Al-Shatrah Veterinary and Biological Sciences Journal
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Page 128 - 141

Molecular and Biochemical Effects of Thyme (*Thymus vulgaris*) Extract on Carbon Tetrachloride (CCl_4)-Induced Hepatic Injury in Rats

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Abstract

Background: Thyme (Thymus vulgaris) is a common plant with anti-inflammatory, antioxidant, and antimicrobial properties, and it is used in the treatment of respiratory diseases. Despite its widespread use, its therapeutic targets and mechanisms of action are still not fully understood. The carbon tetrachloride (CCl_4)-induced liver injury model is widely used as a non-viral hepatitis model. This study aimed to evaluate the effect of thyme extract in alleviating liver damage caused by CCl_4 in rats by examining liver enzymes, oxidative stress indicators, inflammatory cytokines, and the lipid profile. Materials and Methods: Adult male rats were randomly divided into six groups, including the control group, the CCl_4 group (rats treated with CCl_4 only), the silymarin group (rats treated with CCl_4 and then with silymarin at a dose of 100 mg/kg body weight/day), the thyme group (rats treated with CCl_4 and then with thyme extract). Low-dose thyme group: Rats were treated with CCl_4 and then with thyme extract at a dose of (200 mg/kg body weight/day). The high-dose thyme group of rats were treated with CCl_4 and then treated with thyme extract at a dose of (400 mg/kg body weight/day). For 4 weeks. Liver enzymes (ALT, AST), oxidative stress indicators (ROS, MDA), lipid profile (HDL, LDL), inflammatory cytokines (TNF-α, IL-6, IL-10, TGF-β), and antioxidant status (GSH and uric acid levels) were measured. Results: Treatment with thyme extract led to a significant decrease in ALT and AST levels in the injured group compared to the uninjured group, with the effect increasing with higher doses. Thyme also contributed to reducing oxidative stress indicators (ROS and MDA) and improving the lipid profile by increasing HDL and decreasing LDL. The levels of inflammatory cytokines, including TNF-α, IL-6, IL-10, and TGF-β, were regulated. Thyme also restored antioxidant levels, with GSH increasing and uric acid decreasing, and the effects were more pronounced at the high dose. Conclusions: Thyme extract, especially at the high dose, showed a clear protective effect on the liver by reducing oxidative stress, inflammation, and lipid disorder. Its efficacy was similar to that of interferon, suggesting the possibility of considering it a safe natural alternative for treating liver injuries. However, additional clinical studies are needed to confirm its safety and efficacy..

Keywords : Thymus vulgaris ,Hepatic injury ,Oxidative stress , Liver enzymes ,Inflammatory cytokines

الملخص

الخلفية: يُعد نبات الزعتر **(Thymus vulgaris)** من النباتات الشائعة ذات الخصائص المضادة للالتهاب والأكسدة والميكروبات، ويُستخدم في علاج أمراض الجهاز التنفسي. وعلى الرغم من استخدامه الواسع، لا تزال الأهداف العلاجية وآليات عمله غير مفهومة بشكل كامل. ويُستخدم نموذج إصابة الكبد المستحثة بواسطة رباعي كلوريد الكربون **(CCl_4)** على نطاق واسع باعتباره نموذجًا غيرمشابهًا لالتهاب الكبد الفيروسي. هدفت هذه الدراسة إلى تقييم تأثير مستخلص الزعتر في التخفيف من تلف الكبد الناتج عن **CCl_4** في الجرذان من خلال دراسة إنزيمات الكبد، ومؤشرات الإجهاد التأكسدي، وسيتوكينات الالتهاب، وملف الدهون.

