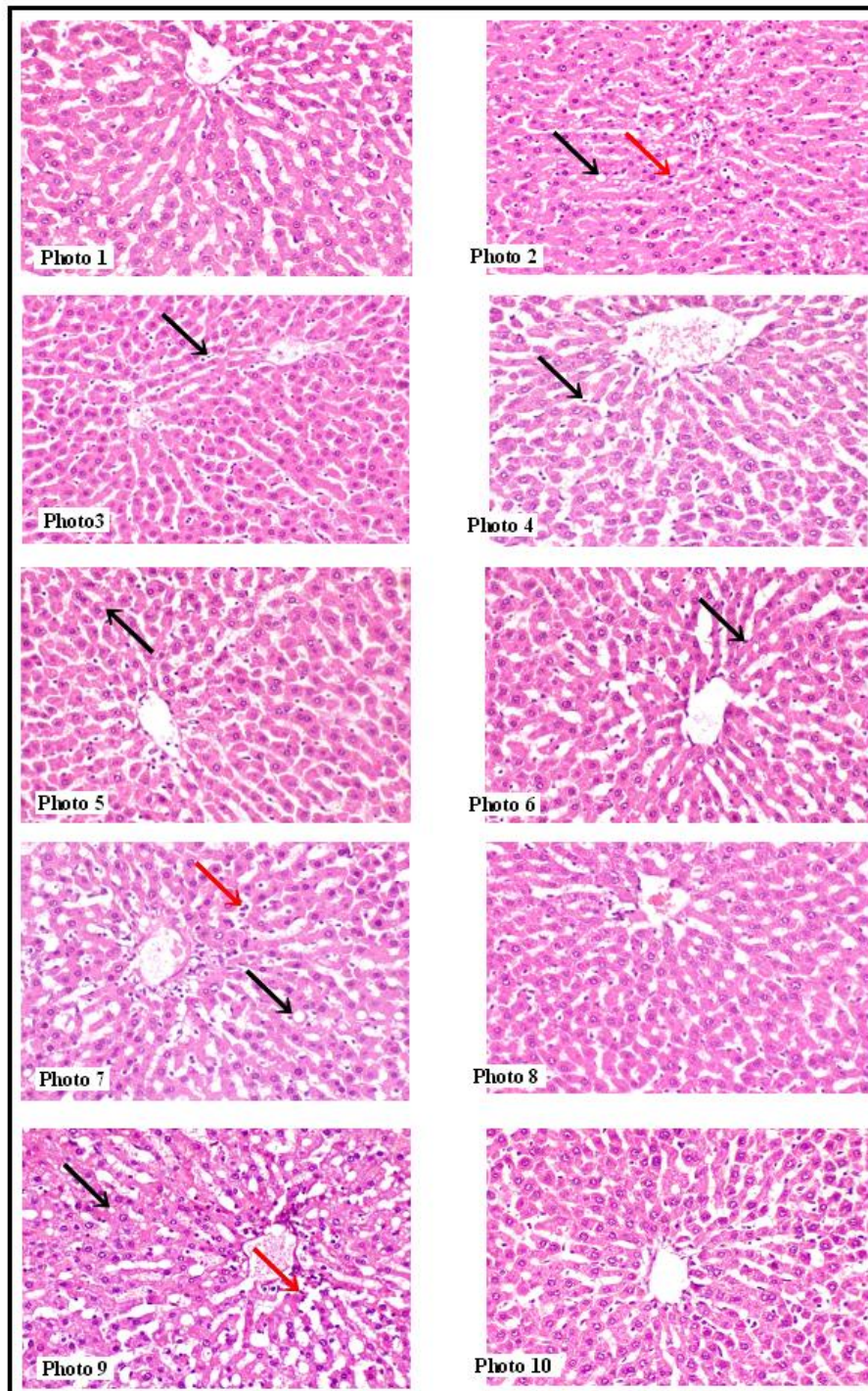
Data from animal studies indicate that when an HFD is administered, inflammation is more likely to occur [91]. Importantly, several studies have reported negative effects of HFD on the expression of certain genes in the hypothalamus [92]. In particular, an HFD led to an upregulation of genes such as toll-like receptor 4 (TLR4), Cd68, NF- $\kappa$ B, Emr1, IL-6, indoleamine 2,3-dioxygenase (IDO), interferon-gamma (IFN- $\gamma$ ), and TNF- $\alpha$  in rodents, suggesting that these changes were connected to diet-induced changes rather than obesity. In line with this, human studies also showed an association between HFD and mild cognitive impairment or dementia, Alzheimer's disease. These associations may be partially explained by the type or composition of dietary fatty acids, which may affect cognitive performance [91].

An important factor in the development of many diseases is oxidative stress [93]. The body constantly produces reactive nitrogen species (RNS) and reactive oxygen species (ROS) through oxidative metabolism and mitochondrial bioenergetics [94]. There is strong evidence that excessive ROS production damages macromolecules, including DNA, proteins, and membrane lipids, over time through oxidative damage [95], leading to neuronal death and affecting the lifespan of several organ systems [96]. Excessive dietary fat intake leads to obesity, which in turn causes white adipose tissue to form, secreting proinflammatory factors and creating a chronic state of inflammation [97]. A prior study showed that proinflammatory cytokines and nuclear factor kappa B (NF- $\kappa$ B) are the primary mediators of increased ROS production by activated immune cells [90].

