Potential Effects of Garlic Oil and Curcumin on Carbon Tetrachloride-Induced Liver Injury in Rats

Ameerah Al-Qahtani[1], Madeha N. Al-Seeni[1*], Hanaa A El-Sherif[1,2]

[1] Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[2] Medical Research Division, Medical Biochemistry Department, National Research Center, Egypt.

ABSTRACT

Many herbal natural products have succeeded in reducing the toxicity of many toxins, such as that caused by carbon tetrachloride. Examples of these natural products are garlic oil and curcumin that are characterized by their high antioxidant activity. The protective effect of garlic oil, curcumin and their combination against CCl_4 hepatotoxicity was investigated in male albino Wistar rats. 40 rats divided into five groups (n=8): 1st group (G1) as the negative control, 4 groups injected by CCl4 to induce liver toxicity. The 2nd Group (G2) (positive control), 3rd group (G3) was treated with garlic oil, 4th group (G4) was treated with curcumin, and 5th group (G5) was treated with a combination of garlic oil and curcumin. The CCl4-induced hepatotoxicity in G2 decreased the antioxidants (reduced glutathione, glutathione peroxidase, glutathione S-transferase, catalase, and superoxide dismutase) and high-density lipoprotein, and increased lipid peroxidation, total cholesterol, triglycerides, low-density lipoprotein-cholesterol, the very low-density lipoprotein-cholesterol and the atherogenic index that severely injured live tissue as revealed by the elevated liver function parameters and liver histology. Hepatotoxicity of G3, G4, and their combination in G5 was attenuated by the administration of garlic oil, curcumin, and their combination, respectively. Garlic oil, curcumin and their combination showed significant hepatotoxic activities by restoring the altered biochemical and histopathological parameters. In G5, the garlic oil and curcumin combination showed better protection than either garlic oil or curcumin, separately.

Key Words: Liver, Hepatoxicity, CCl_4, Garlic oil, Curcumin.

eIJPPR 2020; 10(1):155-163

HOW TO CITE THIS ARTICLE: Ameerah Al-Qahtani, Madeha N. Al-Seeni, Hanaa A El-Sherif (2020). "Potential Effects of Garlic Oil and Curcumin on Carbon Tetrachloride-Induced Liver Injury in Rats", International Journal of Pharmaceutical and Phytopharmacological Research, 10(1), pp.155-163.

INTRODUCTION

The liver protects the body against foreign substances by detoxifying and eliminating drugs and other xenobiotics to prevent liver diseases, which is considered a serious health problem [1-4]. The hepato-toxicological problem is an injury caused by toxic chemicals and some drugs [3, 4]. The hepatotoxicity results from the oxidative stress occurred by the accumulation of reactive oxygen species (ROS) that influence macromolecules in the liver [5]. This oxidative stress results from the imbalance between the increased production of oxidizing species and the compromised effectiveness of antioxidant defenses mechanisms that fail in eliminating the increased levels of reactive oxygen species [6, 7]. The overproduction of free radicals resulting from various liver disorders [8-10].

Carbon tetrachloride (CCl_4) is a hepatotoxic agent used to cause liver injury in many models into illustrated mechanisms behind hepatotoxicity [3, 4]. The liver injury caused by this solvent occurs due to the free radical reactions to the metabolism of CCl_4 inside the liver and also the initiation of lipid peroxidation [11]. The excessive lipid peroxidation leading to disruption in CCl_4 induced liver damage [12].

Corresponding author: Madeha N. Al-Seeni
Address: Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia.
E-mail: ✉ mnalsiny @ ut.edu.sa
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 15 October 2019; **Revised:** 02 February 2020; **Accepted:** 10 February 2020

Because of the high costs and side effects of allopathic medicines, many people started to return to herbal medicines that have fewer side effects [13]. Extracted oils from plant sources function as a scavenger of various reactive oxygen species, like superoxide anion and hydroxyl radicals [3, 4]. The presence of antioxidant components in the oils leads to the elimination of free radicals [14, 15].

Garlic oil is extracted from garlic (*Allium sativum*, Family *Alliaceae*), which has been consumed by a human for over 10,000 years by the Ancient Egyptians as a medicine for different ailments [16]. It provides some biological activities, as it is composed of organosulfur compounds [17]. It also exhibits anti-cancer activity, and anti-atherosclerotic activity, anti-hypertensive activity, anti-microbial activity, immune modulator, and radio-protective activity. These benefits are attributed to the anti-oxidative activity of garlic oil [18].

Curcumin or diferuloylmethane is derived from *Curcuma longa* [19]. It is used as an effective remedy for a variety of disorders such as asthma and hepatic disease since it has various pharmacological effects and acts as an antioxidant, anti-inflammatory, cardio-protective, hepato-protective, and anti-fibrosis [20].

We aimed to investigate the possible ameliorative effects of garlic oil, curcumin, and their combination of oxidative stress resulted from CCl_4 induced hepatotoxicity in male rats.

