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Research Article

TNF-α and IL-1β in Diabetes-Induced Liver Damage: The Relationship between *Trachyspermum ammi* Seeds Methanol Extract and Inflammatory Cytokine Inhibition

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The objective of this study was to determine the effect of *Trachyspermum ammi* (*T. ammi*) on the liver of streptozotocin (STZ)-induced diabetic rats. This study began by identifying the methanolic composition of *T. ammi*. The presence of three compounds was determined using gas chromatography-mass spectrometry (GC-MS), with Thymol (60.705%), Y-Terpinene (22.216%), and P-cymene (17.078%) being the most prevalent. Afterwards, diabetic rats were treated with *T. ammi* (200–500 mg/kg) or losartan (20 mg/kg) daily for 60 days. In the diabetic rats treated with *T. ammi*, levels of hepatic indicators, lipid peroxidation markers, and proinflammatory mediators decreased significantly. In the liver of *T. ammi*-treated diabetic rats, glutathione-S-transferase (GST), catalase (CAT), and glutathione (GSH) increased significantly. Despite the fact that proinflammatory cytokine levels were significantly increased, interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) levels decreased in the groups administered the extract. From these results, it can be concluded that *T. ammi* significantly restored the liver's antioxidant balance and inflammation caused by hyperglycemia.

1. Introduction

Diabetes is a chronic metabolic illness defined by high blood glucose levels, or hyperglycemia, which is typically caused by a lack of insulin secretion and/or insulin hormone action in pancreatic beta cells [1]. Diabetes is seen as a health threat in both developed and developing countries. The complications of this disease can affect several vital tissues in the body and cause pathological and irreversible conditions such as hepatopathy, retinopathy, cardiovascular diseases, neuropathy, and nephropathy [2].

Studies have shown that elevated blood sugar is associated with liver damage such as fibrosis, hepatocellular carcinoma cirrhosis, glycogen deposition, nonalcoholic fatty liver disease, and severe elevations of liver enzymes. In addition, the excessive accumulation of fat in the liver may cause severe metabolic disorders [3].

The primary processes implicated in the development of diabetes-induced liver disorders are reactive oxygen species (ROS) generation and hyperglycemia-induced oxidative stress. Hyperglycemia-induced ROS generation and oxidative stress increase nitric oxide (NO) synthesis in diabetic liver injury. When NO interacts with ROS, it activates numerous cellular signaling pathways, resulting in lipid peroxidation and protein nitration, leading to diabetes-induced tissue damage [4].

The liver plays a crucial role in carbohydrate metabolism regulation and is one of the organs most affected by oxidative stress caused by high blood sugar [5]. Both hyperglycemia and oxidative stress could lead to liver tissue

necrosis and increased ROS and/or reactive oxygen species such as hydroxyl radicals, superoxide, hydrogen peroxide, and singlet oxygen [3].

Trachyspermum ammi (L.) Sprague seeds (*T. ammi*) are an *Apiaceae* plant containing carbohydrates, proteins, lipids, glycosides, minerals, and phenolic compounds with diverse medicinal characteristics [6]. The seeds of *T. ammi* are small and brown in color (Figure 1). It is frequently considered that *T. ammi* seeds are safe because they have been used for a long time to treat inflammatory diseases and digestive disorders. It has an advantage over other plant products that exhibit antioxidant activity due to its ease of availability and low cost [6].

Many studies on animals have shown that *T. ammi* has hypoglycemic, hypocholesterolemic, antiarthritic, antirheumatic, and antidiabetic effects [8–10]. The present study aims to determine if this plant can restore the liver's antioxidant defense system in streptozotocin (STZ)-induced diabetic rats.

2. Materials and Method

2.1. Materials. Gallic acid, 2, 2-diphenyl-1-picrylhydrazyl, ascorbic acid, and quercetin were purchased from Sigma-Aldrich. Sodium Citrate Mono, STZ, Xylazine, Ketamine, and Methanol were obtained and procured from Sigma-Aldrich (Steinheim, Germany). The Folin–Ciocalteu, phenol regent, Potassium acetate, sodium carbonate, and aluminum chloride were obtained from Merck (Darmstadt, Germany).

2.2. Plant Materials and Extract Preparation. Seeds of *T. ammi* after collection were detected at the Islamic Azad University branch in Falavarjan, Isfahan (Iran) (Herbarium Accession No. 091/122/001). First, *T. ammi* seeds were dried and powdered. 50 grams of *T. ammi* seeds were mixed with 500 ml of methanol and placed on a rotary (Irankhodsaz, Iran) for 72 h. The solution was filtered next. Finally, Heidolph Rotavapor dried the extract (Germany).

2.3. Total Phenolic Assay. The Folin–Ciocalteu technique was used to determine the total phenolic content (TPC) of *T. ammi* seed extract [11]. In this method, the Folin–Ciocalteu reagent is reduced by phenolic compounds in an alkaline environment and yields a blue complex. It was incubated at 40°C for 30 min; afterwards, a concentration series was prepared. The absorption was read at a wavelength of 760 nm by a spectrophotometer (SHIMADZU, 240A-T, Japan). Samples were examined in three replications. Gallic acid was the total phenol standard sample. The TPC was reported in mg GAE/g DW (milligram Gallic acid equivalents/gram dry weight) extract.

