https://doi.org/10.31925/farmacia.2026.1.6

ORIGINAL ARTICLE

IN VIVO EVALUATION OF THE HEPATOPROTECTIVE, THERAPEUTIC, AND ANTIOXIDANT EFFECTS OF THYMOL IN A CARBON TETRACHLORIDE-INDUCED ACUTE LIVER INJURY MODEL IN FEMALE WISTAR RATS

HANANE KHITHER [1]*, SORAYA MADOUI [1], SELMA HOUCHI [1], AMINA SAFSAF [2], ASMA MOSBAH [3]

[1]Laboratory of Applied Biochemistry, Faculty of Nature and Life Sciences, Ferhat Abbas Sétif 1 University, Sétif, Algeria
[2]Pathology Laboratory, Sétif University Hospital, Sétif, Algeria
[3]Laboratory of Applied Biochemistry, Faculty of Natural and Life Sciences, University Constantine 1, Constantine, Algeria

**corresponding author: khither.hanane@univ-setif.dz*

Manuscript received: November 2025

Abstract

This study evaluated the hepatoprotective and antioxidant effects of thymol in female Wistar albino rats using a carbon tetrachloride (CCl_4)-induced liver injury model. Rats were treated for seven days with thymol at doses of 100 and 200 mg/kg/day under both preventive and curative regimens. CCl_4 administration induced marked hepatic damage, characterized by elevated liver enzymes, altered lipid profiles, and increased oxidative stress. Thymol treatment markedly attenuated CCl_4-induced liver injury, as evidenced by improved hepatic biochemical parameters, normalization of lipid metabolism, and enhanced antioxidant defense mechanisms. The protective and curative effects were dose-dependent, with the higher dose showing superior efficacy. Overall, thymol reduced lipid peroxidation and strengthened endogenous antioxidant enzyme activities. These findings demonstrate that thymol exhibits significant hepatoprotective, therapeutic, and antioxidant properties in experimental models of liver toxicity, supporting its potential therapeutic application in chemically induced hepatic disorders.

Rezumat

Acest studiu a evaluat efectele hepatoprotectoare și antioxidante ale timolului la șobolani Wistar albino femele, utilizând un model de leziune hepatică indusă de tetraclorură de carbon (CCl_4). Animalele au fost tratate timp de șapte zile cu timol, în doze de 100 și 200 mg/kg/zi, atât în regim preventiv, cât și curativ. Administrarea de CCl_4 a indus leziuni hepatice semnificative, evidențiate prin creșterea enzimelor hepatice, alterarea profilului lipidic și amplificarea stresului oxidativ. Tratamentul cu timol a atenuat semnificativ leziunile hepatice induse de CCl_4, fapt demonstrat prin ameliorarea parametrilor biochimici hepatici, normalizarea metabolismului lipidic și intensificarea mecanismelor de apărare antioxidantă. Efectele protectoare și curative au fost dependente de doză, doza mai mare prezentând o eficacitate superioară. În concluzie, timolul a redus peroxidarea lipidică și a stabilizat activitatea enzimelor antioxidante endogene. Aceste rezultate demonstrează că timolul prezintă proprietăți hepatoprotectoare, terapeutice și antioxidante semnificative în modele experimentale de toxicitate hepatică, susținând un potențial terapeutic pentru afecțiunile hepatice induse chimic.

Keywords: thymol, hepatotoxicity, carbon tetrachloride, antioxidant, liver injury

Introduction

The liver is an essential organ that plays a central role in detoxification, metabolism, and maintaining redox equilibrium. Due to its strategic anatomical position and physiological functions, the liver is highly exposed to toxicity caused by xenobiotics, environmental pollutants, and chemical toxins. Carbon tetrachloride (CCl_4) is one of the most widely used experimental hepatotoxins, reproducibly causing liver damage in animal models, enabling investigation of hepatotoxic pathways and assessment of hepatoprotective substances (1). The primary cause of CCl_4-hepatotoxicity is its bioactivation by cytochrome P450 enzymes, leading to the formation of highly reactive trichloromethyl ($\cdot CCl_3$) and trichloromethyl peroxy ($\cdot OOCCl_3$) radicals that initiate oxidative stress and lipid peroxidation within hepatocellular membranes (2).

Oxidative stress is recognized as a central pathogenic pathway in CCl_4-induced liver toxicity. Excessive generation of reactive oxygen species (ROS) overwhelms endogenous antioxidant defense systems, leading to oxidative damage to proteins, lipids, and nucleic acids (3). This imbalance destroys membrane integrity, impairs mitochondrial and endoplasmic reticulum functions, and ultimately leads to hepatocyte necrosis and inflammatory infiltration. Experimental studies consistently report

that CCl_4 administration causes marked elevations in serum transaminases, increased levels of lipid peroxidation products such as malondialdehyde (MDA), and pronounced histopathological alterations, including steatosis, congestion, and hepatocellular necrosis (4). Consequently, the CCl_4-induced hepatotoxicity model is widely accepted as a reliable experimental tool for studying oxidative liver damage and assessing antioxidant-based hepatoprotective strategies (1).

