

Investigating the Protective Roles of Mulberry (*Morus alba* L.) Leaves in Alleviating Benzo[a]pyrene-Induced Liver Damage in Rats

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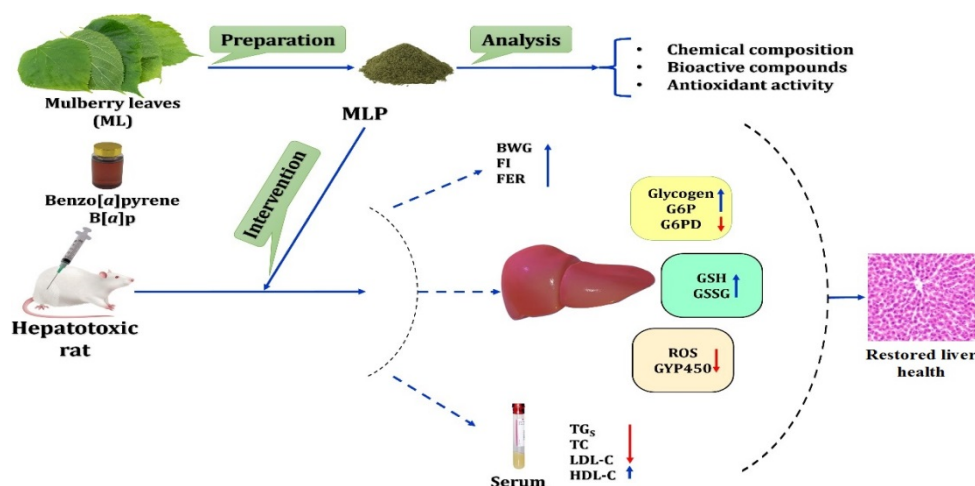
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Received April 03, 2025; Revised May 05, 2025; Accepted May 12, 2025

Abstract This study aimed to evaluate the potential protective effects of Mulberry (*Morus alba* L.) leaf powder (MLP) against benzo[a]pyrene-induced liver damage in rats. MLP was prepared, and its chemical composition, bioactive compounds, and antioxidant properties were analyzed. The results showed that MLP is rich in protein, ash, crude fiber, dietary fiber, carbohydrates, and non-nutrient antioxidants, such as phenolics, carotenoids, flavonoids, polysaccharides, tannins, and kampferol. It also demonstrated strong antioxidant activity, with values of 63.72%, 80.04%, and 82.66% in aqueous, methanol, and ethanol extracts, respectively. For the biological studies, rats were fed MLP at concentrations of 5%, 10%, and 15% (g/100 g) in their basal diet for four weeks following B[a]P-induced hepatotoxicity. Rats exposed to B[a]P showed a significant ($p \leq 0.05$) reduction in glycogen content (-66.5%) and glucose-6-phosphate dehydrogenase (G6PD) activity (-60%), while glucose-6-phosphatase (G6Pase) activity increased substantially by 279.7% compared to the normal control group. Additionally, B[a]P exposure resulted in a significant ($p \leq 0.05$) increase in the activity of drug-metabolizing enzymes (cytochrome P450, CYP450, 64.6%) and reactive oxygen species (ROS, 267.2%), alongside a significant ($p \leq 0.01$) decrease in serum triglycerides (TGs), high-density lipoprotein cholesterol (HDL-c), and glutathione (GSH) levels, with reductions of -15.6%, -37.3%, and -58.4%, respectively, compared to the normal control group. In contrast, the inclusion of MLP in the rat diets for 28 days resulted in significant improvements in liver function, antioxidant status, and lipid metabolism, along with positive changes in liver histopathology. In conclusion, these results suggest that MLP may have potential as a hepatoprotective agent for mitigating liver damage.



Keywords: Chemical composition, bioactive compounds, liver functions, CYP450, serum lipid profile, glutathione

Cite This Article: Yousif A. Elhassaneen, Sobhi I. Hassab El-Nabi, Nazeha A. Khalil, and Sara A. Abd ElMaksoud, "Investigating the Protective Roles of Mulberry (*Morus alba* L.) Leaves in Alleviating Benzo[a]pyrene-Induced Liver Damage in Rats." *American Journal of Public Health Research*, vol. 13, no. 3 (2025): 117-132. doi: 10.12691/ajphr-13-3-3.

1. Introduction

The liver, being the largest solid organ in the body, performs numerous critical functions that are essential to overall health. These include the production of bile, the absorption and metabolism of bilirubin, regulation of blood clotting, and the breakdown of carbohydrates and fats. Additionally, the liver is responsible for storing vitamins and minerals, assisting in protein metabolism, filtering blood, supporting immune functions, producing albumin, and synthesizing angiotensinogen [1]. The liver plays a pivotal role in biotransformation, helping to eliminate toxins and xenobiotics from the body [2,3,4]. Despite its remarkable ability to compensate for early damage, this functional reserve may obscure the initial signs of liver dysfunction. However, when liver diseases progress or bile flow becomes obstructed, the consequences can rapidly become life-threatening. Consequently, liver diseases present a major global health challenge [5,6].

In Egypt, the incidence of liver diseases has significantly increased over the past decade, with the rate of cases doubling in this period. This rise is attributed to both biological factors, such as viral infections, particularly hepatitis, and environmental exposures to toxins like pesticides, aflatoxins, and polycyclic aromatic hydrocarbons [7,8,9,10,11,12,13,14]. Other contributing factors include tobacco use, exposure to hazardous chemicals and heavy metals, as well as endemic infections such as schistosomiasis, all of which play a substantial role in the development and progression of liver disease [15,16,17]. Recent data indicates that hepatitis C continues to be one of the primary causes of liver diseases in Egypt, with approximately 10 million people infected [18]. Additionally, liver cancer has become a leading cause of cancer-related deaths in the country, with Egypt now having one of the highest rates of liver cancer worldwide. Studies show that liver cancer now accounts for 11% of all cancer deaths, a significant increase from 4% in 1993 [19,20]. Moreover, schistosomiasis remains a prevalent concern and is a key risk factor for liver cirrhosis, while Non-Alcoholic Fatty Liver Disease (NAFLD) is also increasing, with studies indicating that 30% of Egyptians are affected by some form of liver fat accumulation [21,22]. It is well-established that food processing and cooking can generate harmful compounds, especially when the necessary precursors are present [23,24,25]. Among these compounds, polycyclic aromatic hydrocarbons (PAHs), which form during incomplete combustion, are found in a variety of foods, particularly those that are charcoal-broiled, roasted, fried, or smoked [26,27,28,29].

Benzo[a]pyrene (B[a]P), a specific member of the PAH family, is produced as a by-product of the incomplete combustion of organic (carbon-containing) materials such as cigarettes, gasoline, and wood. B[a]P is commonly present in cigarette smoke, grilled, fried, and broiled foods, and as a by-product of various industrial processes [9,25]. In addition, B[a]P can be found in ambient outdoor air, indoor air, and some water sources [30]. Several PAHs, including B(a)P, have been demonstrated to be toxic, mutagenic, and carcinogenic through extensive *in vivo*

[31,32,33] and *in vitro* [2,34] studies. Moreover, exposure to B[a]P is linked to liver toxicity and cancer development across vertebrate species [2,31,33,34]. The carcinogenic, mutagenic, and tumorigenic effects of B[a]P are primarily associated with its metabolic conversion into reactive intermediates, such as arene oxides, phenols, quinones, dihydrodiols, and epoxides, which can bind covalently to DNA, forming DNA adducts [2,31,35]. This biochemical interaction is followed by cell proliferation, which is considered an essential step in the fixation of these changes. The mutagenic activity of B[a]P depends on its metabolic activation, and thus, B[a]P is regarded as a promutagen [2].