2.4. Total Flavonoid Assay. The total flavonoid content (TFC) was measured by aluminum chloride [12]. The aluminum chloride calorimeter is based on the formation of an acidic aluminum chloride complex with a hydroxyl flavonoid group. The maximum absorption wavelength is 420 nm. The standard calibration curve chemical was flavonol quercetin. In 0.5 ml of methanol, *T. ammi* seed methanolic extract was dissolved, and 0.1% aluminum chloride was added. 0.1 ml of potassium acetate (1 M) and 2.8 ml of distilled water were added. Stoke's solution was diluted. It was incubated for 30 minutes at room temperature. Absorption at a wavelength of 415 nm was measured by spectrophotometry (SHIMADZU, 240A-T, Japan). The TFC level with standard curves was reported in mg QE/g DW (milligram quercetin/gram dry weight).

Figure 1: *Trachyspermum ammi* (L.) Sprague seeds.

2.5. Antioxidant Activity. The antioxidant level of the methanolic extract of *T. ammi* was determined by the free radical inhibition technique of 1,1-diphenyl-2-picrylhydrazyl (DPPH) measurement [13]. 100, 200, 300, 400, and 500 μg/ml of *T. ammi* seed methanolic extract were combined with 3.8 ml of DPPH solution and incubated at room temperature in the dark for 1 h. In the end, the mixture's absorbance was measured at 517 nm. Ascorbic acid was a positive control. Radical scavenging activity was determined using the following formula:

A0: Absorbance of the control, At: Absorbance of the extract

$$\%\text{Inhibition} = \frac{A0 - At}{A0} \times 100. \quad (1)$$

2.6. Chromatography-Mass Spectrometry (GC-MS) Analysis. The GC-MS analysis of the extract was performed using an Agilent 6890 GC system (Technologies, USA) equipped with an MS detector 5973 (Technologies, USA), with an HP-5MS 5% capillary column (30 m × 0.25 mm). The mass spectrometer was used with an ionization voltage of 70 eV by an EI ionization method at an ionization temperature of 220°C. The spectra were recognized based on their inhibitory index and compared with NIST 2018 indicators [14].

2.7. Experimental Animals. Male Wistar albino rats with a weight of 220 to 250 grams were obtained from the Animal House of Islamic Azad University, Falavarjan branch. The rats were kept in clean polypropylene cages with six rats per cage and a (27 ± 2°C), 12-hour light/12-hour dark cycle. During the trial, rats had free access to food and water. All experimental procedures were approved by the Ethics Committee of

the evaluated Islamic Azad University, Falavarjan branch (Approval No. IR.IAU.FALA.REC.1398, 026).

2.8. Induction of Diabetes and Grouping. After 24 hours of fasting, the animals received a single intraperitoneal injection of 55 mg/kg of STZ in 0.1 M cold citrate buffer (pH 4.5). Blood glucose values above 250 mg/dl three days after an STZ injection characterize diabetes. Methanolic *T. ammi* extract was diluted in distilled water and given orally (200 and 500 mg/kg) for 60 days. The losartan group was given 20 mg/kg for 60 days orally. Daily physiological examinations were performed. Healthy control group cages were cleaned and changed every three days. Every 12 hours, the diabetes cages were cleaned. During therapy, all groups ate normally. The rats were treated as follows: G1 normal control received normal saline. G2 diabetic control received normal saline and 55 mg/kg STZ. G3 diabetic + *T. ammi* (200 mg/kg). G4 diabetic + *T. ammi* (500 mg/kg). G5 diabetic + losartan (20 mg/kg).

2.9. Serum Analysis. This study biochemically analyzed the blood, serum, and liver tissue. The rat's blood was taken from the heart. The blood samples were centrifuged at 1000 g for 15 minutes at 4°C. Fasting blood glucose (FBS) levels (after 8 h) were measured using the glucose oxidase method. Animal weight changes were measured. Aspartate transaminase (AST) and alanine transaminase (ALT) levels were measured using Pars Azmun kits (Iran), an enzymatic colorimetric assay. The activities of serum acid phosphatase (ACP) and alkaline phosphatase (ALP) were measured using the *p*-nitrophenyl phosphate method [15].

2.10. Enzyme Assay. At the end of the trial, the animals' livers were removed under ether anesthesia after an 18-hour fast. The liver tissue samples were cleaned with physiological saline and frozen until the experiment. With a glass homogenizer and cold 0.9% NaCl, the livers were mixed together to make a 10 percent homogenate (w/v). Malondialdehyde (MDA), catalase (CAT), glutathione-S-transferase (GST), and glutathione (GSH) levels were determined using Zellbio's colorimetric/fluorometric test kit (Germany). Results were obtained after estimating the absorbance of each sample using an ELISA plate reader (Epoch, USA).

2.11. Inflammatory Cytokines Assay. To thoroughly remove extra blood, the liver tissues were washed in ice-cold PBS. The tissues were fragmented into small pieces and then homogenized in a brand-new lysis buffer. According to the instructions provided by the kit's Zellbio's colorimetric/fluorometric test (Germany) manufacturer, the ELISA reader (Epoch, USA) method was carried out for interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α).