In recent years, increasing attention has been directed toward natural products and medicinal plants as alternative or complementary strategies for the prevention and treatment of liver diseases. A wide range of plant-derived bioactive compounds has demonstrated hepatoprotective effects, largely attributed to their antioxidant properties. These include free radical scavenging activity, inhibition of lipid peroxidation, and modulation of endogenous antioxidant defense systems such as superoxide dismutase (SOD) and catalase (CAT) (1). Experimental investigations employing CCl_4-induced hepatotoxicity models have consistently shown that various medicinal plant extracts can attenuate biochemical alterations and histopathological damage associated with liver injury; such findings provide strong experimental evidence supporting the therapeutic potential of phytochemicals in hepatic protection (1).

Among aromatic and medicinal plants, *Thymus vulgaris* has gained significant attention for its abundance of bioactive phenolic compounds. One of its main active constituents, thymol, a monoterpenoid phenol, has been widely studied for its antioxidant and hepatoprotective potential. Experimental findings suggest that thymol can reduce lipid peroxidation, restore antioxidant enzyme activities, and limit the rise of liver function markers following CCl_4-induced toxicity (5).

Comparative studies with synthetic antioxidants such as butylated hydroxytoluene (BHT) further highlight thymol's protective role against oxidative stress-related liver damage. These effects are reflected by decreased MDA levels, improved antioxidant defense systems, and preservation of normal liver tissue architecture (5).

Beyond thymol-specific research, studies on medicinal plant extracts in general consistently support the protective role of natural antioxidants against CCl_4-induced hepatotoxicity. Improvements in serum biochemical markers, recovery of hepatic antioxidant defenses, and reduced histopathological damage have been reported following treatment with plant-derived compounds (3). Histological observations further demonstrate reduced hepatocellular necrosis, inflammatory cell infiltration, and lipid accumulation, emphasizing the central role of oxidative stress modulation in liver protection (4).

Despite these promising findings, important questions remain, particularly regarding thymol's effectiveness under different treatment strategies and dosing conditions. In addition, studies directly comparing its preventive and therapeutic effects in the same standardized liver injury model remain limited. Clarifying these aspects is essential for a better understanding of thymol's true hepatoprotective potential.

Therefore, this study aimed to evaluate both the protective and therapeutic effects of thymol in a CCl_4-induced liver toxicity model in *Wistar albino* rats. This was achieved by assessing liver function biomarkers, lipid profile, oxidative stress parameters, and histopathological changes. The originality of this work lies in examining thymol's effects through both preventive and curative approaches within a single experimental design.

Materials and Methods

Animals

We conducted an *in vivo* study on female Wistar albino rats obtained from the Pasteur Institute of Algeria. On arrival, the animals weighed 150 to 160 g. After an adaptation period, the mean body weight reached approximately 200 g at the start of the experimental procedures. We randomly assigned the animals to experimental groups of seven rats *per* group. We balanced the groups for baseline body weight. During the adaptation and experimental periods, the rats had free access to standard laboratory chow supplied by the National Office of Animal Feed (ONAB) in Bejaia. They also had free access to tap water ad libitum. We housed the animals under controlled conditions at 23 ± 2°C. We maintained a natural light-and-dark cycle. We performed all handling and experimental procedures in accordance with internationally accepted guidelines for the care and use of laboratory animals.

At the start of the experiment, the rats were approximately 10-12 weeks old. Animals were housed in groups of seven rats *per* cage under standard laboratory conditions.

Chemical reagents

All chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich. The main reagents included 5-methyl-2-(1-methylethyl) phenol or thymol (CAS No. 89-83-8) with catalog number 16254, carbon tetrachloride or CCl_4 (CAS No. 56-23-5) with catalog number 319961, Tween 80 (CAS No. 9005-65-6) under catalog number P4780, formaldehyde (10%), potassium chloride (KCl), hydrogen peroxide (H_2O_2), potassium phosphate ($KHPO_4$), ethylenediaminetetraacetic acid (EDTA), pyrogallol, trichloroacetic acid (TCA), thiobarbituric acid (TBA), Tris base, and *n*-butanol. All reagents

were prepared and stored according to the manufacturers' recommendations.

The chemicals were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Thymol (CAS No. 89-83-8) was purchased from Sigma-Aldrich (Riedel-de Haën brand) with catalog number 16254. Carbon tetrachloride (CCl_4) (CAS No. 56-23-5) was obtained as reagent grade (≥99.9%) from Sigma-Aldrich, catalog number 319961. Tween 80 (Polysorbate 80) (CAS No. 9005-65-6) was supplied by Sigma-Aldrich under catalog number P4780.

Evaluation of the hepatoprotective and therapeutic effect of thymol

Induction of hepatotoxicity by CCl4

The CCl_4-induced liver injury model used in this study represents an acute hepatotoxicity model. We induced hepatic damage with a single intraperitoneal injection of carbon tetrachloride. Researchers use this approach to examine early oxidative stress and hepatocellular injury. They also use it to capture initial biochemical and histopathological changes linked to toxic liver damage.

Following a 2 h fast, hepatotoxicity was induced by intraperitoneal injection of carbon tetrachloride at 3 mL/kg body weight (2385 mg/kg), previously diluted in olive oil (50% v/v) (6).

Treatment of animals

To evaluate the hepatoprotective and therapeutic effects of thymol against CCl_4-induced liver injury, we randomly assigned the animals to six experimental groups. Each group included seven rats. We administered thymol at 100 mg/kg and 200 mg/kg body weight. We prepared the dosing solutions fresh each day. We administered thymol for seven consecutive days. We used 0.9% NaCl saline containing 0.1% Tween 80 as the vehicle.

