

Aged garlic extract enhances paraoxonase 1 activity and suppress oxidative stress in CCl₄-intoxicated rats

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Abstract

The current study was undertaken to evaluate the hepatoprotective potential of aged garlic extract (AGE) against hepatotoxicity induced by CCl₄ in adult male rats. CCl₄ intoxication (1ml/kg b.w., twice a week, i.p. for 4 weeks) significantly increased oxidative stress as indicated by increased hepatic MDA formation, decreased both serum total and reduced glutathione contents, and inhibition of hepatic glutathione reductase and serum paraoxonase 1 (POX1) activities. In addition, treatment with CCl₄ produced a significant increase in the activities of serum ALT, AST and albumin level with a significant decrease in total protein level. Moreover, treatment with CCl₄ resulted in significant increase in the levels of total cholesterol, LDL-cholesterol, triglycerides as well as creatine kinase isoenzyme and lactate dehydrogenase activities, and a significant decrease in HDL-cholesterol compared to control group. Oral treatment with AGE at doses of 100 and 200 mg/kg/day in combination with CCl₄ significantly ameliorated the severity of CCl₄-induced changes in the above mentioned parameters in a dose-dependent manner. In conclusion, the present study indicated that AGE improves POX1 activity and attenuates liver and cardiac dysfunction induced by CCl₄. The protective effects of AGE against CCl₄ toxicity may be attributed to its antioxidant and free radical scavenging activities due to its higher contents of organosulphur compounds.

Key Words: Garlic, hepatotoxicity, CCl₄, heart function, antioxidant

Extrato envelhecido de alho incrementa a atividade de paraoxonase 1 e suprime o estresse oxidativo em ratos intoxicados com CCl₄

Resumo

O presente estudo foi realizado para avaliar o potencial hepatoprotetor do extrato de alho envelhecido (AGE) contra hepatotoxicidade induzida por CCl₄ em ratos machos adultos. CCl₄ intoxicação (1ml/Kg no peso corporal, duas vezes por semana, ip durante 4 semanas) aumentou significativamente o Estresse oxidativo, tal como indicado pelo aumento da formação de MDA hepática, diminuição tanto de soro total e conteúdo de glutathione, e inibição da atividade de glutathione reductase hepática e paraoxonase soro 1 (POX1). Além disso, o tratamento com CCl₄ produziu um aumento significativo nas atividades séricas de ALT, AST e nível de albumina com uma diminuição significativa no nível de proteína total. Ademais, o tratamento com CCl₄ resultou num aumento significativo dos níveis de colesterol total, colesterol-LDL, triglicérides, bem como isoenzima creatina quinase e atividades da lactato desidrogenase, e uma diminuição significativa nos níveis de HDL-colesterol, se comparado com o grupo controle. O tratamento oral com AGE em doses de 100 e 200 mg / kg / dia em combinação com CCl₄ demonstrou melhoras significativas na gravidade das alterações de CCl₄ induzidas nos parâmetros acima mencionados, de uma maneira dose-dependente. Em conclusão, o presente estudo indicou que a idade aumenta a atividade de POX1 e atenua a disfunção no fígado e no coração induzidas por CCl₄. Os efeitos protetores de idade contra a CCl₄ toxicidade podem ser atribuídos aos seus antioxidantes e radicais livres, devido aos elevados teores de compostos organosulfurados.

Palavras-chave: Alho, hepatotoxicidade CCl₄, função cardíaca, antioxidante

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Introduction

Plants used in traditional medicine for the treatment of liver disorders are of great interest, as they may serve as potential sources for new therapeutic agents that could be applied in the management and prevention of hepatic injury (Liu et al., 1995). Garlic (*Allium sativum* L., Alliaceae) originates from Central Asia and has been used universally as a flavoring agent and phytomedicine. The beneficial effects of garlic consumption in treating a wide variety of human diseases and disorders have been known for centuries. In ancient Egypt, it was used to promote general health and was mentioned in the Codex (Rahman, 2010). In addition, garlic has the ability to act as an antioxidant (Chowdhury et al., 2008), antihypertensive (Silagy & Neil, 1994), antitumor (Galeone et al., 2009), hypolipidemic (Rahman, 2010), hypocholesterolemic (Elmahdi et al., 2008) and antiatherosclerotic (Choudhary, 2008) agent. It also presents anticoagulant (Chan et al., 2007) and immunomodulatory (Chandrashekar & Venkatesh, 2009) properties.