Mulberry extracts or components, especially flavonoids such as quercetin, rutin, and isoquercitrin, scavenge free radicals and have potential against oxidative stress. The presence of prenylated flavonoids further strengthened its antioxidant claims; all parts of *M. alba* are of great therapeutic worth, and their main mechanism of action involves their antioxidant activities [52]. Finally, it was shown that mulberry fruit polysaccharides exerted a concentration-dependent effect on body weight gain in rats. Mulberry can currently enhance the antioxidant levels in the blood and liver while lowering lipid peroxidation [60] (pp. 8).

*3.6. Histopathological examination of the liver.*

Light microscopic examination (H and E X 400) of liver sections of rats from the negative control group revealed normal histoarchitecture of the hepatic lobule (Photo 1).



**Figure 1.** Histopathological examination of the liver from Photo 1 to Photo 10.

In contrast, the liver of rats from the positive control group exhibited histopathological damage characterized by hepatocellular steatosis and Kupffer cell activation (Photo 2). However, the liver of a rat that received BMLE 150 mg/kg showed Kupffer cell activation (Photo 3). Meanwhile, the liver of a rat that received BMLE 300 mg/kg showed Kupffer cell activation (Photo 4). Furthermore, hepatic tissue from rats treated with WMLE150mg/kg showed small cytoplasmic vacuoles in some hepatocytes (Photo 5). Likewise, the liver of a rat that received WMLE300mg/kg exhibited slight Kupffer cell activation (Photo 6). Examined sections from group rats that received BMFE150mg/kg revealed vacuolization of some hepatocytes and the presence of a few inflammatory cells in the hepatic sinusoids (Photo 7).

On the other hand, some examined sections from group rats that received BMFE300mg/kg exhibited apparent normal hepatic parenchyma (Photo 8). Meanwhile, the liver of group rats that received WMFE150mg/kg revealed hepatocellular vacuolization (Photo 9). Likewise, some hepatic sections of rats that received WMFE300mg/kg exhibited no histopathological alterations (Photo 10). According to [98], liver histological examinations in the HF group revealed both macro- and microvesicular steatosis, while liver fat deposition was decreased in the MLE and MFE groups. In contrast, Treatments with MLE and MFE significantly reduced adipocyte size and the number of immature adipocytes, as shown in Figure 1.

#### **4. Conclusions**

The current study investigated the favorable effects of mulberry fruit and leaf extracts on the serum lipid profile and oxidative abnormalities in rats fed a high-cholesterol diet for 6 weeks. Treated rats with oral injections of BMFE and WMFE once daily at 300 mg/kg showed significant decreases in serum TC, TG, and LDL-C levels, and effectively reduced the atherogenic index. Our results suggest that treatments with BMFE and WMFE successfully inhibited adipocyte hypertrophy and fatty liver and reduced oxidative stress. This study offers valuable insights into the development of effective treatments for hypercholesterolemia within the scientific community.

#### **Author Contributions**

Supervision, F.E.A. and E.A.M.H.; methodology, A.S.M.M.; investigation, A.S.M.M.; resources, F.E.A. and E.A.M.H.; data curation, A.S.M.M.; formal analysis, A.S.M.M.; visualization, A.S.M.M.; writing—original draft preparation, A.S.M.M.; writing—review and editing, F.E.A. and E.A.M.H. All authors have read and agreed to the published version of the manuscript.

#### **Institutional Review Board Statement**

This is to certify that the research work presented by Miss Aya Sayed Mohammed Mohammed has been considered and approved by the institutional Animal Care and Use Committee (IACUC)- Menoufia University, Egypt, Approval No: MUFHE IS/NFS/16/23.

#### **Informed Consent Statement**

There is no such thing, because the research used experimental procedures on laboratory rats.

## Data Availability Statement

The data will be made available upon request.

## Funding

The research was self-funded and did not receive any external financial support.

## Acknowledgments

The authors would like to express their sincere gratitude for the invaluable assistance offered by Professor Dr. Fatma EL-zahra Amin, Nutrition Professor in the Nutrition and Food Science Department and EX. Dean from the Faculty of Home Economics at Menoufia University, for her kind, direct supervision and cooperation throughout this work, support, and assistance during all steps of performing this work, and for reviewing the research plan for the present study. Professor Eslam Ahmed Mahmoud Header, Nutrition Professor in the Nutrition and Food Science Department at the Faculty of Home Economics, Menoufia University, deserves sincere thanks and appreciation. His support and assistance throughout all steps of this work, and his insightful comments, have significantly enriched the research study, adding scientific value and providing valuable insights for researchers.

## Conflicts of Interest

The authors have stated that they have no conflicts of interest to declare. The design, data collection, analysis, interpretation, manuscript preparation, and publication of results were conducted independently of any influence from the funding sources.