The experimental groups were organized as follows. Group 01 (negative control) received 0.9% NaCl by oral gavage for seven days. Group 02 (positive control) received 0.9% NaCl by gavage for seven days, followed by intraperitoneal injection of CCl_4. Groups 03 and 04 (post-treatment groups) were injected with CCl_4 and, after 30 min, treated with thymol by oral administration at doses of 100 mg/kg (C100) and 200 mg/kg (C200), respectively, for seven days. Groups 05 and 06 (preventive groups) were pretreated with thymol at doses of 100 mg/kg (P100) and 200 mg/kg (P200), respectively, for seven days before CCl_4 injection.

At the end of the treatment period, all rats were sacrificed, and blood and liver samples were collected for biochemical, oxidative stress, and histopathological analyses.

Sacrifices, blood sampling, and liver recovery

On the eighth day, rats were sacrificed under anesthesia using diethyl ether. Blood samples were collected by puncturing the retro-orbital sinus into heparinized tubes for biochemical analyses. Samples were centrifuged at 3000 rpm for 10 min, and the plasma was collected and stored at −4 °C until biochemical parameter (ASAT, ALAT) and oxidative stress marker (SOD, CAT, MDA) analyses. Following dissection, livers were carefully excised, rinsed with physiological saline (0.9% NaCl), cleaned of adhering fat, weighed, and stored appropriately. Portions of liver tissue were used to prepare a homogenate to assess hepatic oxidative stress parameters.

Determination of body weight and liver relative weight

Body weight was recorded at the beginning of the experiment and followed throughout the study employing a precision balance. Relative liver weight (RLW) was calculated as the ratio of liver weight to total body weight and expressed as a percentage using the following formula:

$$RLW = \frac{Liver\ weight_{(g)}}{Body\ weight_{(g)}} \times 100$$

Biochemical parameter assays

Plasma biochemical markers, including aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), cholesterol, and triglycerides, were measured using commercially available diagnostic kits commonly used in Algerian clinical laboratories (Spinreact®). Each assay was carried out according to the manufacturer's specific guidelines. After an overnight of fasting, blood samples were collected and centrifuged to separate the plasma.

ASAT and ALAT activities were measured using an enzymatic kinetic technique in accordance with the International Federation of Clinical Chemistry (IFCC) guidelines (7,8). To assess the plasmatic level of triglycerides, an enzymatic colorimetric technique based on the GPO-PAP (glycerol-3-phosphate oxidase-peroxidase) principle was used (9). Total cholesterol levels were assessed using an enzymatic colorimetric technique based on the CHOD-PAP (cholesterol oxidase-peroxidase) principle (10). All analyses were performed at Ain Arnat's Medical Analysis Laboratory in Setif, Algeria.

Measurement of oxidative stress markers

Preparation of the homogenate.

On the analysis day, liver homogenates were prepared by homogenizing 500 mg of hepatic tissue in 5 mL of 0.15 M KCl buffer (pH 7.4) at 4 °C using a Potter-Elvehjem tissue homogenizer. As protease inhibitors, 1 mM Phenylmethylsulfonyl fluoride (PMSF) was added to prevent protein hydrolysis. Liver homogenates were centrifuged at 3000 × *g* for 10 min, and the resulting supernatants were collected to determine oxidative stress status.

Protein concentration in liver homogenates was determined using the Biuret method, the Spinreact Kit (BSIS30-1). Antioxidant enzyme activities were

normalized to protein content and expressed as international units *per* milligram of protein.

Total Protein Assay

Plasmatic and hepatic total protein were determined by the Biuret method using the Spinreact Kit (BSIS30-1). The principle of this assay is based on the formation of a violet-blue complex between copper salts and proteins in an alkaline medium. In this test, the color intensity produced is proportional to the total protein amount in the sample, as determined by measuring absorbance at 540 nm using a calibration curve (11). Briefly, twenty-five µL of the standard, homogenate, or plasma was added to 1 mL of the reagent. After 10 min of incubation at room temperature, the absorbance was recorded at 540 nm.

Measurement of superoxide dismutase enzymatic activity

Superoxide dismutase (SOD) activity was determined according to the method of Nandy et al (12). The principle of this assay is based on the inhibition of pyrogallol auto-oxidation by SOD. Absorbance was measured at 420 nm for 60 s in the presence or absence of plasma or liver homogenate samples. The amount of enzyme required to inhibit 50% of pyrogallol auto-oxidation was considered as one unit of SOD activity. The enzymatic activity was expressed as international units *per* milligram of hepatic tissue protein or *per* milliliter of plasma.

Measurement of catalase enzymatic activity

Plasmatic and hepatic catalase activities were assessed by following the decrease in absorbance at 240 nm, which reflects the breakdown of hydrogen peroxide (H_2O_2). The H_2O_2 solution was prepared in 0.1 M potassium phosphate buffer (pH 7.2) and added to the biological sample. Enzymatic activity was calculated using the molar extinction coefficient of 43.6 $M^{-1} \cdot cm^{-1}$ and expressed as µmol/min/mg of tissue protein or µmol/min/mL of plasma (13).

