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BIOCHEMICAL AND HISTOPATHOLOGICAL EVALUATION OF THE HEPATOPROTECTIVE EFFECTS OF DIETARY FOENICULUM VULGARE POWDER IN CCL_4-INDUCED TOXICITY IN RATS

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ABSTRACT
Carbon tetrachloride (CCl_4)-induced hepatotoxicity is widely used as an experimental model to evaluate natural hepatoprotective agents. This study aimed to investigate the protective effects of Foeniculum vulgare powder against liver injury in rats using biochemical and histopathological assessments. A total of twenty-four male albino rats (150 ± 10 g) were randomly assigned into four experimental groups (n = 6 each): a healthy control group, a group exposed to CCl_4, and two CCl_4-exposed groups receiving dietary supplementation with 2% and 4% Foeniculum vulgare powder, respectively. for 28 days. At the end of the experiment, blood samples were collected for biochemical analyses, and liver tissues were processed for histological examination. Results indicated that the hydromethanolic extract of Foeniculum vulgare seeds contained high levels of phenolic and flavonoid compounds, reflecting significant antioxidant potential. Dietary supplementation improved growth performance and dose-dependently restored liver function, serum protein levels, and lipid profiles. Moreover, it mitigated renal dysfunction markers and preserved normal liver histoarchitecture, demonstrating its protective biological effects. These findings suggest that Foeniculum vulgare exerts dose-dependent antioxidant and organ-protective effects, supporting its potential therapeutic application. Nevertheless, further studies are required to confirm its efficacy and safety in broader experimental and clinical settings.

KEYWORDS: Foeniculum vulgare, Hepatoprotective effect, Carbon tetrachloride, Liver toxicity, Oxidative stress.

INTRODUCTION

Liver diseases pose a substantial global health burden, largely attributable to the liver's pivotal roles in metabolism, detoxification, and the maintenance of physiological homeostasis. Prolonged exposure to xenobiotics and environmental toxicants renders hepatic tissue particularly vulnerable to injury, which is commonly manifested by oxidative stress, inflammation, and programmed cell death. Carbon tetrachloride (CCl_4)-induced hepatotoxicity is a well-established experimental model frequently used to explore the molecular mechanisms of liver injury and to assess the effectiveness of potential hepatoprotective compounds. As depicted in Figure 1, CCl_4 is metabolically activated within hepatocytes by cytochrome P450 enzymes, resulting in the generation of highly reactive trichloromethyl (CCl_3) and trichloromethyl peroxy ($OOCCl_3$) radicals (Fareed et al., 2024). These reactive intermediates stimulate the overproduction of reactive oxygen species (ROS), thereby initiating lipid peroxidation, compromising membrane integrity, and impairing mitochondrial function.Collectively, these processes amplify oxidative stress and inflammatory responses, ultimately leading to hepatocellular injury that can advance to necrosis and apoptosis (Begum et al., 2022).

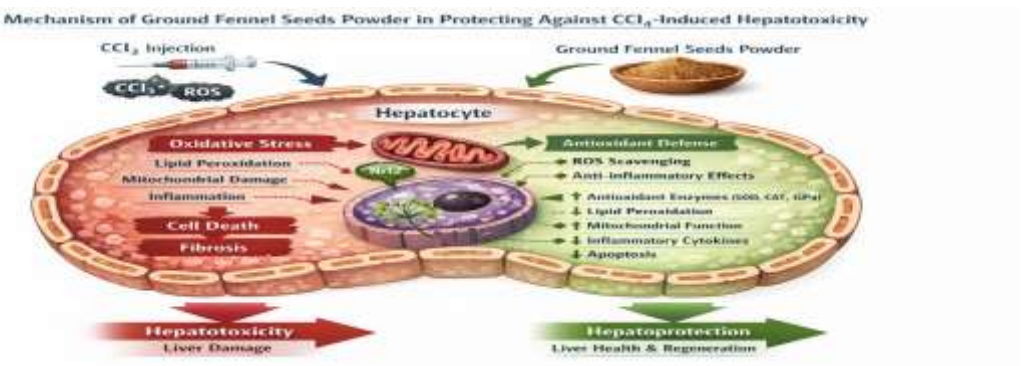

Figure 1. Diagrammatic illustration of the hepatoprotective effects of Foeniculum vulgare seed powder against carbon tetrachloride (CCl_4)-induced oxidative stress, inflammatory responses, and liver cell injury.

The oxidative damage cascade induced by carbon tetrachloride (CCl_4) is strongly linked to the excessive production of reactive oxygen species (ROS), which further aggravates mitochondrial dysfunction and stimulates inflammatory signaling pathways. Collectively, these disturbances lead to elevated serum biomarkers indicative of liver injury, along with marked histopathological changes. Owing to the multifactorial nature of this process, increasing attention has been directed toward identifying safe and effective hepatoprotective agents capable of modulating multiple pathological pathways simultaneously. In this regard, natural products derived from medicinal plants have attracted considerable interest due to their abundance of bioactive constituents—such as polyphenols, flavonoids, and volatile compounds which exhibit pronounced antioxidant, anti-inflammatory, and cytoprotective activities (Gonfa et al., 2024). These compounds contribute to hepatoprotection by scavenging free radicals, enhancing endogenous antioxidant defense systems, and stabilizing cellular structures, thereby reducing hepatic injury. Foeniculum vulgare (fennel) is a widely recognized aromatic medicinal plant traditionally used in the management of gastrointestinal, respiratory, and liver-related disorders. Phytochemical analyses have identified a variety of active constituents, including trans-anethole, flavonoids, phenolic acids, and essential oils, which underlie its potent antioxidant and anti-inflammatory properties. Accumulating evidence indicates that fennel exerts significant hepatoprotective effects across different experimental models of oxidative stress (Barakat et al., 2022; Badgujar et al., 2014). Notably, fennel seed powder has demonstrated a strong capacity to mitigate CCl_4-induced liver injury through multiple integrated mechanisms at the cellular level. These protective effects are largely attributed to the upregulation of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which collectively alleviate oxidative stress and restore redox balance (Zahi et al., 2025; Rudrapal et al., 2022; Barakat et al., 2022). In addition to its antioxidant activity, fennel has been shown to attenuate hepatotoxicity by reducing serum levels of liver enzymes and improving histopathological features, thereby preserving both hepatic function and structural integrity (Barakat et al., 2022; Ozbek et al., 2003). These beneficial outcomes are also associated with the modulation of intracellular signaling pathways involved in oxidative stress and inflammation. In particular, fennel-derived bioactive compounds may activate cytoprotective pathways such as nuclear factor erythroid 2–related factor 2 (Nrf2), while concurrently inhibiting pro-inflammatory mediators. Through these coordinated mechanisms, fennel reduces lipid peroxidation, maintains mitochondrial integrity, and suppresses apoptosis, ultimately supporting liver recovery and regeneration. Despite its well-established ethnopharmacological significance, comprehensive investigations into the dietary application of F. vulgare in CCl_4-induced hepatotoxicity remain limited. Evaluating fennel in its dietary form offers a more practical and translational perspective, as it reflects real-life nutritional interventions that may confer hepatoprotective benefits with fewer adverse effects compared to high-dose extracts or isolated compounds. Dietary phytochemicals are increasingly recognized for their role in modulating endogenous antioxidant systems, including SOD, CAT, and GPx, thereby enhancing cellular resilience to oxidative stress (Zahi et al., 2025; Rudrapal et al., 2022). Biochemical markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin fractions are widely employed as sensitive indicators of liver function and integrity. Elevated levels of these parameters reflect hepatocellular damage and impaired liver function following toxic exposure. Furthermore, histopathological evaluation provides critical insight into structural alterations within hepatic tissue such as fatty changes, necrosis, inflammatory cell infiltration, and sinusoidal dilation allowing for a comprehensive assessment of liver injury and therapeutic efficacy (Begum et al., 2022). Given the complex and interconnected mechanisms underlying CCl_4-induced liver injury including oxidative stress, lipid peroxidation, mitochondrial dysfunction, and inflammation it is essential to explore therapeutic strategies capable of targeting these multiple pathways. Incorporating fennel into the diet may not only improve biochemical indices but also preserve hepatic architecture, highlighting its potential as a preventive nutritional approach (Liao et al., 2024).