## References

1. Fularski, P.; Hajdys, J.; Majchrowicz, G.; Stabrawa, M.; Młynarska, E.; Rysz, J.; Franczyk, B. Unveiling Familial Hypercholesterolemia—Review, Cardiovascular Complications, Lipid-Lowering Treatment and Its Efficacy. *International Journal of Molecular Sciences* **2024**, *25*, 1637, <https://doi.org/10.3390/ijms25031637>.
2. Brandts, J.; Ray, K.K. Familial Hypercholesterolemia. *Journal of the American College of Cardiology* **2021**, *78*, 1831–43, <https://doi.org/10.1016/j.jacc.2021.09.004>.
3. Barquera, S.; Pedroza-Tobías, A.; Medina, C.; Hernández-Barrera, L.; Bibbins-Domingo, K.; Lozano, R.; Moran, A.E. Global overview of the epidemiology of atherosclerotic cardiovascular disease. *Archives of medical research* **2015**, *46*, 328–338, <https://doi.org/10.1016/j.arcmed.2015.06.006>.
4. WHO. HEARTS: Technical Package for Cardiovascular Disease Management in Primary Health care: Risk-based CVD Management. Available online: <https://www.who.int/publications/i/item/9789240001367>.
5. Stone, N.J.; Robinson, J.G.; Lichtenstein, A.H.; Bairey Merz, C.N.; Blum, C.B.; Eckel, R.H.; Goldberg, A.C.; Gordon, D.; Levy, D.; Lloyd-Jones, D.M. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Journal of the American College of Cardiology* **2014**, *63*, 2889–2934, <https://doi.org/10.1016/j.jacc.2013.11.002>.
6. Desforges, J.F.; Gordon, D.J.; Rifkind, B.M. High-density lipoprotein--the clinical implications of recent studies. *The New England Journal of Medicine* **1989**, *321*, 1311–6, <https://doi.org/10.1056/nejm198911093211907>.
7. Barter, P.; Gotto, A.M.; LaRosa, J.C.; Maroni, J.; Szarek, M.; Grundy, S.M.; Kastelein, J.J.; Bittner, V.; Fruchart, J.-C. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *New England Journal of Medicine* **2007**, *357*, 1301–1310, <https://doi.org/10.1056/nejmoa064278>.
8. Rye, K.A.; Barter, P. J. Regulation of High-Density Lipoprotein Metabolism. *Circulation Research* **2014**, *114*, 143–156, <https://doi.org/10.1161/circresaha.114.300632>.

9. Rosenson, R.S.; Brewer, H.B.; Ansell, B.J.; Barter, P.; Chapman, M.J.; Heinecke, J.W.; Kontush, A.; Tall, A.R.; Webb, N.R. Dysfunctional HDL and atherosclerotic cardiovascular disease. *Nature Reviews Cardiology* **2016**, *13*, 48-60, <https://doi.org/10.1038/nrcardio.2015.124>.
10. Lee, S.; Lee, M.-S.; Chang, E.; Lee, Y.; Lee, J.; Kim, J.; Kim, C.-T.; Kim, I.-H.; Kim, Y. Mulberry fruit extract promotes serum HDL-cholesterol levels and suppresses hepatic microRNA-33 expression in rats fed high cholesterol/cholic acid diet. *Nutrients* **2020**, *12*, 1499, <https://doi.org/10.3390/nu1205149>.
11. Ahmed, G.M. Comparison Between Some Mulberry Varieties on Silkworm, *Bombyxmori* L Economic Traits. *Egyptian Academic Journal of Biological Sciences A, Entomology* **2020**, *13*, 147–55, <https://doi.org/10.21608/eajbsa.2020.124328>.
12. Krishna, H.; Singh, D.; Singh, R.S.; Kumar, L.; Sharma, B.; Saroj, P.L. Morphological and antioxidant characteristics of mulberry (*Morus* spp.) genotypes. *Journal of the Saudi Society of Agricultural Sciences* **2020**, *19*, 136–45, <https://doi.org/10.1016/j.jssas.2018.08.002>.
13. Chen, W.; Li, Y.; Bao, T.; Gowd, V. Mulberry Fruit Extract Affords Protection against Ethyl Carbamate-Induced Cytotoxicity and Oxidative Stress. *Oxidative Medicine and Cellular Longevity* **2017**, *2017*, 1594963, <https://doi.org/10.1155/2017/1594963>.