METHODS

Chemicals and biological materials

All chemicals used in this study were of analytical grade. Carbon tetrachloride (CCl_4) was purchased from Sigma-Aldrich (USA). CCl_4 was dissolved in paraffin oil and injected intraperitoneally. Garlic Oil (GO) was obtained from the local market in Jeddah, Saudi Arabia. Curcumin (CUR) was purchased from Sigma-Aldrich (USA). It was suspended in 5% carboxymethyl cellulose (CMC) [21].

Study design

A study conducted on forty (40) male Wistar Albino rats (250-270 g), obtained from the Animal House of King Fahad Medical Research Center, Jeddah, Kingdom of Saudi Arabia. Animals housed in standard cages and left to be acclimatized to laboratory conditions for 14 days before the commencement of the experiment. They were maintained on standard laboratory diet and water *ad libitum*.

Experimental animals were carried out under protocols approved by Institutional Animal House, University of King Abdulaziz, Jeddah, Saudi Arabia. Cages, bedding, and glass water bottles replaced twice a week. Stainless steel feed containers changed every week.

40 male rats were divided into five groups (n=8) as follows:

Group 1 (G1) was the negative untreated control group given the basal diet and distilled water only.

Group 2 (G2) was the positive CCl_4-treated control group injected intraperitoneally with carbon tetrachloride dissolved in paraffin oil (1 ml/kg b.w.) every 72 hours at 3 doses to induce liver toxicity as described by Jain et al. [22].

Group 3 (G3) was intraperitoneally injected with carbon tetrachloride as in G2 every 72 hours at 3 doses and concurrently treated daily with garlic oil at a dose of 200 mg/kg b.w.as described by Wu et al. [23] using oral gavage for 4 weeks.

Group 4 (G4) was intraperitoneally injected with carbon tetrachloride as in G2 every 72 hours at 3 doses and concurrently treated daily with curcumin at a dose of 100 mg/kg b.wt. as described by Rodríguez-Rivera et al. [21] using oral gavage for 4 weeks.

Group 5 (G5) was intraperitoneally injected with carbon tetrachloride as in G2 every 72 hours at 3 doses and concurrently treated daily with a mixture of garlic oil (200 mg/kg b.w.) and curcumin (100 mg/kg b.w.) using oral gavage/4 weeks.

Blood samples collection

At the end of the experimental period and after 12 hours of fasting, blood was withdrawn from the heart of euthanized animals by cervical dislocation. The liver rapidly dissected out and washed with ice-cold saline. Furthermore, half of the liver was kept in ice for liver tissue homogenate preparation and other half fixed in 10% formalin for histological preparations. The serum was separated by centrifugation of blood (3000 xg / 10 min).

Liver tissue homogenate preparation

The liver tissue homogenate was prepared from the ice-cold liver tissues as described in Al-Seeni et al. [3].

Biochemical analysis

Antioxidants

The following antioxidants were estimated in the liver tissue homogenate. Reduced glutathione (GSH) was assayed using Abcam® Reduced Glutathione assay kit (UK), glutathione peroxidase (GPx) was assayed using Abcam® glutathione peroxidase assay kit (UK), glutathione S-transferase (GST) was assayed using Elabscience® glutathione S-transferase assay kit, catalase (CAT) was assayed using Abcam® catalase assay kit (UK), superoxide dismutase (SOD) was assayed using Elabscience® Superoxide Dismutase assay kit (Texas, USA). All analyses were done according to the instruction of the supplier.

Oxidative Stress

Determination of lipid peroxidation

The malondialdehyde (MDA) was assayed in the liver tissue homogenate as an indication of the lipid peroxidation using the Abcam® lipid peroxidation assay kit.

Liver Enzymes Activities

Human® kits (Germany) were used to determine the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and L-γ-Glutamyl transferase (GGT) in the serum according to the instructions of the supplier.

Lipid Profile

Human® kits (Germany) were used in the determination of total cholesterol (CT), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) in the serum. The very low-density lipoprotein-cholesterol (VLDL)was estimated by dividing the triglyceride value by 5 (TG/5), atherogenic index of plasma was calculated as log(TG/HDL-C) [24].

Histopathological investigations

A piece of the liver was washed in sterile saline and fixed in 10% neutral formalin for histopathological studies. Dehydrating in gradual ethanol (50 – 99%), cleared in xylene, and embedded in paraffin. Then 5µ sections were prepared and stained with hematoxylin and eosin (H&S) dye for microscopic investigation.

Statistical Analysis

Data were analyzed using SPSS, version23. Data are represented as M ± SD. T-test was done between G1 and all other groups. ANOVA (analysis of variance) was calculated between groups 2, 3, 4 and 5, means with different superscript (a, b or c) are significantly different from each other at $P<0.05$, same superscripts are non-significant. LSD: least significant difference (Post-Hoc test for ANOVA).