Measurement of malondialdehyde

Malondialdehyde (MDA) levels, used as an indicator of lipid peroxidation, were measured using the thiobarbituric acid reactive substances (TBARS) assay. Samples were treated with trichloroacetic acid and thiobarbituric acid under acidic conditions and high temperature, followed by extraction with *n*-butanol. The absorbance was recorded at 532 nm, and the MDA level was calculated using a molar extinction coefficient of 1.56×10^5 $M^{-1} \cdot cm^{-1}$. Results were expressed as nmol MDA *per* gram of tissue or *per* milliliter of plasma (14).

Histopathological Examination

Liver tissues were fixed in 10% neutral buffered formalin, dehydrated, and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin (15). Histopathological examination was performed under a light microscope, and photomicrographs were obtained at the pathology laboratory of Setif University Hospital, Algeria.

Statistical analysis

Data obtained from *in vivo* experiments were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism 7 software. Comparisons between groups were conducted using Student's t-test for simple comparisons or one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p \leq 0.05$.

Results and Discussion

Effect on body weight and relative liver weight

The results show no significant difference in body weight between the initial and final measurements across the experimental groups. Intraperitoneal injection of carbon tetrachloride (CCl_4) led to a slight decrease in final body weight compared to initial weight in rats. Similarly, prophylactic and curative pretreatment with thymol at 100 and 200 mg/kg/day for 7 days also resulted in a non-significant reduction in final body weight relative to initial values. However, the difference between initial and final weight in the thymol-pretreated and treated groups was significantly lower compared to the CCl_4-intoxicated group (Table I).

As for relative liver weight (RLW), defined as the ratio of liver weight (g) to total body weight (g), only slight variations were observed among the groups (Table I). The RLW values in thymol-treated rats (both doses) closely resembled those of the normal control group and were slightly lower than those in the CCl_4-intoxicated group. This difference was not statistically significant.

The slight reduction in body weight recorded following CCl_4 intoxication is consistent with its hepatotoxic effects, which can disrupt metabolic homeostasis. In thymol-treated groups, body weight variation was not statistically significant, suggesting that thymol treatment had no measurable effect on body weight under the present experimental conditions. These observations are consistent with previous reports and are cautiously interpreted, without extending mechanistic evidence (16, 17). Similarly, no significant differences were recorded in relative liver weight among the experimental groups. This finding is expected in an acute CCl_4 intoxication model, where a single administration is unlikely to induce marked liver enlargement. The slight variations observed in relative liver weight are therefore more likely to reflect changes in overall body weight rather than true hepatic edema or swelling.

Table I

Variation in body weight, liver weight, and relative liver weight in control and thymol-treated rats

Experimental Groups	Parameters				
	Initial weight (g)	Final weight (g)	Δ Weight	Liver weight (g)	Relative liver weight (%)
Healthy	168.33 ± 11.09	176.00 ± 10.53	7.67	6.74 ± 1.08	3.83 ± 0.86
CCl_4 group	203.43 ± 9.73	200.85 ± 11.14	-2.58	8.59 ± 1.06*	4.278 ± 0.45 *
Cur 200 mg	197.57 ± 3.18	183.00 ± 4.08	-14.57	6.34 ± 0.36###	3.46 ± 0.20 ###
Cur 100 mg	184.00 ± 5.11	166.66 ± 11.81	-17.34	6.67 ± 1.23 ##	4.00 ± 0.73 ##
Pre 200mg	181.14 ± 3.65	162.50 ± 20.00	-18.64	7.11 ± 0.72 #	4.37 ± 0. 15 #
Pre 100 mg	**182.85 ± 6.16**	**174.00 ± 8.18**	-8.85	**7.41 ± 1.10** #	**4.26 ± 0.39** #

The values represent the mean ± SEM (n=7). The values are expressed as mean ± SEM, (n = 7); *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$ significant difference compared to the healthy control group, #: $p \leq 0.05$, ##: $p \leq 0.01$, ###: $p \leq 0.001$ significant difference compared to the CCl_4 group

Hepatoprotective and therapeutic effect of thymol

Effect on hepatic biochemical parameters

As illustrated in Figure 1, plasma alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activities were significantly increased in rats exposed to carbon tetrachloride compared with the healthy control group ($p < 0.001$), confirming hepatocellular injury.

Preventive administration of thymol at both tested doses was associated with lower transaminase activities compared with the CCl_4-treated group, with statistically significant differences observed at specific doses. Post-treatment with thymol also resulted in a significant reduction in ALAT and ASAT activities relative to the CCl_4 group. Although enzyme activities were reduced following thymol administration, they were not fully normalized.

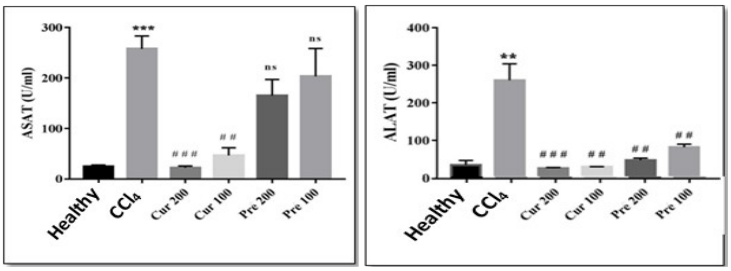


Figure 1.