Liver recent pharmacological therapy is costly and associated with several side effects resulting in patient non-compliance. Thus, there is a need to explore alternative therapies particularly from herbal/plant sources as these are cost effective and possess minimal side effects. Also, it was reported that some plant parts exercise various bioactivities, including antioxidant, anti-inflammatory, antidiabetic, anticarcinogenic, antimutagenic etc., [36,37,38,39]. One of the less studied plant is the mulberry (*Morus alba* L.). Different parts of the mulberry plant have been used over the centuries in traditional medicine as a common agent to treat a variety of conditions including diabetes, cardiovascular diseases, and cancer and for activating the immune system through potent antioxidant activity [40]. The mulberry leaves are nutritious, palatable and nontoxic. Several studies indicated that mulberry leaves contain many nutrients such proteins, dietary fiber and carbohydrates; minerals such iron, zinc, calcium, magnesium and phosphorous and vitamins such A, B, C, D and E [41,43]. Also, many bioactive compounds such flavonoids, phenolics acids, quercetin, isoquercetin and alkaloids have been found in mulberry leaves [42,43]. Such bioactive compounds found in mulberry leaves possesses medical benefits, including diuretic, hypoglycemic, antibacterial, antiviral, hypotensive properties and neuroprotective functions [44]. Unfortunately, there is a dearth of information regarding the effect of mulberry leaves on liver diseases. The objective of this study is to explore the potential protective effects of mulberry (*Morus alba* L.) leaves in mitigating liver damage induced by benzo[a]pyrene in experimental rats. Additionally, the study will assess the bioactive compounds present in mulberry leaves and evaluate their antioxidant properties.

2. Materials and Methods

2.1. Materials

Mulberry leaves (*Morus alba* L.) were sourced from wild trees growing along public irrigation canals in Mit Ghoarb Village, Sinbellaween Center, Dahliya Governorate, Cairo, Egypt, through special arrangements. Standards for benzo[a]pyrene and bioactive compounds (gallic acid, catechin and resveratrol), and antioxidants (α -tocopherol, and butylated hydroxytoluene), were procured from Sigma Chemical Co. (St. Louis, MO, with a local agent in Cairo, Egypt). Casein was obtained from Morgan Chemical Co., Cairo, Egypt. All other chemicals, vitamins,

salt mixtures, reagents, and solvents (unless otherwise specified) were of analytical grade and were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals, and Medical Instruments, Cairo, Egypt. Assay kits for glucose, glucose-6-phosphate dehydrogenase activity (G6Pase), glucose-6-phosphatase activity (G6PD), and malondialdehyde (MDA) were supplied by BIODIAGNOSTIC, Dokki, Giza, Egypt. Additionally, kits for triglycerides (TGs), total cholesterol (TC), HDL-cholesterol, and LDL-cholesterol were obtained from El-Nasr Pharmaceutical Chemicals.

2.2. Methods

2.2.1. Chemical Studies

2.2.1.1. Preparation of Mulberry Leaves Powder (MLP)

Mulberry leaves were carefully handpicked to discard any damaged leaves, washed thoroughly with water, and subsequently dried using a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., PA) in two stages. Initially, the leaves were dried at 60°C for 3 hours, followed by drying at 40°C for 10 hours, until the moisture content of the final product reached approximately 8%. After drying, the leaves were ground into a fine powder using a high-speed mixer (Moulinex Egypt, Al-Araby Co., Benha, Egypt). The resulting powder was then passed through an 80-mesh sieve, and the retained powder was collected for further use.

2.2.1.2. Preparation of Mulberry Leaves Powder (MLP) Extracts

Twenty grams of dried mulberry leaf powder were mixed with 180 mL of water and homogenized. The mixture was then placed in a beaker and agitated at 200 rpm for 1 hour at room temperature using an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany). Afterward, the extract was filtered through Whatman No. 1 filter paper to separate the liquid from the solid residue. The remaining residue was subjected to two additional extractions, and the resulting extracts were combined. The solvent was then removed using a rotary evaporator at 55°C under reduced pressure (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). This extraction process was repeated using 80% methanol and ethanol as solvents, and the residual solvent was removed under reduced pressure at 45°C.

2.2.1.3. Determination of Proximate of MLP

MLP samples were chemically analyzed for moisture, protein, fat, ash, fiber dietary fiber and essential oil contents were determined using the methods described in the A.O.A.C. [45]. Carbohydrates calculated by differences using the following equation: carbohydrates (%) = 100 - (% moisture + % protein + % fat + % Ash + % fiber).

2.2.1.4. Bioactive Compounds Determination in MLP Extracts

The total phenolic content in the MLP extracts was quantified using the Folin-Ciocalteu reagent, following the procedures outlined by singleton and Roosi, [46] and Wolfe et al., [47]. For carotenoid determination, the

method described by Lichtenthaler, [48] was applied, utilizing an 80% acetone extract. Flavonoid content was measured through a colorimetric assay as outlined by Zhisen et al., [49] and the results were expressed in terms of catechin equivalent (CAE) per gram of dry extract using the equation from the standard curve: $y = 0.0003x - 0.0117$ ($R^2 = 0.9827$). Polysaccharide content was evaluated through spectroscopic analysis with a UV-visible spectrophotometer, based on the method described by Vazirian et al., [50] with starch serving as the standard. Tannin content was determined using the procedure by Van- Burden et al., [51] and expressed as catechin equivalents per gram of dry weight. The colorimetric method described by Kumari et al., [52] was employed to detect kaempferol. This method involves the reaction of kaempferol with $AlCl_3$ to form a yellow complex, which is then analyzed spectrophotometrically at 425 nm, with results compared to known standards. For total chlorophyll measurement in mulberry plant parts, the spectrophotometric technique by Porra et al., [53] was utilized. This involved chlorophyll extraction with 80% acetone and measuring the absorbance at 663 nm and 645 nm, with the total chlorophyll content being the sum of chlorophyll a and b.

2.2.1.5. Antioxidant Activity of MLP

Antioxidant activity of MLP extracts and standards (α -tocopherol, BHA, and BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the β -carotene bleaching method following a modification of the procedure described by Marco, [54].

2.2.2. Biological Experiments

2.2.2.1. Animals

The study utilized adult male albino rats (180 ± 10 g each), which were sourced from the Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt.

2.2.2.2. Basal Diet

The basal diet was prepared following the formula outlined by Reeves et al. [55]. and consisted of the following components: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride (0.2%), methionine (0.3%), cellulose (5%), with the remaining portion made up of corn starch (69.5%). The compositions of the salt and vitamin mixtures used in the basal diet were also based on the same reference.

2.2.2.3. Experimental Design

All animal experiments adhered to the guidelines set by the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council by NRC, [56]. Thirty rats were housed individually in wire cages in a controlled environment at $24 \pm 3^\circ\text{C}$, with relative humidity maintained at $54 \pm 3\%$, a 12-hour light/dark cycle, and kept under normal health conditions. The rats were provided with a basal diet for one week before starting the experiment to allow for acclimatization. After this period, the rats were divided into two main experimental groups. The first group (Group 1, 6 rats) served as the negative control, receiving the basal diet and

an injection of NaCl solution containing 0.1% Tween 20 (5 ml/kg body weight), which was used as the vehicle for treating the B[a]P group. The second group (24 rats) was injected intraperitoneally with B[a]P (100 mg/5 ml/kg body weight) dissolved in a 0.9% NaCl solution containing 0.1% Tween 20, inducing liver damage according to the protocol of Hasegawa et al., [57]. The B[a]P-treated rats were further divided as follows: Group 2 (model control) remained on the basal diet without treatment, and Groups 3–5 were fed the basal diet supplemented with 5%, 10%, and 15% (w/w) mulberry leaf powder (MLP) for 28 days, respectively. The concentrations of MLP were selected based on preliminary experiments (data not shown).