RESULTS

Antioxidants

Table 1 shows the effect of garlic oil, curcumin, and garlic oil with curcumin on CCl_4- induced hepatotoxicity in male rats for four weeks on antioxidants in the liver tissue homogenate. CCl_4 significantly induced hepatotoxicity in male rats of the positive control by decreasing the values of reduced glutathione (GSH), glutathione peroxidase (GPX), glutathione S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) compared to the negative control group (G1).

Treating CCl_4-induced hepatotoxicity in G3 with garlic oil significantly increased the mean of GPX, GST, SOD, GSH, and CAT as compared with the positive control group (G2). Similarly, CCl_4-induced hepatotoxicity in G4 with curcumin significantly increasing in the values of GPX, SOD, GST, GSH, and CAT comparing to the positive control (G2). In addition, treating the CCl_4 induced hepatotoxicity in G5 with the combination of garlic oil and curcumin significantly increased the mean values of GPX, GST, SOD, CAT, and GSH compared to that of the positive control group (G2). Moreover, treating the rats with garlic oil in G3 was more efficient than treating with curcumin in G4, whereas treating hepatotoxicity in G5 with the combination of garlic oil and curcumin was more efficient than garlic oil and curcumin alone in ameliorating oxidative stress.

Table 1: Effect of treating CCl_4-induced hepatotoxicity in male rats with garlic oil and curcumin on antioxidants in the liver tissue homogenate.

Antioxidants	Statistics	Group 1 negative control)	Group 2(CCl_4+ve control)	Group 3 (CCl_4+Garlic oil)	Group 4 (CCl_4+ Curcumin)	Group 5 (CCl4+Garlic oil- Curcumin)
CAT U/ml	Mean ± SD	0.36 ± 0.14^{a}	0.26 ± 0.05^{b}	0.32 ± 0.06^{c}	0.29 ± 0.06^{d}	0.35 ± 0.09^{e}
	LSD 0.05=0.07					
	T- test	-	1.929^{***}	0.204^{***}	0.679^{***}	1.285^{***}
SOD U/dl	Mean ± SD	2394.88 ± 34.6^{a}	2012.03 ± 131.7^{b}	2340.49 ± 18.36^{c}	2328.26 ± 20.96^{d}	2364.59 ± 21.36^{e}
	LSD 0.05=47.967					
	T- test	-	7.951^{***}	4.656^{***}	2.106^{***}	3.925^{***}
GSH ug/dl	Mean ± SD	1.59 ± 0.6^{a}	0.94 ± 0.48^{b}	1.3 ± 0.35^{c}	1.1 ± 0.24^{d}	1.39 ± 0.17^{e}
	LSD 0.05=0.34					
	T- test	-	2.388^{***}	1.183^{***}	2.106^{***}	2.882^{***}

GPX ug/dl	Mean ± SD	114.98±9.88[a]	99.66±16.88[b]	123.03±6.36[c]	122.33±6.94[d]	135.24±5.97[e]
	LSD 0.05=17.22					
	T- test	-	2.217***	-4.964***	-1.936***	-1.72***
GST U/dl	Mean ± SD	87.97±11.33[a]	71.71±13.25[b]	102.88±8.5[c]	94.4±15.66 [d]	106.67±13.69[e]
	LSD 0.05=11.54					
	T- test	-	2.638**	-2.976***	-0.943***	-2.975***

SD: standard deviation, t-test values; *: significant at P<0.05, **: highly significant at P<0.01 and ***: very highly significant at P<0.001. Means with different superscript (a, b, c, d or e) are significantly different from each other at P<0.05, LSD: least significant difference (Post-Hoc test for ANOVA).

Lipid peroxidation

Table 2 shows the effect of garlic oil, curcumin, and garlic oil with curcumin on CCl_4- induced hepatotoxicity on lipid peroxide of the liver tissue homogenate in male rats. The CCl_4-induced hepatotoxicity increased lipid peroxidation as revealed by the significantly increased value of MDA in the positive control as a result of liver damage compared to that of the negative control group (G1). Treating the CCl_4-induced hepatotoxicity with garlic oil in G3, curcumin in G4, garlic oil with curcumin in G5 significantly decreased the lipid peroxidation compared to the positive control group (G2). Curcumin in G4 was more efficient than garlic oil in G3 in decreasing the lipid peroxidation, whereas treating hepatotoxicity with the combination of garlic oil and curcumin in G5 was more efficient than treating with garlic oil in G3 or curcumin in G4 in decreasing the lipid peroxidation.

Table 2: Effect of treating CCl4-induced hepatotoxicity in male rats with garlic oil and curcumin on lipid peroxidation in the liver tissue homogenate.

lipid peroxidation	Statistics	Group 1 negative control)	Group 2 (CCl_4+ve control)	Group 3 (CCl_4+Garlic oil)	Group 4 (CCl_4+Curcumin)	Group 5 (CCl_4+Garlic oil- Curcumin)
MDA nmol/ml	Mean ± SD	0.19±0.04[a]	0.27±0.05[b]	0.19±0.06[c]	0.18±0.06[d]	0.15±0.05[e]
	LSD 0.05=0.06					
	T- test	-	-3.161***	0.325***	0.675***	2.283***

SD: standard deviation, t-test values; *: significant at P<0.05, **: highly significant at P<0.01 and ***: very high significant at P<0.001. Means with different superscript (a, b, c, d or e) are significantly different from each other at P<0.05, LSD: least significant difference (Post-Hoc test for ANOVA).

