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Effects of Raw Materials from *Green Fenugreek* and *Black Mulberry* on Oxidative Stress Biomarkers Induced by Carbon Tetrachloride Toxicity in Experimental Rats

Lobna Saad Mohammed Abd Elmeged[1,2]* and Ali Khalaf Ahmed Albaggar[3]

[1]Department of Nutrition, Applied College, AL-Baha University, Al-Makhwa, Saudi Arabia.
[2]Department of Nutrition and Food Sciences, Faculty of Home Economics, Menoufia University, Shibin el Kom, Menofia Governorate, Egypt.
[3]Department of Public Health, Faculty of Applied Medical Sciences, AL-Baha University, Al-Baha, Saudi Arabia.
*Corresponding Author E-mail: lobna_lolo_2007@yahoo.com

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Plant phytochemicals are widely recognized for their hepatoprotective and antioxidant properties, playing an important role in protecting the liver from chemically induced oxidative damage. This study aimed to evaluate the protective effects of dietary green fenugreek and black mulberry against carbon tetrachloride (CCl4)-induced oxidative stress in rats. Thirty male rats were divided into five groups: an untreated control, a CCl4-exposed control, and three groups receiving diets supplemented with green fenugreek, black mulberry, or a combination of both. The effects of supplementation were assessed on antioxidant enzyme activity, liver and kidney function, and lipid profiles. Additionally, histopathological examination of the liver was conducted to determine tissue-level protection. The results showed that plant supplementation significantly enhanced antioxidant enzyme activity, normalized liver and kidney function, and restored lipid balance compared with CCl4-treated controls. Histological analysis confirmed better preservation of liver architecture, with the combined supplementation of green fenugreek and black mulberry providing the most pronounced protective effect. These findings indicate that dietary green fenugreek and black mulberry effectively counteract oxidative stress, improve organ function, and may offer synergistic benefits when used together, supporting their potential as functional dietary interventions for liver health.

Keywords: Carbon tetrachloride-induced hepatotoxicity; Metabolic homeostasis; Morus nigra; oxidative stress; Trigonella foenum-graecum.

Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the capacity of the body's antioxidant defenses, resulting in cellular and tissue damage. In experimental hepatotoxicity models, carbon tetrachloride (CCl_4) is frequently used to induce oxidative injury, as it generates free radicals that target lipids, proteins, and DNA. This leads to lipid peroxidation, depletion of glutathione (GSH), and reduction in the activity of key antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), ultimately elevating serum markers of liver injury, including ALT and AST.[1]

Due to potential side effects of conventional hepatoprotective medications, natural plant-derived compounds are gaining attention as

safer and effective alternatives. Many medicinal plants are rich in polyphenols, flavonoids, and anthocyanins, which neutralize ROS, strengthen endogenous antioxidant systems, and modulate inflammatory pathways.[2]

Fenugreek (Trigonella foenum-graecum) and black mulberry (Morus nigra) were selected for this study based on their well-documented antioxidant and hepatoprotective properties. Fenugreek seeds contain high levels of polyphenols, flavonoids, and polysaccharides that contribute to ROS scavenging and maintenance of liver function under oxidative stress.[3] Supplementation with fenugreek in CCl,, -treated animals has been shown to reduce liver enzyme elevations and preserve antioxidant enzyme activities, indicating hepatoprotective effects.t

Black mulberry is also recognized for its strong antioxidant activity. Its leaves and fruits are rich in phenolic acids, flavonoids, and anthocyanins that effectively reduce oxidative damage in liver tissues. Experimental studies have demonstrated that M. nigra supplementation can normalize liver enzyme levels, enhance SOD and glutathione peroxidase (GPx) activities, and decrease oxidative markers, thereby mitigating hepatocellular injury.u Òv

The hepatoprotective activity of fenugreek and black mulberry is primarily linked to their bioactive compounds, which counteract ROS, inhibit lipid peroxidation, and enhance antioxidant enzyme function. Fenugreek's polyphenols and flavonoids donate electrons or hydrogen atoms to neutralize free radicals, limit chain reactions of lipid peroxidation, and support SOD and CAT activity, maintaining cellular redox balance. Specific constituents, including vitexin, isovitexin, trigonelline, and diosgenin, contribute additional antioxidant and anti-inflammatory effects.w Similarly, M. nigra's diverse phenolic profile, including flavonols, anthocyanins, and caffeoylquinic acids, stabilizes reactive radicals, chelates pro-oxidant metal ions, and supports endogenous antioxidant defenses, reducing lipid peroxidation and preserving hepatocyte structure.x

Thus, the selection of fenugreek and black mulberry for this study is scientifically justified by their complementary bioactive profiles, which are expected to provide synergistic antioxidant and hepatoprotective effects against CCl,, -induced liver oxidative damage.

MATERIALS AND METHODS

Materials

Green fenugreek *(Trigonella foenum-graecum)* and black mulberry *(Morus nigra)* were procured from local markets in Al-Baha City, Kingdom of Saudi Arabia (KSA). In the case of green fenugreek, the leaves were used, whereas the fruits were used from black mulberry for the preparation of plant materials. The plant samples were thoroughly washed with distilled water to remove debris, dirt, and other contaminants. The cleaned materials were then air-dried at ambient temperature (25 ± 2 °C) for 7–10 days until a constant weight was achieved. The dried samples were ground into a fine powder using a mechanical grinder and sieved through a 40-mesh screen. Crushed plant raw materials were used instead of standardized extracts to more accurately simulate the natural dietary consumption of green fenugreek leaves and black mulberry fruits. This approach allows the evaluation of the protective effects of the whole plant matrix, including all phytochemicals, fibers, and minor compounds, as they would be ingested in a normal diet. The resulting powders were stored in airtight containers at 4 °C until use in experimental diets and biochemical analyses, following standardized plant preparation protocols.y

Experimental Animals and Housing

Thirty adult male Sprague Dawley rats, weighing 150 ± 10 g, were obtained from the Institute of Nutrition, Cairo, Egypt. Animals were housed in plastic cages with stainless steel covers under controlled conditions: temperature 22 ± 2 °C, relative humidity 50–60%, and a 12-hour light/dark cycle. Rats were provided ad libitum access to water and standard basal diet throughout a seven-day acclimatization period. All experimental procedures were conducted in compliance with institutional ethical guidelines for the care and use of laboratory animals.

