

EFFECT OF GARLIC CONSUMPTION ON BLOOD LIPID AND OXIDANT/ANTIOXIDANT PARAMETERS IN RAT MALES EXPOSED TO CHLORPYRIFOS

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ABSTRACT

Chlorpyrifos (CPF) is one of the most widely used organophosphate insecticides. In this study the effects of CPF and/or garlic (G) on acetylcholinesterase (AChE) and lipid peroxidation (LPO) were investigated. Following groups were arranged in the experiment: control group (no treatment), a group treated with G, two groups treated with a single dose of chlorpyrifos: low dose (LD) and high dose (HD), respectively, two groups treated with chlorpyrifos following the treatment with G (LD+G) and (HD+G), respectively. Pre-treatment of rats with G prior to administration of CPF reduced total lipid, cholesterol, triglyceride levels, low density lipoprotein (LDL), whereas high density lipoprotein (HDL) was elevated. Albumin was significantly decreased in CPF groups, G reversed this decline. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate dehydrogenase (LDH) activities were increased in CPF groups, whilst G reversed these parameters to control values. Increase in urea and creatinine levels due to the treatment with CPF and garlic afforded strong protection against CPF. Chlorpyrifos administration decreased AChE activity but increased thiobarbituric acid reactive substances (TBARS). Administration of G before a CPF treatment restored both AChE and TBARS. In conclusion, CPF caused liver damage and lipid peroxidation. Garlic increased blood antioxidant capacity and improved lipid profile.

Keywords: Garlic; Oxidative stress; Chlorpyrifos; Acetylcholinesterase; Lipid profile

INTRODUCTION

Organophosphorus insecticides (OPs) are widely used for a variety of agricultural and public health applications (Maroni et al., 2000; Goel et al., 2007). OPs produce a wide range of toxicity in mammals by inhibiting acetylcholinesterase (AChE), and the consequent accumulation of the neurotransmitter acetylcholine (ACh) in synaptic junctions leads to excessive stimulation of postsynaptic cells leading to cholinergic toxicity (Attia, 2000; Pope et al., 2005; Ncibi et al., 2008). Chlorpyrifos-ethyl (CPF) (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) belongs to the phosphorothioate class of organophosphorus insecticides and metabolically activated to its corresponding oxygen analogue, oxon,

in the liver (Kousba et al., 2004). Chlorpyrifos-oxon potently binds to and inhibits AChE to elicit cholinergic toxicity. The bulk of the activation of chlorpyrifos occurs in the liver and its detoxification takes place in the liver and plasma. This biotransformation of organophosphorus insecticides is catalyzed by cytochrome P450 and associated enzymes (Jokanovic, 2001).

Cardiovascular disease is the leading cause of mortality and morbidity worldwide. Oxidation of cholesterol fractions, in particular of low-density lipoprotein cholesterol (LDL), has been accepted as playing an important role in atherosclerosis (Durak et al., 2004). Cholesterol, cholesterol esters, and triglyceride components of the lipoprotein fractions can be oxidized by toxic radicals and can lose their chemical structures

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and cellular functions. Lipid peroxidation is accepted to be a free radical process implicated in the formation of atherosclerosis (Wen et al., 1996). Various studies were performed to establish a possible protective role of garlic in the atherosclerosis process. In this regard, some properties such as antilipidemic, antioxidant, antiplatelet, hemostatic and hemodynamic activities of garlic were established (Helen et al., 1999; Dillon et al., 2003). Relating to the antioxidant activity of garlic, it has been demonstrated that components of aged garlic extract inhibit the in vitro oxidation of LDL by chelating Cu^{+2} , scavenging superoxide ions and thus inhibiting the oxidation of protein and lipid moiety of human LDL (Dillon et al., 2003). Lau (2001) has reported that garlic supplementation in human subjects leads to increased resistance of LDL to oxidation, and it has been suggested that suppressed LDL to oxidation may be one of the powerful mechanisms accounting for anti-atherosclerosis properties of garlic.

This study was aimed firstly at examining the chlorpyrifos effect on the liver through some serum biochemical parameters; secondly to evaluate the safety and efficacy of garlic to ameliorate the deleterious effects of this insecticide.

MATERIALS AND METHODS

Chemicals

Chlorpyrifos, CPF (purity = 98%) was purchased from Chemical Service (Philadelphia, PA, USA). Thiobarbituric acid and all other chemicals were purchased from Sigma Chemical Company (Saint Louis, USA). Garlic powder was obtained from The Arab Gelatin Pharmaceutical Products Company (NAPHA), Egypt. Use of chlorpyrifos was approved by the Animal Care Committee and met all guidelines for its use.

Animals

Thirty six albino rat males weighing 100-120 g were obtained from National Research Institute, Cairo, Egypt and acclimatized for 2 weeks prior to the experiment. They were assigned to six groups and housed in Universal galvanized wire cages at room temperature (22-25 °C) and a photoperiod of 14 h light/10 h dark per day. Animals received standard laboratory balanced commercial diet and water *ad libitum*.

Experimental design

The animals were housed in groups of 6 rats each and divided randomly into 6 groups. The first group served as control (C) and was treated with corn oil orally, group 2 was administered with garlic (G) (90 mg/kg) for 15 days (3 times a week), groups 3 and 4 were treated orally with a single dose of chlorpyrifos; low dose (LD) (1/25; 5.4 mg/kg; of LD_{50}) and high dose (HD) (1/10; 13.5 mg/kg; of LD_{50}), respectively, 24 h prior to decapitation.

