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IN VITRO ANTIOXIDANT ACTIVITY AND HEPATOPROTECTIVE EFFECT OF GARLIC EXTRACT (*ALLIUM SATIVUM* L.) IN CARBON TETRACHLORIDE-INDUCED LIVER INJURY IN MICE

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Summary

***In vitro* Antioxidant Activity and Hepatoprotective Effect of Garlic Extract (*Allium sativum* L.) in Carbon Tetrachloride-Induced Liver Injury in Mice**

The study was conducted to evaluate *in vitro* antioxidant properties and *in vivo* hepatoprotective effect of the garlic extract (*Allium sativum* L.). Direct assessment of antioxidant activity through *in vitro* tests revealed that garlic extract possesses antioxidant capacity by scavenging free radicals: DPPH, $ABTS^{\bullet+}$, and hydroxyl radical ($OH^{\bullet}$). Additionally, garlic extract also effectively inhibited the process of oxidation through chelating Fe^{2+}. The hepatoprotective activity of the garlic extract then was evaluated in a mouse model of carbon tetrachloride (CCl_4)-induced hepatotoxicity. Liver damage was induced in mice by intraperitoneal injection of 10% CCl_4 at a dose of 0.5 mL/kg body weight. Garlic extract at doses of 500 and 1000 mg/kg body weight was administered for eight consecutive days prior to CCl_4 administration. Silymarin suspension at a dose of 100 mg/kg body weight was used as a positive control. The present study showed that garlic extract at the tested doses significantly improved the parameters of CCl_4-induced liver injury, including levels of serum transaminases (ASAT, ALAT), bilirubin, lipid peroxidation (MDA), and glutathione (GSH) ($p < 0.05$). The results indicated that garlic extract at doses of 500 and 1000 mg/kg body weight exhibited a hepatoprotective effect against CCl_4-induced acute hepatotoxicity in mice.

Keywords: *Garlic extract; Antioxidant activity; Carbon tetrachloride (CCl_4); Hepatoprotective activity.*

1. Introduction

The liver is a key organ in the detoxification of drugs and toxic chemicals. It contains a high concentration of enzymes that metabolize toxins, transforming xenobiotics into less toxic compounds for excretion. Liver disease is a leading cause of mortality and morbidity globally. Therefore, medicinal herbs with hepatoprotective properties have gained significant interest from researchers. Among many medicinal plants, Garlic (*Allium sativum* L.; Amaryllidaceae) is widely grown in Hai Duong and is used as a popular spice in Vietnam. It is also used as a remedy against several common diseases such as cold, influenza, snake bites, and hypertension. Several previous studies have reported that garlic extract possesses substantial antioxidant activity that can be responsible for the prevention of reactive oxygen species-mediated disorders including inflammation and liver damage [1]. Gedlik et al. demonstrated that an aqueous garlic extract (250 mg/kg i.p for 28 days) alleviated bile duct ligation (BDL) and scission-induced oxidative liver injury, improving both hepatic structure and function [2]. Garlic extract 500 mg/kg alleviates trastuzumab-induced hepatotoxicity in rats [3]. The methanol garlic extract (75 mg/kg) reduced significantly serum levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate transaminase, superoxide dismutase (SOD), glutathione peroxidases (GPx), and glutathione (GSH) in animals induced with CCl_4 [1]. Additionally, in a rat model of liver injury induced by D-galactosamine (GalN) and LPS, the lactic acid-fermented garlic extract reduced liver injury markers such as necrosis, histological changes, and serum levels of AST and ALT [4]. However, up until now, there has been no study on antioxidant and the liver-protective effects of garlic grown in Vietnam. In this study, we aimed to investigate antioxidant activity and the hepatoprotective effect of garlic extract which was collected in Vietnam, against CCl_4-induced hepatic damage in mice.