المواد وطرائق العمل: تم تقسيم الجرذان الذكور البالغة عشوائيًا إلى ست مجموعات شملت مجموعة السيطرة، و مجموعة **CCl_4** جرذان عوملت بـ **CCl_4** فقط. ، مجموعة السليمارين جرذان عوملت بـ **CCl_4** ثم عولجت بالسليمارين بجرعة (100 ملغم/كغم من وزن الجسم/يوميًا). ، مجموعة الزعتر جرذان عوملت بـ **CCl_4** ثم عولجت بمستخلص الزعتر. مجموعة الجرعة الواطئة من الزعتر جرذان عوملت بـ **CCl_4** ثم عولجت بمستخلص الزعتر بجرعة (200 ملغم/كغم من وزن الجسم/يوميًا). مجموعة الجرعة العالية من الزعتر جرذان عوملت بـ **CCl_4** ثم عولجت بمستخلص الزعتر بجرعة (400 ملغم/كغم من وزن الجسم/يوميًا). لمدة 4 أسابيع. تم قياس إنزيمات الكبد**(ALT)** ، **(AST)**، ومؤشرات الإجهاد التأكسدي**(ROS)** ، **(MDA)**، وملف الدهون**(HDL)** ، **(LDL)**، وسيتوكينات الالتهاب**(TNF-α)** ، **IL-6**، **IL-10**، **TGF-β)**، وحالة مضادات الأكسدة **(GSH)** ومستويات حمض اليوريك.

النتائج: أدى العلاج بمستخلص الزعتر إلى انخفاض معنوي في مستويات **ALT** و **AST**في مجموعة الإصابة مقارنة بالمجموعة الغير مصابة، مع ازدياد التأثير بزيادة الجرعة. كما أسهم الزعتر في خفض مؤشرات الإجهاد التأكسدي **(ROS)** و**(MDA)**، وتحسين ملف الدهون من خلال زيادة **HDL** وتقليل **LDL.** وتم تنظيم مستويات السيتوكينات الالتهابية، بما في ذلك **TNF-α** و **IL-6**و **IL-10**و **TGF-β.**كما استعاد الزعتر مستويات مضادات الأكسدة، حيث ارتفع **GSH** وانخفض حمض اليوريك، وكانت التأثيرات أكثر وضوحًا في الجرعة العالية.

الاستنتاجات: أظهر مستخلص الزعتر، خاصة عند الجرعة العالية، تأثيرًا واقيًا واضحًا للكبد من خلال تقليل الإجهاد التأكسدي والالتهاب واضطراب الدهون. وتشابهت فعاليته مع تأثير الإنترفيرون، مما يشير إلى إمكانية اعتباره بديلاً طبيعياً آمناً لعلاج إصابات الكبد. إلا أن هناك حاجة إلى دراسات سريرية إضافية لتأكيد سلامته وفعاليته.

الكلمات المفتاحية: نبات الزعتر ، إصابة الكبد، الإجهاد التأكسدي، إنزيمات الكبد، السيتوكينات الالتهابية.

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 128 - 141

1. Introduction

Over the past few decades and associated with this trend was an obvious increase in the use of herbal products, some of which have shown encouraging in vitro and in vivo therapeutic activities for the potential treatment of a varieties of diseases. Nevertheless, the hypoglycemic effects of a large number of the preparations are largely unverified in terms of rigorous scientific trials or firm regulation, the information available on their safety is also scarce [1]. It is a common myth that 'natural' is synonymous with 'safe', however, as is demonstrated, this is an incorrect and false premise [2] The liver is one of the most drug-sensitive organs of the body because of its importance in drug metabolism. Pharmaceutical-Induced Hepatobiliary Toxicology Even though the exact physiological pathogenic mechanisms of DILI are not completely understood, they start with the formation of toxic metabolites in the initiation phase of metabolism, which result in mitochondrial inhibition and generation of ROS in a variety of cases. This disturbs the redox steady state of hepatocytes and eventually leads to apoptosis or necrosis in these cells [3, 4]. Considering the implication of oxidative stress in the pathogenesis of a variety of hepatic diseases, natural antioxidants and, in particular, phytochemicals, have been considered as a remedy. It has been reported that about 50% of drugs used for liver disorders are natural products or their derivatives [5]. In comparison, the prevalence of liver damage due to natural and dietary supplements has significantly increased during the last decade, in part due to increased utilization [2].