2.12. Histopathological Assay. The liver tissues were fixed for 48 hours in 4% paraformaldehyde. After that, using standard methods, the paraffin-embedded tissues were cut into 5 μm thick pieces. Hematoxylin and eosin (H&E) were used to stain sections following deparaffinization and dehydration. A pathologist used a 400x magnification light microscope to examine tissue samples.

3. Statistical Analysis

The statistical analysis was performed using SPSS 17.0. Each value is the mean ± SD for six rats in each group ($n = 6$). Groups were compared by the Kruskal–Wallis test. Differences between the two groups were identified by the Mann–Whitney test. $p < 0.05$ were considered significant.

4. Result

4.1. Total Phenolic and Flavonoid. The hydroxyl groups on phenolic substances can prevent free radicals from doing their damage by acting as antioxidants. The presence of free OH groups, particularly 3-OH, is necessary for the antioxidant action of flavonoids. TFC and TPC concentrations can be used as a basis for measuring antioxidant activity [16]. The TFC of the methanolic extract of *T. ammi* seeds was 12.09 ± 1.46 mg QE/g DW, and the R2 value of the calibration curve was 0.995%. The TPC was 42.581 ± 27 mg GAE/g DW, and the R2 value of the calibration curve was 0.998 (Table 1).

4.2. Analysis of DPPH Radical Scavenging. Plants are rich in antioxidant properties due to the presence of phenolic and flavonoid compounds. DPPH is used in the measurement of free radical scavenging activity due to the ease of the reaction [12]. In DPPH radicals, the methanolic extract of *T. ammi* seeds exhibited potent antioxidant activity. At a concentration of 500 μg/ml, the DPPH inhibitor activity of *T. ammi* seed extract was assessed to be 77.35 percent, while the relevant figure for ascorbic acid was 87.93 percent (Figure 2).

4.3. Phyto Components Analysis. The chromatogram of *T. ammi* seeds methanolic extract by GC-MS is shown in Figure 3. The GC-MS analysis of *T. ammi* led to the identification and quantification of 3 components (Table 2). The main volatile components of *T. ammi* were found as Thymol (60.705%), γ -Terpinene (22.216%), and P-cymene (17.078%) which comprised 99.999% of the extract.

4.4. Effect of T. ammi on FBS and Body Weight Changes. The FBS level was significantly increased in the diabetic group control (G2) when compared to the normal control group (G1) ($p < 0.05$). Administration of two doses of *T. ammi* (200 and 500 mg/kg) indicated a significant ($p < 0.01$) decline in the fasting blood glucose FBS level in the treated group (G3 and G4) as compared to the diabetic group (G2). In the losartan group (G5), the glucose level was similar to that in the diabetic control group ($p > 0.05$) (G2) (Figure 4(a)). The average weight in the diabetic control group (G2) was significantly reduced compared to the normal control group (G1). The administration of two doses of *T. ammi* (200 and 500 mg/kg) (G3 and G4) and the

Table 1: Total phenolic and flavonoid content of a methanolic extract of T. ammi seeds.

TPC	TFC
42.58 ± 1.27 mg GAE/g DW	12.09 ± 1.46 mg QE/g DW

TPC, total phenolic content; TFC, total flavonoid content. Means ± standard deviations. Values are means of three biological replicates.

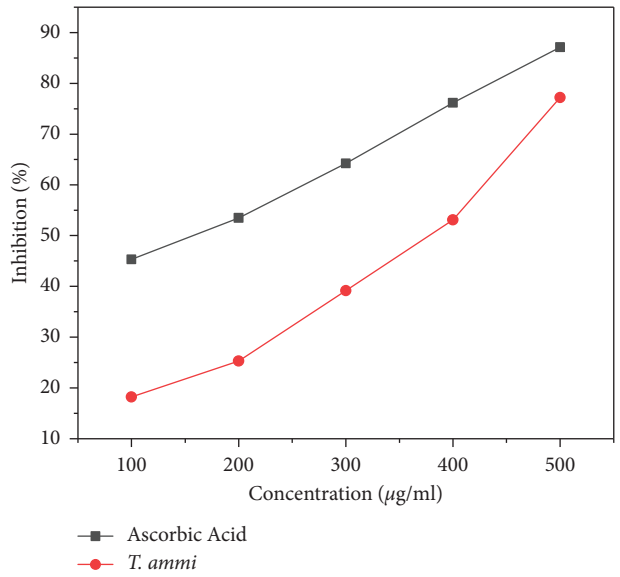


Figure 2: Antioxidant activity (DPPH scavenging method); ascorbic acid was included as a positive control. The values are the means of three biological replicates.