14. Choi, K.H.; Lee, H.A.; Park, M.H.; Han, J.S. Mulberry (*Morus alba* L.) Fruit Extract Containing Anthocyanins Improves Glycemic Control and Insulin Sensitivity via Activation of AMP-Activated Protein Kinase in Diabetic C57BL/Ksj-db/db Mice. *Journal of Medicinal Food* **2016**, *19*, 737–745, <https://doi.org/10.1089/jmf.2016.3665>.
15. Jung, S.; Lee, M.S.; Choi, A.J.; Kim, C.T.; Kim, Y. Anti-Inflammatory Effects of High Hydrostatic Pressure Extract of Mulberry (*Morus alba*) Fruit on LPS-Stimulated RAW264.7 Cells. *Molecules* **2019**, *24*, 1425, <https://doi.org/10.3390/molecules24071425>.
16. Yuan, Q.; Zhao, L. The Mulberry (*Morus alba* L.) Fruit—A Review of Characteristic Components and Health Benefits. *J. Agric. Food Chem.* **2017**, *65*, 10383–10394, <https://doi.org/10.1021/acs.jafc.7b03614>.
17. Ju, W. T.; Kwon, O-Chul.; Lee, M. K.; Kim, H. B.; Sung, G. B.; Kim, Y. S. Quali-Quantitative Analysis of Flavonoids for Mulberry Leaf and Fruit of “Suhyang.” *Korean Journal of Environmental Agriculture*. **2017**, *36*, 249–255, <https://doi.org/10.5338/kjea.2017.36.4.39>.
18. Liu, Z.-Z.; Liu, Q.-H.; Liu, Z.; Tang, J.-W.; Chua, E.-G.; Li, F.; Xiong, X.-S.; Wang, M.-M.; Wen, P.-B.; Shi, X.-Y. Ethanol extract of mulberry leaves partially restores the composition of intestinal microbiota and strengthens liver glycogen fragility in type 2 diabetic rats. *BMC Complementary Medicine and Therapies* **2021**, *21*, 172, <https://doi.org/10.1186/s12906-021-03342-x>.
19. Cao, Y.; Jiang, W.; Bai, H.; Li, J.; Zhu, H.; Xu, L.; Li, Y.; Li, K.; Tang, H.; Duan, W. Study on active components of mulberry leaf for the prevention and treatment of cardiovascular complications of diabetes. *Journal of Functional Foods* **2021**, *83*, 104549, <https://doi.org/10.1016/j.jff.2021.104549>.
20. Horwitz, W. Official Methods of AOAC International, 17<sup>th</sup> Edition; Association of Official Analytical Chemists (AOAC) International: Gaithersburg, USA, **2000**.
21. AOAC Determination of Moisture, Ash, Protein and Fat. Official Method of Analysis of the Association of Analytical Chemists, 18<sup>th</sup> Edition; AOAC: Washington DC, **2005**.
22. Iqbal, S.; Younas, U.; Sirajuddin, Chan, K. W.; Sarfraz, R. A.; Uddin, M. K. Proximate Composition and Antioxidant Potential of Leaves from Three Varieties of Mulberry (*Morus* sp.): A Comparative Study. *International Journal of Molecular Sciences* **2012**, *13*, 6651–6664, <https://doi.org/10.3390/ijms13066651>.
23. Lim, Y.; Oh, J.H.; Park, U.K.; Huh, M.K.; Hwang, S.-Y. Protective Effect of Mulberry Leaf and Yacon Extract Induced Hyperlipidemia in Obese Rats. *Journal of Experimental & Biomedical Sciences/Biomedical Science Letter* **2020**, *26*, 101–8, <https://doi.org/10.15616/bsl.2020.26.2.101>.
24. Huang, J.; Wang, Y.; Ying, C.; Liu, L.; Lou, Z. Effects of mulberry leaf on experimental hyperlipidemia rats induced by high-fat diet. *Experimental and Therapeutic Medicine* **2018**, *6*, 547–556, <https://doi.org/10.3892/etm.2018.6254>.
25. World Health Organization. Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert Consultation. *World Health Organization technical report series* **1985**, *724*, 1–206.
26. Kobayashi, Y.; Miyazawa, M.; Araki, M.; Kamei, A.; Abe, K.; Hiroi, T.; Kojima, T. Effects of *Morus alba* L (Mulberry) leaf extract in hypercholesterolemic mice on suppression of cholesterol synthesis. *J. Pharmacogn. Nat. Prod.* **2015**, *1*, 1000113.
27. Srivastava, S.; Kapoor, R.; Thathola, A.; Srivastava, R. P. Nutritional quality of leaves of some genotypes of mulberry (*Morus alba*). *International Journal of Food Sciences and Nutrition* **2006**, *57*, 305–313, <https://doi.org/10.1080/09637480600801837>.