Hepatic profile (AST and ALT) of rat groups treated with CCl_4 in the presence and absence of thymol (100 and 200 mg/kg for 7 days).1 The values are expressed as mean ± SEM, (n = 7); *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$ significant difference compared to the healthy control group, #: $p \leq 0.05$, ##: $p \leq 0.01$, ###: $p \leq 0.001$ significant difference compared to the CCl_4 group

Effect on lipid biochemical parameters

As shown in Figure 2, carbon tetrachloride administration modestly increased plasma triglyceride and cholesterol levels compared with the healthy control group; however, these differences were not statistically significant. Preventive and post-treatment administration of thymol at both doses did not produce statistically significant changes in plasma lipid parameters compared with the CCl_4-treated group. Therefore, variations in lipid profile were reported descriptively without inferring a hypolipidemic effect.

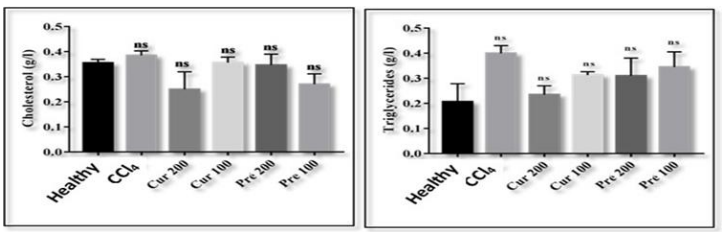


Figure 2.

Lipid profile (triglycerides and cholesterol) of rat groups treated with CCl_4 in the presence and absence of thymol (100 and 200 mg/kg for 7 days).2 The values are expressed as mean ± SEM, (n = 7); *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$ significant difference compared to the healthy control group; #: $p \leq 0.05$, ##: $p \leq 0.01$, ###: $p \leq 0.001$ significant difference compared to the CCl_4 group

Plasmatic ALAT and ASAT activities were significantly increased in CCl_4-intoxicated rats, clearly reflecting hepatocellular injury. These findings may be interpreted as the over-release of these enzymes into the circulation when the hepatocyte membrane is destroyed by reactive free radicals generated during CCl_4 Biotransformation (18-20). Whereas, treatment with thymol significantly decreased ALAT and ASAT activities compared with the CCl_4-treated group. This reflects a significant protective effect against liver toxicity. Enzyme activities were improved but not fully normalized, avoiding overinterpretation of complete functional recovery (21,22).

Variations in plasma lipid parameters observed following CCl_4 administration may reflect disturbances in lipid metabolism induced by oxidative stress (17). Although thymol-treated groups tended to show lower triglyceride and cholesterol levels, these differences were not statistically significant. Therefore, these results should be interpreted with caution, and no definitive hypolipidemic effect can be concluded from the present data.

Evaluation of oxidative stress markers

Oxidative stress markers in plasma

Plasma oxidative stress status is presented in Figure 3. CCl_4- intoxication induces a significant increase in plasma malondialdehyde (MDA) levels compared with the control group, indicating enhanced lipid peroxidation. While plasma catalase (CAT) and superoxide dismutase (SOD) activities decreased following CCl_4 administration, the changes were not statistically significant.

Thymol administration was associated with lower plasma MDA levels than in the CCl_4-treated group, with statistically significant differences observed at selected doses. Modulation of CAT and SOD activities was also observed following thymol treatment. These findings were interpreted as attenuation of oxidative stress without attributing specific molecular mechanisms.

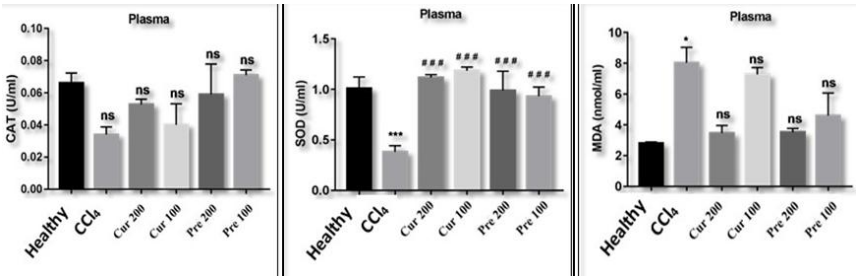


Figure 3.

Effect of thymol on the status of oxidative stress markers in plasma. The values are expressed as mean ± SEM, (n = 7); *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$ a significant difference compared to the healthy control group, #: $p \leq 0.05$, ##: $p \leq 0.01$, ###: $p \leq 0.001$ a significant difference compared to the CCl_4 group

Oxidative stress markers in liver homogenate

The hepatic oxidative stress status is shown in Figure 4. Rats exposed to carbon tetrachloride exhibited a significant increase in hepatic malondialdehyde levels compared with the healthy control group, reflecting marked lipid peroxidation in liver tissue. Hepatic catalase and superoxide dismutase activities were also significantly altered following CCl_4 exposure. Both preventive and post-treatment administration of thymol was associated with lower hepatic MDA levels than in the CCl_4-treated group. Antioxidant enzyme activities in liver homogenates were modulated following thymol treatment. These changes were described without inferring enzyme depletion, compensatory upregulation, or direct restoration mechanisms.