2. Materials and methods

2.1. Preparation of crude ethanol garlic extract

The fresh garlic samples were cultivated and collected in Kinh Mon district, Hai Duong province, in 2022 and authenticated by the Faculty of Pharmacognosy and Traditional Medicine, Ha Noi University of Pharmacy. The voucher specimen (HNIP/18773/23) was deposited at the Faculty of Pharmacognosy and Traditional

Medicine, Ha Noi University of Pharmacy. The fresh garlic samples were kept dry to maintain a moisture content below 60%. The garlic skins were removed. The cloves were then dried in a cold air dryer at 50°C for 12 hours. The cloves were then ground and extracted with 70% ethanol using ultrasound-assisted extraction. The solvent-to-sample ratio was 5:1 (v/w), with the extraction conducted at room temperature below 30°C for 24 hours, repeated twice. The extract was filtered, evaporated under vacuum (at low pressure), and dried to obtain the crude extract. The quality of garlic extract was evaluated at the Hi-Tech Applied Research and Testing Product Center. The garlic extract had a moisture content of 17.48% and a total polyphenol content of 6.28%, pH 5.39; high extraction efficiency of 40.4%. Garlic extract is referred to as GE in this study.

2.2. Chemicals and reagents

5,5′- Dithiobis (2-nitrobenzoic acid); thiobarbituric acid (TBA); ammonium molybdate; tetramethoxypropane; 1,1-diphenyl-2-picrylhydrazyl (DPPH); 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS); 1,10-phenanthroline monohydrate; L-Ascorbic acid; Quercetin (Sigma Aldrich); Na_2HPO_4, KH_2PO_4, dimethyl sulfoxide (Merck); silymarin (Legalon® (MEDAUS GmbH, Germany, lot number B2108012); kits for ASAT - aspartate aminotransferase and ALAT - alanine aminotransferase, bilirubin (Biosystems, Spain). Carbon tetrachloride (CCl_4) and other chemicals were high-grade products for research.

2.3. Experimental procedure

Animals

Adult male *Swiss* mice were sourced from National Institute of Hygiene and Epidemiology, weighing approximately 20–25 g. The animals were acclimatized to the laboratory animal room (Department of Pharmacology, Hanoi University of Pharmacy) for a minimum of 7 days prior to any experimental manipulations. They were housed in the animal room under controlled environmental conditions (at 24 ± 2°C, with 55-60% humidity and a 12-hour light-dark cycle) with a diet supplied by the National Institute of Hygiene and Epidemiology.

Experimental design

In vitro antioxidant activity

DPPH radical scavenging assay

The ability of the prepared extract to scavenge DPPH radicals was determined by the method described by Nguyen et al. [5]. Briefly, a 20 µL solution of GE in different concentrations was added to 180 µL methanol solution of DPPH (0.1 mM). Quercetin was used as a positive control and methanol was used as a blank. All reaction mixtures were shaken and incubated in the dark for 30 min at room temperature. The absorbance was then measured at 517 nm using a UV-Vis spectrophotometer. All samples were analyzed in triplicate. The DPPH scavenging ability of the plant extracts was calculated using the following equation:

$$\%\text{Scavenging Activity} = (\text{Abs control} - \text{Abs sample}) \times 100/(\text{Abs control})$$

where Abs control is the absorbance of the blank and Abs sample is the absorbance of the sample.

The antioxidant activity was reported in terms of IC_{50} (concentration of extract necessary to scavenge 50% DPPH radical).