2- Aim of study

This study investigated the protective effect of Foeniculum vulgare powder on liver damage in rats using biochemical and histopathological approaches

3- MATERIALS AND METHODS

3.1. Materials

3.1.1. Preparation of F. vulgare Powder: Dried seeds of Foeniculum vulgare were procured from local markets in Al-Baha City, Kingdom of Saudi Arabia. The plant material was taxonomically authenticated and thoroughly cleaned to eliminate any extraneous matter. The seeds were then dried in a hot air oven at 50 °C until a constant weight was achieved, ensuring complete removal of residual moisture. Subsequently, the dried seeds were pulverized using a mechanical grinder to obtain a fine powder. The resulting fennel seed powder was incorporated into the experimental diets at concentrations of 2% and 4% throughout the study period

3.1.2. Experimental Animals : In this study, twenty-four (24) male albino rats of the Sprague Dawley strain, with an average body weight of 150 ± 10 g, were utilized. The animals were maintained under standardized laboratory conditions, including a 12-hour light/dark cycle, and were provided with a

standard basal diet and water ad libitum. Prior to the initiation of the experimental procedures, all rats were acclimatized to the laboratory environment for one week to ensure physiological adaptation and stability.

3.1.3. Hepatotoxic Agent: Carbon Tetrachloride (CCl_4) : High-purity carbon tetrachloride (CCl_4) was obtained from El-Gomhouria Company for Chemical Industries (Cairo, Egypt) in a 10% liquid formulation. The reagent was supplied in one-liter opaque plastic containers and was employed in this study as a chemical inducer of experimental liver injury, following the methodology described by Passmore and Eastwood (1986). Before administration, the CCl_4 solution was diluted with pharmaceutical-grade paraffin oil to achieve the desired concentration for inducing hepatotoxicity in the experimental rat models.

3.2. METHODS

3.2.1. Biological Experiment and Dietary Intervention

The experimental diets were prepared by supplementing the basal diet with powdered Foeniculum vulgare at inclusion levels of 2% and 4% (w/w). The basal diet composition was maintained to meet standard nutritional requirements, with the fennel powder incorporated to assess its dose-dependent hepatoprotective effects. The detailed composition and proportions of the dietary ingredients for both the basal and experimental diets are provided in Table 1

Table (1): Composition of the Basal and Experimental Diets (g/100g).

Component (g)	Basal Diet (Control)	2% F. vulgare	4% F. vulgare
Fennel Powder (FVP)	---	2.0	4.0
Casein (Protein)	10.0	10.0	10.0
Corn Oil (Fat)	4.7	4.7	4.7
Mineral Mixture	3.5	3.5	3.5
Vitamin Mixture	1.0	1.0	1.0
Cellulose	5.0	5.0	5.0
Choline Chloride	0.2	0.2	0.2
Sucrose	10.0	10.0	10.0
Corn Starch	Up to 100	Up to 100	Up to 100

3.2.2. Experimental Animals and Ethical Considerations

Twenty-four clinically healthy adult male Sprague–Dawley albino rats, aged 14–16 weeks and weighing 150 ± 10 g, were acquired from the Animal House of the National Research Centre in Giza, Egypt. The sample size was determined based on previously published models of chemically induced hepatic injury to ensure sufficient statistical power while adhering to the principles of Replacement, Reduction, and Refinement (3Rs) for ethical animal use. The study was conducted in strict compliance with internationally recognized guidelines for animal welfare and received formal approval from the Research Ethics Committee of Al-Baha University (Approval No. 46123022; April 17, 2025).

Upon arrival, the rats were individually housed in sanitized, well-ventilated polypropylene cages with stainless-steel covers under controlled environmental conditions, including a 12-hour light/dark cycle. A seven-day acclimation period was implemented to ensure physiological stability, during which the animals had ad libitum access to a standard basal diet and clean drinking water. Food was provided via spill-resistant feeders to minimize waste and cross-contamination, while water was supplied through specialized bottles with stainless-steel sippers. At the conclusion of the acclimation period, all animals were confirmed to be in optimal health, with no mortality or adverse events observed. The rats were then randomly assigned into four experimental groups using a simple randomization method.

3.2.3. Induction of Experimental Hepatotoxicity

Chronic hepatic injury in rats was induced using a standardized carbon tetrachloride (CCl_4) intoxication protocol, as described by Jayasekhar et al. (1997). CCl_4 was diluted with pharmaceutical-grade paraffin oil to prepare a 50% (v/v) solution. The solution was administered via intramuscular (I.M.) injection at a dose of 2 ml/kg body weight, twice weekly, over a period of two consecutive weeks. At the end of the induction phase, blood samples were carefully collected from the retro-orbital plexus to evaluate liver function and confirm the successful establishment of hepatic injury through the measurement of specific biochemical markers.

3.2.4. Experimental Design and Group Allocation:

Following the acclimatization period, twenty-four (24) male Sprague-Dawley rats were randomly assigned to four experimental groups (n=6 per group). The study was designed to evaluate the prophylactic efficacy of dietary Foeniculum vulgare powder against CCl_4 induced chronic hepatotoxicity over a period of four weeks. The groups were allocated as follows:

- Group 1 (Negative Control): Rats were maintained on a standard basal diet and received intramuscular (I.M.) injections of paraffin oil (vehicle) twice weekly.