Extensive studies carried out on garlic have reported the presence of two main classes of antioxidant components, namely flavonoids (Bozin et al., 2008) and sulfur-containing compounds (diallyl sulfide, trisulfide and allyl-cysteine) (Kodera et al., 2002), which are believed to play an important role in the widely demonstrated biological effects of garlic. Nevertheless, its impact on paraoxonase-1 (PON1) is remains obscure.

Paraoxonase 1 (PON1) is a hydrolase enzyme located on HDL-cholesterol and has been postulated to play a protective effect on low density lipoprotein oxidation (Turk et al., 2008). PON1 has also been reported to protect from oxidation and it is likely to be related to the high density lipoprotein antiapoptotic function, since the ability to protect against apoptosis is completely lost when the lipoprotein is oxidized (Krzystek-Korpacka et al., 2008). Liver plays a key role in the synthesis of PON1, as its gene of expression has been observed only in the liver (Leviev et al., 1997). The decrease in PON1 activity in chronic liver diseases is related to the degree of hepatic dysfunction not to allelic or genotypic differences (Marsillach et al., 2009). Also, it was suggested that there is an active role of PON1 in the regulation of oxidative stress, fibrosis and hepatic cell apoptosis in chronic liver diseases (Ferré et al., 2006).

The aim of the present study was to evaluate the effects of chronic administration of AGE on PON1 in CCl₄-intoxicated rats. The *in vivo* potential hepatoprotective effect of AGE against CCl₄-induced hepatotoxicity was detected by measuring the end product of hepatic lipid peroxidation, the activities of some hepatic and blood antioxidative enzymes and some markers of liver function. Also, the study was extended to shed light on the remodulating actions of AGE

administration on some indices related to heart injury such as serum creatine kinase isoenzyme (CK-MB), lactate dehydrogenase (LDH) and lipid profile.

Material and Methods

Chemicals

Aged garlic extract (AGE) (Kyolic®) was purchased from Wakunaga of America Co. (Mission Viejo, CA, USA). It is prepared by soaking sliced raw garlic (*Allium sativum*) in 15–20% aqueous ethanol for 20 months at room temperature. Carbon tetrachloride (CCl₄) was obtained from SISCO Research Laboratories PVT LTD, Mumbai, India. All other chemicals used in the chemical methods were of reagent grade and were obtained from Sigma (Poole, Dorset, UK).

Animals and experimental design

Adult male albino Sprague Dawley rats were used throughout the present investigation. They were obtained from the animal house of the National Research Center, Dokki, Cairo, Egypt, and acclimatized for at least one week prior to the experiments. Animals were housed as groups in plastic cages with the condition of the experimental room. Animals were allowed free standard laboratory diet and drinking water *ad libitum*. All animals received human care in compliance with the guidelines of the Animals Care and Use Committee of National Research Centre, Egypt.

The used animals were divided into six groups (8 rats each) and treated for four weeks as follows: Group 1 was injected intraperitoneally with olive oil (1ml/kg b.w. twice a week) and served as control; Groups 2 and 3 were received AGE (dissolved in distilled water) orally in a single daily dose of 100 mg/kg b.w. (Fallon et al., 1998) and 200 mg/kg b.w. (Sheen et al., 1999), respectively; group 4 was injected intraperitoneally with CCl₄ in olive oil (V/V) at a dose of 1ml CCl₄/kg b.w. (Luckey & Petersen, 2001) twice a week; Groups 5 and 6 were administered daily with AGE at a dose of 100 mg/kg b.w. and 200 mg/kg b.w., respectively, once daily in combination with CCl₄ injection (1ml CCl₄/kg b.w. twice a week).