ABTS radical scavenging assay

The ABTS assay was based on a previously described method [6]. The $ABTS^{\bullet+}$ radical was prepared by mixing 10 mL of the ABTS stock solution (7 mM) with 10 mL of 2.45 mM potassium persulfate solution. This mixture was kept in the dark at room temperature for 16 hours. On the day of analysis, the $ABTS^{\bullet+}$ radical solution was diluted with ethanol to an absorbance of 0.700 ± 0.02 at 734 nm. In 200 µL reaction mixture, 20 µL solution of GE in different concentrations was added to 180 µL $ABTS^{\bullet+}$ radical solution. Trolox was used as standard and methanol was used as a control. All the reaction mixtures were shaken and incubated in the dark for 5 min at room temperature. The absorbance was then measured at 734 nm using a UV-Vis spectrophotometer. All samples were analyzed in triplicate. The $ABTS^{\bullet+}$ radical scavenging ability of GE was calculated using the following equation:

$$\%\ \text{ABTS radical scavenging} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} x100$$

The antioxidant activity was expressed as IC_{50}, defined as the concentration (in µg/mL) of GE and Trolox needed to scavenge 50% $ABTS^{\bullet+}$.

Hydroxyl radical scavenging activity assay

The hydroxyl radical scavenging activity was performed following the method described by Zhang and Li [7] with slight modifications. Both 1,10-phenanthroline (1.5 mM) and $FeSO_4$ (1.5 mM) were dissolved in phosphate buffer (pH 7.4) and mixed thoroughly. The reaction mixture contained 60 µL 0.2 M phosphate buffer (pH 7.8), 20 µL solution of GE in different concentrations, 120 µL mixture of 1,10-phenanthroline (1.5 mM) and $FeSO_4$ (1.5 mM), 30 µL H_2O_2 (80 mM) were added. The mixture was incubated at 37°C for 60 min, and the absorbance was measured at 536 nm. Quercetin was used as standard and distilled water as a control.

Hydroxyl radical scavenging activity is calculated using the following equation:

Hydroxyl radical scavenging activity %= (A_s-A_1) *100/ (A_0-A_1)

A_s is the absorbance of the sample; A_1 is the absorbance of the control solution containing 1, 10-phenanthroline, $FeSO_4$, and H_2O_2; and A_0 is the absorbance of the blank solution containing 1, 10-phenanthroline, and $FeSO_4$.

Metal ion (Fe^{2+}) chelating activity assay

Metal chelating activity of GE was measured using the method previously described by Lima and J.Santos [8],[9], with some modifications. In a microplate, aliquots of 50 µL solution of GE in different concentrations were added to 100 µL of water and 20 µL of $FeSO_4$ (0.3 mM). After incubation at room temperature for 5 min, the reaction was started by adding 30 µL of ferrozine (0.8 mM). The absorbance was recorded at 562 nm. EDTA was used as standard and distilled water as a control. All the experiments were carried out in triplicate. The chelating activity was calculated using the equation below:

$$\%\ Fe^{++}\ \text{chelating Activity} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})}\ x\ 100$$

The antioxidant activity was expressed as IC_{50}, defined as the concentration (in µg/mL) of GE and EDTA needed to chelate 50% metal ion (Fe^{2+}).

Total Antioxidant Capacity (TAC) assay

TAC was measured using the method previously described by I. Bianca with some modifications [10]. 50 µL sample - GE in different concentrations (5, 10, 25, 50, 100, 250, 500 µg/mL) were mixed with 500 µL of the reagent solution (0.6 M HCl, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 minutes. Tubes then were cooled to room temperature, and the absorbance was measured at 695 nm. Ascorbic acid (1 mg/mL) was used as a standard and methanol was used as a control. All samples were analyzed in triplicate. The total antioxidant capacity of the GE was calculated using the following equation and was expressed as total antioxidant capacity (TAC) relative to ascorbic acid.

$$\text{TAC }\% = \frac{(\text{Abs sample} - \text{Abs control})}{(\text{Abs ascorbic acid} - \text{Abs control})} * 100$$

where Abs sample is the absorbance of the sample, Abs control is the absorbance of the control and Abs ascorbic is the absorbance of ascorbic acid at 1 mg/mL.