- El Boushy ARY, van der Poel AFB. Handbook of Poultry Feed from Waste: Processing and Use: 2nd Edition. *Journal of Applied Poultry Research*. 2002 Jul;11(2):223
28. Reeves, P.G.; Nielsen, F.H.; Fahey Jr, G.C. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *The Journal of Nutrition* **1993**, *123*, 1939–1951, <https://doi.org/10.1093/jn/123.11.1939>.
  29. Lassoued, I.; Trigui, M.; Ghilissi, Z.; Nasri, R.; Jamoussi, K.; Kessis, M.; Sahnoun, Z.; Rebai, T.; Boualga, A.; Lamri-Senhadj, M.; Nasri, M.; Barkia, A. Evaluation of hypocholesterolemic effect and antioxidant activity of Boops boops proteins in cholesterol-fed rats. *Food & Function*. **2014**, *5*, 1224–1231, <https://doi.org/10.1039/c3fo60705d>.
  30. Azemi, N.A.; Azemi, A.K.; Abu-Bakar, L.; Sevakumaran, V.; Muhammad, T.S.T.; Ismail, N. Effect of Linoleic Acid on Cholesterol Levels in a High-Fat Diet-Induced Hypercholesterolemia Rat Model. *Metabolites* **2023**, *13*, 53, <https://doi.org/10.3390/metabo13010053>.
  31. Parasuraman, S.; Raveendran, R.; Kesavan, R. Blood sample collection in small laboratory animals. *Journal of Pharmacology and Pharmacotherapeutics* **2010**, *1*, 87–97, <https://doi.org/10.4103/0976-500x.72350>.
  32. Chapman, D.G.; Castillo, R.; Cambell, J.A. Evaluation of protein in foods. I. A method for the determination of protein efficiency ratios. *Canadian journal of biochemistry and physiology*. **1959**, *37*, 679–686.
  33. Wang, L.; Guo, W.; Haq, S.U.; Guo, Z.; Cui, D.; Yang, F.; Cheng, F.; Wei, X.; Lv, J. Anticoccidial activity of Qinghao powder against Eimeria tenella in broiler chickens. *Frontiers in Veterinary Science* **2021**, *8*, 709046, <https://doi.org/10.3389/fvets.2021.709046>.
  34. Allain, C.C.; Poon, L.S.; Chan, C.S.; Richmond, W.; Fu, P.C. Enzymatic determination of total serum cholesterol. *Clinical chemistry* **1974**, *20*, 470–475.
  35. Fossati, P.; Principe, L. Serum triglycerides determined calorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry* **1982**, *28*, 2077–80, <https://doi.org/10.1093/clinchem/28.10.2077>.
  36. Burstein, R.; Cliffer, K.D.; Giesler, G.J. Cells of origin of the spin hypothalamic tract in the rat. *The Journal of Comparative Neurology* **1990**, *291*, 329–44, <https://doi.org/10.1002/cne.902910302>.
  37. Lee, R.; Nieman, D. Nutritional Assessment, 2<sup>nd</sup> Edition; Mosby Missouri, USA, **1996**.
  38. Bernert, J.T.; Turner, W.E.; Patterson, D.G.; Needham, L.L. Calculation of serum “total lipid” concentrations for the adjustment of persistent organ halogen toxicant measurements in human samples. *Chemosphere* **2007**, *68*, 824–831, <https://doi.org/10.1016/j.chemosphere.2007.02.043>.
  39. Friedewald, W.T.; Levy, R.I.; Fredrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*. **1972**, *18*, 499–502.
  40. Niroumand, S.; Khajedaluae, M.; Khadem-Rezaiyan, M.; Abrishami, M.; Juya, M.; Khodae, G.; Dadgarmoghaddam, M. Atherogenic Index of Plasma (AIP): A marker of cardiovascular disease. *Medical Journal of the Islamic Republic of Iran* **2015**, *29*, 240.
  41. Nakabayashi, A.; Kitagawa, Y.; Suwa, Y.; Akimoto, K.; Asami, S.; Shimizu, S.; Hirose, N.; Sugano, M.; Yamada, H. Alpha-Tocopherol enhances the hypocholesterolemic action of sesamin in rats. *Int J Vitam Nutr Res*. **1995**, *65*, 162–168.
  42. Li, J.; Lei, J.; He, L.; Fan, X.; Yi, F.; Zhang, W. Evaluation and Monitoring of Superoxide Dismutase (SOD) Activity and its Clinical Significance in Gastric Cancer: A Systematic Review and Meta-Analysis. *Medical Science Monitor* **2019**, *25*, 2032–2042, <https://doi.org/10.12659/MSM.913375>.
  43. Aebi, H. [13] Catalase in vitro. In *Methods in Enzymology*; Academic Press: **1984**; Volume 105, pp. 121–126, [https://doi.org/10.1016/s0076-6879\(84\)05016-3](https://doi.org/10.1016/s0076-6879(84)05016-3).
  44. McCord, J.M.; Fridovich, I. Superoxide dismutase. An enzymic function for erythrocyte (hemocuprein). *The Journal of Biological Chemistry* **1969**, *244*, 6049–6055.
  45. Östman, E.; Granfeldt, Y.; Persson, L.; Björck, I. Vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects. *European Journal of Clinical Nutrition* **2005**, *59*, 983–8, <https://doi.org/10.1038/sj.ejcn.1602197>.
  46. Macsween R.N.M. Theory and Practice of Histological Techniques. *Journal of Clinical Pathology* **1977**, *30*, 1089.
  47. Kattil, A.; Hamid; Dash, K.K.; Shams, R.; Sharma, S. Nutritional composition, phytochemical extraction, and pharmacological potential of mulberry: A comprehensive review. *Future Foods* **2024**, *9*, 100295, <https://doi.org/10.1016/j.fufo.2024.100295>.
  48. Hao, J.; Gao, Y.; Xue, J.; Yang, Y.; Yin, J.; Wu, T.; Zhang, M. Phytochemicals, pharmacological effects and molecular mechanisms of mulberry. *Foods* **2022**, *11*, 1170, <https://doi.org/10.3390/foods11081170>.