Liver enzymes

Table 3 shows the effect of garlic oil, curcumin, and garlic oil with curcumin on CCl_4- induced hepatotoxicity on liver enzymes.CCl_4 induced hepatotoxicity in rats of positive control (G2) (P<0.001) increasing AST, ALT, ALP, and GGT compared to the negative control (G1). Treating CCl_4 led to hepatotoxicity with garlic oil, curcumin, and garlic oil with curcumin in G3, G4, and G5 decreased activity of AST, ALT, ALP, and GGT compared to the positive control (G2). Best results were obtained in G5 when CCl_4 hepatotoxicity was treated with the combination of garlic oil and curcumin.

Table 3: Effect of treating CCl_4-induced hepatotoxicity in male rats with garlic oil and curcumin on liver enzymes.

Liver enzymes	Statistics	Group 1 negative control)	Group 2(CCl_4+ve control)	Group 3 (CCl_4+Garlic oil)	Group 4 (CCl_4+Curcumin)	Group 5 (CCl_4+Garlic oil-Curcumin)
AST U/L	Mean ± SD	25.03±5.88[a]	75.08±17.63[b]	34.04±8.7[c]	28.01±5.88[d]	22.39±2.06[e]
	LSD 0.05=10.56					
	T- test	-	-7.619***	-2.424***	1.017***	-1.198***
ALT U/L	Mean ± SD	12.09±3.04[a]	24.02±5.63[b]	11.82±3.04 [c]	10.71±2.01[d]	10.24±1.55[e]
	LSD 0.05=3.53					
	T- test	-	-5.271***	0.182***	1.076***	1.545***

GGT U/L	Mean ± SD	1.91±0.22[a]	4.69±0.65[b]	2.24±0.43[c]	2.24±0.16[d]	1.91±0.22[e]
	LSD 0.05=0.42					
	T- test	-	-11.403***	-1.898***	3.372**	-1.580***
ALP U/L	Mean ± SD	63.64±8.11[a]	86.39±8.37[b]	61.69±5.78[c]	66.39±21.53[d]	57.32±9.97[e]
	LSD 0.05=13.22					
	T- test	-	-5.521***	0.555***	-0.339***	1.39***

SD: standard deviation, t-test values; *: significant at P<0.05, **: highly significant at P<0.01 and ***: very high significant at P<0.001. Means with different superscript (a, b, c, d or e) are significantly different from each other at P<0.05, LSD: least significant difference (Post-Hoc test for ANOVA).

Lipid profile

Table 4 shows the effect of garlic oil, curcumin, and garlic oil with curcumin on CCl_4- induced hepatotoxicity on the serum lipid profile. The total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL), very-low-density lipoprotein (VLDL), and atherogenic index of plasma (AIP) in the positive control (G2) increased compared to the negative control (G1). In contrast, high-density lipoprotein-cholesterol (HDL), in the positive control (G2) significantly decreased compared to the negative control (G1).

The combination of garlic oil and curcumin in G5 was most effective in reducing the dangerous lipid levels and increasing the HDL (the useful lipids) compared to garlic oil in G3 or curcumin in G4.

Table 4: Effect of treating CCl_4-induced hepatotoxicity in male rats with garlic oil and curcumin on lipid profile and the atherogenic index of plasma (AIP).

Lipid profile	Statistics	Group 1 negative control)	Group 2(CCl_4+ve control)	Group 3 (CCl_4+ Garlic oil)	Group 4 (CCl_4+ Curcumin)	Group 5 (CCl_4+Garlic oil-Curcumin)
TC (ug/dl)	Mean ± SD	27.28±5.03[a]	38.21±4.95[b]	35.1±8.25[c]	35.11±3.42[d]	29.06±4.96[e]
	LSD 0.05=4.94					
	T- test	-	-4.386***	-2.291*	-3.664**	-0.716***
TG (ug/dl)	Mean ± SD	26.11±4.9[a]	45.63±11.41[b]	36.83±11.86[c]	34.28±6.09[d]	32.44±6.38[e]
	LSD 0.05=9.56					
	T- test	-	-4.444***	-2.362*	-2.953**	-2.223***
HDL (ug/dl)	Mean ± SD	19.62±8.01[a]	12.86±4.49[b]	21.7±6.78[c]	21.11±7.01[d]	24.38±8.59[e]
	LSD 0.05=7.04					
	T- test	-	2.083	-0.561	-0.395	-1.144
LDL (ug/dl)	Mean ± SD	15.05±4.74[a]	25.23±2.09 [b]	22.1±2.48 [c]	22.58±3.45[d]	17.49±2.25[e]
	LSD 0.05=2.68					
	T- test	-	-5.554***	-3.728**	-3.63**	-1.316
VLDL **(ug/dl)**	Mean±SD	5.22±1.217[a]	9.13±2.281[b]	7.365±2.371[c]	6.85±0.980[d]	6.487±1.276[e]
	LSD 0.05= 1.558					
	T-test	-	-0.591***	-2.171***	2.618***	0.843***
AIP	Mean±SD	0.0910±0.217[a]	0.1305±0.193[b]	0.2000±0.200[c]	0.2177±0.235[d]	0.2191±0.241[e]
	LSD 0.05= 0.1914					
	T-test	-	0.404**	-3.398 ***	0.856***	0.665***