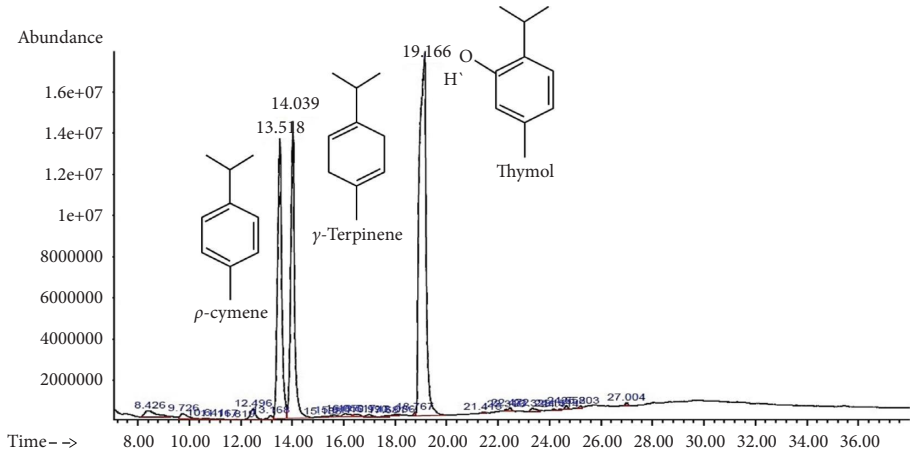


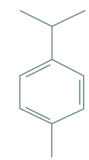
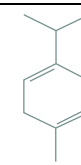
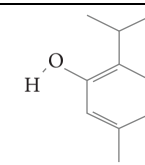
Figure 3: Chromatogram obtained from the GC/MS with the extract of methanolic *T. ammi* seeds. Thymol, γ-Terpinene, and *p*-cymene were the most common compound.

losartan group (G5) showed an increase in average weight in the treated diabetic group compared to the diabetic control group (G2), though this increase was not significant ($p > 0.05$) (Figure 4(b)).

4.5. Effect of T. ammi and ALT, AST, ALP, and ACP. As shown in Figure 5, the activities of ALT, AST, ALP, and ACP in the STZ-induced diabetic group (G2) were significantly increased compared to the normal group (G1) ($p < 0.05$). During 8 weeks of treatment with *T. ammi* (200 and 500 mg/kg) (G3 and G4) and drug control losartan (20 mg/kg) (G5), the activity of ALT, AST, ALP, and ACP compared to the diabetic group caused by STZ ($p < 0.01$, $p < 0.001$) decreased significantly. Significant reductions in ALT, AST, ALP, and ACP levels were observed in the diabetic group receiving a methanolic extract of *T. ammi* and in the losartan group.

4.6. Effect of T. ammi on GSH, CAT, GST, and MDA. The effects of *T. ammi* and losartan on liver GSH, CAT, GST, and MDA are shown in Figure 6. The GSH, CAT, GST, and MDA

Table 2: GC-MS spectral analysis of the methanolic extract of T. ammi seed.

S/no	RT (min)	Library/ID	MF	MW (g/mol)	Total	Chemical structure	Pharmacological actions
1	13.518	*p*-cymene	$C_{10}H_{14}$	134.22	17.078		Anti-inflammatory [17] Antimicrobial [18]
2	14.039	γ-terpinene	$C_{10}H_{16}$	136.23	22.216		Antinociceptive [19] Anti-inflammatory [20] Antimicrobiocidal [21] Antioxidant [22] Anticancer [20] Analgesic [23]
3	19.166	Thymol	$C_{10}H_{14}O$	150.22	60.705		Anti-inflammatory [24] Antileishmanial [25] Antioxidant [26] Antifungal [27] Antihemolytic [28] Antibacterial [24]

RT = retention time; MF = molecular formula; MW = molecular weight.

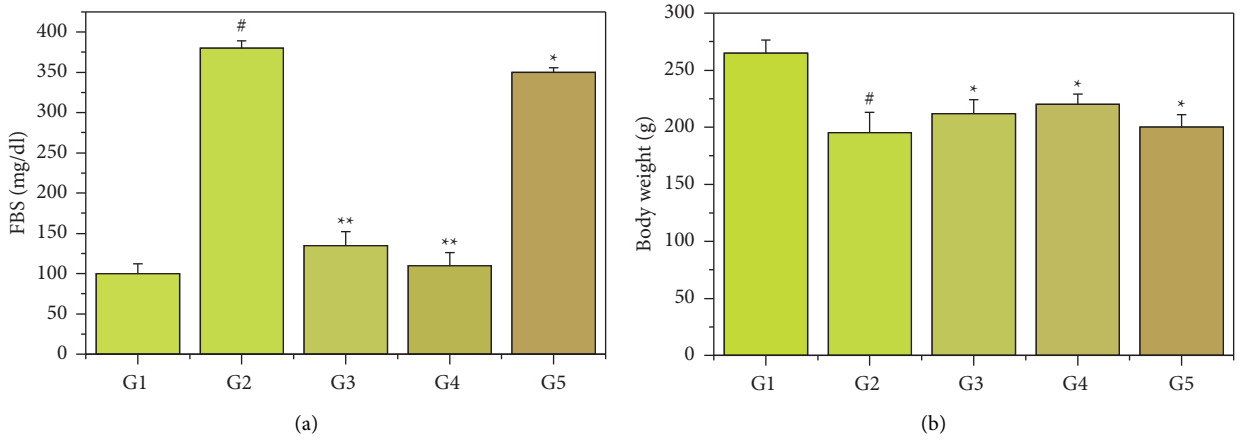


Figure 4: FBS levels and body weight assays in diabetic rats. Normal control (G1); diabetic control (G2); diabetic rat + *T. ammi* low dose 200 mg/kg (G3); diabetic rat + *T. ammi* high dose 500 mg/kg (G4); diabetic rat + losartan (20 mg/kg). Each value is the mean ± SD for six rats in each group ($n = 6$). $^{\#}p < 0.05$ significant with normal control (G1), *nonsignificant with diabetic control (G2), $^{**}p < 0.01$ significant, and $^{***}p < 0.001$ most significant with diabetic control (G2).