Blood and tissue sampling

After the end of the experimental period, rats were fasted for over night and the blood samples were collected from retroorbital venous plexus of the rats in all groups under diethyl ether anesthesia. Blood samples were left to clot then centrifuged at 3000 r.p.m for 15 minutes to separate the sera, which were stored at -20°C until biochemical analysis. After blood collection, all animals were rapidly killed and liver tissues were dissected and immediately homogenized in 50 mM ice cold- phosphate buffer solution (pH 7.4) to give 20% (w/v) homogenate (Lin et al., 1998). The homogenate was centrifuged at 3000 r.p.m

at 0°C for 10 minutes and the supernatant was used for the determination of malondialdehyde (MDA) and glutathione reductase activity (GR).

Analytical determinations

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined colorimetrically using kits purchased from Randox Laboratories LTD Co., UK. Total protein and albumin concentrations were determined in serum using kits produced by Biodiagnostic Co., Cairo, Egypt. Globulin concentration was calculated by subtracting the concentration of albumin from the concentration of the total protein. Lipid peroxidation in liver homogenate was estimated by measurement of malondialdehyde (MDA) by the spectrophotometric method of Ruiz-Larnea et al. (1994). Determinations of total glutathione (tGSH) and reduced glutathione (rGSH) concentrations in serum were carried out following the methods described by Saville (1958) and Prins & Loose (1969), respectively. The oxidized glutathione (oxGSH) in serum was calculated by subtracting the concentration of rGSH from the concentration of tGSH. Serum paraoxonase (PON1) activity was determined according to the kinetic spectrophotometric method described by Eckerson et al. (1983). Glutathione reductase (GR) activity in liver homogenate was determined using a kit purchased from Oxis Research™ Co., USA. Colorimetric determinations of serum levels of triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) were assayed using kits produced by Sentinel CH, Milan, Italy. Kinetic determinations of CK-MB and LDH activities were carried out using kits purchased from Centronic

GmbH, Germany and Teco Diagnostic, CA, USA, respectively.

Statistical analysis

The obtained data were subjected to one way analysis of variance (ANOVA); correlation analysis was also performed. All analyses were performed using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA. Tukey test was used to clarify the significance between the individual groups at probability level $P < 0.01$ (Steel & Torrie, 1980).

Results

The effects of daily aged garlic extract (AGE) intake on the markers of liver function in CCl_4 -intoxicated rats are presented in table (1). CCl_4 treatment resulted in significant increase in ALT and AST activities ($P < 0.01$) compared to the control group. At the same time, it caused significant decrease ($P < 0.01$) in the level of serum total protein with a significant elevation in albumin level but did not affect globulin level. The administration of AGE to rats succeeded in ameliorating significantly, in a dose dependent-manner, the CCl_4 -induced changes in the mentioned parameters.

As shown in table (2), CCl_4 intoxication significantly increased MDA and oxGSH formation, lowered serum levels of tGSH and rGSH, and lowered hepatic GR and serum PON1 activities; however, the intake of AGE in combination with CCl_4 significantly lowered MDA formation, alleviated GSH depletion, and enhanced GR and PON1 activities ($P < 0.01$, as compared with CCl_4 -treated group).

Table 1. Serum markers of liver function in different studied groups.

Group	Control	AGE (100 mg/kg)	AGE (200 mg/kg)	CCl_4 (1ml/kg)	AGE (100 mg/kg) + CCl_4	AGE (200 mg/kg) + CCl_4
ALT (IU/l)	61.5±1.84 ^D	63±1.97 ^D	58±1.87 ^D	154±4.19 ^A	100±3.15 ^B	81±2.54 ^C
AST (IU/l)	122±1.80 ^D	120±1.73 ^D	119±1.81 ^D	453±10.38 ^A	297±7.94 ^B	202±5.35 ^C
T.Proteins (g/dl)	7.56±0.12 ^A	7.39±0.12 ^A	7.24±0.12 ^A	6.77±0.10 ^C	7.15±0.12 ^{AB}	7.30±0.11 ^{AB}
Albumin (g/dl)	3.67±0.08 ^A	3.59±0.06 ^A	3.46±0.05 ^A	3.15±0.11 ^B	3.49±0.07 ^A	3.56±0.08 ^A
Globulin (g/dl)	3.89±1.57 ^A	3.80±0.10 ^A	3.78±0.07 ^A	3.62±0.13 ^A	3.66±0.13 ^A	3.74±0.14 ^A