Chemicals and Reagents

Dietary components, including casein, cellulose, and a vitamin–mineral premix were purchased from Morgan Co., Cairo, Egypt.

Additional chemicals such as formalin, ethanol, and EDTA were obtained from El-Nasr Pharmaceutical Chemicals, El-Amereia, Cairo, Egypt.

Basal and experimental Diets

Experimental diets were prepared by supplementing the basal diet with 5% powdered green fenugreek (GF), 5% powdered black mulberry (BM), or a 5% combination of GF and BM powders. Diets were freshly prepared on a weekly basis, stored at 4 °C, and provided to animals ad libitum throughout the experimental period. [1]p The composition of the experimental diets is summarized in Table 1.

Carbon Tetrachloride (CCl,,)

Carbon tetrachloride (CCl,,) was acquired as a 10% liquid solution from El-Gomhoria Company for Chemical Industries, Cairo, Egypt. The solution was stored in 1-L white plastic containers and employed as a hepatotoxic agent to induce liver injury. For experimental administration, CCl,, was diluted in paraffin oil obtained from a local pharmacy during the induction period.[13]

Animal Housing and Maintenance

Male Sprague Dawley albino rats, aged 14–16 weeks and weighing 150–160 g, were obtained from the Animal Laboratory. Animals were housed in sanitized plastic cages with stainless steel tops under controlled laboratory conditions: temperature 22 ± 2 °C, relative humidity 50–60%, and a 12-hour light/dark cycle. Rats were provided with ad libitum access to basal diet and water via drinking bottles equipped with metallic spouts. All rats were acclimated to housing conditions and basal diet for seven days prior to the start of the experimental procedures.

Induction of Oxidative Stress

Oxidative stress was experimentally induced by intraperitoneal administration of CCl,, diluted in a suitable vehicle, such as paraffin or olive oil, at a dose of 2 mL/kg body weight. Injections were administered twice weekly for two consecutive weeks. Once metabolized in the liver through cytochrome P450 enzymes, CCl,, generates highly reactive trichloromethyl and trichloromethylperoxyl radicals. These species initiate lipid peroxidation, compromise cellular membranes, and trigger oxidative damage, leading to impaired hepatic and renal function.[1]t

Experimental Design and Grouping

Thirty rats were randomly allocated into five groups, each containing six animals, as follows:

• G1 (Positive Control): Healthy rats maintained on the basal diet for 28 days without any treatment.
• G2 (Negative Control): Rats subjected to CCl,, -induced oxidative stress and fed the basal diet for 28 days without supplementation.
• G3: Rats exposed to CCl,, and fed a basal diet supplemented with 5% black mulberry (BM).
• G4: Rats exposed to CCl,, and fed a basal diet supplemented with 5% green fenugreek (GF).
• G5: Rats exposed to CCl,, and fed a basal diet supplemented with a 5% mixture of black mulberry and green fenugreek (BM + GF).

All diets were administered ad libitum, and animals were monitored daily for health, feed intake, and behavior throughout the experimental period.

Determination of Total Phenolics, Flavonoids, and DPPH Radical Scavenging Activity

The phytochemical composition and antioxidant activity of the plant materials were evaluated by determining total phenolic content, total flavonoid content, and DPPH radical scavenging activity using standard spectrophotometric methods. Total phenolic content (TPC) was measured using the Folin–Ciocalteu colorimetric method, where the plant extract was mixed with diluted Folin–Ciocalteu reagent followed by sodium carbonate solution, incubated for 30 min in the dark, and the absorbance was recorded at 765 nm. Results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) according to the method . t w Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric assay, in which the extract was reacted sequentially with sodium nitrite, aluminum chloride, and sodium hydroxide, and the absorbance was measured at 510 nm. The results were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW) following the method .t x Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, where the plant extract was mixed with a methanolic DPPH solution and incubated in the dark for 30 min, after which the absorbance was measured at 517 nm.

The radical scavenging activity was expressed as percentage inhibition according to the method. t y

Biological Assessment

Throughout the 28-day experimental period, daily feed consumption was recorded for each group. Body weights were measured on a weekly basis to monitor growth patterns. Feed efficiency ratio (FER), percentage body weight gain (BWG%), and relative organ weights were calculated according to established protocols.[1]u

Blood Collection and Organ Harvesting

At the conclusion of the study, animals were fasted for 12 hours prior to sample collection. Blood was drawn via the retro-orbital plexus using microcapillary tubes and allowed to clot for 30 minutes at 37/ °C. Samples were then centrifuged at 3000 rpm for 10 minutes to obtain serum, which was aliquoted into sterile polypropylene tubes and stored at "20/ °C until further biochemical analyses. The liver, kidney, heart, and spleen were excised, rinsed in physiological saline, weighed, and fixed in 10% neutral buffered formalin for subsequent histological examination.[1]v

Biochemical Analyses

Liver Function Markers: Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using commercial spectrophotometric kits (BioMerieux) following Reitman and Frankel.[1]w Alkaline phosphatase (ALP) activity was measured colorimetrically .[1]

Bilirubin and Protein: Serum total bilirubin was quantified colorimetrically at 578/ nm,[1]y and total protein (TP) concentration was determined.[2]

Lipid Profile: Total cholesterol was measured following Ratliff and Hall.[21] Triglycerides and HDL cholesterol were quantified using enzymatic colorimetric assays.[22] VLDL and LDL cholesterol levels were calculated according to the method.[23]

Histopathological Study

Liver tissues were excised from all rats immediately after sacrifice and fixed in 10% neutral buffered formalin. Samples were dehydrated, cleared, and embedded in paraffin, then sectioned at 4–5 µm. Sections were stained with hematoxylin and eosin (H&E)t w , where nuclei appear faint and cytoplasm light pink. Stained sections were examined under a light microscope to assess hepatocyte morphology, vacuolation, inflammation, fibrosis, and overall liver architecture.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Statistical evaluation was performed using one-way analysis of variance (ANOVA) for a completely randomized design.[2]t Post hoc comparisons among group means were conducted using Duncan's multiple range test, with significance set at $p < 0.05$.