- Group 2 (Positive Control - CCl_4): Rats were fed a standard basal diet and subjected to CCl_4 intoxication (50% v/v in paraffin oil) via I.M. injection at a dosage of 2 ml/kg B.Wt., twice weekly for the duration of the study to induce chronic liver injury, according to Jayasekhar et al. (1997).
- Group 3 (CCl_4 + 2% FVP): Rats were fed a basal diet supplemented with 2% (w/w) F. vulgare powder and concurrently received CCl_4 injections as described for Group 2.
- Group 4 (CCl_4 + 4% FVP): Rats were fed a basal diet supplemented with 4% (w/w) F. vulgare powder and concurrently received CCl_4injections as described for Group 2.

At the end of the experimental period, blood samples were collected via the retro-orbital plexus technique for biochemical analysis, followed by the excision of liver tissues for histopathological examination to confirm the protective capacity of the dietary intervention."

3.2.6. Blood Sampling and Serum Preparation

At the end of the 28-day study, the rats were fasted for 12 hours with water available ad libitum. Blood was collected from the retro-orbital plexus using sterile capillary tubes and placed into clean, dry centrifuge tubes. Samples were allowed to clot at 37 °C for 30 minutes, followed by centrifugation at 3000 rpm for 10 minutes to separate the serum. The collected serum was carefully transferred into labeled polypropylene tubes and stored at −20 °C until subsequent biochemical analyses, including measurements of glucose and liver function markers.

3.2.7. Organ Collection and Histopathological Preparation

Immediately following blood collection, the animals were euthanized, and the liver, kidneys, heart, and spleen were carefully excised. The harvested organs were thoroughly rinsed in ice-cold physiological saline (0.9% NaCl) to remove any residual blood and subsequently weighed to determine their relative organ weights. For histopathological analysis, the organs were fixed in 10% neutral buffered formalin, following the standardized protocol described by Drury and Wallington (1980).

3.2.6. Biological evaluation:

Throughout the 28-day experimental period, daily food intake was carefully recorded, and body weight measurements were taken weekly for all rats. At the end of the study, key nutritional and growth parameters were calculated according to the methodology described by Chapman et al. (1959). These parameters included:

- **Body Weight Gain (BWG %):** Assessed to evaluate the overall growth performance of the animals.
- **Food Efficiency Ratio (FER):** Calculated to determine the efficiency of dietary utilization relative to body weight gain.
- **Relative Organ Weight:** The liver and other relevant organs were excised and weighed at the end of the experiment to calculate the organ-to-body weight ratio. The following formulas were applied for the respective calculations::

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

$$\text{FER} = \frac{\text{Body Weight Gain (g)}}{\text{Total Food Intake (g)}}$$

3.2.8. Phytochemical Screening

3.2.8. 1. Total Phenolic Content (TPC)

The total phenolic content (TPC) of the hydromethanolic extract of Foeniculum vulgare seeds was determined using the Folin–Ciocalteu colorimetric method, following the procedure described by Singleton and Rossi (1965). In brief, the extract was mixed with Folin–Ciocalteu reagent and 7.5% sodium carbonate (Na_2CO_3). After incubation at room temperature for 30–60 minutes, the absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content (TPC) was quantified and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g), offering a precise measure of the extract's phenolic constituents.

3.2.8. 2. Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the extract was measured using the aluminum chloride ($AlCl_3$) colorimetric assay, following the method of Zhishen et al. (1999). In this procedure, the extract was mixed with 10% $AlCl_3$ solution and 1 M potassium acetate, then incubated for 30 minutes. The absorbance was recorded at 415 nm, and the flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g). This assay provides a reliable estimation of the total flavonoid compounds in the extract, which are recognized for their antioxidant and bioactive properties.

3.2.9. Biochemical Analysis

The biochemical parameters in the collected serum were quantified using commercially available diagnostic kits and measured via a spectrophotometer. The following methodologies were employed:

3.2.8.1. Liver Function Biomarkers

- **Aminotransferase Activities (ALT and AST):** Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using a colorimetric assay based on the method of Reitman and Frankel (1957).
- Alkaline Phosphatase (ALP): Serum ALP activity was evaluated colorimetrically following the enzymatic procedure established by Roy (1970).
- Total Bilirubin (T.BIL): The concentration of total bilirubin was quantified at a wavelength of 578 nm using the colorimetric approach described by Doumas et al. (1973).

3.2.8.2. Serum Lipid Profile

- Total Cholesterol (TC) and Triglycerides (TG): Serum TC and TG levels were assessed using enzymatic colorimetric methods according to Ratliff and Hall (1973) and Jacobs and Van Denmark (1960), respectively.
- High-Density Lipoprotein (HDL-c): Serum HDL-c was estimated following the standardized enzymatic techniques of Jacobs and Van Denmark (1960).
- VLDL-c and LDL-c Fractions: The concentrations of very-low-density lipoprotein (VLDL-c) and low-density lipoprotein (LDL-c) were mathematically calculated based on the primary lipid parameters according to the formulas described by Lee and Nieman (1996).

3.2.8.3. Estimation of Blood Glucose Concentration

Serum glucose levels were determined using the enzymatic glucose oxidase (GOD-PAP) technique, following the methodology established by Trinder (1969). This specific 'Trinder method' relies on the catalytic oxidation of glucose to produce gluconic acid and hydrogen peroxide. The latter reacts with a chromogenic oxygen acceptor to yield a colored product, the absorbance of which was recorded at 505 nm via a spectrophotometer to calculate the glucose concentration in mg/dL.

3.2.9 . Statistical Analysis

All data were expressed as mean ± standard deviation (Mean ± SD), and differences between experimental groups were evaluated using one-way analysis of variance (ANOVA). For pairwise comparisons, the Least Significant Difference (LSD) post-hoc test was applied to identify specific differences between group means at a significance level of $p < 0.05$. All statistical analyses were conducted using SPSS software (Version 25.0; IBM Corp., Armonk, NY, USA), following the procedures outlined by Snedecor and Cochran (1967).

3.2.10. Ethical Approval and Compliance

All experimental procedures involving live animals were carried out in strict compliance with institutional guidelines and international standards for the ethical care and use of laboratory animals. The experimental protocol, encompassing all animal handling and study procedures, received formal approval from the Research Ethics Committee of Al-Baha University (Approval No. 46123022; April 17, 2025). Every effort was made to minimize animal suffering and to use the smallest number of animals required to achieve statistically meaningful results, in accordance with established ethical principles.