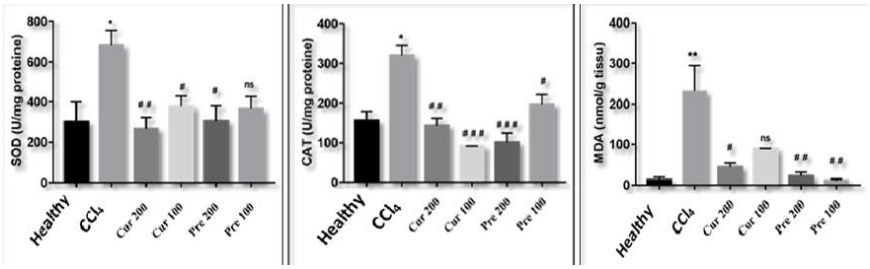


Figure 4.

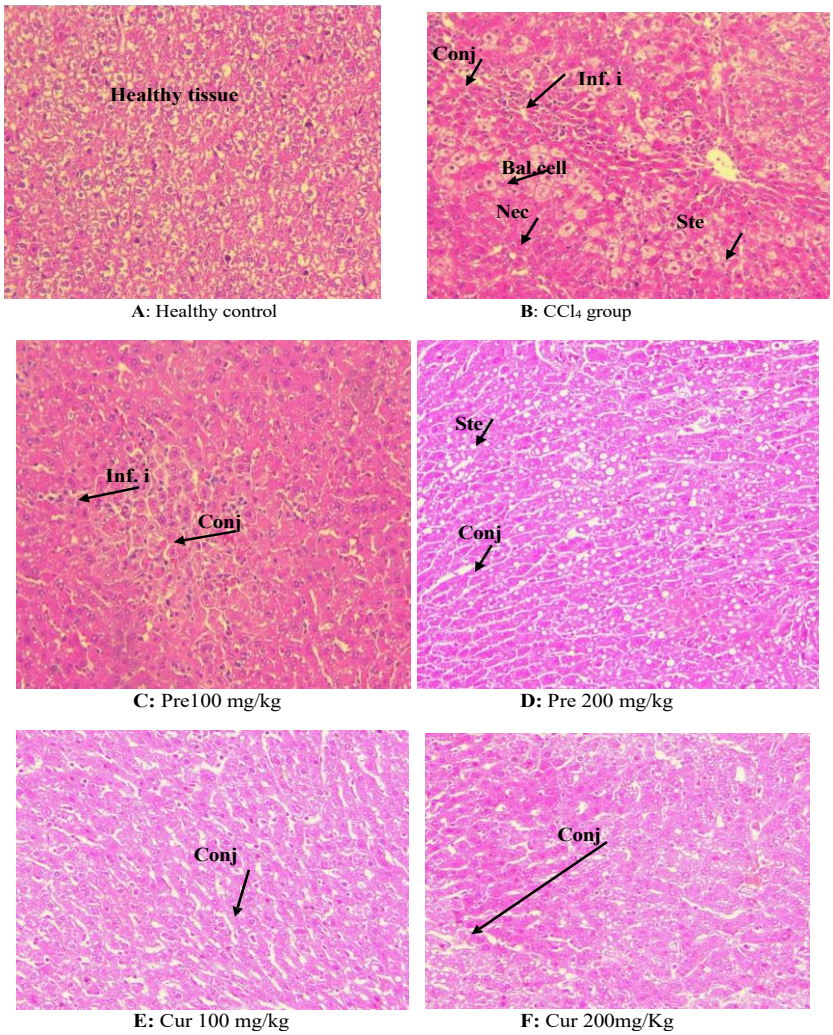
Effect of thymol on the hepatic status of oxidative stress markers. Values are expressed as mean ± SEM, (n = 7); *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$ significant difference compared to the healthy control group, $^{\#}$: $p \leq 0.05$, $^{\#\#}$: $p \leq 0.01$, $^{\#\#\#}$: $p \leq 0.001$ significant difference compared to the CCl_4 group

Evaluation of oxidative stress status revealed a significant increase in plasma MDA levels following CCl_4 exposure, reflecting enhanced lipid peroxidation. Changes in Catalase and SOD activities were observed in both plasma and liver samples. While plasma antioxidant enzyme activities decreased after CCl_4 administration, hepatic enzyme activities responded differently, highlighting tissue-specific variations in oxidative stress. No mechanistic conclusions regarding enzyme depletion, compensatory, or regulation were drawn, as such interpretations require additional experimental evidence (23, 24). Thymol treatment was associated with lower MDA levels and modulated CAT and SOD activities compared with the CCl_4-treated group. These findings suggest an attenuation of oxidative stress, although these effects were interpreted cautiously, without assuming direct enzyme restoration or specifying molecular mechanisms involved (23,24).

Histopathological study

Representative histological sections are presented in Figure 5. Liver tissues from the healthy control group exhibited normal hepatic architecture, with well-organized hepatocytes and intact sinusoids. In contrast, the CCl_4-treated group showed marked hepatic alterations, including hepatocellular degeneration, inflammatory cell infiltration, vascular congestion, necrosis, ballooned hepatocytes, and steatosis.

In the thymol pre-treatment groups, liver tissues demonstrated a noticeable reduction in the severity of histopathological lesions, particularly at the higher dose, with partial preservation of normal hepatic architecture. Likewise, post-treatment with thymol resulted in less tissue damage than in the CCl_4-treated group, although mild alterations remained.