In vivo hepatoprotective activity

The experiment was performed following the method described by Tung et al. [11]. The animals were randomly divided into five groups, each consisting of ten mice. Group I (normal) served as the normal control and was administered 0.1 mL/10 g/day saline. Group II (control) acted as the disease control and also received 0.1 mL/10 g/day saline. Group III (Sylimarin) served as the positive control and was given silymarin at a dose of 100 mg/kg/day. Groups IV and V were the treatment groups, receiving GE oral doses of 500 and 1000 mg/kg, respectively. Groups I to V received saline, GE, and silymarin for 8 days. On the 8th day, the final day of the treatment period, after oral administration of the last dose for 1 hour, animals in groups II, III, IV, and V were administered CCl_4 10% intraperitoneally (i.p.) at a dose of 0.5 mL/kg body weight. Following oral administration of the last dose for 24 hours, 1 mL blood sample was collected from the retro-orbital venous sinus of the mice using a capillary tube. The animals were then sacrificed, and liver samples were collected for analysis of various parameters.

Serum analyses: Serum was separated by centrifugation at 3000 rpm for 20 mins. Enzyme activities of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT), and bilirubin were measured by the spectrophotometric method according to instructions of the kit supplier (Biosystems S.A – Barcelona, Spain), using semi-auto biochemistry analyzer TC-3300 Plus (Teco Diagnostics, USA).

Liver fraction assay: Liver tissues were collected, and liver fractions were immediately isolated. Catalase (CAT), reduced glutathione

(GSH), and lipid peroxidation were determined from freshly isolated mitochondria. In detail, the liver samples (10%, w/v) were homogenized in ice-cold phosphate buffer (100 mM, pH 7.4) and then centrifuged at 10 000 rpm for 30 min at 0 °C (5702R, Eppendorf, Germany). The supernatant (PMS) was subsequently collected and used for the measurement of biochemistry parameters.

Hepatic lipid peroxidation level was assayed by the thiobarbituric acid reactive substance (TBARS) method. Specifically, 0.15 mL PMS was mixed with 2 mL thiobarbituric acid (0.25% in acetic acid, pH 2.4–2.6) in the presence of saturated butylated hydroxytoluene (BHT). The reaction mixture was placed in a boiling water bath for 60 min. The formed colored adduct was extracted by *n*-butanol and then measured spectrophotometrically at 532 nm. The result was expressed as malondialdehyde (MDA) equivalent (nmol MDA/mg protein), using freshly prepared tetramethoxypropane as standard.

GSH level in liver tissue was estimated by a spectrophotometric method using Ellman's reagent. PMS was precipitated by the addition of one volume of 4% sulfosalicylic acid. The supernatant obtained after centrifugation (20 µL) was mixed with 10 mM 5,5′-Dithiobis (2-nitrobenzoic acid) (20 µL) and phosphate buffer (160 µL). The absorbance of the product was immediately recorded at 412 nm. GSH content was expressed as µmol reduced GSH/mg protein.

CAT activity was assessed using the method described by Mahmoud [12]. The reaction mixture contained diluted PMS (replaced with distilled water in standard and blank wells), hydrogen peroxide (H2O2) 20 mM in sodium-potassium phosphate buffer (50 mM) pH7.4 as described in Table 1.

Table 1. The components of the reaction mixture for measuring catalase activity

Reagents	Test	Control-test	Standard	Blank
PMS	20 µL	20 µL	-	-
Distilled water	-	200 µL	20 µL	220 µL
H_2O_2	200 µL	-	200 µL	-

The mixture was incubated at 37°C in three minutes. The reaction was stopped with ammonium molybdate (32.4 mmol/L). The absorbance of the yellow complex of molybdate and hydrogen peroxide is measured at 374 nm against the blank.