4. RESULTS

4.1 Phenolic Profile

The hydromethanolic extract of Foeniculum vulgare seeds was analyzed for its total phenolic and flavonoid content, and the results are summarized in Figure 3. The extract exhibited a high total phenolic content of 42.10 mg GAE/g, indicating a substantial presence of bioactive phenolic compounds capable of contributing to antioxidant activity. In parallel, the flavonoid content was measured at 125.8 mg QE/g, reflecting the abundance of flavonoid compounds, which are known to exert significant free radical scavenging and protective effects in biological systems.

These findings suggest that the hydromethanolic extract of F. vulgare seeds is rich in phenolic and flavonoid compounds, supporting its potential use as a natural source of antioxidants and its therapeutic relevance in mitigating oxidative stress. The elevated levels of these bioactive compounds are consistent with the observed biological activities reported in previous studies and provide a chemical basis for the pharmacological properties of fennel.

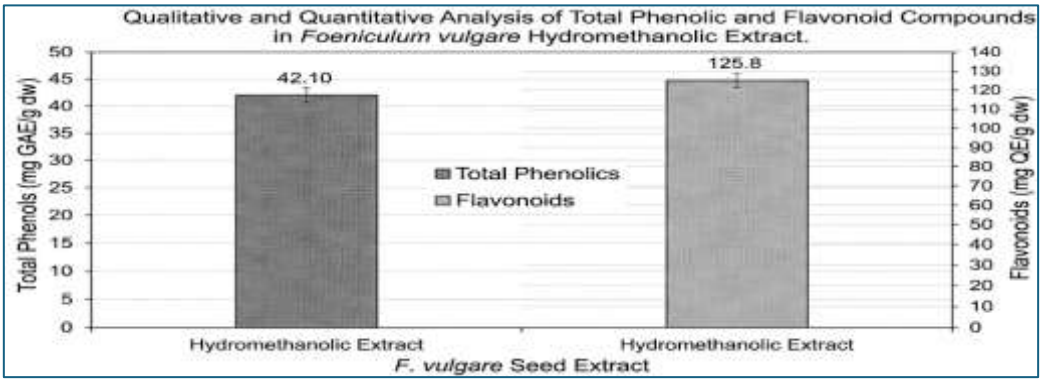

Figure 3. Quantitative phytochemical analysis of the hydromethanolic extract of Foeniculum vulgare seeds. (A) (A) Total phenolic content, reported as milligrams of gallic acid equivalent per gram of extract (mg GAE/g). (B) Total flavonoid content, reported as milligrams of quercetin equivalent per gram of extract (mg QE/g).

4.2 Biological changes:

The effects of dietary supplementation with Foeniculum vulgare on body weight gain (BWG), food intake (FI), and feed efficiency ratio (FER) are summarized in Figure 4. The negative control group exhibited the highest BWG, FI, and FER values (1.102 ± 0.003 g/28 days, 22.63 ± 0.09 g/day, and 0.910 ± 0.004, respectively), reflecting normal growth performance.

In contrast, the positive control group showed a significant reduction ($p \leq 0.05$) in all measured parameters, with the lowest values recorded for BWG (0.829 ± 0.002 g/28 days), FI (8.34 ± 0.04 g/day), and FER (0.038 ± 0.001), indicating the detrimental effects of the experimental condition.

Dietary supplementation with F. vulgare notably improved growth performance relative to the positive control group. Rats receiving 2% F. vulgare (Group 3) demonstrated moderate increases in BWG, FI, and FER (0.951 ± 0.005 g/28 days, 12.41 ± 0.07 g/day, and 0.060 ± 0.002, respectively), whereas those fed 4% F. vulgare (Group 4) exhibited more pronounced improvements (1.048 ± 0.004 g/28 days, 15.52 ± 0.06 g/day, and 0.101 ± 0.003), approaching the values observed in the negative control group.

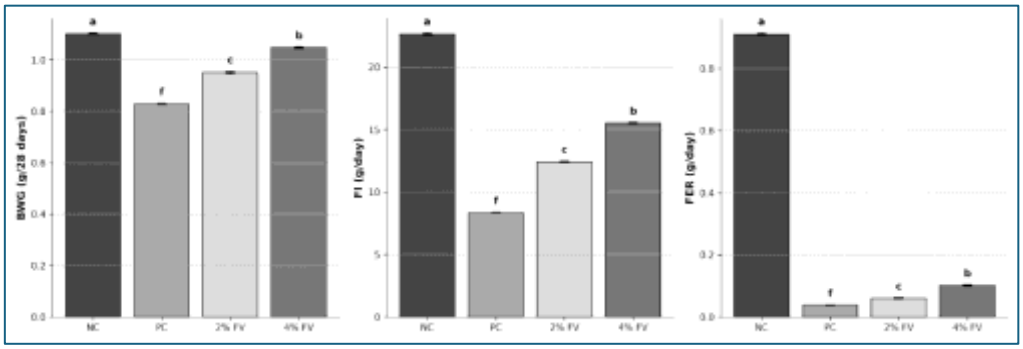

Figure 4. Growth performance parameters, including body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FER) of experimental groups. Data are presented as Mean ± SD (n = 6). Bars labeled with different superscript letters (a, b, c, f) represent statistically significant differences at P < 0.05, as determined by one-way ANOVA followed by Duncan's multiple range test.

4.3.Biochemical Analysis

The effects of Foeniculum vulgare supplementation on liver enzyme activities, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), are presented in **Figure 5**. The negative control group exhibited the lowest levels of ALT, AST, and ALP (21.8 ± 1.70, 21.9 ± 1.80, and 66.5 ± 2.30 U/L, respectively), reflecting normal hepatic function. In contrast, the positive control group showed a marked and statistically significant elevation ($p \leq 0.05$) in all measured enzymes, with values reaching 71.2 ± 2.90 U/L for ALT, 72.4 ± 2.70 U/L for AST, and 89.3 ± 2.80 U/L for ALP, indicating severe hepatic injury. Administration of Foeniculum vulgare resulted in a significant attenuation of liver enzyme levels compared to the positive control group. Rats treated with 2% F. vulgare (Group 3) demonstrated a moderate reduction in ALT, AST, and ALP levels (44.1 ± 3.90, 53.8 ± 5.20, and 77.4 ± 2.90 U/L, respectively). Meanwhile, the group receiving 4% F. vulgare (Group 4) showed a more pronounced improvement, with enzyme levels (30.6 ± 3.20, 40.8 ± 2.90, and 72.3 ± 1.90 U/L, respectively) approaching those of the negative control group.