2.2.2.4. Biological Evaluation

Daily records of food intake were maintained throughout the 28-day period, with body weight measurements taken weekly. Body weight gain (BWG%), food intake (FI), and food efficiency ratio (FER) were evaluated using the methods of El-khawaga et al., [58]. Calculations were made using the following formulas:

$$\text{BWG (\%)} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$$

$$\text{FER} = \frac{\text{grams of body weight gained (g over 28 days)}}{\text{grams of feed consumed (g over 28 days)}}$$

2.2.2.5. Blood Sampling and Preparation

At the end of the 4-week experiment, blood samples were collected following a 12-hour fasting period. The rats were anesthetized with ether, and blood was drawn via the abdominal aorta. The blood was placed in clean, dry centrifuge tubes, allowed to clot at room temperature, and then centrifuged at 3000 rpm for 10 minutes to separate the serum by Drury and Wellington., [59]. The serum was transferred to clean tubes and stored at -20°C for subsequent analysis.

2.2.2.6. Organ Sampling and Preparation

Liver samples were carefully dissected from the rats after euthanasia, and any residual blood was removed. The organs were washed with cold saline, dried with filter paper, weighed, and fixed in a 10% formalin solution for histological examination, as described by Dury and wallington ., [59]. Liver homogenates were prepared as described by El-khawaga et al., [60]. A piece of liver tissue was accurately weighed and homogenized in ice-cold 0.9% saline using a Teflon pestle attached to a homogenizer motor. The homogenate was diluted to obtain a 5% (w/v) liver homogenate, then centrifuged at 5000 rpm for 30 minutes at 4°C to remove cell debris and nuclei. The resulting supernatant was used for biochemical analysis.

2.2.2.7. Hematological Analysis

a. Liver Functions

Glycogen content in liver homogenates was measured according to Damsbo et al., [61]. The activity of hepatic glucose-6-phosphate dehydrogenase (G6PD) was assessed as per the method of chan et al., [62] while hepatic glucose-6-phosphatase (G6Pase) activity was determined using the method described by Rossetti et al., [63].

b. Serum Lipid Profile

Serum lipid profile [triglycerides (TGs), total cholesterol (TC), high density lipoprotein- cholesterol, HDL-c, and low density lipoprotein-cholesterol, LDL-c] was determined in serum using the methods of Ahmadi et al., [64] and Fossati and Prenape.,[65].

c. Redox Status

Reduced (GSH) and oxidized (GSSG) glutathione levels in serum samples were quantified colorimetrically according to Elman et al., [66]. Malondialdehyde (MDA) levels in the serum were measured using the thiobarbituric acid (TBA) method described by Buege et al., [67].

d. Drug Metabolizing Enzymes (Cytochrome P-450)

Cytochrome P-450 activity was determined by carbon monoxide difference spectrophotometry of dithionite-reduced samples, following the method of Omura et al., [68].

2.2.2.8. Histopathological Investigation

Small liver tissue samples were taken from all experimental groups and fixed in 10% neutral-buffered formalin. The samples were then dehydrated through increasing ethanol concentrations (70%, 80%, and 90%), cleared in xylene, and embedded in paraffin. Sections with a thickness of 4-6 µm were prepared and stained with Hematoxylin and Eosin, following the methodology outlined to Bancroft et al., [69].

2.3. Statistical Analysis

All measurements were carried out in triplicate and presented as mean ± standard deviation (SD). Statistical analysis was conducted using the Student's t-test and the MINITAB 12 software (Minitab Inc., State College, PA). Data were expressed as mean ± SD. To assess the significance of differences, one-way ANOVA was performed followed by Duncan's test for multiple comparisons, using the MINITAB 12 software (Minitab Inc., State College, PA). A p-value of ≤ 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Chemical Studies

3.1.1. Proximate Chemical Composition of Mulberry (*Morus alba* L.) Leaves Powder

The proximate chemical composition of Mulberry (*Morus alba* L.) leaves powder (MLP) offers valuable insights into its nutritional profile and potential health benefits. The moisture content of MLP ranged from 6.42% to 7.19%, with a mean value of $6.89 \pm 0.29\%$, which is relatively low and consistent with findings from other dried plant materials such as Moringa and Fenugreek [70,71]. This low moisture content is crucial for preserving the leaves, reducing microbial growth, and extending shelf life. Regarding protein content, MLP demonstrated a range of 13.54% to 15.04%, with a mean of $14.09 \pm 0.82\%$, making it a good plant-based protein source. This aligns with studies on other leafy vegetables like Spinach and Amaranth suggesting that MLP can

contribute significantly to protein intake, particularly in vegetarian and vegan diets [72]. The crude fat content in MLP ranged from 1.30% to 1.56%, with a mean value of $1.48 \pm 0.11\%$, indicating that MLP is low in fat, making it an ideal choice for health-conscious consumers and weight management diets, similar to other plant sources like Spinach and Cabbage [72,73]. Additionally, the crude fiber content of MLP, ranging from 8.92% to 10.52% (mean $9.73 \pm 0.72\%$), is comparable to other fiber-rich greens like Moringa highlighting its role in promoting digestive health and supporting weight management [74,75]. The ash content of MLP, ranging from 1.32% to 1.57%, with a mean of $1.45 \pm 0.09\%$, falls within the typical range for green leafy vegetables [73,76]. The carbohydrate content, determined by difference, ranged from 35.65% to 39.12%, with a mean of $36.67 \pm 2.15\%$, suggesting that MLP contains moderate carbohydrates primarily in the form of sugars and starch, which aligns with similar findings in Moringa and Spinach [71,74]. Overall, MLP's proximate chemical composition aligns well with other commonly consumed green leafy plants, showing its promise as a nutritious source of protein, fiber, and low fat [77,78,79,80,81,82]. The higher protein and fiber content compared to many other leafy greens positions mulberry leaves as a valuable functional food that can support muscle maintenance, digestive health, and weight management, particularly in plant-based diets. The potential health benefits of MLP highlight its importance as a dietary component for those seeking plant-based alternatives for protein and fiber intake, aligning with the growing interest in plant-based nutrition.

Table 1. Proximate chemical composition (g/100) of Mulberry (*Morus alba* L.) leaves powder (MLP)

Factor	Range	Mean $\pm$ SD
Moisture	6.42 – 7.19	6.89 $\pm$ 0.29
Total protein	13.54 – 15.04	14.09 $\pm$ 0.82
Crude fat	1.30 – 1.56	1.48 $\pm$ 0.11
Crude fiber	8.92 – 10.52	9.73 $\pm$ 0.72
Ash	1.32 – 1.57	1.45 $\pm$ 0.09
Carbohydrates (by difference)	35.65 – 39.12	36.67 $\pm$ 2.15

Values represent the mean $\pm$ SD (n=3).

3.1.2. Some Bioactive Compounds Content of Mulberry (*Morus alba* L.) Leaves Powder (MLP)

The bioactive compound content of Mulberry (*Morus alba* L.) leaves powder (MLP) presented in Table 2 reveals its potential as a functional food with notable health benefits. The total phenolic content in MLP ranged from 692.66 to 706.78 mg gallic acid/100g, with a mean of 711.45 ± 12.67 mg/100g, reflecting its strong antioxidant properties that help reduce oxidative stress and the risk of chronic diseases like cancer and cardiovascular conditions [76,83]. Carotenoids, particularly beta-carotene, were also found in substantial amounts, with total carotenoids ranging from 309.78 to 330.67 mg catechin/100g (mean: 321.56 ± 9.17 mg/100g), which are beneficial for improving vision health and potentially reducing cancer risks [74]. The flavonoid content, ranging from 621.78 to 645.89 mg RE/100g (mean: 647.52 ± 21.76 mg/100g), highlights the presence of compounds

like quercetin and kaempferol, known for their antioxidant, anti-inflammatory, and anticancer properties [75,72]. Polysaccharides, important for immune modulation and gut health, were also present, though at relatively low levels (mean: 8.63 ± 0.43 g/100g), which suggests a modest contribution compared to other plant sources [84]. Tannins, with a mean of 817.43 ± 20.08 mg catechin/100g, provide additional antioxidant and anti-inflammatory effects, while kaempferol (mean: 49.42 ± 1.91 mg/100g) offers potential benefits in reducing the risk of heart disease and providing neuroprotective effects [73]. The chlorophyll content (mean: 112.67 ± 3.65 mg/100g) further supports mulberry leaves' detoxifying and antioxidant properties, aiding liver function and detoxification [83]. Essential oils, which possess antimicrobial, antifungal, and antioxidant properties, were also found in MLP (mean: 61.78 ± 2.17 mg/100g) and the dietary fiber content (mean: 21.61 ± 1.98 g/100g) contributes to gastrointestinal and cardiovascular health [76, 84]. Overall, MLP's bioactive compounds content aligns well with other commonly consumed green leafy plants, showing its promise as a rich source of their constituents [79,80,81,82,85]. In conclusion, the bioactive compounds in mulberry leaves powder, including phenolics, carotenoids, flavonoids, tannins, and essential oils, emphasize its health-promoting properties, particularly through antioxidant, anti-inflammatory, and anticancer effects. These findings confirm the potential of mulberry leaves as a valuable functional food ingredient for human nutrition and health.