Thyme *(Thymus vulgaris L., Lamiaceae*) is a small herbal shrub, originating from the western Mediterranean region and is widely used not only as an aromatic herb but also in a variety of traditional medicine practices. Its primary therapeutic effects are anti-tussive and expectorant effects as well as antispasmodic, making the herb especially valuable for the treatment of upper respiratory tract diseases, either in its own right, or in combination with other medicinal plants [6]. According to the German Commission E, thyme formulations are suitable for the internal treatment of bronchitis, pertussis, and common cold in the upper respiratory tract [7]. Other medicinal effects of thyme include its antioxidant [8], antimicrobial [9], genotoxic [10], anti-inflammatory [11], analgesic and antipyretic [12], and antidiabetic [13] activities. Thyme preparations come in different forms, including dry herb, liquid extracts (juice, elixir or as tinctures), and can be part of complex herbal formulations such as syrups and tablets [14].

The application of thyme in the food industry is essentially associated with the flavor, scent and antioxidative and antimicrobial activities of the plant. Still, its primary contribution to the food industry continues to be its flavor- modulating effect, but its other functional properties are also advantageous in thyme- containing food products [15] .Thyme medicinal effects are due to its antioxidant capacity, which stem from its monoterpene phenolic compounds, such as thymol and carvacrol, which are some of the main ingredients in its essential oil. Other components, including rosmarinic acid and flavonoids (i.e., quercetin, eriocitrin, luteolin, and apigenin) are responsible for the antioxidant activity of alcoholic and aqueous extracts [16]. Thyme plants also differ in their chemical profile (i.e., chemical pattern), reflecting the distribution of secondary metabolites, at least seven chemical patterns were described according to the dominant monoterpenoids such as thymol, carvacrol, geraniol, α-terpineol, sabinene hydrate, linalool, 1,8-cineole [17]. Thus, chemical composition variations should be taken into account in the appraisal of the pharmacological actions of thyme treatments .In terms of the liver, the available evidence for the

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 128 - 141

impact of thyme is inconsistent. Although generally regarded as safe (GRAS) for use in food, chronic ingestion or excessive amounts of its preparations (especially tinctures and essential oils rich in thymol and carvacrol) can result in hepatotoxicity [18] and even worsen a pre-existent hepatic pathology in humans [19]. On the other hand, liver protective effects have been reported in several experimental models; ethanol and methanol extracts of thyme exerted their preventive effect on oxidative liver toxicity induced by aflatoxin and N-nitrosodiethylamine (NDEA) in the liver, respectively [20, 21]. Moreover, thyme extracts and essential oils have been also reported to reduce the liver damage in mice induced by carbon tetrachloride (CCl_4) [22]. In addition, other researches had reported the hepatoprotective effects of thyme in models of paracetamol-induced liver injuries in rat using respectively the water extract [23] and the essential oil [24] sources of thyme. To this end, in the present study, we assessed molecular and biochemical alterations induced by the extract used as thyme (*Thymus vulgaris*) extract on CCl_4-induced liver damage by measuring liver enzymes (ALT, AST) as indirect markers of liver function, besides oxidative stress (ROS, MDA), lipid profile (HDL, LDL), cytokines (TNF-α, IL-6 as proinflammatory, IL-10, TGF-β as antiinflammatory), antioxidant status (GSH), and uric acid levels as markers of oxidative status. Further we compared the efficacy of thyme extract to the conventional treatment (silymarin-treated group)

2. Materials and Methods

1.2Experimental Animals

Adult male white rats (Rattus norvegicus, 180-200 g body weight) were used for the experiments. The rats were kept in cages made of plastic at a room temperature of 22 ± 2°C and 50–60% relative humidity in a 12 h light/dark cycle. All the animals received standard chow and drinking water.

2.2Preparation of Thyme Extract

The plant thyme (*Thymus vulgaris*) was gathered, dried in the shade, and was pulverized. For each test, 100 g of the powder extracted in 70% ethanol at room temperature for 72 h by maceration. The extract was filtered and concentrated in vacuum on a rotary evaporator to afford a thick extract. The extract was kept at 4°C until use.