Values are mean ± SE for 8 rats per group. Within each row, means with different letters are significantly different ($P < 0.01$) using one way (Tukey) ANOVA test.

Table 2. Oxidant-antioxidant status in different studied groups.

Group	Control	AGE (100 mg/kg)	AGE (200 mg/kg)	CCl_4 (1ml/kg)	AGE (100 mg/kg) + CCl_4	AGE (200 mg/kg) + CCl_4
L.MDA (nmol/g liver)	28.1±0.37 ^D	26.8±0.63 ^D	24.1±0.57 ^D	64.88±0.99 ^A	45.13±0.69 ^B	35.13±1.65 ^C
S. tGSH (nmol/ml)	12.36±0.22 ^A	11.72±0.21 ^{AB}	12.07±0.24 ^A	8.05±0.44 ^D	9.61±0.17 ^C	10.44±0.19 ^{BC}
S. rGSH (nmol/ml)	7.95±0.09 ^A	8.26±0.14 ^A	8.20±0.19 ^A	2.32±0.25 ^D	5.14±0.17 ^C	7.31±0.18 ^B
S.oxGSH (nmol/ml)	4.41±0.21 ^B	3.46±0.25 ^{BC}	3.87±0.12 ^{BC}	5.73±0.27 ^A	4.47±0.21 ^B	3.12±0.15 ^C
L.GR (mU/g liver)	290±10.23 ^{AB}	294±3.97 ^{AB}	311±7.31 ^A	249±4.64 ^C	262±4.58 ^{BC}	298±5.56 ^A
S.PON1 (IU/l)	629±10.38 ^{AB}	650±10.70 ^{AB}	668±12.52 ^A	474±17.78 ^D	541±8.92 ^C	603±9.90 ^B

Values are mean ± SE for 8 rats per group. Within each row, means with different letters are significantly different ($P < 0.01$) using one way (Tukey) ANOVA test. L: liver; S: serum; GR: glutathione reductase; GSH: glutathione.

The effects of AGE and CCl₄ on some parameters of lipid profile are depicted in table (3). The data show that treatment with CCl₄ resulted in significant (P < 0.01) increases in total cholesterol, LDL-c, triglycerides levels as well as LDL/HDL ratio, and a significant (P < 0.01) decrease in HDL-c level compared with the control group.. Co-treatment with AGE and CCl₄ resulted in a significant protection of these parameters against the above mentioned effects of CCl₄. Meanwhile, the high dose of AGE (200 mg/kg b.w.) was found to be more effective in normalizing the markers of lipid

profile in CCl₄ - treated rats. On the other hand, the activities of CK-MB and LDH were significantly increased by CCl₄ treatment (Table 4, P < 0.01); and AGE administration significantly reduced CK-MB and LDH activities (P < 0.01) towards the normal values of the controls.

The present work (Table 5) declared that serum PON1 activity correlates inversely with serum ALT, AST activities, serum triglycerides, total cholesterol, LDL-c, oxGSH and hepatic MDA levels; and correlates directly with serum HDL-c, total proteins, rGSH and hepatic GR activity.

Table 3. Serum total cholesterol, triglycerides, HDL-c and LDL-c levels in different studied groups.