SD: standard deviation, t-test values; *: significant at P<0.05, **: highly significant at P<0.01 and ***: very high significant at P<0.001. Means with different superscript (a, b, c, d or e) are significantly different from each other at P<0.05, LSD: least significant difference (Post-Hoc test for ANOVA).

Histopathology of the liver

The histopathology of the liver tissues of the studied rat groups is shown in Fig.1 (A, B, C, D, E). Fig.1A shows the hepatic tissues of the negative control with the normal structure of the liver in which the hepatocytes have formed lobules surrounding the central vein separated by blood

sinusoid. In addition, it shows normal hepatic architecture with portal tracts composed of the normal bile duct, portal vein, normal hepatic strands of cells, normal hepatic artery, and blood sinusoids, normal kupffer cells, and normal central vein. Whereas, Fig. 1B shows the hepatic tissues of the positive control CCl_4-treated group (G2) with drastic changes such as inflammation and congestion of the hepatic tissues and the central vein. Moreover, congestion in vascular spaces and necrosis were also seen. Clear accumulation of hepatic granules with well-seen cytoplasm and prominent nucleus were also seen. The CCl_4 toxicity also showed distorted hepatic structure, distorted vein, and distorted kupffer cells.

The concurrent administration of garlic oil to G3 rats showed that garlic oil alleviated the toxicity of CCl_4 (Fig.1C). The hepatic tissues were near to the normal structure and mild congestion around the central vein. Nearly normal hepatic cells, blood sinusoids, and kupffer cells were also seen. In addition, the concurrent administration of curcumin alleviated the toxic effects in G4 rats (Fig.1D). The hepatic tissues of G4 rats showed nearly normal structure with mild congestion around the central vein, mildly congested and inflamed hepatic strands of cells, mildly congested hepatic central vein and artery, and mildly congested kupffer cells were also seen. Furthermore, the liver of rats from the garlic oil-and curcumin-treated group (G5) showed that garlic oil and curcumin alleviated the toxicity of the CCl_4. The hepatic tissues showed the nearly normal structure of the hepatic tissues. Nearly normal hepatic cell strands, nearly normal hepatic artery, nearly normal blood sinusoids, and nearly normal kupffer cells were also seen (Fig. 1E). This result shows that garlic oil and curcumin have a protective effect against CCl_4-induced impaired liver damage in male rats. Garlic oil was more efficient than curcumin in ameliorating the liver tissues in G3 and G4, respectively. Whereas the combination of garlic oil and curcumin in G5 was more efficient than garlic oil in G3 and curcumin in G4 in ameliorating the liver tissues.