RESULTS

Phytochemical Analysis

Table 2 presents the total phenolic and flavonoid contents of *green fenugreek* and *black mulberry* powders, along with their corresponding DPPH radical scavenging activities. The data demonstrate that *black mulberry* powder contained substantially higher levels of flavonoids (47.10/ ±/ 1.90/ µg/mL) than *green fenugreek* powder (26.98/ ±/ 1.60/ µg/mL). Likewise, the total phenolic content was considerably higher in *black mulberry* (340.32/ ±/ 24.3/ µg/mL) than in *green fenugreek* (109.90/ ±/ 15.60/ µg/mL).

Regarding antioxidant capacity, as assessed by the DPPH radical scavenging assay, *black mulberry* exhibited superior activity (15.10/ ±/ 1.50) relative to *green fenugreek* (12.45/ ±/ 1.70). All measurements represent the mean/ ±/ standard deviation of three independent replicates, indicating consistent and reproducible results.

Biological evaluation

Supplementation of rats' diets with 5% *black mulberry*, 5% *green fenugreek*, or their combination influenced body weight gain and daily food intake under carbon tetrachloride-induced oxidative stress. Rats that were not exposed to CCl,, showed the highest body weight gain (13.99/ ±/ 0.99%), while those subjected to CCl,, without treatment exhibited a marked decrease in weight gain (7.4/ ±/ 0.55%). Weight gain percentage was calculated using the formula:

$$\text{Weight gain (\%)} = [\text{Final body weight} - \text{Initial body weight} / \text{Initial body weight}] \times 100$$

Inclusion of *black mulberry* alone did not significantly improve weight gain (7.37/ ±/

0.97%), whereas *green fenugreek* supplementation provided moderate recovery (9.2/ ±/ 0.32%). The combined administration of both supplements led to a notable enhancement in weight gain (12.17/ ±/ 0.58%), approaching the levels observed in untreated control rats.

Food intake was similarly affected by oxidative stress. The positive control group recorded a significant reduction in daily consumption (12.26/ ±/ 0.25/ g/day/rat) compared with the negative control (16.46/ ±/ 0.91/ g/day/rat). Daily food intake was measured as the average amount of feed consumed per rat per day. Diets supplemented with *black mulberry* increased intake to 17.13/ ±/ 1.21/ g/day/rat, while *green fenugreek* restored consumption to 16.23/ ±/ 0.70/ g/day/rat. The combination of the two supplements effectively normalized food intake (16.06/ ±/ 0.80/ g/day/rat).

All data are presented as mean/ ±/ standard deviation of three independent replicates. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to determine

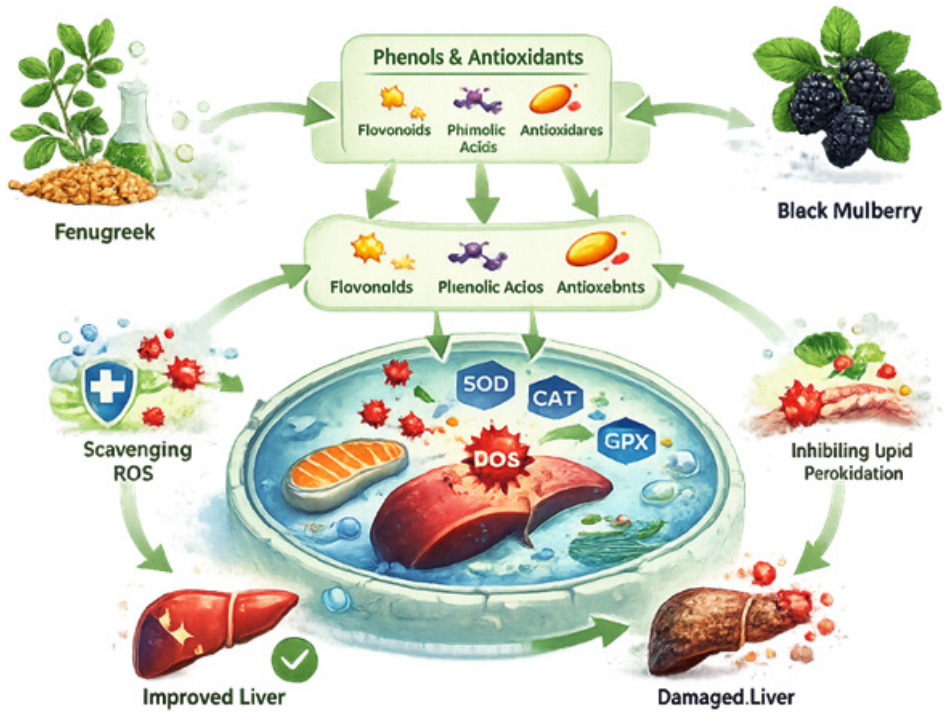

Fig. 1. Possible Cellular Mechanism of Fenugreek and Black Mulberry in Reducing Oxidative Stress and Improving Liver Function. (Source: Original data obtained from current study)

Table 1. The basic and experimental diets' compositions

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

significant differences among groups, with p/ </ 0.05 considered statistically significant.

These results suggest that co-supplementation with *black mulberry* and *green fenugreek* exerts a synergistic effect on body weight recovery, whereas both individual and combined treatments are capable of restoring food intake to near-normal levels in rats experiencing chemically induced oxidative stress.