49. Khakwani, E.; Rizwan, B.; Noreen, S.; Amjad, A.; Shahzadi, M.; Rashid, N.; Ijaz, A. Functional and nutraceutical characterization of mulberry leaves. *Pakistan BioMedical Journal* **2022**, *5*, 90-95, <https://doi.org/10.54393/pbmj.v5i4.366>.
50. Shaheen, K.A.; Hashem, S.W. Study the Potential Effect of Mulberry Leaves and Fruits on Experimental Animals Infected with Hyperlipidemia. *Journal of Home Economics- Minufiya University* **2020**, *30*, 337-350, <https://doi.org/10.21608/mkas.2020.160372>.
51. Butt, M.S.; Nazir, A.; Sultan, M.T.; Schroën, K. Morus alba L. nature's functional tonic. *Trends in Food Science & Technology*. **2008**, *19*, 505–512, <https://doi.org/10.1016/j.tifs.2008.06.002>.
52. Sánchez-Salcedo, E, M.; Mena, P.; García-Viguera, C.; Hernández, F.; Martínez, J, J. (Poly)phenolic compounds and antioxidant activity of white (*Morus alba*) and black (*Morus nigra*) mulberry leaves: Their potential for new products rich in phytochemicals. *Journal of Functional Foods* **2015**, *18*, 1039–1046, <https://doi.org/10.1016/j.jff.2015.03.053>.
53. Jiang, Y.; Nie, W.J. Chemical properties in fruits of mulberry species from the Xinjiang province of China. *Food Chemistry* **2015**, *174*, 460–466, <https://doi.org/10.1016/j.foodchem.2014.11.083>.
54. Imran, M.; Khan, H.; Shah, M.; Khan, R.; Khan, F. Chemical composition and antioxidant activity of certain *Morus* species. *Journal of Zhejiang University Science B* **2010**, *11*, 973–80, <https://doi.org/10.1631/jzus.B1000173>.
55. Ercisli, S.; Orhan, E. Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. *Food Chemistry*. **2007**, *103*, 1380–1384, <https://doi.org/10.1016/j.foodchem.2006.10.054>.
56. Lim, H.H.; Lee, S.O.; Kim, S.Y.; Yang, S.J.; Lim, Y. Anti-inflammatory and antiobesity effects of mulberry leaf and fruit extract on high fat diet-induced obesity. *Experimental Biology and Medicine* **2013**, *238*, 1160–1169, <https://doi.org/10.1177/1535370213498982>.
57. Cui, W.; Luo, K.; Xiao, Q.; Sun, Z.; Wang, Y.; Cui, C.; Chen, F.; Xu, B.; Shen, W.; Wan, F.; Cheng, A. Effect of mulberry leaf or mulberry leaf extract on glycemic traits: a systematic review and meta-analysis. *Food & Function* **2023**, *14*, 1277–1289, <https://doi.org/10.1039/d2fo02645g>.
58. Noh, D.J.; Yoon, G.A. Mulberry (*Morus alba* L.) ethanol extract attenuates lipid metabolic disturbance and adipokine imbalance in high-fat fed rats. *Nutrition Research and Practice* **2022**, *16*, 716–728, <https://doi.org/10.4162/nrp.2022.16.6.716>.
59. Liu, Y.; Liu, Y.; Mu, D.; Yang, H.; Feng, Y.; Ji, R.; Wu, R.; Wu, J. Preparation, structural characterization and bioactivities of polysaccharides from mulberry (*Mori Fructus*). *Food Bioscience* **2022**, *46*, 101604, <https://doi.org/10.1016/j.fbio.2022.101604>.
60. Chan, K.-C.; Yang, M.-Y.; Lin, M.-C.; Lee, Y.-J.; Chang, W.-C.; Wang, C.-J. Mulberry leaf extract inhibits the development of atherosclerosis in cholesterol-fed rabbits and in cultured aortic vascular smooth muscle cells. *Journal of Agricultural and Food Chemistry* **2013**, *61*, 2780–2788, <https://doi.org/10.1021/jf305328d>.
61. Klop, B.; Elte, J.; Cabezas, M. Dyslipidemia in Obesity: Mechanisms and Potential Targets. *Nutrients*. **2013**, *5*, 1218–1240, <https://doi.org/10.3390/nu5041218>.
62. Marsh, J.B. Lipoprotein Metabolism in Obesity and Diabetes: Insights from Stable Isotope Kinetic Studies in Humans. *Nutrition Reviews* **2003**, *61*, 363–375, <https://doi.org/10.1301/nr.2003.nov.363-375>.
63. Saleh, J.; Sniderman, A.D.; Cianflone, K. Regulation of plasma fatty acid metabolism. *Clinica Chimica Acta* **1999**, *286*, 163–180, [https://doi.org/10.1016/s0009-8981\(99\)00099-6](https://doi.org/10.1016/s0009-8981(99)00099-6).
64. Chen, C.C.; Liu, L.K.; Hsu, J.D.; Huang, H.P.; Yang, M.Y.; Wang, C.J. Mulberry extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. *Food Chemistry* **2005**, *91*, 601–607, <https://doi.org/10.1016/j.foodchem.2004.06.039>.
65. Yang, X.; Yang, L.; Zheng, H. Hypolipidemic and antioxidant effects of mulberry (*Morus alba* L.) fruit in hyperlipidemia rats. *Food and Chemical Toxicology* **2010**, *48*, 2374–2379, <https://doi.org/10.1016/j.fct.2010.05.074>.
66. Venkatesan, N.; Niranjali, Devaraj, S.; Devaraj, H. Increased binding of LDL and VLDL to apo B, E receptors of hepatic plasma membrane of rats treated with Fibernat. *European Journal of Nutrition*. **2003**, *42*, 262–271, <https://doi.org/10.1007/s00394-003-0420-8>.
67. Kunutsor, S.K.; Zaccardi, F.; Karppi, J.; Kurl, S.; Laukkanen, J.A. Is High Serum LDL/HDL Cholesterol Ratio an Emerging Risk Factor for Sudden Cardiac Death? Findings from the KIID Study. *Journal of Atherosclerosis and Thrombosis* **2017**, *24*, 600–608, <https://doi.org/10.5551/jat.37184>.
68. White, C.R.; Datta, G.; Mochon, P.; Zhang, Z.; Kelly, O.; Curcio, C.; Parks, D.; Palgunachari, M.; Handattu, S.; Gupta, H. Vasculoprotective effects of apolipoprotein mimetic peptides: an evolving paradigm in Hdl therapy (Vascular Disease Prevention, In Press.). *Vascular disease prevention* **2009**, *6*, 122, <https://doi.org/10.2174/1567270000906010122>.