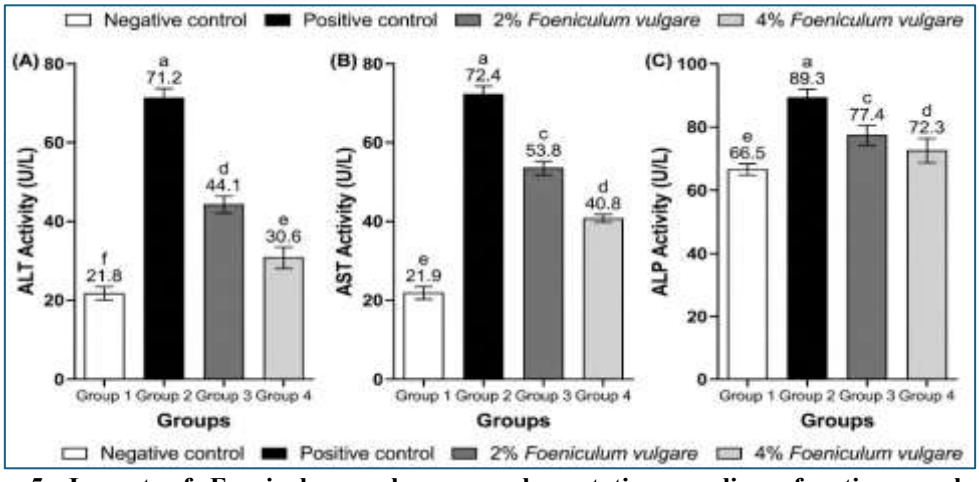

Figure 5. Impact of Foeniculum vulgare supplementation on liver function markers in experimental rats. Panels show: (A) Alanine aminotransferase (ALT) activity, (B) Aspartate aminotransferase (AST) activity, and (C) Alkaline phosphatase (ALP) activity. Data are expressed as Mean ± SD (n = 6). Bars with different superscript letters (a, c, d, e, f) indicate significant differences at P < 0.05, based on one-way ANOVA followed by Duncan's multiple range test.

The effects of Foeniculum vulgare supplementation on serum albumin and total protein levels are presented in **Figure 6**. The negative control group exhibited the highest values of serum albumin and total protein (5.48 ± 0.34 and 7.36 ± 0.25 mg/dl, respectively), reflecting normal hepatic synthetic function. In contrast, the positive control group showed a significant decline ($p \leq 0.05$) in both parameters, recording 2.60 ± 0.35 mg/dl for albumin and 4.98 ± 0.30 mg/dl for total protein, indicating

impaired liver function. Dietary administration of Foeniculum vulgare resulted in a significant improvement in serum protein profiles compared to the positive control group. Rats treated with 2% F. vulgare (Group 3) demonstrated a moderate increase in albumin and total protein levels (4.22 ± 0.21 and 6.52 ± 0.22 mg/dl, respectively). Meanwhile, the group receiving 4% F. vulgare (Group 4) showed a more pronounced restoration, with albumin and total protein values (5.18 ± 0.26 and 7.02 ± 0.20 mg/dl, respectively) approaching those observed in the negative control group.

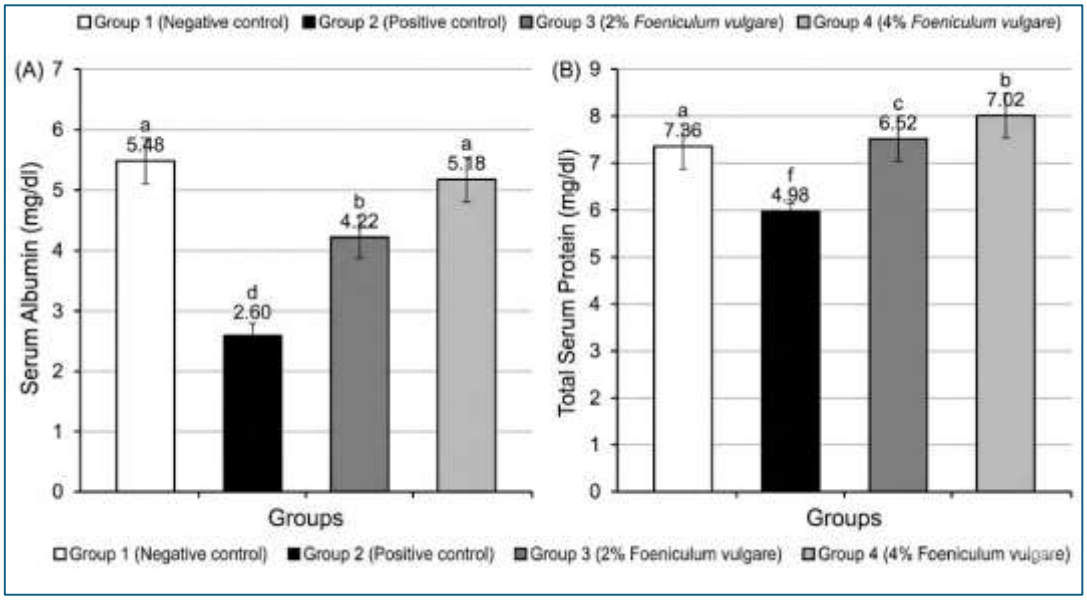

Figure 6. Effects of Foeniculum vulgare supplementation on serum protein parameters in experimental rats. (A) Serum albumin (mg/dl) and (B) Total serum protein (mg/dl). Values are expressed as Mean ± SD (n = 6). Columns labeled with different superscript letters (a–f) represent statistically significant differences at p ≤ 0.05, as determined by one-way ANOVA followed by Duncan's multiple range test.

Figure 7 summarizes the impact of Foeniculum vulgare supplementation on serum lipid parameters, including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and very-low-density lipoprotein cholesterol (VLDL-c). In the negative control group, rats exhibited the lowest total cholesterol (122.5 ± 3.40 mg/dl) and triglycerides (105.2 ± 5.10 mg/dl), along with the highest HDL-c concentration (42.6 ± 2.80 mg/dl). LDL-c and VLDL-c levels were 46.10 ± 4.90 mg/dl and 21.40 ± 1.10 mg/dl, respectively, indicating normal lipid homeostasis. These baseline values reflect intact hepatic lipid metabolism and efficient clearance of atherogenic lipoproteins under physiological conditions. Conversely, the positive control group displayed significant dyslipidemia, characterized by markedly elevated total cholesterol (242.8 ± 18.20 mg/dl) and triglyceride concentrations (215.6 ± 5.30 mg/dl). HDL-c was significantly reduced to 27.5 ± 2.60 mg/dl, whereas LDL-c and VLDL-c increased substantially to 145.30 ± 3.90 mg/dl and 43.20 ± 1.10 mg/dl, respectively. These alterations indicate impaired lipid metabolism, likely reflecting hepatocellular dysfunction and enhanced synthesis or decreased clearance of atherogenic lipoproteins, consistent with a pathological state. Dietary supplementation with F. vulgare demonstrated a dose-dependent ameliorative effect on lipid profiles. Administration of 2% F. vulgare (Group 3) partially restored lipid homeostasis, with total cholesterol decreasing to 158.7 ± 6.10 mg/dl and triglycerides to 126.4 ± 5.80 mg/dl. HDL-c increased to 34.9 ± 3.10 mg/dl, while LDL-c and VLDL-c were reduced to 66.80 ± 2.70 mg/dl and 25.40 ± 1.30 mg/dl, respectively. These results suggest moderate improvement in cholesterol transport and a partial reduction in atherogenic lipoproteins. A higher dose of 4% F. vulgare (Group 4) induced a more pronounced correction of dyslipidemia. Total cholesterol and triglyceride levels declined further to 141.2 ± 4.90 mg/dl and 119.3 ± 2.60 mg/dl, respectively, approaching the values observed in the negative control. HDL-c increased to 38.2 ± 2.90 mg/dl, while LDL-c and VLDL-c decreased to 58.30 ± 4.20 mg/dl and 24.30 ± 0.60 mg/dl, respectively. These findings indicate enhanced reverse cholesterol transport, reduced synthesis or enhanced clearance of LDL and VLDL, and overall improvement in lipid metabolism.