Biochemical Analysis

Effect of *Black Mulberry*, *Green Fenugreek*, and Their Combination on Liver Enzymes in CCl,, -Treated Rats

The impact of dietary supplementation with 5% *black mulberry* (BM), 5% *green fenugreek* (GF), and a 5% combined mixture on hepatic enzyme activities alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline

Table 2. Total Phenolic and Flavonoid Contents and DPPH Radical Scavenging Activity of *black mulberry and green fenugreek*

Parameter	*Green fenugreek* Powder	*Black mulberry* Powder
Total flavonoid(µg/ml)	26.98±1.60	47.10±1.90
Total phenolic compounds (µg/ml)	109.90±15.60	340.32±24.3
DPPH(%)	12.45±1.70	15.10±1.50

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

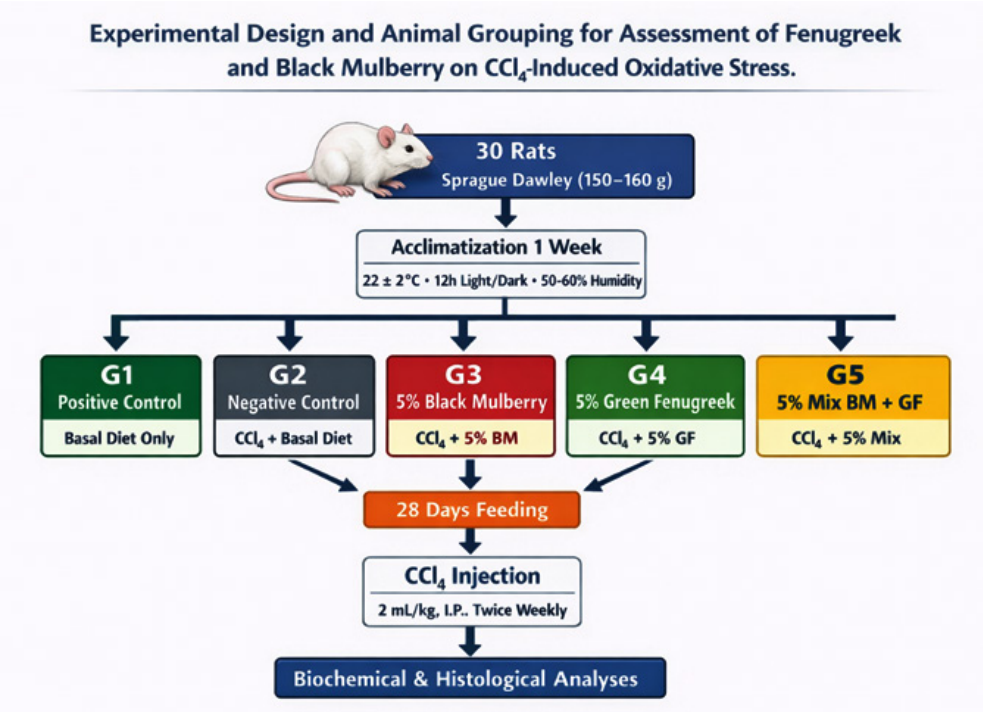

Fig. 2. Experimental Design and Animal Grouping for the Evaluation of Fenugreek and Black Mulberry against CCl,, -Induced Oxidative Stress. (Source: Original data obtained from current study)

phosphatase (ALP)was assessed in rats subjected to oxidative stress induced by carbon tetrachloride ($CCl_{,,}$).

ALT (GPT): Rats exposed to $CCl_{,,}$ without supplementation exhibited a marked increase in ALT levels (34.9/ ±/ 1 U/L) relative to the negative control (27.87/ ±/ 1.53 U/L), indicating hepatocellular injury. The percentage change was calculated as:

Percentage change (%) = [Treated value – Control value / Control value] × 100

Supplementation with BM, GF, or their combination attenuated this elevation. The most substantial reduction was observed in the BM-supplemented group (28.99/ ±/ 0.57 U/L, 17.11% decrease), while the combined mixture resulted in a modest reduction (32.33/ ±/ 0.5 U/L, 8.41% decrease), indicating partial hepatoprotective effects.

AST (GOT): $CCl_{,,}$ exposure significantly increased AST activity to 228.0/ ±/ 1.52 U/L compared to 136.0/ ±/ 1 U/L in the negative control. Dietary interventions reduced AST levels, with GF supplementation showing the greatest decrease (172.66/ ±/ 1.53 U/L, 24.21% reduction) compared to BM alone (181.66/ ±/ 1.53 U/L, 20.28% reduction). The combination group produced an intermediate effect (196.33/ ±/ 1.53 U/L, 13.71% reduction), indicating that both BM and GF can mitigate oxidative hepatocyte damage, with GF exhibiting slightly stronger protective activity.

ALP: $CCl_{,,}$ significantly elevated ALP activity (142.33/ ±/ 1.53 U/L) relative to the negative control (99.66/ ±/ 1.53 U/L). All supplemented groups demonstrated reductions in ALP, with the combination treatment showing the most notable decrease (121.33/ ±/ 1.53 U/L, 14.84% reduction), followed by BM (131.33/ ±/ 2.52 U/L, 7.84% reduction) and GF (132.66/ ±/ 1.53 U/L, 6.88% reduction).

All data are presented as mean/ ±/ standard deviation of three independent replicates. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to determine significant differences among groups, with p/ d"/ 0.05 considered statistically significant.

Summary: Dietary supplementation with BM, GF, or their combination provided partial hepatoprotection against $CCl_{,,}$ -induced oxidative stress, as evidenced by decreased ALT, AST, and ALP activities. While single supplements showed stronger effects on ALT and AST, the combined supplementation exhibited a synergistic benefit in reducing ALP activity, confirming the protective role of these dietary interventions.

Effect of *Black Mulberry*, *Green Fenugreek*, and Their Combination on Renal Function in $CCl_{,,}$ -Treated Rats

Figure 5 presents the influence of dietary supplementation with 5% *black mulberry* (BM), 5% *green fenugreek* (GF), and their 5% combined mixture on renal biomarkers in rats exposed to oxidative stress induced by carbon tetrachloride ($CCl_{,,}$). In the positive control group, which received $CCl_{,,}$ without any supplementation, serum creatinine, urea, and uric acid levels were significantly elevated compared to the negative control, indicating pronounced renal dysfunction.