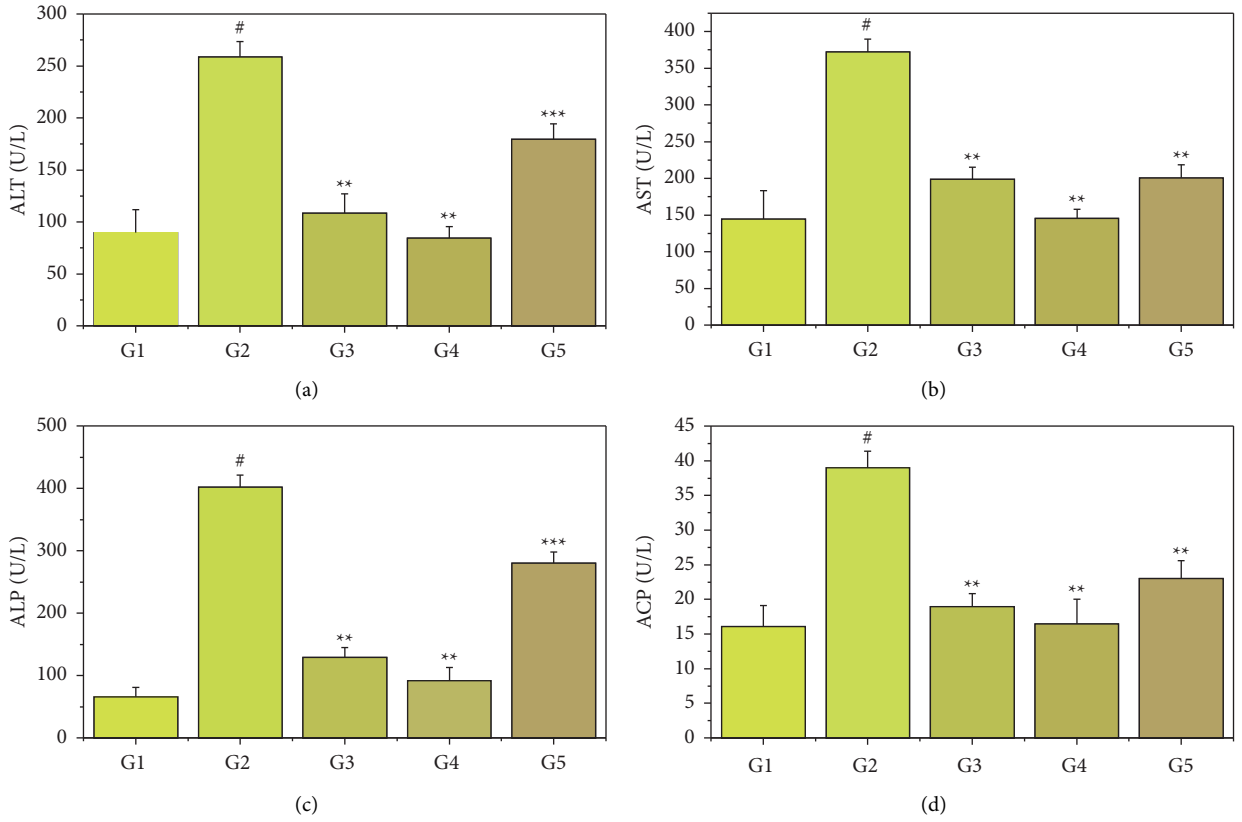


Figure 5: ALT, AST, ALP, and ACP serum assays on liver function indicators in diabetic rats. Normal control (G1); diabetic control (G2); diabetic rat + *T. ammi* low dose 200 mg/kg (G3); diabetic rat + *T. ammi* high dose 500 mg/kg (G4); diabetic rat + losartan (20 mg/kg). Each statistic is the mean standard deviation for six rats in each group ($n = 6$). $^{\#}p < 0.05$ significant with normal control (G1), *nonsignificant with diabetic control (G2), $^{**}p < 0.01$ significant, and $^{***}p < 0.001$ most significant with diabetic control (G2).

levels in the diabetic group (G2) were significantly lower than in the normal group (G1) ($p < 0.05$). The levels of GSH, CAT, GST, and MDA in the liver of diabetic groups treated with *T. ammi* extract (G3 and G4) were much higher than in the diabetic group (G2) that were not treated ($p < 0.01$, $p < 0.001$). Administration of losartan for 60 days significantly increased the liver GSH, CAT, GST, and MDA levels in diabetic rats. Losartan was less effective than *T. ammi* extract.