Table 2. Some bioactive compounds content of Mulberry (*Morus alba* L.) leaves powder (MLP)

Compounds	Range	Mean $\pm$ SD
Total phenolics (mg gallic acid.100 g ⁻¹)	692.656 – 706.78	711.45 $\pm$ 12.67
Total carotenoids (mg catechin.100 g ⁻¹)	309.78 – 330.67	321.56 $\pm$ 9.17
Total flavonoids (mg resveratrol, RE.100 g ⁻¹)	621.78 – 645.89	647.52 $\pm$ 21.76
Polysaccharides (g starch. 100 g ⁻¹)	8.12 – 829.15	8.63 $\pm$ 0.43
Tannins (mg catechine. 100 g ⁻¹)	801.78 – 841.09	817.43 $\pm$ 20.08
Kampferol (mg.100g ⁻¹)	47.02 – 51.98	49.42 $\pm$ 1.91
Total chlorophyll (mg. 100g ⁻¹)	109.11 – 115.20	112.67 $\pm$ 3.65
Essential oil (mg. 100g ⁻¹)	58.34 – 63.19	61.78 $\pm$ 2.17
Dietary fiber (g.100g ⁻¹)	19.67 – 23.49	21.61 $\pm$ 1.98

Values represent the mean $\pm$ SD (n=3).

3.1.3. Antioxidant Activity of Mulberry (*Morus alba* L.) Leaves Powder (MLP)

The antioxidant activity (AA) of MLP was evaluated using different solvents (water, methanol, and ethanol) and compared to standard antioxidants, such as BHT (butylated hydroxy toluene) and α -tocopherol (vitamin E). The results presented in Table 3 demonstrate that the antioxidant activity of MLP varies with the solvent used for extraction, highlighting the importance of selecting an appropriate solvent for maximizing the extraction of bioactive compounds. In water, MLP exhibited a moderate antioxidant activity of $63.72 \pm 1.23\%$, which may be attributed to the solubility of water-soluble compounds like phenolics and flavonoids known for their free radical scavenging activity [74]. However, the activity in water

was lower compared to methanol and ethanol, suggesting that some bioactive compounds in mulberry leaves are more effectively extracted using organic solvents. The methanol extract showed an antioxidant activity of $80.04 \pm 2.01\%$, consistent with the ability of methanol to extract polyphenolic compounds, which are key contributors to antioxidant properties [71]. The highest antioxidant activity ($82.66 \pm 0.88\%$) was observed in the ethanol extract, which can solvate a wider range of both polar and non-polar compounds, such as flavonoids and tannins, that contribute significantly to antioxidant activity [75]. These results align with findings from other studies, where ethanol was found to be particularly effective in extracting antioxidant compounds [83]. In comparison with the standard antioxidants, BHT showed antioxidant activity ranging from 71.61% to 95.06%, which is similar to the activity observed in the methanol and ethanol extracts of mulberry leaves. α -Tocopherol, a natural antioxidant, exhibited the highest activity at 96.93%, but the antioxidant activity of mulberry leaf extracts, while lower, still demonstrated significant potential compared to many other plant-based antioxidants [74]. Statistical analysis ($p \leq 0.05$) revealed significant differences in antioxidant activity across the different solvents, further supporting the conclusion that the choice of solvent plays a crucial role in maximizing antioxidant extraction from plant materials [73]. Numerous studies have highlighted a strong and significant positive relationship ($p < 0.01$) between the bioactive compounds found in Moringa leaves powder (MLP) and its antioxidant activity across various plant parts [39,86,87,88]. Antioxidants play a crucial role in shielding cells from oxidative stress, a physiological process that can potentially cause cellular damage. Oxidative stress is believed to be linked to the onset of various chronic diseases, including cancer, cardiovascular diseases, diabetes, rheumatoid arthritis, obesity, and age-related conditions such as neurodegenerative disorders like Parkinson's and Alzheimer's disease [38,39,89,90]. The phytochemical composition and antioxidant properties of MLP suggest that this food could be effectively utilized as a functional food. The current findings partially align with observations from several researchers who reported that various plant parts, including leaves, food processing by-products and agricultural residues, exhibit high antioxidant activity due to their substantial bioactive compound content [10,11,12] [91,92,93,94] [95,96,97,98] [99,100,101] [102,103,104]. For instance, Elbasouny et al., [92] reported that MLP demonstrates significant antioxidant capacity and free radical inhibition, attributed to its polyphenolic content. In conclusion, the antioxidant activity of mulberry leaves powder is promising, particularly when extracted with ethanol and methanol, making them valuable natural sources of antioxidants with potential health benefits. These findings underscore the significant antioxidant potential of mulberry leaves, which can be considered a natural alternative to synthetic antioxidants like BHT and even compare favorably with well-known natural antioxidants like α -tocopherol.

Table 3. Antioxidant activity (AA, %) of Mulberry (*Morus alba* L.) leaves powder (MLP)

Factor	In water (H ₂ O)	In methanol (MeOH)	In ethanol (EtOH)
<u>Antioxidant activity (AA):</u>			
AA (%)	63.72 ± 1.23^b	80.04 ± 2.01^a	82.66 ± 0.88^a
AA [% of BHT (50 mg/ml)]	71.61 ± 0.78^b	89.95 ± 1.09^a	92.90 ± 0.65^a
AA [% of BHT (100 mg/ml)]	67.03 ± 1.17^b	84.20 ± 0.88^a	86.96 ± 0.34^a
AA [% of α -tocopherol (50 mg/ml)]	65.74 ± 0.98^b	82.58 ± 1.11^a	85.28 ± 0.56^a
<u>Standards:</u>			
BHT (50 mg/ml)	88.98 ± 0.12	88.98 ± 0.12	88.98 ± 0.12
BHT (100 mg/ml)	95.06 ± 0.09	95.06 ± 0.09	95.06 ± 0.09
α -tocopherol (50 mg/ml)	96.93 ± 0.03	96.93 ± 0.03	96.93 ± 0.03

Values represent the mean $\pm$ SD (n=3). Means in the same raw with the different superscript letters were significantly ($p \leq 0.05$).