Black Mulberry (BM)

Administration of BM powder partially mitigated $CCl_{,,}$ -induced renal alterations. Serum creatinine decreased to 0.92/ ±/ 0.072/ mg/dL, representing an 11.10% reduction relative to the positive control. Urea levels declined to 35.67/ ±/ 1.52/ mg/dL (17.04% reduction), and uric acid was reduced to 4.46/ ±/ 0.15/ mg/dL (4.28% reduction), reflecting a moderate nephroprotective effect.

Green Fenugreek (GF)

Supplementation with *Green Fenugreek* (*GF*) powder improved renal function in $CCl_{,,}$ -treated rats. Creatinine decreased to 0.80/ ±/ 0.10/ mg/dL (20.8% reduction), urea dropped to 33.67/ ±/ 0.58/ mg/dL (21.7% reduction), and uric acid was markedly lowered to 2.40/ ±/ 0.10/ mg/dL (48.5% reduction), demonstrating strong nephroprotective effects of *GF* against oxidative stress.

Combination (BM + GF)

The mixture of BM and GF powders exhibited the strongest restorative effect on kidney function. Serum creatinine was reduced to 0.77/ ±/ 0.025/ mg/dL (24.5% reduction), urea decreased to 36.33/ ±/ 1.53/ mg/dL (15.51% reduction), and uric acid reached 2.46/ ±/ 0.15/ mg/dL (48.62% reduction). This suggests a potential synergistic interaction between the two supplements, enhancing protection against $CCl_{,,}$ -induced nephrotoxicity.

In conclusion, all supplemented groups

demonstrated significant improvements in renal biomarkers compared to the positive control. The combination of BM and GF was most effective in lowering creatinine levels, whereas GF alone had the strongest impact on uric acid reduction, underscoring the complementary nephroprotective properties of these plant-based interventions under oxidative stress conditions.

Effect of *Black Mulberry, Green Fenugreek*, and Their Combination on Serum Lipid Profile in CCl_4 -Treated Rats

Figure 6 illustrates the effects of dietary supplementation with 5% *black mulberry* (*BM*), 5% *green fenugreek* (*GF*), and their 5% combined mixture on serum lipid parameters in rats exposed to oxidative stress induced by carbon tetrachloride (CCl_4).

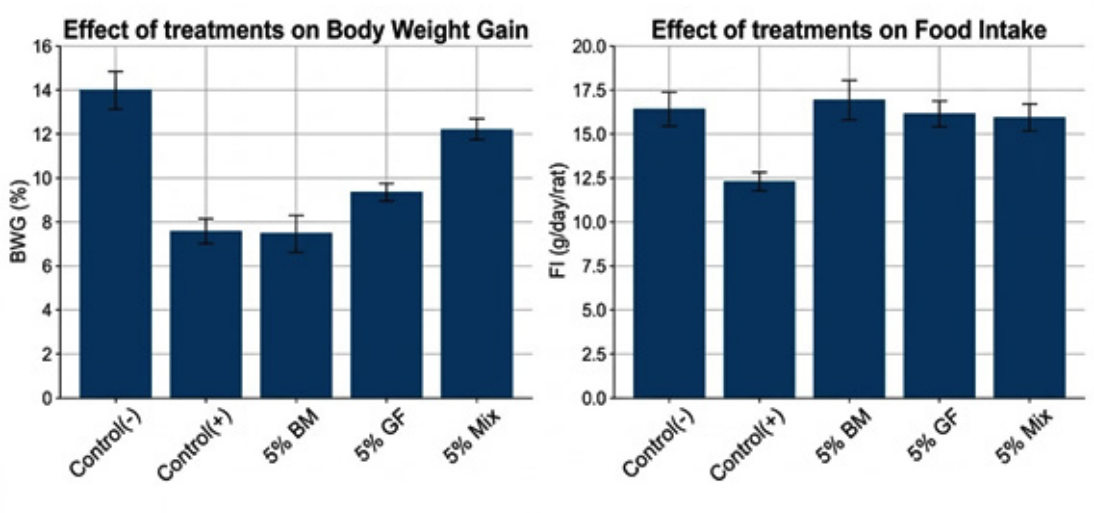

Fig. 3. Effect of dietary supplementation with 5% *Morus nigra* powder, 5% *green fenugreek* powder, and their 5% mixture on body weight gain in rats exposed to carbon tetrachloride (CCl_4)–induced oxidative stress. (Source: Original data obtained from current study)

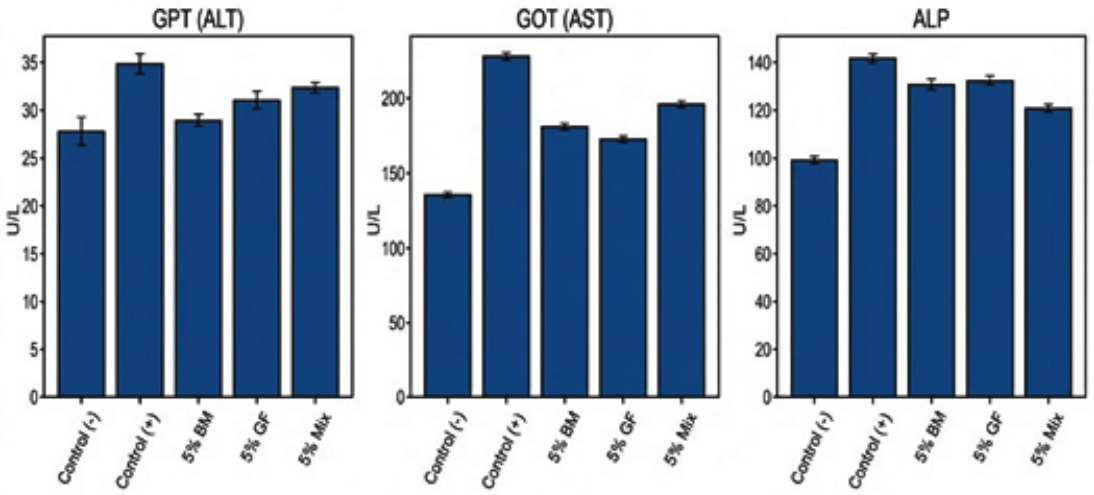

Fig. 4. Effect of dietary supplementation with 5% *Morus nigra* powder, 5% *green fenugreek* powder, and their 5% mixture on liver enzymes (ALT, AST, and ALP) in rats subjected to carbon tetrachloride-induced oxidative stress. (Source: Original data obtained from current study).