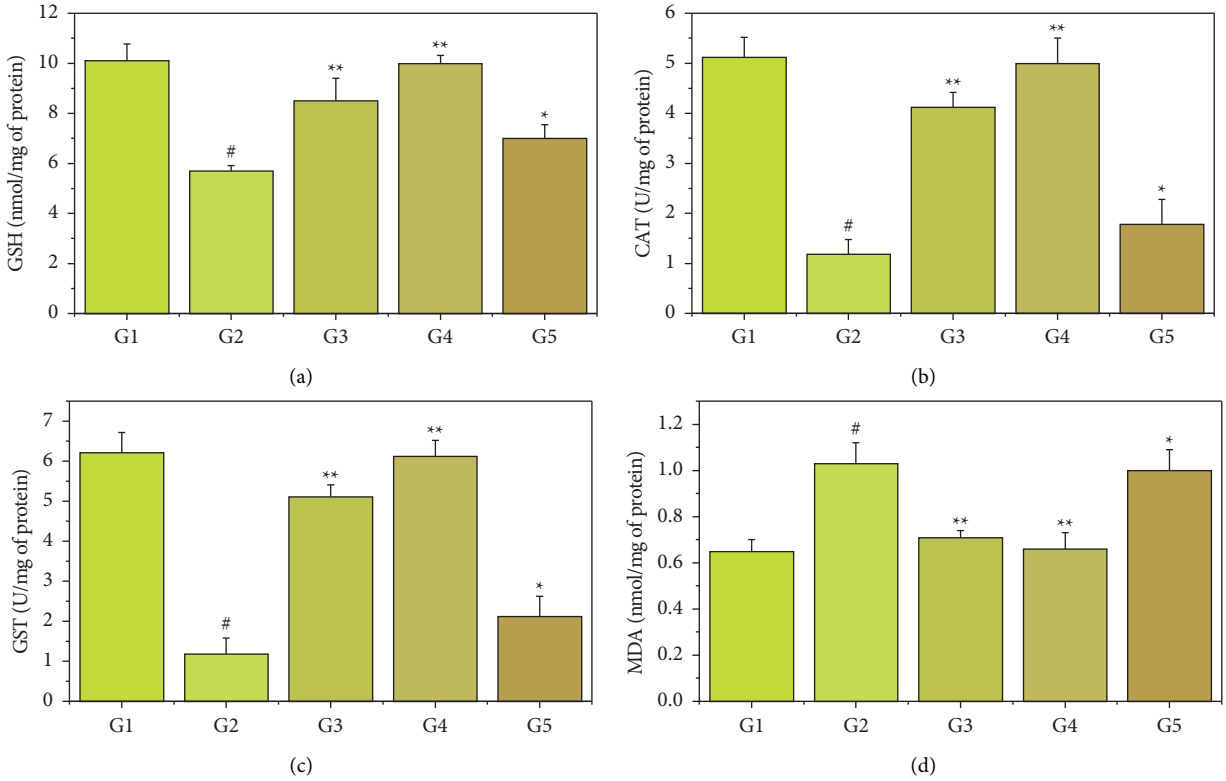


Figure 6: GSH, CAT, GST, and MDA assays in the liver of diabetic rats. Normal control (G1); diabetic control (G2); diabetic rat + *T. ammi* low dose 200 mg/kg (G3); diabetic rat + *T. ammi* high dose 500 mg/kg (G4); diabetic rat + losartan (20 mg/kg). Each statistic is the mean standard deviation for six rats in each group ($n = 6$). $^{\#}p < 0.05$ significant with normal control (G1), *nonsignificant with diabetic control (G2), $^{**}p < 0.01$ significant, and $^{***}p < 0.001$ most significant with diabetic control (G2).

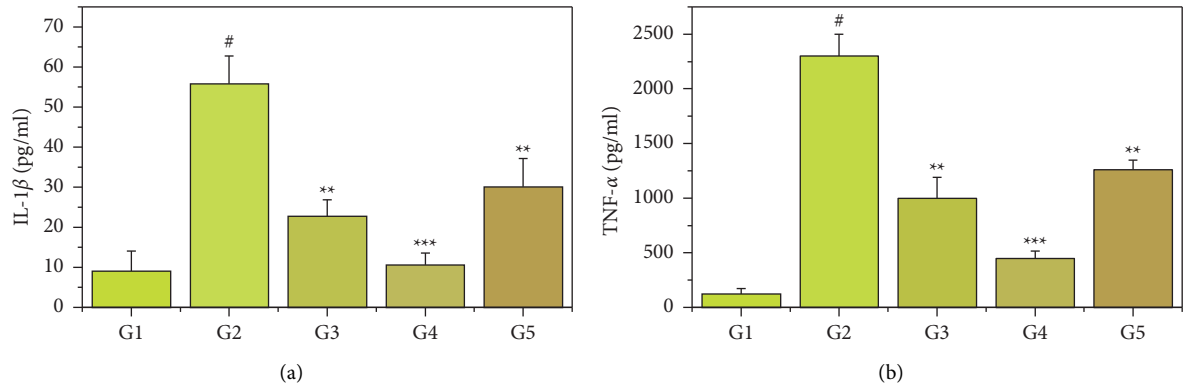


Figure 7: IL-1β and TNf-α assays in the liver of diabetic rats. Normal control (G1); diabetic control (G2); diabetic rat + *T. ammi* low dose 200 mg/kg (G3); diabetic rat + *T. ammi* high dose 500 mg/kg (G4); diabetic rat + losartan (20 mg/kg). Each statistic is the mean standard deviation for six rats in each group ($n = 6$). $^{\#}p < 0.05$ significant with normal control (G1), *nonsignificant with diabetic control (G2), $^{**}p < 0.01$ significant, and $^{***}p < 0.$ 01 most significant with diabetic control (G2).