3.2. Biological Studies

3.2.1. Effect of Four Weeks Treatment with Mulberry (*Morus alba* L.) Leaves Powder (MLP) on BWG, FI and FER in Rats with of Liver Disorders Induced by B[a]P

The effects of MLP treatment on BWG, FI, and FER in rats with B[a]P-induced hepatotoxicity are presented in Tables (4 and 5). The results revealed that rats treated with B[a]P showed a significant ($p \leq 0.05$) reduction in BWG (-51.4%), FI (-40.5%), and FER (-43.1%) when compared to the normal control group. In contrast, administration of MLP (at doses of 5, 10, and 15 g/100g diet) for 28 days led to a significant ($p \leq 0.05$) improvement in all three parameters: BWG, FI, and FER. The increase in these measures was observed to be dose-dependent with the MLP treatment. The results from this study suggest that Mulberry leaves powder has a beneficial effect in improving BWG, FI, and FER in rats with liver disorders induced by B[a]P. This is likely due to the potent hepatoprotective and antioxidant properties of *Morus alba*, as reported in previous studies [105]. The active compounds in Mulberry leaves, including polyphenols, flavonoids, and anthocyanins, are thought to play a crucial role in mitigating oxidative stress and promoting liver regeneration [106]. In terms of dosage, the highest beneficial effects were observed with a dose of 15 g/100 diet, which aligns with the findings of other studies that have demonstrated dose-dependent effects for plant-derived hepatoprotective agents [107,108]. Other previous studies reported that injected rats by carbon tetrachloride or B[a]P caused decrease in both FER and BWG which significantly improved by consumption of plant parts contains bioactive compounds such as found in MLP [109,110,111] [112,113,114,115]. Therefore, future studies should focus on optimizing the dosage and assessing long-term effects, as well as exploring the molecular mechanisms through which MLP mediates these beneficial effects.

3.2.2. Effect of Four Weeks Treatment with Mulberry (*Morus alba* L.) leaves Powder (MLP) on Glycogen Content, G6PD and G6P in Rats with of Liver Disorders Induced by B[a]P

The effects of MLP on liver functions in rats with B[a]P-induced hepatotoxicity are shown in Tables (6 and 7). At the end of the 28-day treatment period, rats exposed to B[a]P exhibited a significant ($p \leq 0.05$) reduction in glycogen content (-66.5%) and G6PD activity (-60.0%), while G6Pase activity notably increased by 279.7% when compared to the normal control group. In contrast, MLP treatment at doses of 5, 10, and 15 g/100g diet significantly ($p \leq 0.05$) improved glycogen content and G6PD activity, while simultaneously decreasing G6Pase activity. The effectiveness of MLP treatment varied with the dose, showing a dose-dependent response where the improvements in glycogen content and G6PD activity, along with the reduction in G6Pase activity, became more pronounced at higher doses of MLP. The results of this study are consistent with previous investigations that emphasize the harmful impact of B[a]P on liver function, leading to progressive damage in experimental models [100,114,116,117] [118,119,120,121]. The liver injury resulting from B[a]P exposure is attributed to its metabolic conversion into reactive intermediates such as arene oxides, phenols, quinones, and dihydrodiols, which

bind to DNA and form genotoxic adducts [2,31,102,103] [104,122,123,124]. Additionally, oxidative stress plays a critical role in inducing cytotoxicity in liver cells, leading to mitochondrial and lysosomal dysfunction, as well as damage to the cell membrane [2,9,25,34] [101,102,103,104] [125,126,127]. Moreover, the findings suggest that B[a]P-induced hepatotoxicity disrupts carbohydrate and lipid metabolism, which are critical for liver function [100,101,116] [117,118,119,120]. This is reflected in the decrease in hepatic glycogen, G6PD activity, and HDL-cholesterol levels, along with an increase in G6Pase activity, total cholesterol, and LDL-cholesterol compared to the normal-control group. The marked reduction in hepatic glycogen in the hepatotoxic group points to impaired glycogenesis due to liver dysfunction [101,102,116]. The restoration of glycogen content, which signals liver regeneration, aligns with previous studies demonstrating the hepatoprotective properties of MLP [107,108]. The recovery of G6PD activity further suggests that MLP may possess antioxidant properties that help mitigate the oxidative damage caused by B[a]P. Additionally, the reduction in G6Pase activity implies a potential normalization of gluconeogenesis, which is vital for the regulation of liver metabolism [128].

Table 4. Effect of four weeks treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on BWG, FI and FER in rats with of liver disorders induced by B[a]P

Groups	BWG (%)		FI (g/day/rat)		FER	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
G1: Normal control	0.94 – 1.03	1.01 $\pm$ 0.03 ^a	13.07 – 15.59	14.4 $\pm$ 1.03 ^a	0.077- 0.084	0.082 $\pm$ 0.004 ^a
G2: Model control	0.43 – 0.52	0.49 $\pm$ 0.04 ^c	8.01 – 10.03	8.56 $\pm$ 0.87 ^d	0.047 – 0.058	0.054 $\pm$ 0.006 ^d
G3: MLP (5 g/100 diet)	0.58- 0.61	0.59 $\pm$ 0.02 ^c	9.05 – 10.55	9.36 $\pm$ 0.69 ^{cd}	0.067- 0.065	0.061 $\pm$ 0.003 ^c
G4: MLP (10 g/100 diet)	0.74- 0.85	0.81 $\pm$ 0.06 ^b	10.23 – 12.05	10.98 $\pm$ 0.72 ^c	0.069- 0.074	0.070 $\pm$ 0.002 ^b
G5: MLP (15 g/100 diet)	0.86- 0.96	0.88 $\pm$ 0.05 ^b	11.51 – 12.41	12.08 $\pm$ 0.47 ^b	0.068 – 0.076	0.074 $\pm$ 0.004 ^b

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. MLP, Mulberry leaves powder, G1, normal group/healthy rats group; G2, model (hepatotoxic) rats group; G3, G4 and G5, model group treated with 5, 10 and 15 g/100 diet of MLP. BWG, body weight gain; FI, feed intake; FER, feed efficiency ratio.

Table 5. Effect of four weeks treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on BWG, FI and FER (as a percent of change) in rats with of liver disorders induced by B[a]P

Groups	BWG	FI	FER
G1: Normal control	0.00	0.00	0.00
G2: Model control	-51.4	-40.5	-43.1
G3: MLP (5 g/100 diet)	+20.4	+9.3	+12.9
G4: MLP (10 g/100 diet)	+65.3	+28.2	+29.6
G5: MLP (15 g/100 diet)	+79.5	+41.1	+37.03

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. MLP, Mulberry leaves powder, G1, normal group/healthy rats group; G2, model (hepatotoxic) rats group; G3, G4 and G5, model group treated with 5, 10 and 15 g/100 diet of MLP. BWG, body weight gain; FI, feed intake; FER, feed efficiency ratio.

Table 6. Effect of four weeks treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on glycogen content, G6PD and G6P in rats with of liver disorders induced by B[a]P

Groups	Glycogen content (mg/g wet tissue)		Glucose-6-phosphate dehydrogenase activity (G6PD, U/g wet tissue)		Glucose-6-phosphatase activity (G6Pase, μ mole/min/g wet tissue)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
G1: Normal control	11.64-15.93	13.78 $\pm$ 1.75 ^a	14.10-17.54	15.68 $\pm$ 1.41 ^a	2.76-3.21	3.01 $\pm$ 0.18 ^d
G2: Model control	3.89-5.76	4.61 $\pm$ 0.81 ^d	5.76-7.11	6.27 $\pm$ 0.59 ^d	9.89-13.84	11.43 $\pm$ 1.72 ^a
G3: MLP (5 g/100 diet)	5.46-6.13	5.9 $\pm$ 0.31 ^d	8.32-8.62	8.49 $\pm$ 0.132 ^c	9.11-10.34	9.77 $\pm$ 0.50 ^{ab}
G4: MLP (10 g/100 diet)	5.67-8.18	7.01 $\pm$ 1.02 ^c	9.34-10.54	9.94 $\pm$ 0.48 ^{bc}	6.42-7.39	6.89 $\pm$ 0.39 ^b
G5: MLP (15 g/100 diet)	7.86-9.61	8.81 $\pm$ 0.72 ^b	10.98-12.65	11.56 $\pm$ 0.76 ^b	5.11-6.62	5.83 $\pm$ 0.61 ^c

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. MLP, Mulberry leaves powder, G1, normal group/healthy rats group; G2, model (hepatotoxic) rats group; G3, G4 and G5, model group treated with 5, 10 and 15 g/100 diet of MLP.