Group Parameter	Control	AGE (100 mg/ kg)	AGE (200 mg/kg)	CCl ₄ (1ml/kg)	AGE (100 mg/kg) + CCl ₄	AGE (200 mg/kg) + CCl ₄
T.Chol (mg/dl)	58.88±1.78 ^C	56.53±1.71 ^C	53.58±1.62 ^C	92.27±4.11 ^A	75.34±3.39 ^B	65.23±2.93 ^{BC}
Trigl (mg/dl)	33.80±1.58 ^C	32.45±1.11 ^C	30.76±1.05 ^C	64.22±2.19 ^A	52.02±1.78 ^B	46.88±1.60 ^B
HDL-c (mg/dl)	19.85±0.59 ^{BC}	21.42±0.68 ^A	24.39±0.80 ^B	14.09±0.43 ^F	15.80±0.45 ^{DE}	17.41±0.57 ^{CD}
LDL-c (mg/dl)	32.28±2.13 ^C	28.68±1.95 ^C	26.50±2.19 ^C	64.94±4.21 ^A	49.14±3.33 ^B	38.45±3.08 ^{BC}
LDL-c/HDL-c ratio	1.65±0.14 ^{CD}	1.31±0.11 ^{CD}	1.86±0.10 ^D	4.63±0.31 ^A	3.12±0.20 ^B	2.23±0.20 ^{BC}

Values are mean ± SE for 8 rats per group. Within each row, means with different letters are significantly different (P < 0.01) using one way (Tukey) ANOVA test.

Table 4. Serum lactate dehydrogenase (LDH) and creatinine kinase (CK-MB) activities in different studied groups.

Group Parameter	Control	AGE (100 mg/ kg)	AGE (200 mg/kg)	CCl ₄ (1ml/kg)	AGE (100 mg/kg) + CCl ₄	AGE (200 mg/kg) + CCl ₄
LDH (IU/l)	393±3.97 ^D	320±3.87 ^D	390±5.97 ^D	846±8.49 ^A	643±6.47 ^B	465±13.04 ^C
CK-MB (IU/l)	491±33.99 ^C	478±33.12 ^C	454±31.47 ^C	821±16.29 ^A	691±13.63 ^B	531±10.49 ^C

Values are mean ± SE for 8 rats per group. Within each row, means with different letters are significantly different (P < 0.01) using one way (Tukey) ANOVA test.

Table 5. The statistical correlation coefficient (R) between PON 1 activity and other parameters.

	Correlation	R value	P value	
PON 1	ALT	- ve	-0.97644	0.1385
	AST	- ve	-0.71836	0.4898
	T. protein	+ ve	0.58795	0.5999
	L. MDA	- ve	-0.99236	0.0787
	tGSH	+ ve	0.92188	0.2533
	rGSH	+ ve	0.96334	0.1729
	oxGSH	- ve	-0.81087	0.3980
	L.GR	+ ve	0.98881	0.0953
	Cholesterol	- ve	-0.99783	0.0420
	Triglyceride	- ve	-0.98572	0.1077
	HDL-c	+ ve	0.93666	0.2278
	LDL-c	- ve	-0.99993	0.0078
	LDH	- ve	-0.95882	0.1833
	CK	- ve	-0.53365	0.6416

Discussion

Liver injury can be caused by toxic chemicals, drugs, and virus infiltration from ingestion or infection. Carbon tetrachloride (CCl₄) has been widely used in animal models to investigate chemical toxin-induced liver

damage. The most remarkable pathological characteristics of CCl₄-induced hepatotoxicity are fatty liver, cirrhosis and necrosis, which have been thought to result from the formation of reactive intermediates such as trichloromethyl free radicals (CCl₃) metabolized by the mixed

function of cytochrome P450 in the endoplasmic reticulum (Wang et al., 2008). Therefore one of the therapeutic strategies against liver injury is to find antioxidant compounds that are able to block liver injury through scavenging of trichloromethyl free radical generated by CCl_4 .