The rate constant of a first-order reaction (k) equation is used to determine catalase activity:

Catalase activity of test

$$kU = \frac{2.303}{t} \times \frac{Vt}{Vs} * [\log \frac{S0}{S-M}]$$

t: time

S0: absorbance of standard tube

S: absorbance of the test tube

M: absorbance of control test

Vt: total volume of reagents in test tube

Vs: volume of PMS

2.4. Statistical analysis

For *in vitro* experiments, data are expressed as the mean ± standard deviation (SD). Concentration providing 50% inhibition (IC_{50}) and 95% confidence intervals for IC_{50} values was calculated by non-linear regression using GraphPad Prism software (San Diego, CA, USA).

For *in vivo* experiments, all data are expressed as the mean ± standard error of the mean (SEM). Statistical analyses were performed by a one-way analysis of variance (ANOVA) followed by Dunnett's test to compare the differences. Statistical significance was set at $p < 0.05$.

3. Results

3.1 In vitro antioxidant effects of the garlic extract

The *in vitro* antioxidant capacity of garlic extract (GE) is shown in Tables 2, 3, and 4. The positive controls, quercetin, and trolox, exhibited strong antioxidant activity, with IC_{50} values of 5.28 µg/mL for the DPPH assay, 19.01 µg/mL for the hydroxyl radical assay, and 61.50 µg/mL for the ABTS assay. In comparison, garlic extract showed antioxidant activity with IC_{50} values of 1210 µg/mL for $DPPH^{\bullet}$, 892.60 µg/mL for hydroxyl radicals, and 227.10 µg/mL for $ABTS^{\bullet+}$. Additionally, the concentration of GE required to chelate 50% of Fe^{2+} was 14.82 µg/mL. When compared to ascorbic acid at 1 mg/mL, the total antioxidant capacity of GE at concentrations of 312, 625, 1250, 2500, and 5000 µg/mL was 4.80%, 7.88%, 13.36%, 22.06%, and 57.76%, respectively.

Table 2. Free-radicals scavenging capacity of garlic extract

Samples	IC_{50} (µg/mL)		
	$DPPH^{\bullet}$	$OH^{\bullet-}$	$ABTS^{\bullet+}$
Garlic extract	1210.00 (774 - 1919)	892.60 (522.70 - 1524.00)	227.10 (209.10 - 246.60)
Quercetin	5.28 (3.79 - 7.35)	19.01 (9.01 - 40.10)	
Trolox			61.50 (56.03 - 67.49)

(Data represents the mean and 95% confidence intervals for IC_{50} values).

Table 3. Metal ion (Fe^{2+}) chelating ability of garlic extract

Samples	EC_{50} (µg/mL)	R^2
Garlic extract	14.82 (12.14-18.08)	0.9855
EDTA	16.47 (12.49-21.72)	0.9671

(Data represents the mean and 95% confidence intervals for IC_{50} values).

Table 4. Total Antioxidant Capacity (TAC) of garlic extract

Garlic extract (µg/mL)	TAC (%)
312	4.80 ± 0.05
625	7.88 ± 0.10
1250	13.36 ± 0.15
2500	22.06 ± 0.15
5000	57.76 ± 0.75

3.2. In vivo hepatoprotective activity

3.2.1. Effects of GE administration on hepatic ASAT, ALAT:

The serum activities of ASAT, and ALAT of normal, control, and experimental animals were given in Fig. 1.