Overall, the results indicate that Foeniculum vulgare supplementation exerts dose-dependent hypolipidemic effects, characterized by an increase in HDL-c and a reduction in total cholesterol, triglycerides, LDL-c, and VLDL-c. These effects likely reflect both hepatoprotective activity and modulation of lipid metabolism, underscoring the potential of F. vulgare as a functional dietary intervention for the management of dyslipidemia.

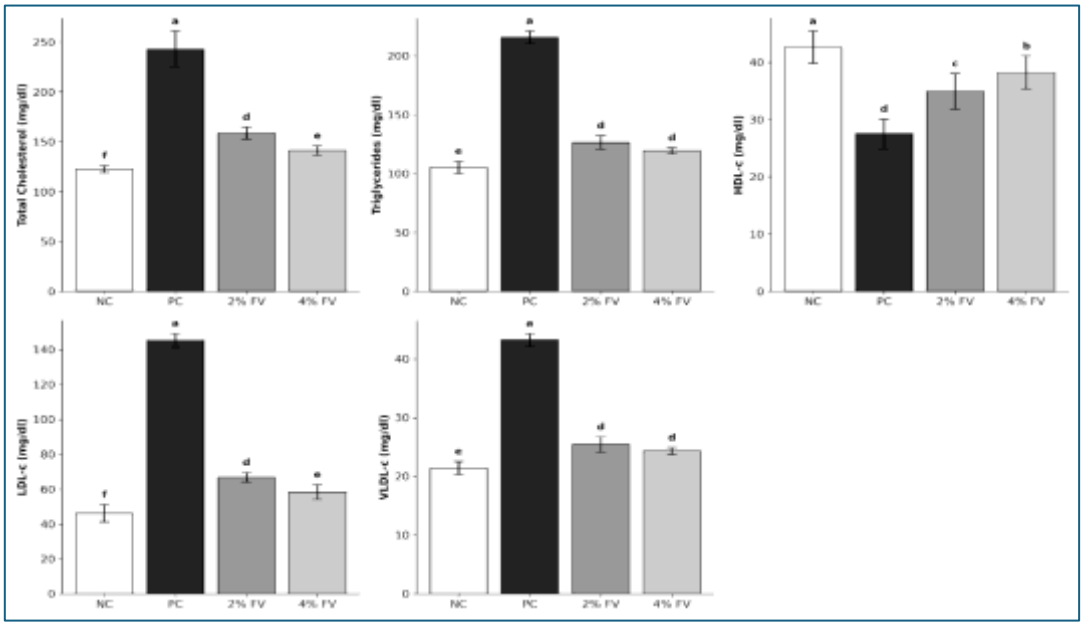

Figure 7. Effects of Foeniculum vulgare supplementation on serum lipid profile parameters in experimental rats. Panels represent: (A) Total cholesterol (TC), (B) Triglycerides (TG), (C) High-density lipoprotein cholesterol (HDL-c), (D) Low-density lipoprotein cholesterol (LDL-c), and (E) Very low-density lipoprotein cholesterol (VLDL-c). Data are shown as Mean ± SD (n = 6). Bars with different superscript letters (a–f) indicate significant differences at $p \leq 0.05$, as determined by one-way ANOVA followed by Duncan's multiple range test.

The effects of Foeniculum vulgare supplementation on renal function markers, including serum urea, uric acid, and creatinine, are presented in Figure 8. Rats in the negative control group exhibited the lowest levels of urea (18.8 ± 1.60 mg/dl), uric acid (2.10 ± 0.08 mg/dl), and creatinine (0.67 ± 0.04 mg/dl), indicating normal kidney function. In contrast, the positive control group showed a significant elevation ($p \leq 0.05$) in all renal markers, with urea reaching 36.5 ± 2.05 mg/dl, uric acid 3.82 ± 0.14 mg/dl, and creatinine 1.02 ± 0.12 mg/dl, reflecting renal impairment likely due to experimental toxicity. Dietary supplementation with F. vulgare significantly ameliorated these alterations in a dose-dependent manner. Administration of 2% F. vulgare (Group 3) reduced serum urea, uric acid, and creatinine levels to 27.3 ± 2.75 mg/dl, 2.88 ± 0.11 mg/dl, and 0.82 ± 0.08 mg/dl, respectively, indicating partial restoration of renal function. A higher dose of 4% F. vulgare (Group 4) further improved renal parameters, with urea at 22.1 ± 3.00 mg/dl, uric acid at 2.52 ± 0.16 mg/dl, and creatinine at 0.74 ± 0.10 mg/dl, approaching the values observed in the negative control group. These findings suggest that F. vulgare supplementation exerts a **protective effect on renal function**, mitigating the toxic effects observed in the positive control group.