3.2 Induction of Hepatic Injury by CCl_4

Hepatic damage was then induced by intraperitoneal CCl_4 (1 mL/kg body weight with 1:1 dilution in olive oil) twice a week for 4 weeks. This model is commonly employed to reproduce hepatic injury that is This model is commonly used to induce experimental hepatic injury and oxidative stress in rodents. in rodents, as previously reported.

4.2Experimental Design

The animals were randomly divided into six groups (n = 6 per group):

- **Control group**: healthy untreated rats.
- **CCl_4-treated group**: rats administered CCl_4 only.
- **Silymarin-treated group**: rats administered CCl_4 and treated with silymarin (100 mg/kg body weight/day).
- Thyme-treated group: rats administered CCl_4 and treated with thyme extract.
- **Low-dose thyme group**: rats administered CCl_4 and treated with thyme extract at a dose of 200 mg/kg body weight/day.
- **High-dose thyme group**: rats administered CCl_4 and treated with thyme extract at a dose of 400 mg/kg body weight/day.

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 128 - 141

All treatments were administered orally once daily for four weeks...

5.2Biochemical and Molecular Assessments

On the last day of treatment (4 weeks), blood and liver tissues were obtained, and the following analyses were conducted :

- **Analyses of Liver Enzymes (ALT, AST):** Quantitative analyses were conducted with the available kits (ELISA kits, BioSystems S.A., Barcelona, Spain) according to the manufacturer's protocol.
- **Oxidative Stress Markers** :ROS: Detected with a fluorescence spectrophotometer (Shimadzu RF-5301PC, Japan) . Malondialdehyde (MDA): Assessed by TBARS assay with commercial kits (Cayman Chemical, Ann Arbor, MI, USA).
- **Lipoproteins (HDL, LDL),** both were measured using colorimetric enzyme kits (RandoxLaboratories Ltd., Crumlin, UK).
- **Analysis of Molecular Markers (Cytokines):** The expression levels of TNF-α, IL-6, IL-10 and TGF-β were measured using ELISA kits (R&D Systems, Minneapolis, MN, USA) and read at 450 nm using a microplate reader (BioTek ELx800, Winooski, VT, USA).
- **Antioxidant Levels:** Glutathione (GSH): Determined by using test kits (Sigma-Aldrich, St. Louis, MO, USA). Uric Acid: Assessed by an enzymelinking colorimetric assay with readymixed reagents (Spinreact, Girona, Spain).

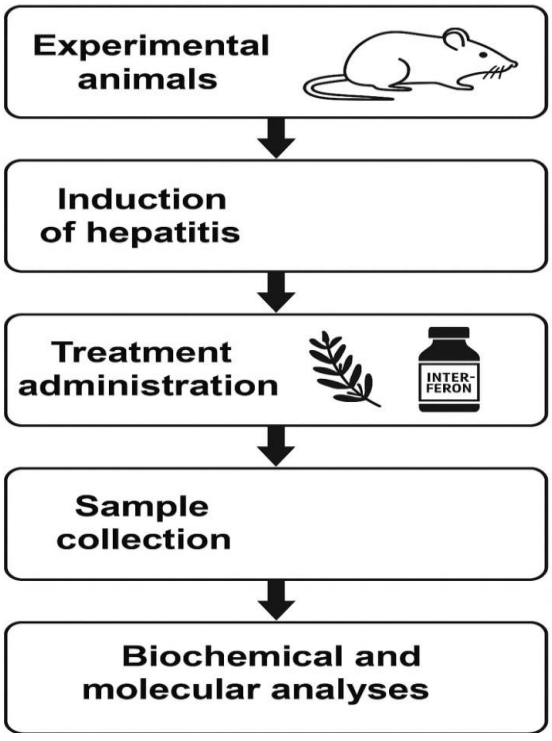

Figure 1. Flow chart illustrating the experimental design: grouping of rats, induction of hepatitis, treatment administration (thyme extract/ silymarin-treated), sample collection, and subsequent biochemical and molecular analyses.