4.7. Effect of T. ammi on IL-1β and TNF-α. The mean level of inflammatory cytokines IL-1β and TNF-α in the diabetic control group (G2) increased significantly ($p < 0.05$) compared to the healthy control group (G1) (Figure 7). Observations showed that the level of IL-1β and TNF-α in the treatment groups receiving two doses of *T. ammi* (G3 and G4), and the drug group receiving losartan (G5) were significantly decreased compared to the diabetic control group (G2) ($p < 0.01$, $p < 0.001$).

4.8. Histopathological Liver Tissue Analysis. In parts of the normal control (G1) group, regular hepatocytes extended radially around the central vein. The sinusoids and Kupffer cells were normal in this group, with no indications of hemorrhage or infiltration. In the diabetic control (G2) group, the number of hepatocytes as well as the way they were arranged went down; besides, the deformation and vacuolization of hepatocytes were observed. Compared to other groups, the findings for the G4 group were closest to

those of the normal control group. There was no difference in the number of hepatocytes, their regulation, or their sinusoids between the G3 and G4 groups. However, there was an increase in the number of Kupffer cells and infiltration. The G5 group showed a decreased radial configuration. Compared to the diabetes group (G2), the losartan group (G5) exhibited minimal improvement (Figure 8).

5. Discussion

The primary mechanisms implicated in the development of diabetes-induced liver disorders are ROS generation and hyperglycemia-induced oxidative stress [4]. Superoxide is a reactive oxygen species that can harm cells and DNA, resulting in various disorders. Due to their chemical structures and redox properties, plants that have a lot of secondary metabolites like phenolics and flavonoids can act as antioxidants [29]. *T. ammi* methanolic extract showed significant antioxidant activity against the free radicals tested. The high concentration of phenolics and flavonoids in these extracts could be what makes them bioactive. Flavonoids stop the production of reactive oxygen, bind to trace elements that are needed to make free radicals, get rid of reactive species, and protect antioxidant defenses. Similarly, phenolics confer oxidative stress tolerance. Given the therapeutic applications of these compounds, studying their effects could help identify medicinal plants [30].

The GC-MS analysis of *T. ammi* seed methanolic extract revealed the presence of three bioactive compounds. Based on the results of the present study, the compounds shown by the GC-MS have biologically active and pharmacological properties, which can be useful for the treatment of various diseases. Thymol has been shown to induce antioxidant effects, and the antimicrobial effects of *p*-cymene have been demonstrated [18, 24, 31]. It has been shown that γ -Terpinene could be used as an analgesic [23]. Also, the anticancer effect of this compound was revealed in the study [20]. Other studies have shown that Thymol, *p*-cymene, and γ-terpinene have anti-inflammatory properties [20, 24]. These compounds can inhibit inflammatory cytokines and prevent apoptosis by hindering the production of reactive oxygen species via increasing antioxidant enzymes [32]. The biological activities of the compounds in the methanolic extract of *T. ammi* seeds offer evidence for the medicinal application of this plant.

Glucose homeostasis is the balance between insulin and glucagon that keeps blood glucose levels steady [5]. The liver, muscles, and fat cells need glucose homeostasis to use glucose. A high concentration of glucose induces hyperglycemia and liver damage. It has been reported that *T. ammi* has an antidiabetic and glucose-lowering effect [33, 34]. In this investigation, a diabetic-induced state resulted in a rise in serum glucose concentration (hyperglycemia). A methanolic extract of *T. ammi* can reduce blood glucose levels due to its high level of phenol and flavonoid and can be used as an antidiabetic agent.

In the present study, diabetic rats had significantly less body mass than nondiabetic rats. The decreased body weight reported in diabetic rats could be a result of increased protein degradation. These results are consistent with those previously reported when diabetic rats were treated with STZ [15]. In our study, 60 days of losartan medication increased the body weight of diabetes participants. In the diabetes + *T. ammi* group, there was no significant change in body weight. This could be due to the diuretic properties of *T. ammi* [35].

The liver is the most significant organ for drug and chemical metabolism. Destruction of liver cells manifests primarily as an impairment in the permeability of liver cell membranes, resulting in the release of tissue contents into the bloodstream [11, 36]. In this investigation, STZ-treated rats developed hyperglycemia to a large degree, resulting in hepatotoxicity as an interconnected mechanism, as seen by higher levels of ALP, ACP, ALT, and AST. The rats with diabetes caused by STZ had necrotized livers, corroborating our findings. Therefore, the rise in ALP, ACP, ALT, and AST activity in serum is mostly attributable to the leakage of these enzymes from the cytosol of the liver into the bloodstream, which indicates the hepatotoxic effect of STZ. In contrast, the treatment of STZ-diabetic mice with *T. ammi* extract and losartan decreased ALP, ACP, ALT, and AST activity to normal levels. Safhi et al. [14] reported that STZ-treated rats had elevated serum enzyme levels. As determined by the study, *T. ammi* administration for 60 days revealed dose-dependent, protective effects against STZ-induced hepatotoxicity.