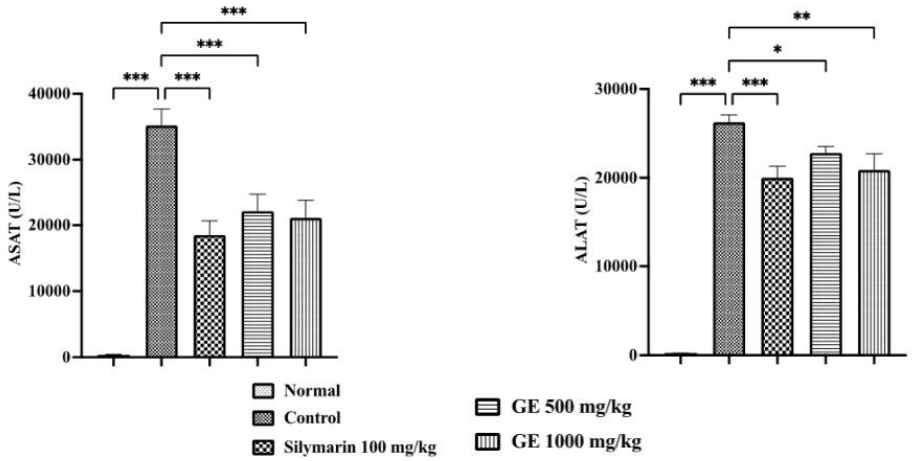

Fig. 1. Effects of GE administration on hepatic ASAT, ALAT

*(Data shown are mean values ± SEM; *p< 0.05; **p < 0.01; ***p< 0.001: compared to control group; n=9).*

The results indicated that the injection of CCl_4 into mice induced liver injury, which was represented by markedly elevated serum levels of ASAT and ALAT compared to the normal group ($p < 0.05$). Silymarin at a dose of 100 mg/kg resulted in a significant decrease in the CCl_4-induced elevation of serum enzymes ASAT and ALAT compared to the control group ($p < 0.001$). Treatment with GE significantly reduced enzyme activities when compared to the control group. The activities of ASAT and ALAT (37.2% and 22.3%, respectively) were significantly lower in the group treated with GE at 500 mg/kg/day compared to the control group. The activities of ASAT and ALAT (40% and 23.3%, respectively) were significantly lower in the group treated with GE at 1000 mg/kg/day compared to the control group. The effect of GE is comparable to that of

silymarin treatment. These results indicate a protective effect of GE against CCl_4-induced liver injury in mice.

3.2.2. Effects of GE on total bilirubin:

The serum total bilirubin levels of normal, control, and experimental animals were given in Fig. 2.

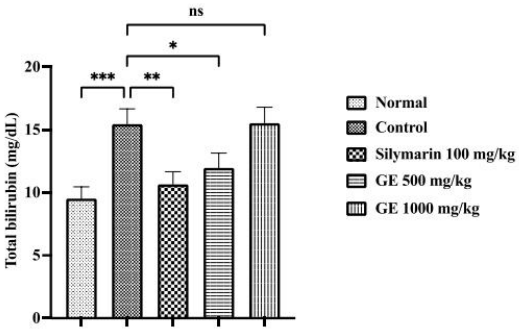

Fig. 2. Effects of GE administration on hepatic bilirubin *(Data shown are mean values ± SEM; *p< 0.05; **p < 0.01; ***p< 0.001; ns, the difference was not significant: compared to control group (p<0,01); n=8).*

As shown in Fig. 2, the administration of CCl_4 resulted in a significant increase in total bilirubin levels (p<0.001), indicating impaired liver excretory function. Conversely, treatment with GE at doses of 500 mg/kg and silymarin at 100 mg/kg led to a significant decrease in total bilirubin levels compared to the untreated group (p<0.05). However, no significant differences were noted in total bilirubin levels between the GE 1000 mg/kg group and the control group (p>0.05).

3.2.3 Effects of GE administration on lipid peroxidation

To evaluate the protective effect of GE against lipid peroxidation, MDA content was measured in liver homogenate (Fig. 3).

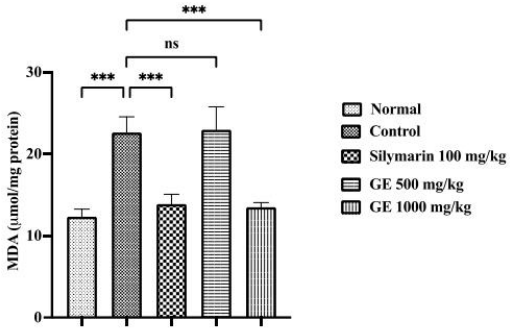

Fig. 3. Effects of GE administration on MDA content *(Values are mean ± SEM; ***p< 0.001; ns, the difference was not significant: compared to control group; n=8).*

The administration of CCl_4 alone induced a significant increase in MDA content compared to the normal control group (p<0.001). GE at 1000 mg/kg and silymarin at 100 mg/kg significantly inhibited the formation of MDA induced by CCl_4 treatment (p<0.001).