The present results showed that CCl_4 causes significant increases in ALT and AST values which are cytoplasmic in location and are released into circulation after cellular damage so the increase in this enzyme may be attributed to the damage in the structural integrity of the liver (Recknagel et al., 1991). Similar to the present results, Bhattacharjee & Sil (2007) reported that CCl_4 administration to rats or mice caused hepatotoxicity, which is accompanied by elevations of ALT and AST enzymes. The hypofunction of the liver was further confirmed in the present study by the decrease in serum total protein level accompanied by increases in albumin level and albumin/globulin ratio. CCl_4 as a xenobiotic induces lipid peroxidation (LP) and toxicity (Jeon et al., 2003). It is well established that CCl_4 is metabolized in the liver to highly reactive trichloromethyl radical ($\text{CCl}_3\cdot$) by the activation of cytochrome P450. $\text{CCl}_3\cdot$ and its highly reactive derivative, the trichloro-methyl peroxy radical ($\text{CCl}_3\text{Coo}\cdot$), are assumed to initiate free radical – mediated LP of the cytoplasmic membrane phospholipids causing functional and morphological changes in the cell membrane leading to accumulation of lipid – derived oxidants and liver injury (Badger et al., 1996). The peroxidation process has been suggested to result in the release of soluble products that may affect cell membrane. Cell membranes integrity is broken and enzymes in the cell plasma leak out (Martin-Aragon et al., 2001). This may explain the increase in the activities of ALT and AST in serum of CCl_4 -treated group.

In the present study, hepatic malondialdehyde (MDA) was found to be significantly higher in animals treated with CCl_4 alone. It has been shown that lipid peroxides are not only noxious to the living organism, but also some of their stable breakdown products such as MDA have been recognized to cause some cell alterations by modifying protein structure. The reaction between MDA and the primary amino groups of proteins forms Schiff base compounds, which give rise to intra and intermolecular linkages which can lead to polymerization and inactivation of enzymes. In addition, MDA reactivity towards amino groups can result in inhibition of DNA and RNA protein synthesis (Sreenivas Rao et al., 2004). So the decreased total protein in CCl_4 treated group may be due to the liver damage caused by CCl_4 which may affect protein synthesis.

Alterations in hepatic antioxidant status may therefore manifest oxidative stress caused by CCl_4 and its metabolites. The CCl_4 -induced escalation of lipid peroxidation in the liver might be a consequence of increased formation of free

radicals as well as the inhibition of the activities of antioxidant enzymes such as glutathione reductase. The results obtained indicate that at the used level of CCl_4 treatment, the antioxidant defense system in the liver and serum was insufficient to give complete protection against lipid peroxidation. This might result from the fact that the liver is the main site of CCl_4 biotransformation and at the same time the main site of free radicals formation.

CCl_4 treatment increased serum triglyceride, total cholesterol, LDL- c and LDL/HDL values but decreased HDL- c level. These results are in agreement with those recorded by Torres-Durán et al. (1998). Cholesterol concentrations are determined by metabolic functions, which are influenced by integrity of vital organs such as the liver and kidney (Marcus & Milton, 1982). The elevation of triglycerides level may be due to impaired removal and destruction of triglycerides rich in lipoproteins such as very low density lipoprotein, low density lipoprotein and remnants (Recknagel & Lombardi, 1961), resulting in increased plasma levels of very low density lipoprotein triglycerides (Ginsberg & Grundy 1982), which is a risk factor in atherosclerosis. Extrahepatic lipoprotein lipase such as in the heart is involved in the uptake of triglycerides rich lipoproteins from the circulation (Nestel et al., 1963). Hyperlipidemia and lipoprotein abnormalities (Rahman, 2010) and oxidative stress (Jayakumar et al., 2008) are well-established risk factors in heart disease. This suggestion asserted the increment in both serum CK-MB and LDH recorded in the current study. Theoretically, CK-MB is a marker of heart failure. The mechanism for the release of this marker seems to be from ventricular remodeling, ongoing myocyte degeneration, the presence of coronary artery disease and reduced coronary reserve (Potluri et al., 2004). LDH is an enzyme found in the cytoplasm of almost all body tissues, where its main function is to catalyze the oxidation of L-lactate to pyruvate. It is assayed as a measure of anaerobic carbohydrate metabolism and as one of several serum indicators of myocardial infarction (Piccone et al., 1995).