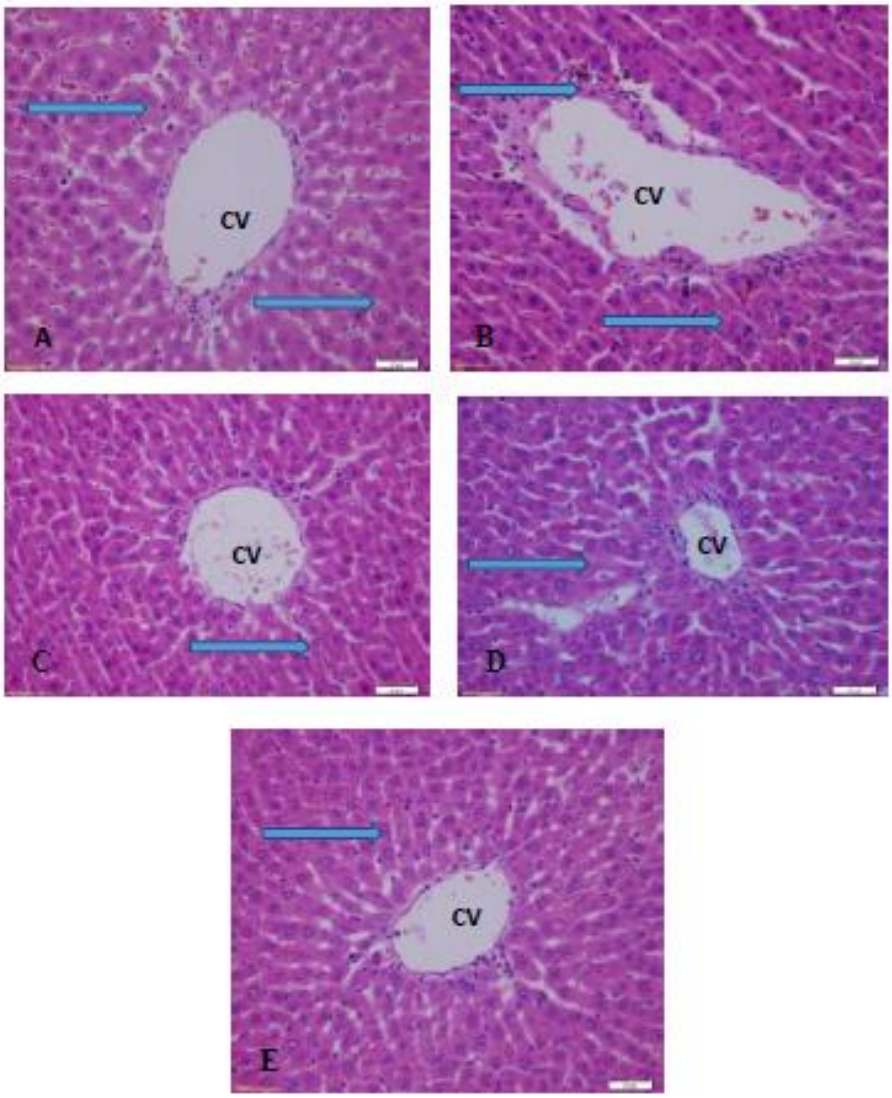

Fig.1. A: Hepatic tissues of the negative control group (G1),**B:** hepatic tissues of CCl_4positive control group (G2),**C:** hepatic tissues of garlic oil treated group (G3),**D:** hepatic tissues of curcumin treated group (G4),**E:** hepatic tissues garlic oil and curcumin treated group (G5). X= 400, (H&E stains).

DISCUSSION

The current study was focused on testing the hepatoprotective activity of garlic oil and curcumin against CCl_4-induced hepatotoxicity in male rats. The CCl_4-induced liver toxicity increases oxidative stress as revealed by the increase of lipid peroxidation and the decrease of antioxidant parameters in the positive CCl_4-treated group. CCl_4 toxicity causes free radical reactions to the metabolism of the CCl_4 inside the liver and also the initiation of lipid peroxidation [11]. The excessive lipid peroxidation leads to disruption in CCl_4 inducing the liver damage [12]. In addition, the increase in lipid peroxidation and the decrease in the antioxidants due to CCl_4 toxicity resulted from the imbalance between antioxidant defense and ROS production [25]. This balance can be restored through dietary antioxidant supplementation [3, 4, 26], which eventually inhibits the hepatotoxicity [4, 27, 28].

In addition, the increase of free radicles and the altered oxidative stress as a result of CCl_4 toxicity affected the lipid profile parameters that were also severely affected by increasing the total cholesterol, triglycerides, and low-density lipoproteins, and the decrease in high-density lipoprotein is considered a risk factors for dyslipidemia and associated diseases [3, 4, 24].

Similarly, the oxidative stress of the CCl_4-induced hepatotoxicity increased liver function parameters; AST, ALT, ALP, and GGT due to enzymes leakage from the liver into the bloodstream due to the death or damage of the hepatocytes [3, 4, 29]. This damage in hepatocytes also appeared in the altered hepatic tissues that showed severe damage to the hepatic cells in the positive control group as a result of CCl_4-induced toxicity. This result is supported by previous studies [3, 4].

Restoring the antioxidant capacities and minimizing of lipid peroxidation as a result of treating the CCl_4-induced toxicity with garlic oil, curcumin, and their combination is supported by other studies stated that glutathione peroxidase, catalase, and glutathione are examples of the detoxifying system of the body, can sweep ROS [30]. Alternative medicines represented in this study by garlic oil, curcumin, and their combination can limit ROS-mediated injuries and thus protect the liver from possible damages [31].

These benefits are attributed to the anti-oxidative activity of garlic oil [32] due to its possession of more than 30 organosulfur compounds; for instance, diallyl sulfide, diallyl disulfide, and diallyl trisulfide [17, 33, 34] that increase the activity of glutathione-related antioxidant system in the liver by supplying protective effects against the oxidative stress [23, 35]. In addition, the ameliorative effect of garlic oil is supported by the fact that extracted oils from plant sources function as a scavenger for various reactive oxygen species, like superoxide anion and hydroxyl radicals that lead to the elimination of free radicals [14, 15]. In addition, garlic oil has several biological benefits because it contains organosulfur compounds, which work synergistically as anti-cancer, anti-atherosclerotic, anti-hypertensive, anti-microbial, immunomodulator, and radio-protective [17]. These organosulfur components of garlic oil increase the activity of glutathione-related antioxidant systems in the liver [32] by supplying protective effects against the oxidative stress agents [35].