3.2.4 Effects of GE administration on CAT activities and GSH content in mice

GSH content and CAT activities were measured as indicators of oxidative stress prevention in both control and experimental animals. The effects of GE on GSH content and CAT activities are presented in Table 5.

Table 5. Effect of GE on GSH content and CAT activities

Groups	GSH (µmol/mg protein)	CAT (kU/g mg protein)
Normal control	4.36 ± 0.34	3.16 ± 0.02
Untreated control	2.30 ± 0.50$^{\#\#}$	3.05 ± 0.05
Silymarin 100 mg/kg	4.13 ± 0.44*	3.10 ± 0.03
GE 500 mg/kg	3.39 ± 0.83	2.90 ± 0.14
GE 1000 mg/kg	3.99 ± 0.38*	3.04 ± 0.05

*(Values are expressed as mean ± SEM; $^{\#\#}p<0.01$ when compared to the normal control group;*p<0.05 when compared to the CCl_4 group (control group).*

In CCl_4-intoxicated mice, hepatic GSH levels were significantly decreased compared to the normal control group (p<0.01). The oral administration of GE at 1000 mg/kg and silymarin at 100 mg/kg effectively protected hepatocytes against this depletion. No significant differences in CAT were detected in the CCl_4-treated group compared to the normal control group.

4. Discussion

Garlic (*Allium sativum*) is an annual spice that has been utilized as both food and a traditional remedy for various ailments. *A. sativum* is abundant in several sulfur-containing phytoconstituents, including alliin, allicin, ajoenes, vinyldithiins, and flavonoids [13]. Reports indicated that extract and isolated compounds of *A. sativum* exhibited a range of biological activities, such as antibacterial,

antiviral, antifungal, antiprotozoal, antioxidant, anti-inflammatory, and anticancer properties, among others [13].

In this study, we assessed the antioxidant potential of GE and its possible antioxidant mechanism using various *in vitro* assays. The results indicated that GE can scavenge free radicals such as hydroxyl radicals ($OH^{\bullet}$), ABTS radical ($ABTS^{\bullet+}$), and $DPPH^{\bullet}$. However, its radical scavenging activity was relatively weak, with IC_{50} values of 1210 µg/mL for DPPH•, 892.60 µg/mL for hydroxyl radicals, and 227.10 µg/mL for $ABTS^{\bullet+}$. The total antioxidant capacity of GE at 5000 µg/mL was only 57.76% that of ascorbic acid at 1 mg/mL. The results are consistent with Almatroodi 's study, the methanol extract of garlic showed a maximum of 67.5% of DPPH by 600 µg/mL [1].

Metal ion chelation is also thought to be an essential antioxidant mechanism. When hydrogen peroxide is present, metal ions generate toxic hydroxyl radicals via Fenton or Fenton-like reactions. Therefore, the chelation of metal ions inhibits free radical generation through the termination of the Fenton reaction [6]. In this study, GE showed strong capacity in metal ion chelation, the concentration of GE required to chelate 50% of Fe^{2+} was 14.82 µg/mL. These results confirm that GE exhibits a wide range of antioxidant properties. The various antioxidant mechanisms of GE may work synergistically to enhance its overall antioxidant activity.