Total Cholesterol (T.C)

In the negative control group, total cholesterol levels were 69.67/ ±/ 1.53/ mg/dL. Administration of CCl,, significantly elevated T.C to 86.00/ ±/ 1.00/ mg/dL, indicating dyslipidemia associated with hepatotoxicity. Supplementation with BM or GF individually partially mitigated this elevation, reducing T.C to 81.00/ ±/ 1.00/ mg/dL and 80.33/ ±/ 1.53/ mg/dL, respectively. Notably, the combined BM + GF treatment resulted in the most pronounced effect, lowering T.C to 77.33/ ±/ 1.53/ mg/dL, equivalent to a 10% reduction relative to the positive control.

Triglycerides (T.G)

Triglyceride levels increased markedly following CCl,, exposure, from 97.67/ ±/ 1.53/ mg/dL in healthy controls to 160.60/ ±/ 3.78/ mg/dL. Both BM and GF supplementation significantly decreased T.G to 112.00/ ±/ 1.00/ mg/dL and 107.33/ ±/ 1.53/ mg/dL, respectively. The combination of BM and GF exhibited the strongest protective effect, reducing triglycerides further to

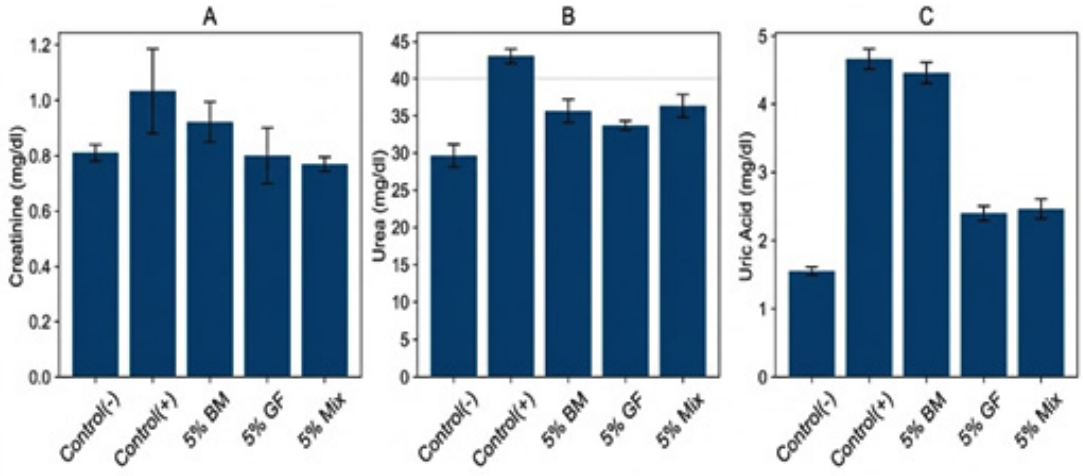

Fig. 5. Effect of dietary supplementation with 5% *Morus nigra* powder, 5% *green fenugreek* powder, and their 5% mixture on kidney function (Creatinine, Uric Acid and Urea) in rats subjected to carbon tetrachloride induced oxidative stress. (Source: Original data obtained from current study).

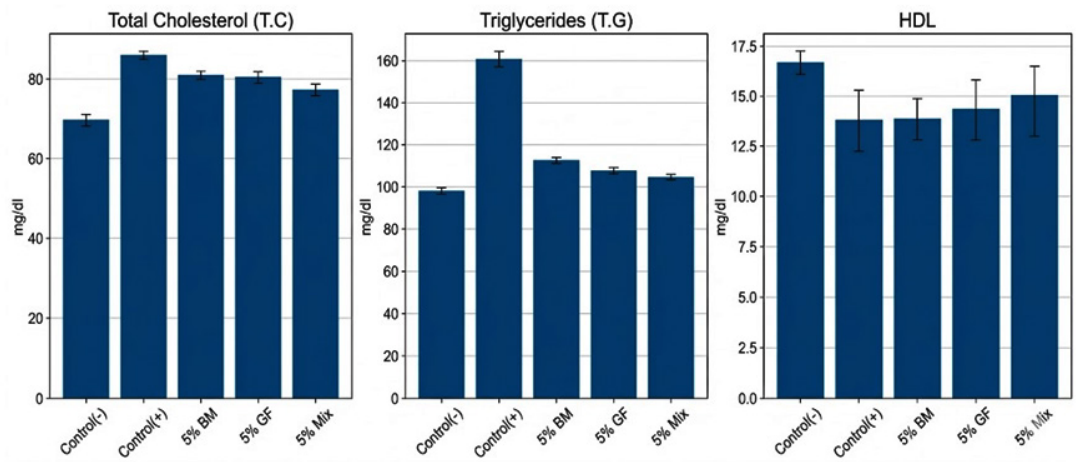

Fig. 6. Effect of dietary supplementation with 5% *Morus nigra* powder, 5% *green fenugreek* powder, and their 5% mixture on T.C, T.G and H.D.L in rats subjected to carbon tetrachloride-induced oxidative stress. (Source: Original data obtained from current study).

104.33/ ±/ 1.53/ mg/dL, indicating enhanced lipid regulatory activity.

High-Density Lipoprotein (HDL)

CCl_4 exposure slightly reduced HDL levels from 16.67/ ±/ 0.58/ mg/dL in controls to 13.80/ ±/ 1.59/ mg/dL. Dietary supplementation with BM or GF produced modest improvements (13.83/ ±/ 1.04/ mg/dL and 14.33/ ±/ 1.53/ mg/dL, respectively). The combined treatment restored HDL to 15.00/ ±/ 2.00/ mg/dL, reflecting partial recovery of protective lipoprotein levels.