The present data revealed a significant decrease in serum PON1 activity of CCl_4 -intoxicated rats. Liver plays the key role in the synthesis of serum PON1, and the gene expression has been observed only in the liver (Leviev et al., 1997) thus, the decrease in PON1 activity here may be due to the hepatic dysfunction induced by CCl_4 toxicity. As PON1 is known to be tightly bound with HDL-c, therefore, the decrease in PON1 activity could be a consequence of an altered synthesis and/or secretion of HDL-c. Alterations in HDL-c structure and concentration associated with decreases in hepatic lecithin cholesterol acyl transferase activity are frequent in chronic liver diseases (Sabesin et al., 1997). Moreover, the disulfide bond (cys-42 and cys-353), in PON1 molecule, was found to be essential

for its activity; while the free thiol (cys-284) was not; this suggesting that cys-284 is the active site responsible for the antioxidant property of PON1 (Josse et al., 2002). The highly reactive free radicals produced by CCl_4 metabolism can react with sulfhydryl groups, such as glutathione and protein thiols (Brattin et al., 1985), and it was stated that there is a close association between PON1 antioxidant activity and the number of $-\text{SH}$ groups within PON1 (Jaouad et al., 2006), therefore the reduction in PON1 antioxidant activity might be due to an alteration in nature and number of free thiol groups in its molecule.

The hepatoprotective effects of aged garlic extracts (AGE) were demonstrated in the current study by restoring the activities of serum ALT and AST enzymes that were significantly raised by CCl_4 administration. These results indicated that AGE has membrane stabilizing effect. This effect may be attributed to the ability of AGE to protect the liver tissues from free radical-mediated toxic damages and reduction of the oxidative stress through decreased hepatic MDA of CCl_4 -intoxication rats. Our present study agreed with the previous study of Hsu et al. (2006) which indicated that the water soluble S-allyl cysteine component of garlic alleviates ALT and AST enzymes and suppress lipid peroxidation in acetaminophen-induced endothelial dysfunction and haemostatic disorder in mice. On the other hand, AGE administration could increase serum total protein by preventing protein oxidation which probably reflects the potential ability of AGE to improve protein synthesis and increasing the immunoglobulin and globulin concentrations (Hassan et al., 2010).

The results of the present study indicate that serum tGSH, rGSH levels and hepatic GR activity were significantly decreased and the oxGSH is increased in response to CCl_4 treatment alone compared with control rats implying increased oxidative damage to the liver. On the contrary, the antioxidants values were significantly elevated by administration of AGE.

The present results demonstrated that the possible hepatoprotective mechanisms of AGE on CCl_4 -induced liver damage in rats might be due to either inhibiting the cytochrome P 450 – dependant oxygenase activity, preventing lipid peroxidation or stabilizing the hepatocyte membrane. Garlic contains a number of organosulphur compounds which are generally believed to be the active constituents responsible for its biological actions (Agarwal, 1996). These can be divided into water-soluble compounds such as S-allyl cysteine and oil-soluble compounds such as diallyl sulphide. The aged garlic extract that we have used is prepared by slicing fresh garlic and soaking it in dilute ethanol for 20 months at room temperature. This process is supposed to cause increased activity of certain new generated compounds like S-allyl cysteine (SAC), S-allyl mercaptocysteine (SAMC), allixine and selenium

which are stable and highly bioactive (Rahman & Billington, 2000).

It is well understood that rGSH is an important cellular antioxidant that is capable of direct or indirect (via glutathione peroxidase) conjugation with reactive oxygen species such as lipid hyperperoxides and hydrogen peroxide (Meister, 1983). In contrast to the toxic activation of CCl_4 via the cytochrome P450 2E1 pathway, the detoxification pathway involves rGSH conjugation of the trichloromethyl radical, a cytochrome P450 2E1 – mediated CCl_4 -metabolite (Recknagel et al., 1991). rGSH is largely mediated through the activity of glutathione S-transferase (GST), and forms adducts with the toxic metabolites of CCl_4 . Moreover, rGSH contributes to the detoxification of CCl_4 , and it has been suggested that one of the principle causes of CCl_4 -induced liver injury is lipid peroxidation caused by its free radical derivatives (Recknagel et al., 1991).