Liver injuries induced by CCl_4 are a commonly used model for investigating hepatoprotective activity. When animals are treated with CCl_4, this compound is metabolized via CYP2E1 in the liver to a highly reactive trichloromethyl free radical, which can initiate a chain reaction of radical species production, resulting in lipid peroxidation, hepatocellular oxidative stress, and necrosis of liver [14]. Therefore, to investigate the hepatoprotective activity of GE, the present study evaluated the effects of this extract on serum transaminase levels, as well as MDA, GSH, and CAT levels in the liver tissue of CCl_4-treated mice.

Our results indicated that ASAT and ALAT were increased in mice with CCl_4 treatment. Elevated or abnormal levels of liver function enzymes are considered to be significant markers of liver damage. In contrast, a significant reduction in plasma activities of ASAT and ALAT was found in mice with GE treatment in comparison with the CCl_4-treated group. This finding suggests that GE protected the liver tissue from CCl_4-induced injury. Besides, GE ameliorated the excretory function of the liver, and this effect was shown by suppressing the elevation of the bilirubin serum level. The findings of the current study were in accordance with a previous study which has also confirmed that levels of ASAT and ALAT increased considerably after CCl_4 or D-galactosamine treatment, and this was suppressed by aged black garlic treatment [15].

It is well-known that chemicals like carbon tetrachloride (CCl_4) can lead to excessive formation of ROS which results in oxidative damage and lipid peroxidation [14]. GE decreased the MDA content in the liver significantly as compared to the control group. This can be explained by antioxidant properties, scavenging free radicals, the inhibition of lipid peroxidation, and its propagation in the liver. VK Nguyen et al. (2020) identified five major compounds in the essential oil extracted from garlic in Kinh Mon (Hai Dương), which are diallyl sulfide, diallyl disulfide, 3-vinyl-1,2-dithiacyclohex-4-ene, 3-vinyl-1,2-dithiacyclohex-5-ene, and diallyl trisulfide. The essential oil is capable of scavenging free radicals, specifically DPPH [16]. Diallyl disulfide (DADS) is the major flavonoid isolated from the garlic essential oil. Some previous studies have indicated that DADS can prevent lipid peroxidation and damage to cells. DADS can reduce deoxycholic acid-induced ROS levels in Barrett's epithelial cells. Treatment with DADS can activate antioxidant enzymes, such as glutathione *S*-transferase (GST), catalase, heme oxygenase-1 (HO-1), and superoxide dismutase. These enzymes work by transforming peroxides into less toxic or harmless substances through redox reactions, thus protecting various cells and tissues from ROS damage [17]. GE contains these compounds, the decrease of lipid peroxidation in the groups treated with GE might be due to the presence of these compounds.

GSH is an indicator of redox status and plays the principal role in scavenging free radicals and ROS. In our study, the oral administration of GE was effective in protecting hepatocytes against GSH depletion induced by CCl_4. These findings were consistent with the findings of Almatroodi et. al.'s study in which they indicated that methanol garlic extract increased the activities of some antioxidant enzymes e.g., superoxide dismutase (SOD), glutathione peroxidases (GPx) and glutathione (GSH) in hepatic tissues of rats [1].

The precise mechanism of hepatoprotection induced by GE and the specific chemical constituents responsible for this effect remain unknown. Recent studies have indicated that compounds such as alliin and allicin exhibit broad-spectrum antioxidant activities by regulating ROS generation and inhibiting mitogen-activated protein kinase (MAPK). Saponins extracted from garlic have been reported to scavenge intracellular ROS and protect mouse-derived C2C12 myoblasts from growth inhibition and H_2O_2-induced DNA damage [13]. These compounds may contribute to hepatoprotective activity against carbon tetrachloride-induced liver damage.

5. Conclusion

Experimental evidence obtained in the present study showed that GE has *in vitro* antioxidant activity. The oral administration of GE exerted favorable hepatoprotective activity against carbon tetrachloride-induced liver damage. These findings provide a pharmacological basis for the use of garlic in the treatment of liver diseases.

Conflict of interest: *The authors declare that there are no conflicts of interest.*

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