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 128 - 141

6.2 Statistical Analysis

Data are expressed as mean ± standard deviation (Mean ± SD). Analysis was performed using ANOVA followed by Tukey's test to determine the differences between groups. A difference was considered statistically significant when $p < 0.01$.

3. Results

1.3 Liver Enzymes

As shown in Table 1, serum levels of ALT and AST were significantly elevated in the hepatotoxic group group (150.44 ± 11.24 U/L and 180.65 ± 9.45 U/L, respectively) compared with the control group (30.23 ± 2.60 U/L and 35.46 ± 1.40 U/L, respectively; $p < 0.01$). Treatment with thyme markedly reduced these levels (ALT: 80.25 ± 7.56 U/L; AST: 100.87 ± 8.27 U/L), while silymarin-treated group treatment demonstrated an even greater improvement (ALT: 60.26 ± 4.78 U/L; AST: 75.54 ± 7.45 U/L). Notably, high-dose thyme (ALT: 70.85 ± 6.65 U/L; AST: 85.43 ± 5.71 U/L) exerted a stronger protective effect than the low-dose regimen (ALT: 110.76 ± 9.08 U/L; AST: 120.98 ± 8.54 U/L).

Table 1. Serum liver enzymes (ALT and AST) levels in experimental groups

Group	ALT (U/L) (Mean ± SD)	AST (U/L) (Mean ± SD)	p-value
Control	30.23 ± 2.60 f	35.46 ± 1.40 f	p < 0.01
CCl_4-treated group	150.44 ± 11.24 a	180.65 ± 9.45 a	
Standard treatment group	80.25 ± 7.56 c	100.87 ± 8.27 c	
Thyme-treated group	60.26 ± 4.78 e	75.54 ± 7.45 E	
Low-dose thyme	110.76 ± 9.08 b	120.98 ± 8.54 B	
High-dose thyme	70.85 ± 6.65 d	85.43 ± 5.71 D	

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 128 - 141

2.3 Oxidative Stress Markers and Lipid Profile

Table 2 illustrates that the hepatotoxic group group exhibited significantly increased ROS (45.21 ± 6.04 µM) and MDA (6.59 ± 1.25 nmol/mg), along with elevated LDL (170.01 ± 6.65 mg/dL) and reduced HDL (30.23 ± 3.39 mg/dL), compared to the control group (ROS: 10.53 ± 2.65 µM; MDA: 1.25 ± 0.65 nmol/mg; LDL: 120.45 ± 9.60 mg/dL; HDL: 60.56 ± 2.45 mg/dL; $p < 0.01$). Thyme administration attenuated oxidative stress and improved lipid parameters (ROS: 20.29 ± 2.12 µM; MDA: 3.10 ± 0.87 nmol/mg; LDL: 140.22 ± 5.25 mg/dL; HDL: 45.87 ± 1.49 mg/dL), whereas silymarin-treated group treatment yielded comparable outcomes (ROS: 15.87 ± 2.77 µM; MDA: 2.54 ± 0.55 nmol/mg; LDL: 130.45 ± 8.33 mg/dL; HDL: 50.47 ± 1.85 mg/dL). Interestingly, high-dose thyme produced superior effects (ROS: 18.87 ± 3.65 µM; MDA: 2.84 ± 0.48 nmol/mg; LDL: 135.56 ± 6.90 mg/dL; HDL: 55.89 ± 1.77 mg/dL) compared to low-dose thyme (ROS: 25.09 ± 3.23 µM; MDA: 4.29 ± 1.22 nmol/mg; LDL: 150.43 ± 9.85 mg/dL; HDL: 40.76 ± 2.87 mg/dL).