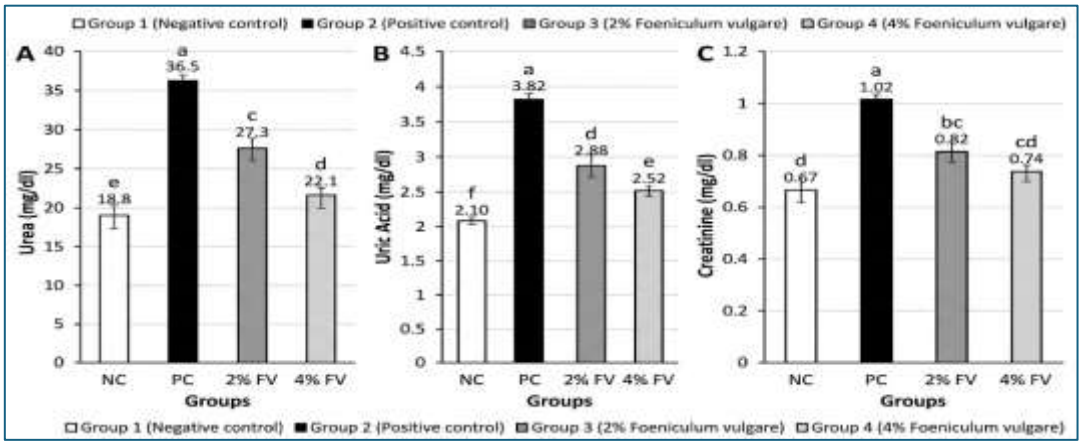

Figure 8. Effects of Foeniculum vulgare supplementation on kidney function biomarkers in experimental rats. Panels represent: (A) Urea (mg/dl), (B) Uric acid (mg/dl), and (C) Creatinine (mg/dl). Values are expressed as Mean ± SD (n = 6). Bars labeled with different superscript letters (a, bc, cd, d, e, f) represent statistically significant differences at $p \leq 0.05$, as determined by one-way ANOVA followed by Duncan's multiple range test.

4.5 Histopathological Analysis of Liver Sections

Liver sections were examined histologically following Hematoxylin and Eosin (H&E) staining at 400× magnification.

(A) Negative Control Group: The hepatic architecture appeared intact and well-organized. The central vein (CV) was clearly defined and surrounded by orderly cords of hepatocytes exhibiting prominent nuclei and distinct sinusoidal spaces. No signs of inflammation, necrosis, or cellular damage were observed, reflecting normal liver morphology.
(B) Positive Control Group (CCl4-intoxicated): Sections revealed severe structural disruption. Dense inflammatory cell infiltration was observed, particularly around the portal tracts and centrolobular regions. Widespread hepatocyte necrosis and cytoplasmic vacuolation were evident, indicating extensive hepatotoxicity induced by CCl4.
(C) CCl4 + 2% F. vulgare: Histological examination showed moderate improvement in hepatic architecture. Inflammatory foci were noticeably reduced, although some residual congestion and localized hepatocellular swelling persisted. The tissue demonstrated an early stage of recovery compared to the positive control group.
(D) CCl4 + 4% F. vulgare: Liver sections exhibited a pronounced hepatoprotective effect. The parenchyma appeared largely restored, with a marked decrease in necrotic areas and inflammatory infiltration. The histological features closely resembled those of the negative control group, indicating a dose-dependent protective effect of fennel powder against chemically induced liver injury.

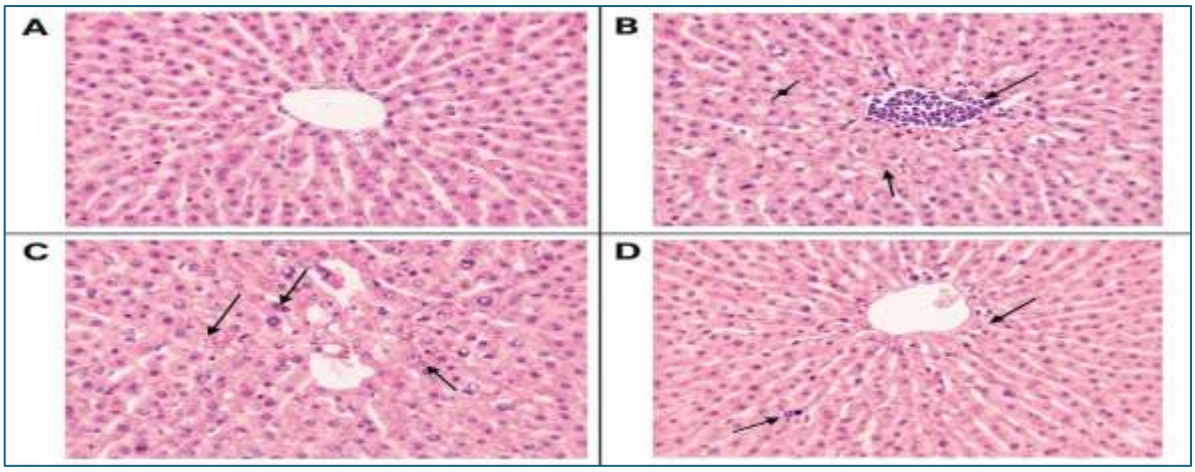

Figure 9. Histological evaluation of rat liver sections using hematoxylin and eosin (H&E) staining at 400× magnification.

5- DISCUSSION

The present study demonstrated that dietary supplementation with Foeniculum vulgare exerts significant protective effects on hepatic and renal functions, improves growth performance, and modulates serum lipid profiles in rats subjected to CCl_4-induced hepatotoxicity in a dose-dependent manner. Phytochemical analysis revealed that the hydromethanolic extract of F. vulgare seeds contains high levels of phenolic and flavonoid compounds, which are well-established antioxidants capable of scavenging reactive oxygen species (ROS), mitigating oxidative stress, and stabilizing cellular membranes (Barakat et al., 2022; see Figure 10). These bioactive compounds may also enhance endogenous antioxidant enzyme activities, including superoxide dismutase (SOD) and catalase, thereby strengthening cellular defenses against oxidative damage (Cherbal et al., 2013). The observed hepatoprotective and renoprotective effects are consistent with the known ability of phenolic compounds to upregulate endogenous antioxidants while inhibiting lipid peroxidation pathways, ultimately maintaining the integrity of hepatic and renal tissues. Furthermore, the reduction in pro-inflammatory cytokines such as TNF-α and IL-6 suggests an additional anti-inflammatory mechanism that contributes to the mitigation of hepatocellular injury (Yakut et al., 2022).

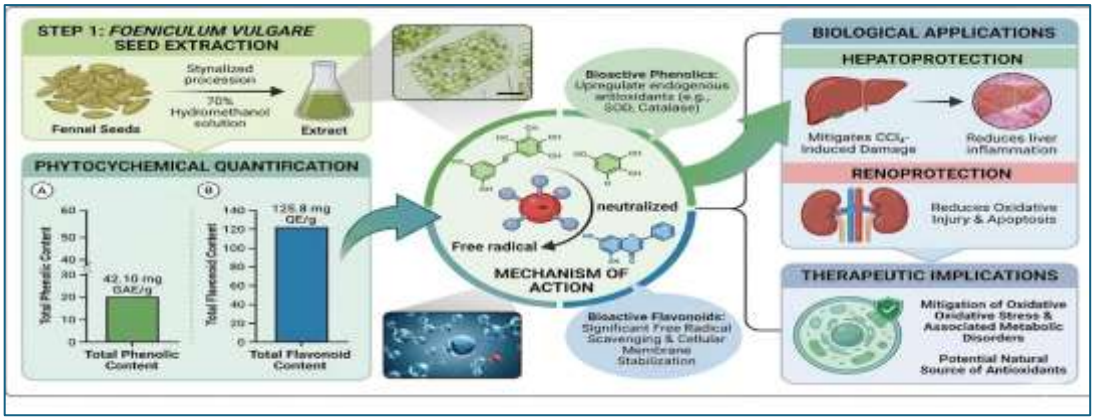

Figure 10: Illustration of how phenolic and flavonoid compounds from F. vulgare seeds mitigate oxidative stress, resulting in liver inflammation reduction.