A: Healthy control **B**: CCl_4 group

C: Pre100 mg/kg **D:** Pre 200 mg/kg

E: Cur 100 mg/kg **F:** Cur 200mg/Kg

Figure 5.

Liver histological sections (A) Healthy control: HT (healthy tissues). (B) CCl_4 group: Inf. I (inflammatory infiltration), Bal. cell (ballooned hepatocytes), Nec (necrosis), Conj (congestion). (C) Pre-treatment (Pre 100 mg/kg): Inf. I (inflammatory infiltration), Conj (congestion). (D) Pre-treatment (Pre 200 mg/kg): Ste (steatosis), Conj (congestion). (E) Curative treatment (Cur 100 mg/kg): Con (congestion). (F) Curative treatment (Cur 200 mg/kg): Conj (congestion).

Histopathological examination corroborated the biochemical findings, revealing pronounced hepatic damage in CCl_4-treated rats, including necrosis, inflammatory infiltration, steatosis, and disruption of normal liver architecture (25). Administration of Thymol alleviated the occurrence of these lesions, especially at the higher dose, suggesting a protective effect at the tissue level. These results reflect histological improvement but do not indicate entire hepatic regeneration beyond the structural changes recorded (25,26).

Overall, the results of this study demonstrate that thymol attenuated the biochemical, oxidative, and histopathological alterations induced by acute CCl_4 exposure. While these findings reveal the thymol hepatoprotective and therapeutic potential, the conclusions are limited to the specific experimental conditions of this study (27-33). Further studies are required to elucidate the underlying mechanisms, assess tissue-specific antioxidant responses, and evaluate the long-term efficacy of thymol in models of liver toxicity.

Conclusions

This study assessed the hepatoprotective and therapeutic effects of thymol at 100 and 200 mg/kg/day, administered either prior to or following CCl_4 exposure, in Wistar rats. Acute CCl_4 intoxication was characterized by significant alterations in biochemical markers of liver toxicity and oxidative stress, including increased plasma transaminases and high lipid peroxidation.

Thymol treatment was associated with an attenuation of CCl_4-induced biochemical and oxidative alterations, as reflected by reduced ALAT and ASAT activities and decreased malondialdehyde levels compared with the CCl_4-treated group. Improvements in antioxidant enzyme activities were observed in both plasma and liver samples following thymol administration; however, these changes were interpreted cautiously and not attributed to specific molecular mechanisms. Although changes in plasma lipid parameters did not reach statistical significance, no adverse effects were observed following thymol administration at the tested doses.

Overall, the results of this study reveal that thymol attenuated biochemical, oxidative, and histopathological alterations induced by acute CCl_4 intoxication. While these findings support the hepatoprotective and therapeutic potential of thymol, further studies are required to further clarify the underlying molecular mechanisms, assess long-term safety, and evaluate its efficacy in other experimental models of liver toxicity.

Acknowledgement

The authors thank the Algerian Ministry of Higher Education and Scientific Research (MESRS) and the General Directorate of Scientific Research and Technological Development (DGRSDT) for supporting this research study.

Funding

Not applicable.

Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

All experimental procedures involving animals were carried out in strict accordance with the ethical principles stated in the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, revised 1985), as well as with applicable national regulations and institutional policies. The study protocol was reviewed and approved by the Institutional Ethics Committee of the Laboratory of Applied Biochemistry, Ferhat Abbas University of Setif 1 (Approval No. IAEC/FAS/2025). All experimental procedures were reviewed and approved by the institutional ethics committee prior to the commencement of the study.

AI use disclosure

During the preparation of this manuscript, the authors used ChatGPT to translate from French to English for editing and language improvement, specifically to improve spelling, grammar, word choice, readability, and clarity. The authors reviewed and revised all outputs from these tools and take full responsibility for the final content of the work. The authors used QuillBot for paraphrasing.

Conflict of interest

The authors declare no conflict of interest.

References

1. Xiao M, Li GY, Zhang CJ, Liu Q, Li HB. Antioxidant activity and hepatoprotective effect of medicinal plants against chemically induced liver injury in mice. World J Gastroenterol. 2020;26(37):5629-5645.
2. Abdalla GE, El-Behairy OA. Protective effects of Thymus vulgaris oil against CCl_4-mediated hepatotoxicity, oxidative stress and immune-suppression in male rats. Adv Anim Vet Sci. 2021;9(7):1053-1062.
3. Sabiu S, Olatunde EO, Ajani EO. Mechanism of hepatoprotective potential of aqueous leaf extract of