Table 2. Oxidative stress markers (ROS, MDA) and lipid profile (LDL, HDL) in experimental groups

Group	ROS (µM) (Mean ± SD)	MDA (nmol/mg) (Mean ± SD)	LDL (mg/dL) (Mean ± SD)	HDL (mg/dL) (Mean ± SD)	p-value
Control	10.53 ± 2.65 f	1.25 ± 0.65 F	120.45 ± 9.60 f	60.56 ± 2.45 A	p < 0.01
CCl_4-treated group	45.21 ± 6.04 a	6.59 ± 1.25 A	170.01 ± 6.65 a	30.23 ± 3.39 E	
Standard treatment group	20.29 ± 2.12 c	3.10 ± 0.87 C	140.22 ± 5.25 c	45.87 ± 1.49 D	
Thyme-treated group	15.87 ± 2.77 e	2.54 ± 0.55 E	130.45 ± 8.33 e	50.47 ± 1.85 C	
Low-dose thyme	25.09 ± 3.23 b	4.29 ± 1.22 B	150.43 ± 9.85 b	40.76 ± 2.87 E	
High-dose thyme	18.87 ± 3.65 d	2.84 ± 0.48 D	135.56 ± 6.90 d	55.89 ± 1.77 B	

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 128 - 141

3.3 Pro-inflammatory Cytokines

As presented in Table 3, virus infection led to a marked elevation in TNF-α (25.23 ± 3.75 ng/mL) and IL-6 (20.24 ± 2.60 ng/mL) relative to the control group (TNF-α: 5.56 ± 0.69 ng/mL; IL-6: 2.21 ± 0.28 ng/mL; $p < 0.01$). Treatment with thyme significantly reduced cytokine levels (TNF-α: 15.38 ± 2.30 ng/mL; IL-6: 10.31 ± 1.06 ng/mL), while silymarin-treated group exerted a stronger suppressive effect (TNF-α: 10.55 ± 1.86 ng/mL; IL-6: 5.28 ± 0.96 ng/mL). High-dose thyme (TNF-α: 12.21 ± 1.58 ng/mL; IL-6: 8.44 ± 0.67 ng/mL) outperformed low-dose thyme (TNF-α: 20.78 ± 1.02 ng/mL; IL-6: 15.90 ± 1.40 ng/mL), indicating a dose-dependent anti-inflammatory response

Table 3. Serum inflammatory cytokines (TNF-α, IL-6) levels in experimental groups

Group	TNF-α (ng/mL) (Mean ± SD)	IL-6 (ng/mL) (Mean ± SD)	p-value
Control	5.56 ± 0.69 F	2.21 ± 0.28 F	
CCl_4-treated group	25.23 ± 3.75 A	20.24 ± 2.60 A	
Standard treatment group	15.38 ± 2.30 C	10.31 ± 1.06 C	
Thyme-treated group	10.55 ± 1.86 E	5.28 ± 0.96 E	p < 0.01
Low-dose thyme	20.78 ± 1.02 B	15.90 ± 1.40 B	
High-dose thyme	12.21 ± 1.58 D	8.44 0.67 D	
		8.45	

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 128 - 141

4.3Antioxidant Marker and Uric Acid

As shown in Table 4, hepatic injury was associated with a significant depletion of GSH (40.33 ± 2.93 µM) and a concomitant rise in uric acid (9.01 ± 1.34 mg/dL), compared to the control group (GSH: 80.62 ± 4.55 µM; uric acid: 5.53 ± 0.72 mg/dL; $p < 0.01$). Thyme treatment restored GSH levels (70.70 ± 5.65 µM) and normalized uric acid (6.07 ± 0.52 mg/dL), while silymarin-treated group showed a comparable effect (GSH: 75.23 ± 8.23 µM; uric acid: 5.78 ± 0.40 mg/dL). Again, the high-dose thyme regimen (GSH: 72.22 ± 4.62 µM; uric acid: 5.89 ± 0.65 mg/dL) was more effective than the low-dose group (GSH: 60.12 ± 6.95 µM; uric acid: 7.02 ± 0.94 mg/dL)