The mechanism by which treatment of rats with AGE increases the hepatic content of rGSH may involve either increased synthesis and /or decreased utilisation. It is doubtful that decreased rGST activity could account for decreased utilisation; indeed, the garlic constituent's S-allyl cysteine (SAC) (Hatono et al., 1996) have been shown to induce some isoforms of GST in liver. Alternatively, Hsu et al. (2004) proposed that SAC, and S-propyl cysteine (SPC) and other cysteine related compounds such as S-methyl cysteine and S-ethyl cysteine that derived from garlic may act as precursors of rGSH and stimulate rGSH synthesis. In previous studies, the intake of SAC and SPC was reported to increase rGSH retention in organs and blood, which consequently elevated glutathione peroxidase and/or catalase activities and suppressed oxidative stress presented in normal and diabetic mice (Hsu et al., 2004) and alleviate acetaminophen-induced depletion of rGSH content in blood and organs, and consequently they reduced oxidative damage in blood and liver (Hsu et al., 2006).

Additionally, there is growing evidence that the hepatoprotective effect of AGE takes place directly at the level of hepatocytes by inhibiting cytochrome P450 2E1 activity (Sumioka et al., 1998). The toxicity of CCl_4 results from its bioactivation to the toxic metabolite trichloromethyl radical via cytochromes P450 2E1 (Recknagel et al., 1991). The protective effect of garlic may be due, at least in part, to inhibition of this bioactivation since some garlic constituents inhibit the 2E1 isoform of cytochrome P450 (Lin et al., 1996).

The decrease in the levels of serum total cholesterol, LDL-c and triglyceride combined with significant elevation in serum HDL-c level in AGE treated groups may be attributed to the water soluble organosulfur compounds of AGE (Gebhardt, 1993) that can inhibit key enzymes in lipid synthesis. In addition, AGE protects liver from oxidative stress and protein deficiency. This in turn improves liver function and consequently

improves lipid metabolism. The normalization of abnormal lipids, lipoproteins, and hypertension, inhibition of platelet aggregation, and an increase in antioxidant status are believed to improve heart disease (Rahman, 2010). This may confirm the improvement in CK-MB and LDH values that were observed in AGE groups in our study. Our results were asserted by the finding of Alkreathy et al. (2010) who demonstrated a significant decrease in the cardiac MDA level with concomitant decrease in serum CK and LDH activities by treatment with AGE in doxorubicin-induced cardiotoxicity in rats.

Rosenblat et al. (2006) stated that intervention, means including the use of potent antioxidants of different sources, may be increase PON1 activity. AGE able to prevent free radical from reaching biomolecules in susceptible biological systems and improve HDL-c, this besides to its antioxidant characteristics exhibited by its organosulfur compounds (Gebhardt, 1993). Many, *in vitro* and *in vivo*, studies have provided initial evidence that antioxidants can increase PON1 activity possibly by protecting the enzyme from oxidative stress (Aviram et al., 1999; Aviram et al., 2000). PON1, an HDL-associated enzyme synthesized in the liver and secreted into the blood (Sabesin et al., 1997). So, the high PON1 activity found in our AGE groups could be asserted also by the significant positive correlation that was found between PON1 activity and HDL-c level.

Conclusions

In conclusion, the present study showed that AGE attenuate the hepatic dysfunction induced by CCl₄ as indicated by decreasing both GPT and GOT; and normalizing PON1 activity as well as lipid peroxidation reaction and its related biochemical changes. The protective effects of AGE against CCl₄-induced liver toxicity may be attributed to its antioxidant and free radical scavenging activities due to its higher contents of organosulphur compounds.

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