Interpretation

These results indicate that both black mulberry and green fenugreek exert beneficial effects on lipid metabolism in rats under oxidative stress. Individually, each supplement improved cholesterol and triglyceride levels and slightly enhanced HDL. However, the combination of BM and GF demonstrated superior efficacy, suggesting that synergistic interactions among their bioactive constituents may enhance lipid-lowering and hepatoprotective effects under CCl_4 -induced oxidative conditions.

Histopathological Evaluation of Liver Tissue

Microscopic evaluation of liver sections revealed distinct histological changes across the experimental groups, as illustrated in Figure 7 (A–E):

Group 1 (Negative Control): Liver tissue displayed normal hepatic architecture. Hepatocytes were polygonal with centrally located nuclei and arranged radially around the central vein, consistent with healthy liver morphology and intact lobular structure (Figure 7A).

Group 2 (CCl_4 -treated): Mild vacuolar changes were observed within hepatocytes (indicated by arrows), suggesting early cellular stress or initial lipid accumulation. Despite these alterations, the overall lobular organization remained largely preserved (Figure 7B).

Group 3 (CCl_4 + 5% Black Mulberry): Pronounced hepatocyte vacuolation and cellular degeneration were evident (arrow), reflecting oxidative stress–induced liver injury. Disruption of cellular morphology indicated early hepatocellular damage (Figure 7C).

Group 4 (CCl_4 + 5% Green Fenugreek): Liver sections exhibited portal fibrosis and infiltration of inflammatory cells surrounding the bile duct (arrow). These histological features are indicative of oxidative stress–related hepatic injury

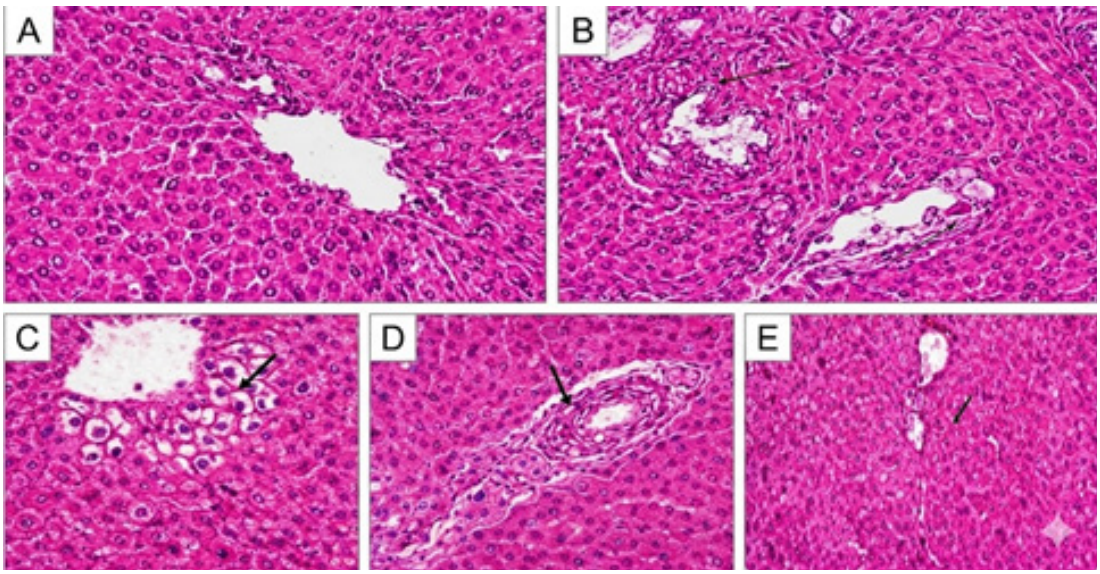

Fig. 7. Histopathological examination of rat liver sections stained with Hematoxylin and Eosin (H&E, 400x). (A) Negative Control group showing normal hepatic architecture and radial arrangement of hepatocytes. (B) CCl_4 -treated group showing mild vacuolar changes (arrows). (C) CCl_4 + 5% Black Mulberry group showing hepatocyte degeneration. (D) CCl_4 + 5% Green Fenugreek group displaying portal fibrosis and inflammatory cell infiltration. (E) CCl_4 + 5% BM + GF mixture group showing vacuolar degeneration and ballooning. *(Source: Original histological sections obtained from the current study).*

accompanied by an inflammatory response (Figure 7D).

Group 5 (CCl_4 + 5% BM + GF mixture): Hepatocytes showed vacuolar degeneration and ballooning (arrow), suggesting fatty change and oxidative damage. These findings indicate the characteristic hepatocellular injury associated with CCl_4 exposure (Figure 7E).

Overall, the histopathological assessment demonstrates that CCl_4 exposure induces progressive liver damage. The supplementation of black mulberry, green fenugreek, or their combination showed varying degrees of histological presentation across the treated groups.

DISCUSSION

The findings of the present study demonstrate that dietary supplementation with *black mulberry* and *green fenugreek* powders provides significant protection against carbon tetrachloride (CCl_4)-induced oxidative stress in rats. These protective effects were reflected in improvements across several physiological and biochemical parameters, including body weight gain, food intake, liver enzyme activities, kidney function markers, and serum lipid profiles. Notably, the combined administration of both plant powders consistently produced the most pronounced protective effects, suggesting a synergistic interaction between their bioactive constituents.[2u] Such synergistic effects are commonly observed when multiple phytochemicals act through complementary antioxidant and metabolic pathways.

Phytochemical analysis revealed that *black mulberry* powder contains higher levels of total phenolic and flavonoid compounds compared with *green fenugreek* powder. This difference was reflected in the stronger DPPH radical scavenging activity observed in *mulberry*, highlighting the crucial role of phenolic compounds in neutralizing reactive oxygen species and interrupting oxidative chain reactions.[2v] Phenolic compounds are well known for their ability to donate electrons or hydrogen atoms, thereby preventing oxidative damage to cellular macromolecules. The high antioxidant potential of *black mulberry* is largely attributed to its rich content of anthocyanins, flavonols, and phenolic acids, which have been widely reported to possess potent free radical–scavenging and anti-inflammatory activities.[2w] In contrast, *fenugreek* contains important bioactive compounds such as flavonoids, saponins, and alkaloids that also contribute to antioxidant activity, although at relatively lower concentrations compared with those present in *mulberry*.[2x] These differences in phytochemical composition may explain the variations observed in the protective efficacy of the two supplements.