Table 4. Antioxidant marker (GSH) and uric acid levels in experimental groups

Group	GSH (µM) (Mean ± SD)	Uric acid (mg/dL) (Mean ± SD)	p-value
Control	**80.62 ± 4.55 A**	**5.53 ± 0.72 F**	**p < 0.01**
CCl_4-treated group	**40.33 ± 2.93 F**	**9.01 ± 1.34 A**	
Standard treatment group	**70.70 ± 5.65 D**	**6.07 ± 0.52 C**	
Thyme-treated group	**75.23 ± 8.23 B**	**5.78 ± 0.40 E**	
Low-dose thyme	**60.12 ± 6.95 E**	**7.02 ± 0.94 B**	
High-dose thyme	**72.22 ± 4.62 C**	**5.89 ± 0.65 D**	

5.3 Anti-inflammatory Cytokines (Table 5).

Table 5 demonstrates that CCl_4-induced hepatic injury significantly decreased IL-10 (5.35 ± 0.33 ng/mL) and elevated TGF-β (8.28 ± 1.20 ng/mL) compared with the control group (IL-10: 12.23 ± 1.38 ng/mL; TGF-β: 2.57 ± 0.69 ng/mL; $p < 0.01$). Thyme administration enhanced IL-10 (10.76 ± 1.62 ng/mL) and reduced TGF-β (4.67 ± 0.28 ng/mL), with silymarin-treated group showing similar improvements (IL-10: 9.85 ± 1.05 ng/mL; TGF-β: 3.29 ± 0.29 ng/mL). High-dose thyme provided better modulation of cytokine levels (IL-10: 11.35 ± 1.65 ng/mL; TGF-β: 3.59 ± 0.75

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 128 - 141

ng/mL) than the low-dose group (IL-10: 8.65 ± 1.20 ng/mL; TGF-β: 5.84 ± 0.87 ng/mL).

Table 5. Anti-inflammatory cytokines (IL-10, TGF-β) levels in experimental groups

Group	IL-10 (ng/mL) (Mean ± SD)	TGF-β (ng/mL) (Mean ± SD)	p-value
Control	12.23 ± 1.38 A	2.57 ± 0.69 F	p < 0.01
CCl_4-treated group	5.35 ± 0.33 F	8.28 ± 1.20 A	
Standard treatment group	10.76 ± 1.62 C	4.67 ± 0.28 C	
Thyme-treated group	9.85 ± 1.05 D	3.29 ± 0.29 e	
Low-dose thyme	8.65 ± 1.20 E	5.84 ± 0.87 b	
High-dose thyme	11.35 ± 1.65 B	3.59 ± 0.75 d	

4. Discussion

The findings of the study also showed a high elevation in liver enzymes (ALT and AST) in the virus inoculated group as compared to control, suggesting liver injury due to infected with CCl_4. These liver cell injury caused enzymes leakage into the blood stream [25]. A marked decrease in these activities was realized after thyme treatment particularly with the high dose, indicating the potent protective effect of thyme in decreasing liver cell damage. This may be attributed to bioactive compounds of thyme like thymol and carvacrol which possess antioxidant and anti-inflammatory effect supporting tissue protection and contribution to the restoring liver function [26]. These results are in accordance with previous studies, which reported that thyme extract could alleviate liver inflammation resulting from different pathogens and lessen the decline in liver functions .In contrast, there was a marked rise in oxidative stress markers such as free radicals (ROS) and lipid peroxidation products (MDA) and an impairment in lipid profile, manifested by high levels of LDL and a low level of HDL of the CCl_4-treated group [27]. This is a sign of

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 128 - 141

severe oxidative stress and cell damage that can cause further liver problems and inflammation. As a result, thyme induced a significant decrease in these markers, which was better at the high dose than with a low one [28]. This translated into a normalisation of potentially liver-disease-causing lipid levels, with a significant increase in beneficial HDL and a reduction in detrimental LDL. This positive impact is the result of thyme's antioxidants, which neutralize free radicals, and its ability to neutralize the toxic effects that result fromCCl_4-induced hepatic injury . Other investigators have also demonstrated the potential of thyme to diminish oxidative stresses and ameliorate fatty livers. Additionally, it has been demonstrated that thyme compounds stimulate the endogenous production of antioxidant species including glutathione, thus further reinforcing the body defences against oxidative challenges[29]