Table 7. Effect of four weeks treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on glycogen content, G6PD and G6P (as a percent of change) in rats with of liver disorders induced by B[a]P

Groups	Glycogen content	Glucose-6-phosphate dehydrogenase activity	Glucose-6-phosphatase activity
G1: Normal control	0.00	0.00	0.00
G2: Model control	-66.5	-60.0	279.7
G3: MLP (5 g/100 diet)	+27.9	+35.4	-14.5
G4: MLP (10 g/100 diet)	+52.06	+58.5	-39.7
G5: MLP (15 g/100 diet)	+91.1	+84.3	-48.9

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. MLP, Mulberry leaves powder, G1, normal group/healthy rats group; G2, model (hepatotoxic) rats group; G3, G4 and G5, model group treated with 5, 10 and 15 g/100 diet of MLP.

3.2.3. Effect of four Weeks Treatment with Mulberry (*Morus alba* L.) Leaves Powder (MLP) on Serum Lipid Profile (mmol/L) in Rats with of Liver Disorders Induced by B[a]P

The effect of MLP on the serum lipid profile, which includes triglycerides (TGs), total cholesterol (Cho), and low-density lipoprotein cholesterol (LDL-c) in rats with B[a]P-induced hepatotoxicity, is shown in Tables (8 and 9). At the conclusion of the four-week study, B[a]P-treated rats exhibited a significant ($p \leq 0.05$) decrease in TGs (-16.6%) and HDL-c (-37.3%), along with a significant increase in Cho (+30.3%) and LDL-c (+71.5%) compared to the normal control group. In contrast, MLP treatment at doses of 5, 10, and 15 g/100g diet for four weeks led to a significant ($p \leq 0.05$) increase in TG and HDL-c levels, while decreasing Cho and LDL-c levels compared to the B[a]P-treated control group. Additionally, the effects of MLP were found to be dose-dependent, with higher doses resulting in more pronounced improvements in lipid metabolism. Previous research has similarly demonstrated that exposure to B[a]P significantly decreases serum triglycerides (TGs) and HDL-c, while increasing cholesterol levels, which is likely a result of liver dysfunction [115,116,129,130]. The improvements in serum lipid profiles observed with MLP treatment, particularly the increase in HDL-c and the reduction in LDL-c and total cholesterol (TCho), suggest that MLP

may have a protective role in lipid metabolism, possibly by enhancing liver function and reducing oxidative stress. The dose-dependent effects seen in the treatment groups further reinforce the idea that MLP may work through mechanisms that regulate lipid synthesis, cholesterol transport, and clearance. Studies on *Morus alba* have shown that its bioactive compounds, including flavonoids and alkaloids, possess both hepatoprotective and lipid-lowering properties [107,108,128]. Additionally, the bioactive compounds found in MLP in this study may contribute to cardiovascular health by improving serum lipid profiles through their antioxidant, anti-inflammatory, and free radical scavenging activities [131,132,133,134]. Furthermore, oxidation of LDL-c and damage to endothelial cells are considered critical factors in the early development of atherosclerosis [135]. Several studies have shown that the phenolics, carotenoids, polysaccharides, and alkaloids present in MLP effectively reduce LDL-c oxidation in vitro through various oxidases [39,102,103] [104,136,137]. In contrast, the results from this study, consistent with previous findings, suggest that the reduction in serum TGs and HDL-c following B[a]P exposure is likely due to impaired secretion of these lipids from the liver into the bloodstream. This dysfunction may stem from structural damage to liver cells and/or energy depletion, as evidenced by a marked decrease in hepatic glycogen content [102,103,104,115,116,121,130].

Table 8. Effect of four weeks treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on serum lipid profile (mmol/L) in rats with of liver disorders induced by B[a]P

Groups	TGs		TCho		HDL-c		LDL-c	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
G1: Normal control	1.27-1.57	1.41 $\pm$ 0.12 ^a	2.68-3.11	2.87 $\pm$ 0.17 ^c	1.34-1.51	1.42 $\pm$ 0.06 ^a	0.81-0.97	0.88 $\pm$ 0.06 ^c
G2: Model control	1.09-1.26	1.19 $\pm$ 0.07 ^c	3.43-4.01	3.74 $\pm$ 0.23 ^a	0.82-0.94	0.89 $\pm$ 0.05 ^c	1.39-1.70	1.51 $\pm$ 0.13 ^a
G3: MLP (5 g/100 diet)	1.20-1.30	1.25 $\pm$ 0.04 ^c	2.98-3.89	3.36 $\pm$ 0.38 ^{ab}	0.99-1.12	1.01 $\pm$ 0.13 ^{bc}	1.29-1.73	1.48 $\pm$ 0.18 ^a
G4: MLP (10 g/100 diet)	1.27-1.33	1.29 $\pm$ 0.02 ^{bc}	2.87-3.45	3.20 $\pm$ 0.24 ^b	0.99-1.29	1.14 $\pm$ 0.12 ^b	1.18-1.50	1.38 $\pm$ 0.14 ^{ab}
G5: MLP (15 g/100 diet)	1.22-1.46	1.36 $\pm$ 0.12 ^a	2.84-3.1	2.98 $\pm$ 0.108 ^c	1.26-1.47	1.35 $\pm$ 0.08 ^{ab}	0.93-1.02	1.10 $\pm$ 0.18 ^b

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. MLP, Mulberry leaves powder, G1, normal group/healthy rats group; G2, model (hepatotoxic) rats group; G3, G4 and G5, model group treated with 5, 10 and 15 g/100 diet of MLP. TGs, triglycerides, TCho, total cholesterol, HDL-c, high density lipoprotein cholesterol, LDL-c, low density lipoprotein cholesterol.

Table 9. Effect of four weeks treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on serum lipid profile (as a percent of change) in rats with of liver disorders induced by B[a]P

Groups	TGs	TCho	HDL-c	LDL-c
G1: Normal control	0.00	0.00	0.00	0.00
G2: Model control	-15.6	+30.3	-37.3	+71.5
G3: MLP (5 g/100 diet)	+5.04	-10.16	+13.48	-1.98
G4: MLP (10 g/100 diet)	+8.4	-14.4	+28.08	-8.60
G5: MLP (15 g/100 diet)	+14.2	-20.3	+51.68	-27.15

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. MLP, Mulberry leaves powder, G1, normal group/healthy rats group; G2, model (hepatotoxic) rats group; G3, G4 and G5, model group treated with 5, 10 and 15 g/100 diet of MLP. TGs, triglycerides, TCho, total cholesterol, HDL-c, high density lipoprotein cholesterol, LDL-c, low density lipoprotein cholesterol.

3.2.4. Effect of four Weeks Treatment with Mulberry (*Morus alba* L.) Leaves Powder (MLP) on Hepatic Glutathione Fractions (as a percent of change) in Rats with of liver Disorders Induced by B[a]P