Exposure to CCl_4 resulted in a marked reduction in body weight gain and food intake, which is consistent with the metabolic disturbances and appetite suppression commonly associated with oxidative stress and hepatic injury.[2y] Interestingly, dietary supplementation with *green fenugreek* improved body weight gain more effectively than *black mulberry* alone, while the combined supplementation restored body weight values close to those of the normal control group.[3p] *Fenugreek* is known to stimulate appetite, improve digestion, and enhance nutrient utilization due to its fiber and saponin content, whereas *mulberry*'s potent antioxidant activity may help reduce oxidative damage and metabolic stress.[31] The complementary actions of these two plants likely contributed to the improved growth performance observed in the combined treatment group.

The hepatotoxic effect of CCl_4 was clearly demonstrated by the significant elevation of serum liver enzymes (ALT, AST, and ALP), which serve as important biomarkers of hepatocellular injury.[32] Supplementation with *black mulberry*, *green fenugreek*, or their combination significantly reduced these enzyme levels, indicating a protective effect against liver damage.[33] *Black mulberry* appeared to exert a stronger effect in reducing ALT levels, whereas *fenugreek* showed greater efficacy in lowering AST levels.[3t] The combined treatment resulted in the greatest reduction in ALP activity, suggesting complementary hepatoprotective mechanisms.[3u] These improvements may be attributed to the antioxidant properties of the phytochemicals present in both plants, which can inhibit lipid peroxidation, stabilize hepatocyte membranes, and enhance endogenous antioxidant defense systems.[3v] Anthocyanins in *mulberry*, together with flavonoids and saponins in *fenugreek*,

are likely responsible for these hepatoprotective effects.[3]w

Renal function parameters were also adversely affected by CCl,, exposure, as evidenced by increased levels of serum creatinine, urea, and uric acid.[3]x Supplementation with *black mulberry* and *green fenugreek* significantly ameliorated these alterations. *Fenugreek* showed a particularly strong effect in reducing uric acid levels, whereas the combined supplementation produced the greatest reduction in creatinine levels.[3]y These findings indicate a potential synergistic nephroprotective effect, which may result from the combined antioxidant and anti-inflammatory properties of the phytochemicals present in both plants. Such compounds can enhance renal antioxidant defenses, reduce oxidative injury, and improve kidney function under conditions of toxic stress.t p

In addition to hepatic and renal protection, CCl,, exposure significantly disrupted lipid metabolism, leading to increased serum total cholesterol and triglycerides and decreased HDL levels.[3]x These alterations are characteristic of oxidative stress–induced metabolic imbalance.t [1] Supplementation with either *black mulberry* or *green fenugreek* improved lipid profile parameters,[3]y while the combined treatment produced the most pronounced reductions in cholesterol and triglycerides and partially restored HDL levels.t p The hypolipidemic effects of *fenugreek* are mainly attributed to its soluble fibers and saponins, which reduce intestinal cholesterol absorption and promote bile acid excretion.t u Meanwhile, polyphenolic compounds in *mulberry* may enhance lipid metabolism and prevent oxidative modification of lipoproteins.[3]w The combined use of these two plants therefore appears to exert additive or synergistic effects in maintaining lipid homeostasis during oxidative stress conditions.t [2] The combined supplementation consistently produced the greatest protective effects, which may be attributed to the complementary phytochemical composition of the two plants. *Black mulberry* provides a rich source of phenolic antioxidants and anthocyanins,t t whereas *fenugreek* contributes saponins, fibers, and flavonoids that support metabolic regulation and organ protection.t [3] These findings support the potential use of these plant materials, either individually or in combination, as functional dietary supplements for the prevention of oxidative stress–related organ damage.t v

Limitations of the Study

Despite the promising findings of the present study, several limitations should be considered. First, the experiment was conducted under controlled laboratory conditions using an animal model, which may not fully reflect physiological responses in humans. Second, the study primarily focused on biochemical parameters without investigating the underlying molecular mechanisms responsible for the observed protective effects. Additionally, the identification and quantification of individual bioactive compounds responsible for these effects were not extensively explored. Therefore, future studies should include molecular investigations, isolation of active compounds, and clinical evaluations to further validate the therapeutic potential of *black mulberry* and *green fenugreek* in preventing oxidative stress related organ damage.

CONCLUSION

This study demonstrates that *black mulberry* and *green fenugreek* powders possess significant antioxidant, hepatoprotective, nephroprotective, and lipid-modulating properties. Their combined administration consistently produced synergistic effects, enhancing individual benefits, reducing oxidative stress, and improving both biochemical and physiological parameters in rats. These findings support the potential application of *black mulberry* and *green fenugreek* as natural dietary supplements for the prevention or mitigation of oxidative stress–related organ damage.

Recommendations for future research

1. Investigate the precise molecular mechanisms underlying the hepatoprotective and nephroprotective effects of these plants.
2. Assess the long-term efficacy and safety of *black mulberry* and *green fenugreek* supplementation in animal models and humans.
3. Explore the dose-response relationship to optimize the therapeutic potential of these plant powders.
4. Evaluate the synergistic interactions of *black mulberry* and *green fenugreek* with other natural antioxidants or dietary interventions.

These recommendations will help guide further studies to fully understand and harness the therapeutic potential of these medicinal plants.

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Conflict of Interest

The author(s) do not have any conflicts of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

All experimental protocols were reviewed and approved by the Research Ethics Committee of Al-Baha University (Ref. No. 46123022) on 17 April 2025. Animal care and handling followed institutional guidelines to ensure welfare and minimize distress.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to Reproduce Material from Other Sources

Not applicable

Author Contributions

Lobna Saad Mohammed Abd Elmeged: Conceptualization, Methodology, Data Collection, Analysis, Writing – Original Draft, and Final Approval of the Manuscript; Ali Khalaf Ahmed Albaggar: Data Review and Analysis, Writing – Review & Editing, Supervision, and Provision of Resources and Technical Support.

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