Eucalyptus obliqua in carbon tetrachloride-intoxicated Wistar rats. J Appl Pharm Sci. 2017;7(8):183-190.
4. Hassan NS, Abbas NF, Sharaf HA. Histopathological and histochemical studies on the effect of taurine in preventing carbon tetrachloride-induced hepatic injury. Egypt J Hosp Med. 2003;10:52-65.
5. Al-Malki AL. Antioxidant properties of thymol and butylated hydroxytoluene in carbon tetrachloride-induced mice liver injury. J King Abdulaziz Univ Sci. 2010;22:239-248.
6. Wang BJ, Liu CT, Tseng CY, Wu CP, Yu ZR. Hepatoprotective and antioxidant effects of Bupleurum kaoi extract and its fractions on CCl_4-induced liver damage. Food Chem Toxicol. 2004;42(4):609-617.
7. Bergmeyer HU, Horder M, Rej R. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3: IFCC method for alanine aminotransferase (L-alanine:2-oxoglutarate aminotransferase). J Clin Chem Clin Biochem. 1986;24(7):481-495.
8. Bergmeyer HU, Horder M, Rej R. Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2: IFCC method for aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase). J Clin Chem Clin Biochem. 1986;24(8):497-510.
9. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982;28(10):2077-2080.
10. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974;20(4):470-475.
11. Koller A. Total serum protein. In Kaplan LA, Presce AJ, eds. Clinical Chemistry, Theory, Analysis, and Correlation. 5th ed, St. Louis: Mosby Company. 1984. p. 1316-1319.
12. Nandy S, Shekhar HP, Ranjan BN, Chakraborty B. In vitro evaluation of antioxidant activity of Leucas plukenetii. Asian J Plant Sci Res. 2012;2(3):254-262.
13. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-126.
14. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 1990;186:421-431.
15. Bancroft JD, Gamble M. Theory and practice of histological techniques. 5th ed. Edinburgh: Churchill Livingstone; 2002. p. 172-175, 593-620.
16. Choi SS, Kim ES, Jung JE, et al. PPARγ antagonist Gleevec improves insulin sensitivity and promotes browning of white adipose tissue. Diabetes. 2016;65(4):829-839.
17. Saravanan S, Pari L. Protective effect of thymol on high-fat diet-induced diabetic nephropathy in C57BL/6J mice. Chem Biol Interact. 2016;245:1-11.
18. Yang BY, Zhang XY, Guan SW, Hua ZC. Protective effect of procyanidin B2 against CCl_4- induced acute liver injury in mice. Molecules. 2015;20(7):12250-12265.
19. Eidi A, Mortazavi P, Tehrani ME, Rohani AH, Safi S. Hepatoprotective effects of pantothenic acid on carbon tetrachloride-induced toxicity in rats. EXCLI J. 2012;11:748-759.
20. Maameri Z, Djerrou Z, Halmi S, Djaalab H, Riachi F, Hamdipacha Y. Evaluation of hepatoprotective effect of Pistacia lentiscus fatty oil in rats intoxicated by carbon tetrachloride. Int J Pharmacogn Phytochem Res. 2015;7(2):251-254.
21. Chao J, Liao JW, Peng WH, Lee MS, Pao LH, Cheng HY. Antioxidant, analgesic, anti- inflammatory and hepatoprotective effects of Mahonia oiwakensis stem extract. Int J Mol Sci. 2013;14(2):2928-2945.
22. Alam K, Nagi MN, Badary OA, Al-Shabanah OA, Al-Rikabi AC, Al-Bekairi AM. Protective action of thymol against carbon tetrachloride hepatotoxicity in mice. Pharmacol Res. 1999;40(2):159-163.
23. Al-Malki AL. Antioxidant properties of thymol and butylated hydroxytoluene in carbon tetrachloride-induced liver injury. J King Abdulaziz Univ Sci. 2010;22:239-248.
24. Abdel-Aziem SH, Hassan AM, El-Denshary ES, Hamzawy MA, Mannaa FA, Abdel-Wahhab MA. Ameliorative effects of thyme and calendula extracts against aflatoxin-induced oxidative stress and genotoxicity in rat liver. Cytotechnology. 2014;66(3):457-470.
25. Lee KY, Bae IY. Protective effect of Protaetia brevitarsis seulensis against CCl_4-induced hepatotoxicity in rats. Entomol Res. 2021;51(3):105-110.
26. Dahiya R, Mohammad A, Kumar S. Antioxidant and hepatoprotective potential of thymol against chemically induced liver toxicity. J Pharmacol Sci. 2020;145(2):125-135.
27. Zhang Y, Zhang L, Sun X. Protective role of thymol against oxidative stress-induced liver damage. J Funct Foods. 2021;79:104420.
28. Cojocaru-Toma M, Pantea V, Popa V, Gudumac V, Spinosu G, Cociug A, et al. Examination of hepatoprotective potential by organ mass evaluation, biochemical and histopathologic analysis of Agrimonia eupatoria L. and Cichorium intybus L. extracts in toxic hepatitis. Farmacia. 2025;73(5):1223-1235.
29. Salehi B, Mishra AP, Shukla I, Sharifi-Rad M, Contreras MDM, Segura-Carretero A, et al. Thymol, thyme and other plant sources: health and potential uses. Phytother Res. 2018;32(9):1688-1706.
30. Goud PR, Das S, Devi US. Thymol mitigates liver damage by suppressing pro-inflammatory cytokines and oxidative stress. Phytother Res. 2019;33(4):785-795.
31. Khan RA, Zubair H, Malik A. Thymol attenuates lipid accumulation and prevents hepatic steatosis in toxin-induced liver injury. Food Chem Toxicol. 2022;165:113141.
32. Pradeep K, Mohan CV, Gobianand K. Protective effect of natural antioxidants against CCl_4- induced hepatotoxicity in rats. J Ethnopharmacol. 2007;112(2):285-291.
33. Sharma N, Dey S, Agarwal S. Curcumin and silymarin as hepatoprotective agents: a comparative study. J Tradit Complement Med. 2020;10(1):50-56.