Exposure to CCl_4 caused significant decreases in body weight gain (BWG), food intake (FI), and feed efficiency ratio (FER), indicating systemic toxicity and catabolic stress. Supplementation with 2% and 4% F. vulgare powder significantly reversed these effects in a dose-dependent manner, likely due to the antioxidant and bioactive properties of fennel, which reduce hepatocellular oxidative damage, improve nutrient absorption, and support energy metabolism. These findings align with previous studies demonstrating the ability of phenolic-rich plant extracts to counteract growth suppression induced by hepatotoxins (Batool et al., 2023). Biochemical analysis revealed marked elevations in serum liver enzymes ALT, AST, and ALP following CCl_4 administration, confirming hepatocellular damage. F. vulgare supplementation significantly reduced these enzyme activities in a dose-dependent manner. Serum albumin and total protein levels, reflecting hepatic synthetic function, were partially restored with 2% supplementation and nearly normalized with 4% supplementation. These results indicate stabilization of hepatocyte membranes, inhibition of lipid peroxidation, and restoration of protein synthesis, consistent with the antioxidative activity of phenolic and flavonoid compounds (Alkinani et al., 2021; Labban & Abd Elmeged 2026). Alterations in lipid metabolism observed in the CCl_4-treated group likely result from oxidative impairment of key enzymes such as HMG-CoA reductase and lipoprotein lipase, a phenomenon documented in rodent models of hepatotoxicity (Elmeged & Albaggar., 2026). Normalization of lipid profiles with F. vulgare suggests modulation of these enzymatic pathways or enhancement of reverse cholesterol transport via upregulation of apolipoprotein A-I synthesis (Mathias et al., 2025; Alzahrani et al., 2026). The partial restoration of serum albumin and total protein also indicates improved hepatic synthetic capacity, reinforcing evidence that antioxidant phytochemicals protect the protein synthesis machinery from CCl_4-induced depletion (Rasha &Lobna , 2026). CCl_4 intoxication induced dyslipidemia characterized by elevated total cholesterol, triglycerides, LDL-c, and VLDL-c, accompanied by decreased HDL-c, reflecting impaired hepatic lipid metabolism including reduced reverse cholesterol transport and increased production or decreased clearance of atherogenic lipoproteins. Dietary F. vulgare supplementation significantly ameliorated these alterations in a dose-dependent manner. Total cholesterol and triglycerides decreased, LDL-c and VLDL-c were reduced, and HDL-c increased toward negative control levels. These effects indicate improved hepatic lipid metabolism and potential cardiovascular protection, possibly through modulation of HMG-CoA reductase activity and enhanced reverse cholesterol transport (Zakernezhad et al., 2021). Renal biomarkers including urea, uric acid, and creatinine were elevated following CCl_4 exposure, indicating renal impairment secondary to oxidative stress and systemic toxicity. F. vulgare supplementation significantly and dose-dependently reduced these markers, highlighting its renoprotective potential. The bioactive phenolics and flavonoids likely mitigate tubular damage by reducing ROS generation, preventing apoptotic pathways, and supporting normal renal function (Kooti et al., 2017). These improvements align with evidence that oxidative stress is central to nephrotoxicity and that phenolic compounds can attenuate renal tubular injury through antioxidant and anti-apoptotic mechanisms (Baptista et al., 2024). Collectively, these findings suggest that F. vulgare confers hepatoprotective, renoprotective, and hypolipidemic effects through complementary mechanisms including antioxidant and anti-inflammatory actions, modulation of lipid-metabolizing enzymes, and stabilization of protein synthesis. These results provide a biochemical rationale for the traditional use of fennel and support its potential as a functional dietary intervention to counteract oxidative stress-related hepatic and renal disorders (Xu et al., 2022).Histopathological examination corroborated the biochemical and phytochemical data. CCl_4 administration caused marked structural disruption of hepatic tissue including centrilobular necrosis, cytoplasmic vacuolation, and dense inflammatory cell infiltration, consistent with elevated liver enzymes and disrupted protein profiles. F. vulgare supplementation protected hepatic architecture in a dose-dependent manner. The 2% FVP group showed moderate improvement with reduced inflammatory foci and partial restoration of hepatocyte integrity, while the 4% FVP group exhibited near-complete restoration of liver parenchyma closely resembling the negative control(Patel & Goyal 2015). These histological improvements corresponded with significant reductions in ALT, AST, ALP, and normalization of serum albumin and total protein. The protective effects are attributable to phenolic and flavonoid compounds, which stabilize membranes, scavenge ROS, and suppress pro-inflammatory cytokines such as TNF-α and IL-6, thereby mitigating hepatocellular injury. The dose-dependent histological recovery further supports the notion that higher concentrations of F. vulgare provide superior hepatoprotection consistent with its phytochemical content.

Overall, the integration of histopathological, biochemical, and phytochemical findings supports the efficacy of Foeniculum vulgare as a natural hepatoprotective agent capable of mitigating chemically induced liver toxicity and provides a mechanistic basis for its traditional medicinal use.

6- CONCLUSION

The present study demonstrates that dietary supplementation with Foeniculum vulgare powder confers significant hepatoprotective, renoprotective, and hypolipidemic effects in CCl_4-induced toxic rats. The observed improvements in growth performance, normalization of liver and kidney biomarkers, restoration of serum protein levels, and modulation of lipid profiles underscore the therapeutic potential of F. vulgare as a functional dietary intervention. These effects are likely mediated by the high content of phenolic and flavonoid compounds, which exert antioxidant, anti-inflammatory, and membrane-

stabilizing actions, thereby mitigating oxidative stress and tissue damage. Dose-dependent responses observed in this study suggest that higher supplementation levels provide greater protective efficacy, highlighting the importance of optimizing intake for maximal therapeutic benefit. The findings support the traditional use of F. vulgare and provide a mechanistic framework for its pharmacological activities. Collectively, this study establishes F. vulgare as a promising natural agent for preventing and alleviating chemically induced hepatic and renal injuries, as well as associated metabolic disturbances, and lays the foundation for further research toward clinical applications and nutraceutical development.

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