69. Gordon, T.; Castelli, W.P.; Hjortland, M.C.; Kannel, W.B.; Dawber, T.R. High density lipoprotein as a protective factor against coronary heart disease. *The American Journal of Medicine* **1977**, *62*, 707–14, [https://doi.org/10.1016/0002-9343\(77\)90874-9](https://doi.org/10.1016/0002-9343(77)90874-9).
70. Hirata, A.; Okamura, T.; Sugiyama, D.; Kuwabara, K.; Kadota, A.; Fujiyoshi, A.; Miura, K.; Okuda, N.; Ohkubo, T.; Okayama, A. The relationship between very high levels of serum high-density lipoprotein cholesterol and cause-specific mortality in a 20-year follow-up study of Japanese general population. *Journal of Atherosclerosis and Thrombosis* **2016**, *23*, 800-809, <https://doi.org/10.5551/jat.33449>.
71. Ikenaga, M.; Higaki, Y.; Saku, K.; Uehara, Y. High-Density Lipoprotein Mimetics: a Therapeutic Tool for Atherosclerotic Diseases. *Journal of Atherosclerosis and Thrombosis* **2016**, *23*, 385–394, <https://doi.org/10.5551/jat.33720>.
72. Packard, C.J.; Ford, I.; Robertson, M.; Shepherd, J.; Blauw, G.J.; Murphy, M.B.; Bollen, E.L.; Buckley, B.M.; Cobbe, S.M.; Gaw, A. Plasma lipoproteins and apolipoproteins as predictors of cardiovascular risk and treatment benefit in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER). *Circulation* **2005**, *112*, 3058-3065, <https://doi.org/10.1161/CIRCULATIONAHA.104.526848>.
73. Nicholls, S.J.; Tuzcu, E.M.; Sipahi, I.; Grasso, A.W.; Schoenhagen, P.; Hu, T.; Wolski, K.; Crowe, T.; Desai, M.Y.; Hazen, S.L. Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *Jama* **2007**, *297*, 499-508, <https://doi.org/10.1001/jama.297.5.499>.
74. Kimura, T.; Itoh, T.; Fusazaki, T.; Matsui, H.; Sugawara, S.; Ogino, Y.; Endo, H.; Kobayashi, K.; Nakamura, M. Low-Density Lipoprotein-Cholesterol/High-Density Lipoprotein-Cholesterol Ratio Predicts Lipid-Rich Coronary Plaque in Patients With Coronary Artery Disease—Integrated-Backscatter Intravascular Ultrasound Study—. *Circulation Journal* **2010**, *74*, 1392-1398, <https://doi.org/10.1253/circj.cj-09-0849>.
75. Kim, J.H.; Jeong, M.H.; Hong, Y.J.; Lee, K.H.; Kim, I.S.; Choi, Y.H.; Lee, M.G.; Park, K.-H.; Sim, D.S.; Kim, J.H. Low density lipoprotein-cholesterol/high density lipoprotein-cholesterol ratio predicts plaque vulnerability in patients with stable angina. *Korean Circulation Journal* **2012**, *42*, 246-251, <https://doi.org/10.4070/kcj.2012.42.4.246>.
76. Yang, F.; Patterson, R.P. A novel impedance-based tomography approach for stenotic plaque detection: A simulation study. *International Journal of Cardiology* **2010**, *144*, 279–283, <https://doi.org/10.1016/j.ijcard.2009.01.059>.
77. Hayashi, M.; Shimizu, W.; Albert, C.M. The Spectrum of Epidemiology Underlying Sudden Cardiac Death. *Circulation Research* **2015**, *116*, 1887–1906, <https://doi.org/10.1161/CIRCRESAHA.116.304521>.
78. Priori, S.G.; Blomström-Lundqvist, C.; Mazzanti, A.; Blom, N.; Borggrefe, M.; Camm, J.; Elliott, P.M.; Fitzsimons, D.; Hatala, R.; Hindricks, G.; Kirchhof, P.; Kjeldsen, K.; Kuck, K.-H.; Hernandez-Madrid, A.; Nikolaou, N.; Norekval, T.M.; Spaulding, C.; Van Veldhuisen, D.J. ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the European Society of Cardiology (ESC) Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC). *European Heart Journal* **2015**, *36*, 2793-2867, <https://doi.org/10.1093/eurheartj/ehv316>.
79. Sirikanchanarod, A.; Bumrungpert, A.; Kaewruang, W.; Senawong, T.; Pavadhgul, P. The effect of mulberry fruits consumption on lipid profiles in hypercholesterolemic subjects: A randomized controlled trial. *Journal of Pharmacy and Nutrition Sciences* **2016**, *6*, 7-14, <https://doi.org/10.6000/1927-5951.2016.06.01.2>.
80. Zhang, H.; Ma, Z.; Luo, X.; Li, X. Effects of Mulberry Fruit (*Morus alba* L.) Consumption on Health Outcomes: A Mini-Review. *Antioxidants* **2018**, *7*, 69, <https://doi.org/10.3390/antiox7050069>.
81. Chobanian, A.V. Single risk factor intervention may be inadequate to inhibit atherosclerosis progression when hypertension and hypercholesterolemia coexist. *Hypertension* **1991**, *18*, 130–131, <https://doi.org/10.1161/01.hyp.18.2.130>.
82. Townsend, N.; Wilson, L.; Bhatnagar, P.; Wickramasinghe, K.; Rayner, M.; Nichols, M. Cardiovascular disease in Europe: epidemiological update 2016. *European Heart Journal* **2016**, *37*, 3232–3245, <https://doi.org/10.1093/eurheartj/ehw334>.
83. Ma, Z. 90. F.; Lee, Y. Y. Virgin Coconut Oil and its Cardiovascular Health Benefits. *Natural Product Communications* **2016**, *11*, 1151–1152.
84. Lu, H.; Pan, W.; Wan, Q.; Cheng, L.; Shu, X.; Pan, C.; Qian, J.; Ge, J. Trends in the prevalence of heart diseases over a ten-year period from single-center observations based on a large echocardiographic database. *Journal of Zhejiang University SCIENCE B* **2016**, *17*, 54–59, <https://doi.org/10.1631/jzus.b1500136>.
85. Jabri, M.A.; Wannas, D.; Hajji, N.; Sakly, M.; Marzouki, L.; Sebai, H. Role of laxative and antioxidant properties of *Malva sylvestris* leaves in constipation treatment. *Biomedicine & Pharmacotherapy* **2017**, *89*, 29–35, <https://doi.org/10.1016/j.biopha.2017.02.020>.