TNF-α is employed as an inflammatory biomarker that appears in a variety of acute and chronic liver illnesses and is thought to have a role in the processes of liver deterioration and repair. In diabetes and hepatic damage, TNF-α regulates apoptosis and inflammatory processes. IL-1β is a predominant cytokine in inflammatory conditions, especially in diabetic mellitus [14]. In this study, TNF-α and IL-1β levels increased significantly in the diabetic group (G2) when compared to the control group (G1) representing the induction of diabetes and hepatic injury. It has been observed that STZ induction causes liver cell damage and death [37]. The level of inflammatory cytokines decreased after administering *T. ammi* extract (200 or 500 mg) to diabetic groups. Examining the histopathological changes in the liver tissue of the experimental groups showed a reduction in inflammation. The results indicated that *T. ammi* possessed anti-inflammatory capabilities due to its phenolic and flavonoid content as well as its powerful antioxidant properties.

The damage that diabetes does to the liver is likely caused by lipid peroxidation, which happens when free radicals are made [38]. In the diabetic control group, due to increased oxidative stress, we saw a sharp decrease in the levels of GSH, CAT, GST, and MDA in the liver tissue. When *T. ammi* extract and losartan were given to the diabetic rats, the levels of GSH, CAT, GST, and MDA went up in their livers. Observations indicating that *T. ammi* extract greatly decreased lipid peroxidation in the liver and prevented hepatic histological damage imply that this plant has a hepatoprotective potential in diabetes.

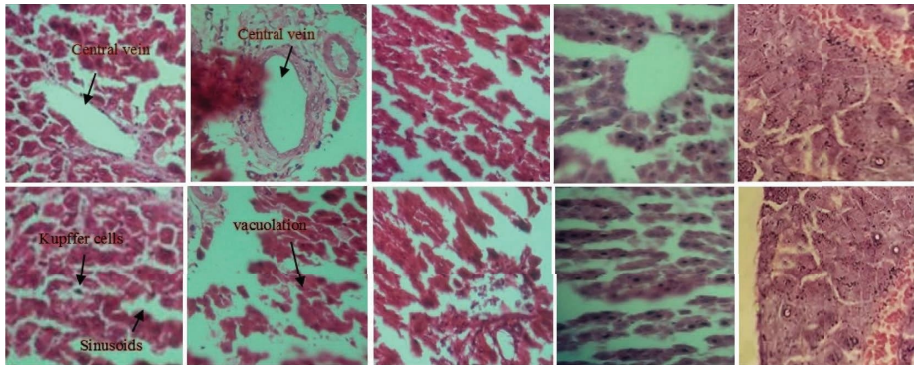


Figure 8: Histopathological liver tissue. *T. ammi* methanolic extract reduces the inflammatory response in the liver of STZ-induced diabetic rats. Normal control (G1); diabetic control (G2); diabetic rat + *T. ammi* low dose 200 mg/kg (G3); diabetic rat + *T. ammi* high dose 500 mg/kg (G4); diabetic rat + losartan (20 mg/kg).

6. Conclusion

Liver damage has been documented as a major complication of diabetes mellitus. Indeed, various studies suggest that mortality due to liver disease in diabetic patients is very high. The pathological effects of liver damage are due to increased blood sugar levels, increased oxidative stress levels, and increased liver enzymes. Treatment of diabetic groups with a methanolic extract of *T. ammi* significantly reduced the levels of fasting blood glucose, liver enzymes, and inflammatory cytokines. It also increased the level of antioxidant enzymes. The methanolic extract of *T. ammi* showed significant effects on liver GSH, CAT, and GST levels and a reduction of pathological changes in liver tissue samples. This study identified *T. ammi* as having the most antioxidant and hepatoprotective properties, which may be attributable to its greater TPC, TFC, and component concentrations as determined by GC-MS analysis. More studies are needed in the field of isolation and identification of the active compounds of the methanol extract of *T. ammi* so that safe and affordable drugs can be provided for the treatment of diabetes complications.

Data Availability

Data are available from the corresponding author upon reasonable request.

Additional Points

(i) The identification of *T.ammi* seed methanolic extract compounds was investigated. (ii) Thymol, γ-Terpinene, and *p*-cymene were the most common compounds. (iii) Due to its phenolic and flavonoid compounds, it has biological activity. (iv) Observations indicating that *T. ammi* extract greatly decreased lipid peroxidation and inflammatory cytokines in the liver and prevented hepatic histological damage imply that this plant has hepatoprotective potential in diabetes.

Ethical Approval

Ethics approval statement (IR.IAU.FALA.REC.1398, 026). Approval Date: 2019-07-15, Evaluated by Islamic Azad University, Falavarjan Branch.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Najimeh Zolfaghari conceptualized the study, performed data curation, carried out formal analysis, provided funding acquisition, performed investigation, developed methodology, project administration, provided resources, developed software, performed supervision, validated and visualized the study, wrote the original draft, and reviewed and edited the study. Ramesh Monajemi performed data curation, methodology, project administration, supervision, validation, and visualized the study. Kahin ShahaniPour performed methodology, project administration, and visualized the study. Ali Mohammad Ahadi performed formal analysis, investigation, and project administration.

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