With respect to inflammation, the CCl_4-treated group ’s contents on pro-inflammatory cytokines, such as TNF-α and IL-6, were found to be higher, suggesting an intense inflammatory reaction in toxic liver injury. On the other hand, thyme decreased the production of these cytokines, and increased the production of anti-inflammatory cytokines, IL-10, and TGF-β [30] . This, in turn, modulated the immune response to the disease and mitigated chronic inflammation. This effect may be induced by thyme’s phenolic compounds and essential oils, suppressing the gene expression of inflammatory mediators and promoting the production of anti-inflammatory-related factors. This is an essential effect in preventing tissue damage and the advancement of chronic oxidative hepatic damage , which is evident from previous research on thyme's immunoregulatory properties [31] [32] .As for antioxidants, a significant reduction in GSH and a significant elevation of uric acid were observed in the CCl_4-treated group reflecting the impaired oxidative stress defenses and the increased inflammation. Thyme was crucial to increasing glutathione and reducing uric acid, and hence biochemical defense against liver damage and damage due to CCl_4-induced hepatic injury . It can be concluded that thyme is an efficient natural stimulant of endogenous antioxidants thus, reversing the oxidative stress and hepatic toxicity [33] .Compared with conventional silymarin-treated group therapy [34], The silymarin-treated group showed greater efficacy in reducing liver enzymes and inflammatory cytokines . Instead, thyme could emerge as an alternative option because of its similarity of effects to that of silymarin-treated group as well as its natural source and consequently fewer potential adverse effects, thus when conventional therapies are less tolerated or in general combined with less hazards for the patients. This also fosters developing conclusion that herbal drug such as thyme can be significant in treatment of the viral hepatic disorders, when the healing is difficult or the patient is confronted by complications related to classical treatment[35] .All in all, the present study underscores the importance of thyme extract in hepatoprotection and attenuation of oxidative stress and inflammation triggered by CCl_4-induced hepatic injury . As demonstrated by the better response in biological markers in high-dose compare to low-dose, it suggests that optimal doses should be established to exert the best therapeutic success. Potential future application of thyme as an effective adjunctive to therapy are promising, but these need to be backed by extensive clinical’s to confirm the quality and the safety especially in chronic patients. Finally, the specific molecular mechanisms of action of the thyme compounds need to be investigated for a better knowledge of their therapeutic effect and their possible role in human health.

Al-Shatrah Veterinary and Biological Sciences Journal
ISSN (Print): 2958-8952
Volume 2 , Issue 3 , June 2026
Page 128 - 141

5. Conclusions

The findings showed that thyme (*Thymus vulgaris*) extract provides protection against CCl_4-induced liver damage due to the significant decrease in the liver enzymes (ALT and AST) which indicates the improvement of liver performance. Moreover, the extract demonstrated that it can regulate the lipid profile through elevation of high density lipoprotein (HDL) and reduction of low density lipoprotein (LDL) indicating a better lipid balance of the body. Additionally, thyme had a beneficial regulatory effect on the levels of reactive oxygen species (ROS), and malondialdehyde (MDA) during the alleviation of oxidative stress, indicating its antioxidant property played a role to protect liver cells from free radical injury. Thyme also helped to regulate the immune response by decreasing the pro-inflammatory cytokines TNF-α and IL-6, and increasing the anti-inflammatory cytokines IL-10 and TGF-β, to mitigate chronic inflammation and restore the body's response to the disease. Regarding antioxidants, the GSH levels and the uric acid were both created by the thyme to be regained and diminish, thereby improving the power of the liver to battle the stress and the toxicity of oxidative. Thyme appeared to be promising as a new alternative to conventional (silymarin-treated group) treatment, as well as for patients who had experienced adverse effects from conventional drugs.

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Volume 2 , Issue 3 , June 2026
Page 128 - 141

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Page 128 - 141

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Volume 2 , Issue 3 , June 2026
Page 128 - 141

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