86. Lee, H.; Choi, E.J.; Park, S.; Lee, J. Laxative and antioxidant effects of ramie (*Boehmeria nivea* L.) leaf extract in experimental constipated rats. *Food Science & Nutrition* **2020**, *8*, 3389–3401, <https://doi.org/10.1002/fsn3.1619>.
87. Jiang, D.Q.; Guo, Y.; Xu, D.H.; Huang, Y.S.; Yuan, K.; Lv, Z.Q. Antioxidant and anti-fatigue effects of anthocyanins of mulberry juice purification (MJP) and mulberry marc purification (MMP) from different varieties mulberry fruit in China. *Food and Chemical Toxicology* **2013**, *59*, 1-7, <https://doi.org/10.1016/j.fct.2013.05.023>.
88. Chen, W.; Lu, Y.; Hu, D.; Mo, J.; Ni, J. Black mulberry (*Morus nigra* L.) polysaccharide ameliorates palmitate-induced lipotoxicity in hepatocytes by activating Nrf2 signaling pathway. *International Journal of Biological Macromolecules* **2021**, *172*, 394–407, <https://doi.org/10.1016/j.ijbiomac.2021.01.059>.
89. Tan, B.L.; Norhaizan, M.E. Effect of High-Fat Diets on Oxidative Stress, Cellular Inflammatory Response and Cognitive Function. *Nutrients* **2019**, *11*, 2579, <https://doi.org/10.3390/nu11112579>.
90. André, C.; Dinel, A.-L.; Ferreira, G.; Layé, S.; Castanon, N. Diet-induced obesity progressively alters cognition, anxiety-like behavior and lipopolysaccharide-induced depressive-like behavior: Focus on brain indoleamine 2,3-dioxygenase activation. *Brain, Behavior, and Immunity* **2014**, *41*, 10-21, <https://doi.org/10.1016/j.bbi.2014.03.012>.
91. Tan, B.L.; Norhaizan, M.E.; Liew, W.P.P. Nutrients and Oxidative Stress: Friend or Foe?. *Oxidative Medicine and Cellular Longevity* **2018**, *2018*, 9719584, <https://doi.org/10.1155/2018/9719584>.
92. Sies, H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: *Oxidative eustress*. *Redox Biology* **2017**, *11*, 613–619. <https://doi.org/10.1016/j.redox.2016.12.035>.
93. Wang, L.; Chen, X.; Du, Z.; Li, G.; Chen, M.; Chen, X.; Liang, G.; Chen, T. Curcumin suppresses gastric tumor cell growth via ROS-mediated DNA polymerase  $\gamma$  depletion disrupting cellular bioenergetics. *Journal of Experimental & Clinical Cancer Research* **2017**, *36*, 47, <https://doi.org/10.1186/s13046-017-0513-5>.
94. Mazon, J.N.; de Mello, A.H.; Ferreira, G.K.; Rezin, G.T. The impact of obesity on neurodegenerative diseases. *Life Sciences* **2017**, *182*, 22–8, <https://doi.org/10.1016/j.lfs.2017.06.002>.
95. Muñoz, A.; Costa, M. Nutritionally Mediated Oxidative Stress and Inflammation. *Oxidative Medicine and Cellular Longevity* **2013**, *2013*, 610950, <https://doi.org/10.1155/2013/610950>.
96. Knight, J.A. Review: Free radicals, antioxidants, and the immune system. *Annals of clinical and laboratory science* **2000**, *30*, 145–158.
97. Lim, H.H.; Yang, S.J.; Kim, Y.; Lee, M.; Lim, Y. Combined treatment of mulberry leaf and fruit extract ameliorates obesity-related inflammation and oxidative stress in high fat diet-induced obese mice. *Journal of Medicinal Food* **2013**, *16*, 673-680, <https://doi.org/10.1089/jmf.2012.2582>.

## Publisher's Note & Disclaimer

The statements, opinions, and data presented in this publication are solely those of the individual Author (s) and contributor(s) and do not necessarily reflect the views of the publisher and/or the editor(s). The publisher and/or the editor(s) disclaim any responsibility for the accuracy, completeness, or reliability of the content. Neither the publisher nor the editor(s) assume any legal liability for any errors, omissions, or consequences arising from the use of the information presented in this publication. Furthermore, the publisher and/or the editor(s) disclaim any liability for any injury, damage, or loss to persons or property that may result from the use of any ideas, methods, instructions, or products mentioned in the content. Readers are encouraged to independently verify any information before relying on it, and the publisher assumes no responsibility for any consequences arising from the use of materials contained in this publication.