On the other hand, curcumin also succeeded in protecting against CCl_4 hepatotoxicity because of its antioxidant, anti-inflammatory, cardio-protective, hepato-protective, and anti-fibrosis activity by scavenging reactive species and eliminating superoxide anion (O_), nitric oxide (NO), peroxynitrite (NOO), hydroxyl (OH_) and peroxyl (ROO) radicals and indirectly stimulating an up-regulation of antioxidant proteins, such as superoxide dismutase, glutathione-S-transferase, catalase, glutathione peroxidase, g-glutamyl cysteine ligase, and glutathione reductase. Furthermore, CUR promotes the synthesis of reduced glutathione (GSH) by the induction of gGCL. Thus, it provides a strong antioxidant effect and protects the body cells from the oxidative damage because of the presence of phenolic groups in this substance, which are responsible for its capacity to deal with reactive species [19, 20, 36].

The combination of garlic oil and curcumin was more efficient in protecting rats under study against CCl_4 toxicity due to the numerous antioxidant components of both of them that synergistically played a crucial role in the protection process [20, 35, 36].

CONCLUSION

Garlic oil and curcumin succeeded in attenuating hepatotoxicity in CCl_4-induced hepatotoxic rats. They could restore the liver function enzymes, antioxidants, and lipid profile to the normal. They also restored the injured liver tissues to their normal as in the negative control. It is also worthy to mention that the combination of garlic oil and curcumin in G5 revealed maximum protection compared to either garlic oil or curcumin, separately.

REFERENCES

[1] EL-Zayadi A, Selim O, Rafik M, EL-Haddad S. Prevalence of hepatitis C virus among non-A, non-B related chronic liver disease in Egypt. J of Hepatol.1992;14: 416-417.

[2] Sofair AN, Barry V., Manos M.M., Thomas A., Zaman A., Terrault N.A., Murphy R.C., Stabach N., Huie S., Van Ness G. The epidemiology and clinical characteristics of patients with newly diagnosed alcohol-related liver disease: results from population-based surveillance. J Clin Gastroenterol. 2010;44: 301- 307.

[3] Al-Seeni MN, El Rabey HA, Zamzami MA, Alnefayee AM. The hepatoprotective activity of olive oil and Nigella sativa oil againstCCl4 induced hepatotoxicity in male rats. BMC Complement Altern Med. 2016; 4-16(1):438.

[4] Elbakry MA, El Rabey HA, Elremaly W, Sakran MI, Almutairi FM. The methanolic extract of Moringa oleifera attenuates CCl4induced hepatonephro toxicity in the male rat. Biomed Res.2019; 30 (1): 23-31.

[5] Lu Y, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. Free Radical Biol Med.2008; 44: 723-738.

[6] Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. Drug Metabol Rev. 2012; 44: 88-106.

[7] Ibrahim ZS, Nassan MA, Soliman MM. Ameliorative effects of pomegranate on carbon tetrachloride hepatotoxicity in rats: A molecular and histopathological study. Mol MedRep. 2016; 13: 3653-3660.

[8] You Y, Park J, Yoon H-G, Lee Y-.H, Hwang K, Lee J, Kim K, Lee K.-W, Shim S, Jun W. Stimulatory effects of ferulic acid on endurance exercise capacity in mice. Biosci, Biotechnol, Bioc.2009;73: 1392-1397.

[9] Lu Y, Sun J, Petrova K, Yang X, Greenhaw J, Salminen WF, Beger RD, Schnackenberg LK. Metabolomics evaluation of the effects of green tea extract on acetaminophen-induced hepatotoxicity in mice. Food Chem Toxicol. 2013; 62: 707-721.

[10] Sumida Y, Niki E, Naito Y, Yoshikawa T. Involvement of free radicals and oxidative stress in NAFLD/NASH. Free Radical Res. 2013; 47: 869-880.

[11] Rasha S, Ashraf A, Aly R. Carbon tetrachloride-induced liver disease in rats: the potential effect of supplement oils with vitamins E and C on the nutritional status. German Med Sci. 2009;7:1612-3174.

[12] Muriel P, Albo N, Perez-Alvarez V. Kupffer cells inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. Comp Biochem Physiol C Toxicol Pharmacol. 2001; 130: 219-226.

[13] Joshi D, Mittal DK, Shukla S, Srivastav SK, Dixit VA. Curcuma longa Linn. extract and curcumin protect CYP 2E1 enzymatic activity against mercuric chloride-induced hepatotoxicity and oxidative stress: A protective approach. Exp Toxicol Pathol.2017;69: 373-382.

[14] Nakbi A, Tayeb W, Grissa A, Issaoui M, Dabbou S, Chargui I, Ellouz M, Miled A, Hammami M. Effects of olive oil and its fractions on oxidative stress and the liver's fatty acid composition in 2, 4-Dichlorophenoxyacetic acid-treated rats. Nutr Metabol. 2010; 7: 80.