The findings in Tables (10 and 11) demonstrate the effects of a four-week MLP treatment on hepatic glutathione (GSH) fractions in rats with liver damage induced by B[a]P. The data reveal a significant decrease in both reduced glutathione (GSH) and oxidized glutathione (GSSG) in the B[a]P-treated model group, with reductions of 58.4% in GSH and 36.2% in GSSG compared to the normal control group. This reduction highlights a disruption in the antioxidant defense system, indicating oxidative stress, which aligns with previous research linking B[a]P-induced hepatotoxicity to increased oxidative damage [102,103,104,116]. Furthermore, GSH is known to function as a nonenzymatic scavenger of reactive oxygen species (ROS), which further explains the decrease in GSH levels due to B[a]P exposure [38,138]. The current study supports earlier findings, including those [118,119] who reported a significant ($p \leq 0.05$) reduction in GSH levels (-38.35%) in rats treated with B[a]P compared to normal rats. Additionally, [9,25]. observed a significant ($p \leq 0.05$) decrease in GSH levels in human erythrocytes following B[a]P exposure. According Mahran and Elhassaneen [114] and Halliwell and Gutteridge [139] the reduction in GSH levels may result from impaired glutathione reductase (GSH-Rd) activity,

which in turn elevates the activity of antioxidant enzymes like glutathione-S-transferase and glutathione peroxidase (GSH-Px), both of which rely on GSH as a cofactor for their functions. Treatment with MLP at 5, 10, and 15 g/100g diet led to significant improvements in the levels of both GSH and GSSG. Specifically, the group receiving 5 g/100g diet exhibited a 15.9% increase in GSH and a 21.4% increase in GSSG. In contrast, the groups receiving higher doses of 10 and 15 g/100g diet showed even greater improvements, with GSH increasing by 47.4% and 91.8%, respectively, and GSSG rising by 24% and 40.3%. These findings indicate that MLP may have a protective effect on the liver by boosting its antioxidant defenses, potentially due to bioactive compounds like flavonoids and polyphenols, which are known to enhance cellular antioxidant systems and mitigate oxidative damage [107,108,128]. The dose-dependent nature of the response further suggests that higher MLP doses result in more substantial improvements in oxidative stress markers, reinforcing the antioxidant potential of Mulberry (*Morus alba* L.) and its bioactive components [39,132]. The increase in GSSG, although indicative of oxidative stress, may also signify the liver's adaptive mechanism to counteract oxidative damage by upregulating GSH production [140,141]. These results align with previous research emphasizing the significance of redox balance in liver protection and regeneration [134]. Furthermore, the outcomes of this study support earlier reports on the therapeutic potential of *Morus alba* in alleviating liver dysfunction through the modulation of oxidative stress [115,116,117,120].

Table 10. Effect of four weeks treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on hepatic glutathione fractions in rats with of liver disorders induced by B[a]P

Groups	Reduced glutathione conc. (GSH, $\mu\text{mol}/\text{mg}$ tissue protein)		Oxidized glutathione conc. (GSSG, $\mu\text{mol}/\text{mg}$ tissue protein)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
G1: Normal control	5.11-7.24	6.18 $\pm$ 0.86 ^a	0.665 – 0.689	0.673 $\pm$ 0.010 ^a
G2: Model control	2.11-2.88	2.57 $\pm$ 0.33 ^c	0.410 – 0.435	0.429 $\pm$ 0.017 ^c
G3: MLP (5 g/100 diet)	2.55-3.42	2.98 $\pm$ 0.35 ^{bc}	0.500 – 0.536	0.521 $\pm$ 0.019 ^b
G4: MLP (10 g/100 diet)	3.65-4.07	3.79 $\pm$ 0.19 ^b	0.524 – 0.538	0.532 $\pm$ 0.008 ^b
G5: MLP (15 g/100 diet)	4.44-5.33	4.93 $\pm$ 0.36 ^{ab}	0.5.90 – 0.616	0.602 $\pm$ 0.014 ^{ab}

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. MLP, Mulberry leaves powder, G1, normal group/healthy rats group; G2, model (hepatotoxic) rats group; G3, G4 and G5, model group treated with 5, 10 and 20 g/100 diet of MLP.

Table 11. Effect of four weeks treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on hepatic glutathione fractions (as a percent of change) in rats with of liver disorders induced by B[a]P

Groups	GSH	GSSG
G1: Normal control	00	00
G2: Model control	-58.4	-36.2
G3: MLP (5 g/100 diet)	+15.9	+21.4
G4: MLP (10 g/100 diet)	+47.4	+24
G5: MLP (15 g/100 diet)	+91.8	+40.3

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. MLP, Mulberry leaves powder, G1, normal group/healthy rats group; G2, model (hepatotoxic) rats group; G3, G4 and G5, model group treated with 5, 10 and 20 g/100 diet of MLP.

3.2.5. Effect of Four Weeks Treatment with Mulberry (*Morus alba* L.) Leaves Powder (MLP) on Hepatic ROS and CYP-p450 in Rats with of Liver Disorders Induced by B[a]P

The data presented in Tables (12 and 13) highlights the impact of a four-week treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on hepatic reactive oxygen species (ROS) and cytochrome P450 (CYP450) enzyme activity in rats with liver disorders induced by B[a]P. The results demonstrate that rats in the B[a]P-exposed model control group experienced a substantial increase in both ROS levels (+267.2%) and CYP450 activity (+64.6%), when compared to the normal control group. These findings suggest that B[a]P exposure causes significant oxidative stress and alterations in hepatic metabolic pathways, consistent with previous studies linking polycyclic aromatic hydrocarbons (PAHs) such as B[a]P to increased oxidative stress and liver dysfunction [114,116]. In contrast, treatment with MLP at 5, 10, and 15 g/100g diet resulted in significant decreases in both

ROS and CYP450 activity. Specifically, the 5 g/100g diet dose of MLP caused a modest reduction of -12.3% in ROS and -9.7% in CYP450 activity. In comparison, higher doses of 10 and 15 g/100g diet led to even greater reductions. The 10 g/100g diet dose resulted in a decrease of -41.08% in ROS and -18.7% in CYP450 activity, while the highest dose of 15 g/100g diet produced a significant reduction of -58.91% in ROS and -33.2% in CYP450 activity. These findings indicate that the effects of MLP on oxidative stress and liver enzyme activity are dose-dependent, suggesting that MLP may play a role in alleviating oxidative damage and regulating enzymes involved in xenobiotic metabolism, thereby offering potential hepatoprotective benefits. The observed decrease in ROS levels following MLP treatment is particularly significant, as it indicates a reduction in oxidative damage, a key factor in liver injury induced by B[a]P exposure [102,103,104,114,121]. Furthermore, the reduction in CYP450 activity suggests a decrease in the liver's ability to metabolize toxic substances, including B[a]P. This polycyclic aromatic hydrocarbon is processed by various CYP enzymes into reactive intermediates such as hydroxy derivatives, phenolic diols, dihydro-diols, quinones, semiquinones, and epoxides, which contribute to hepatic damage [2,10,11,12] [118,119,123] [142,143]. By down regulating CYP450 activity, MLP may limit the production of these harmful metabolites, thereby providing a protective effect against liver injury. These findings are consistent with previous studies highlighting the antioxidant and hepatoprotective effects of MLP and its bioactive compounds, including flavonoids, polyphenols, and alkaloids. Research [117,108,128] has shown that Mulberry can mitigate oxidative stress and regulate enzyme activity, offering protection to the liver from various toxins, including B[a]P. The dose-dependent nature of the observed effects further strengthens the idea that higher doses of MLP provide more significant hepatoprotective benefits, supporting earlier findings on Mulberry's potential to reduce liver dysfunction [140,141].

Table 12. Effect of four weeks treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on hepatic ROS and CYP-p450 in rats with of liver disorders induced by B[a]P

Groups	ROS (U/ml tissue)		CYP450 (nmol/mg protein)	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
G1: Normal control	0.51-0.61	0.55 $\pm$ 0.04 ^d	1.72 – 2.09	1.81 $\pm$ 0.25 ^c
G2: Model control	1.91-2.14	2.02 $\pm$ 0.09 ^a	2.54 – 3.25	2.98 $\pm$ 0.30 ^a
G3: MLP (5 g/100 diet)	1.36-1.99	1.77 $\pm$ 0.29 ^{ab}	2.50 – 2.79	2.69 $\pm$ 0.15 ^{ab}
G4: MLP (10 g/100 diet)	1.06-1.32	1.19 $\pm$ 0.106 ^b	2.28 – 2.68	2.42 $\pm$ 0.32 ^b
G5: MLP (15 g/100 diet)	0.70-1.01	0.83 $\pm$ 0.129 ^c	1.66 – 2.27	1.99 $\pm$ 0.29 ^c

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. MLP, Mulberry leaves powder, G1, normal group/healthy rats group; G2, model (hepatotoxic) rats group; G3, G4 and G5, model group treated with 5, 10 and 20 g/100 diet of MLP. ROS, reactive oxygen species, CYP450, cytochrome P.450