[15] Hamed M, El-Rigal N, Ali S. Effects of black seed oil on the resolution of hepato-renal toxicity induced by bromobenzene in rats. Eur Rev Med Pharmacol Sci.2013; 61: 926-919.

[16] Shaarawy SM, Tohamy AA, Elgendy SM, Elmageed, ZYA, Bahnasy A, Mohamed MS, Kandil E, Matrougui K. Protective effects of garlic and silymarin on NDEA-induced rats hepatotoxicity. Int J Boil Sci. 2009; 5: 549.

[17] Zeng T, Zhang CL, Pan GB, Zhao S, Dou DD, Xin X, Xie KQ. The protective effects of garlic oil on acute ethanol-induced oxidative stress in the liver of mice. J Sci Food Agri.2008; 88: 2238-2243.

[18] Banerjee SK, Mukherjee PK, Maulik SK. Garlic as an antioxidant: the good, the bad and the ugly. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2003; 17: 97-106.

[19] Briones MA. Curcumin Inhibits the IKK: NF-kappa B Pathway in Neural Fear Circuits. Biol Psychol. 2018; 21: 145-151.

[20] Kabirifar R., Safari F, Karimollah A, Moradi, A, Eskandari-Nasab E. Hepatoprotective effects of curcumin in rats after bile duct ligation via downregulation of Rac1 and NOX1. Nutrition.2017;36: 72-78.

[21] Rodríguez-Rivera A, Galicia M, Reyes-Gordillo K et al. Methyl palmitate prevents CCl4-induced liver fibrosis. Appl. Toxicol. 2008; 28: 1021–1026

[22] Jain R, Nandakumar K, Srivastava1 V, Vaidya SK, Patet S, Kumar P. Hepatoprotective activity of ethanolic and aqueous extract of Terminalia belerica inrats. Pharmacologyonline. 2008;2:411–427.

[23] Wu C, Sheen L, Chen H-W, Tsai S-J, Lii C-K. Effects of organosulfur compounds from garlic oil on the antioxidation system in rat liver and red blood cells. Food Chem Toxicol.2001;39: 563-569.

[24] Dobiasova M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in

apoB-lipoprotein-depleted plasma (FER(HDL)). Clin Biochem. 2001;34:583–8.

[25] Albano E. 2008.Oxidative mechanisms in the pathogenesis of the alcoholic liver disease.Molecular aspects of medicine. 29: 9-16.

[26] Witkop JJ. Role of Antioxidant Supplementation and Exercise Regimen in Handling Oxidative Stress from Natural Pm2. 5 Exposure Due to Boreal Forest Fire. The United States, University of Alaska Fairbanks. 2019.

[27] Chen K-H, Li P-C, LinW-H, Chien C-T, Low B-H. Depression by a green tea extract of alcohol-induced oxidative stress and lipogenesis in rat liver. Biosci, Biotech, Bioc.2011; 75: 1668-1676.

[28] Tatiya AU, Surana SJ, Sutar MP, Gamit NH. Hepatoprotective effect of polyherbal formulation against various hepatotoxic agents in rats. Pharmacogn Res.2012; 4:50-56.

[29] Obi F, Omogbai L, Oriafo O, Ovat O. Effect of a short time post carbon tetrachloride treatment interval on rat plasma enzyme levels and percentage mortality. J Appl Sci Environ Manage.2001; 5(1): 5-8.

[30] Mohammadi M, Yazdanparast R. Methoxy VO-Salen complex: in vitro antioxidant activity, cytotoxicity evaluation and protective effect on CCl4-induced oxidative stress in rats. Food ChemToxicol.2009; 47: 716-721.

[31] Kim Y., You Y., Yoon H.-G., Lee Y.-H., Kim K., Lee J., Kim M. S., Kim J.-C. and Jun W. Hepatoprotective effects of fermented Curcuma longa L. on carbon tetrachloride-induced oxidative stress in rats. Food Chemistry. 2014; 151: 148-153.

[32] Banerjee S., Mukherjee PK, Maulik S. Garlic as an antioxidant: the good, the bad and the ugly. Phytother Res: Int J Devoted to Pharmacol Toxicol Evaluation of Natural Product Derivatives. 2003; 17: 97- 106.

[33] Block E. The chemistry of garlic and onions. Sci Am.1985; 252: 114-9.

[34] Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y. Intake of garlic and its bioactive components. J Nutr.2001; 131: 955S-962S.

[35] Agarwal MK, Iqbal M, Athar M. Garlic oil ameliorates ferric nitrilotriacetate (Fe- NTA)-induced damage and tumor promotion: implications for cancer prevention. Food Chem Toxicol.2007;45: 1634-1640.

[36] Trujillo J, Chirino YI, Molina-Jijón E, Andérica-Romero AC, Tapia E, Pedraza- Chaverrí J. Renoprotective effect of the antioxidant curcumin: Recent findings. Redox biology.2013;1: 448-456.