Table 13. Effect of four weeks treatment with Mulberry (*Morus alba* L.) leaves powder (MLP) on hepatic ROS and CYP-p450 (as a percent of change) in rats with of liver disorders induced by B[a]P

Groups	ROS	CYP450
G1: Normal control	0.00	0.00
G2: Model control	+267.2	+64.6
G3: MLP (5 g/100 diet)	-12.3	-9.7
G4: MLP (10 g/100 diet)	-41.08	-18.7
G5: MLP (15 g/100 diet)	-58.91	-33.2

Each value represents the mean $\pm$ SD (n=6) Means under the same column with different superscript letters exhibited significant at $P \leq 0.05$. MLP, Mulberry leaves powder, G1, normal group/healthy rats group; G2, model (hepatotoxic) rats group; G3, G4 and G5, model group treated with 5, 10 and 20 g/100 diet of MLP. ROS, reactive oxygen species, CYO450, cytochrome P.450

3.3. Histological Studies

3.3.1. Effect of Treatment with MLP on Rat Liver Histological Disorders Induced by B[a]P

Effect of treatment with MLP on rat liver histological disorders induced by B[a]P was shown in Figure 1. Light microscopy examination of liver sections of rats from group 1 revealed the normal histoarchitecture of hepatic parenchyma (Photo1). In adverse, liver of rats from group 2 exhibited histopathological alterations characterized by vacuolar degeneration of hepatocytes, infiltration of the portal triad with inflammatory cells (Photos 2 and 3). Meanwhile, liver of rats from group 3 showed small vacuoles in the cytoplasm of some hepatocytes, slight dilatation of hepatic sinusoids (Photo 4). On the other hand, some sections from group 4 exhibited only slight hydropic degeneration of some hepatocytes (Photo 5). Furthermore, liver of rats from group 5 demonstrated slight Kupffer cells proliferation (Photo 6). Consistent with our findings, Elhassaneen and Mahrran [121] observed significant histopathological changes in liver tissue of rats exposed to B[a]P. These alterations included vacuolar degeneration of hepatocytes, focal necrosis of liver cells accompanied by inflammatory cell infiltration, activation of Kupffer cells, fibrosis in the portal triad, and the formation of new bile ductules. Similar liver abnormalities, such as hepatic necrosis, mononuclear cell infiltration, and hepatocyte apoptosis, have been reported by other researchers in rats exposed to B[a]P [144]. Studies have suggested that the liver damage resulting from B[a]P exposure is primarily driven by oxidative stress induced by its reactive metabolites. This oxidative stress predominantly targets liver parenchymal cells, including Kupffer cells, endothelial cells, and stellate cells, contributing to tissue damage. In this context, Hajam et al., [145] highlighted that ROS-induced membrane injury in hepatocytes leads to collagen accumulation, a key factor in the development of liver fibrosis and cirrhosis. Conversely, treatment with the MLP in B[a]P-induced hepatotoxic rats resulted in varying degrees of improvement in liver tissue histopathology. These improvements in liver architecture may be attributed to the bioactive properties of the MLP, such as their antioxidant

capacity, free radical scavenging activity, and ability to inhibit lipid peroxidation. In support of this, several studies demonstrated that plant-based extracts with similar bioactive compounds to this plant part can reduce oxidative stress and protect liver cells from irreversible damage [102,103,104,115]. Our study further suggests that MLP play a significant role in restoring liver function and partially reversing hepatic tissue damage, thereby reinforcing their hepatoprotective potential.

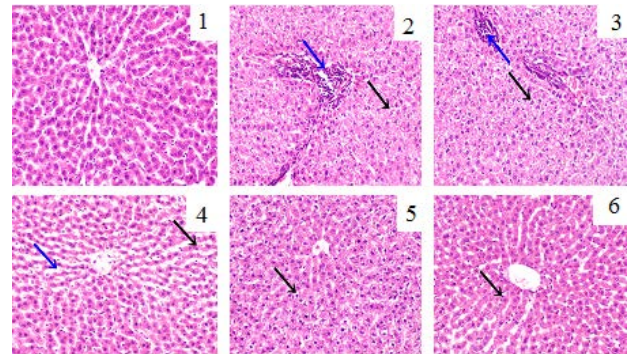


Figure 1. Effect of treatment with MLP on rat liver histological disorders induced by B[a]P

Photo 1. Photomicrograph of liver of rat from group 1 showing the normal histoarchitecture of hepatic parenchyma; **Photo 2.** Photomicrograph of liver of rat from group 2 showing vacuolar degeneration of hepatocytes (black arrow) and infiltration of the portal triad with inflammatory cells (blue arrow); **Photo 3.** Photomicrograph of liver of rat from group 2 showing vacuolar degeneration of hepatocytes (black arrow) and infiltration of the portal triad with inflammatory cells (blue arrow); **Photo 4.** Photomicrograph of liver of rat from group 3 showing small vacuoles in the cytoplasm of some hepatocytes (black arrow) and slight dilatation of hepatic sinusoids (blue arrow); **Photo 5.** Photomicrograph of liver of rat from group 4 showing slight hydropic degeneration of some hepatocytes (black arrow); **Photo 6.** Photomicrograph of liver of rat from group 5 showing slight Kupffer cells proliferation (black arrow) (H & E X 200).

4. Conclusion

Benzo[a]pyrene (B[a]P) is a widespread environmental and food contaminant and is a significant risk factor in the development of liver diseases. Mulberry leaves have been shown to serve as a functional food, rich in various bioactive compounds with antioxidant properties that can help prevent or mitigate liver damage caused by chemical toxins like B[a]P. The hepatoprotective effects of mulberry leaves against B[a]P-induced toxicity may occur through several mechanisms, including: 1) enhancing liver function, 2) modulating drug-metabolizing enzyme regulators (such as cytochrome P450), 3) improving antioxidant activity, 4) improving lipid metabolism, and 5) inducing favorable changes in liver histopathology. These findings indicate that mulberry leaves have the potential to act as a hepatoprotective agent to counteract liver damage. Future research should focus on exploring the

incorporation of mulberry leaves as a natural food additive in everyday meals, beverages, and various other food products.

ACKNOWLEDGMENT

The authors would like to express sincere thanks and appreciation to Some people in the village of Mit Ghoarb, Sinbellaween Center, Dahlia Governorate, Cairo, Egypt, for their efforts and assistance provided during the collection of Mulberry leaves samples. Sincere appreciations were also extended to Staff of Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt for verification the plant part samples.

Conflicting Interests

The authors acknowledge that this is not present in the article for the possibility of publishing it.

Ethical Considerations

The ethical issues of the current work was reviewed and approved by the Scientific Research Ethics Committee, Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval # 14- SREC- 01-2024).

Authors' Contribution

Yousif Elhassaneen contributed to the creation and development of the study protocol, supervised the practical experimental work, gathered conceptual data, reviewed and validated the results and statistical analyses, and assisted in drafting and reviewing the manuscript. Sara Abd ElMaksoud carried out the experimental work, gathered, organized, and analyzed the results, retrieved foundational information and concepts, and wrote the initial manuscript draft. Sobhi Hassab El-Nabi and Nazeha Khalil were involved in preparing the study protocol, overseeing the practical experiments, gathering conceptual insights, confirming the accuracy of the study results, and drafting the manuscript.

Abbreviations

AA, antioxidant activity, Abs, absorbance, BWG, body weight gain, DMSO, FI, feed intake, dimethyl sulfoxide, G6PD, glucose-6-phosphatase, FER, feed efficiency ratio, G6Pase, glucose-6-phosphate dehydrogenase, GSH, reduced glutathione, GSSG, oxidized glutathione, HDL-c, High density lipoprotein-cholesterol, LDL-c, low density lipoprotein-cholesterol, MDA, malondialdehyde, MLP, mulberry leaves powder, ROS, reactive oxygen species, SD, standard deviation, TGs, triglycerides.

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