Groups 5 and 6 were administered orally with LD and HD of chlorpyrifos following the treatment with garlic (LD+G) and (HD+G), respectively. The treatment period was extended for 16 days.

Sample collection

The animals were starved overnight for 12h before the blood was collected. Rats were anaesthetized with ether and venous blood samples were collected by direct heart puncture in sterilized vials. The blood samples were centrifuged at 1000 xg for 15 min at 4 °C and serum was recovered.

Biochemical parameters

Thiobarbituric acid reactive substances (TBARS) were measured according to the method described by Tapel and Zalkin (1959). The color intensity of the TBARS reactants was measured at 532 nm and a molar extinction coefficient of $156,000 \text{ cm}^{-1} \text{ mole}^{-1}$ was used for the calculation of concentrations.

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated in blood serum by the method described by Reitman and Frankel (1957), using commercial kits obtained from Bio M²erieux, France. The activity of alkaline phosphatase (ALP) was measured according to Rosalki et al. (1993), the activity of lactate dehydrogenase (LDH) was estimated according to the method of Friedman and Young (1997), low density lipoprotein cholesterol (LDL) was measured according to the method of Assmann et al. (1984), high density lipoprotein cholesterol (HDL) was measured according to the method of Burstein et al. (1984), and triglycerides were measured according to the method of Fossati and Prencipe (1982), using commercial kits obtained from BioSystems Co., Spain. Acid phosphatase activity (ACP) was determined according to Tietz (1986), and total lipids were measured according to the method of Knight et al. (1972) using commercial kits obtained from Bio ADWIC, Egypt. Serum albumin was measured according to the method of Dumas et al. (1971), and serum creatinine was measured according to the method of Henry (1974) using commercial kits obtained from Biocon® Diagnostik, Marienhagen, Germany. Urea was measured according to the method of Patton and Crouch (1977) using a commercial kit obtained from Diamond Co., Egypt. Serum acetylcholinesterase activity (AChE) was measured according to the method of Blawen et al. (1983) using a commercial kit obtained from Quimica Clinica Aplicada S.A. Spain. Total antioxidant capacity (TAC) was measured according to the method of Koracevic et al. (2001), using a commercial kit obtained from Biodiagnostic Co., Egypt.

Statistical analyses

The mean and standard error values were determined for all the parameters and the results were

expressed as the mean \pm standard error for 6 rats in each group. The data were analyzed using a one-way analysis of variance (ANOVA). The student-Newman-Keuls test was used to compare the treated groups with the control group and the significance is given at $P < 0.05$, $P < 0.01$ and $P < 0.001$ (Newman, 1939 and Keuls, 1952).

RESULTS

Effect of garlic on albumin and lipid profile

Serum total lipid, cholesterol, triglyceride levels and LDL-cholesterol were found to be significantly decreased in rats pre-treated with repeated doses of garlic

prior to the administration of CPF, whereas HDL level was elevated compared to the control group. Albumin levels decreased significantly in rats treated with LD and HD of chlorpyrifos compared to the control, garlic reversed this decline (Table 1).

Effect of garlic on liver function

Results of this part are presented in Table 2. As shown, in groups treated with chlorpyrifos at both tested doses blood levels of AST, ALT, ALP, ACP and LDH were increased compared to those of the control group indicating a liver toxicity. Treatment with garlic prior to the CPF administration showed a reverse of these parameters to control levels.

Table 1: Effects of garlic on blood serum albumin and lipid profile in chlorpyrifos-induced oxidative damage in rats

Parameters	Control	Garlic	Chlorpyrifos		Chlorpyrifos+Garlic	
			LD _(5.4 mg/Kg)	HD _(13.5 mg/Kg)	LD _(5.4 mg/Kg)	HD _(13.5 mg/Kg)
Serum Albumin *	2.82 \pm 0.045	3.45 \pm 0.117 ^z	2.20 \pm 0.023 ^z	2.08 \pm 0.026 ^z	2.46 \pm 0.038 ^c	2.33 \pm 0.038 ^f
Serum Total Lipids *	6.33 \pm 0.076	5.90 \pm 0.062 ^z	7.05 \pm 0.056 ^z	8.84 \pm 0.280 ^z	5.92 \pm 0.030 ^{z,c}	6.38 \pm 0.088 ^f
Serum Cholesterol **	110.1 \pm 4.32	105.6 \pm 1.29	125.1 \pm 1.80 ^y	136.6 \pm 1.02 ^z	111.1 \pm 4.02 ^b	126.8 \pm 1.92 ^{z,f}
Serum Triglycerides **	82.3 \pm 0.45	71.2 \pm 2.81 ^z	88.6 \pm 1.62 ^z	115.9 \pm 5.00 ^z	81.7 \pm 0.78 ^b	92.0 \pm 1.77 ^{z,f}
Serum HDL-Cholesterol**	58.6 \pm 2.80	70.2 \pm 4.60 ^y	48.2 \pm 0.85 ^z	39.1 \pm 2.36 ^z	55.1 \pm 2.70 ^{x,b}	49.0 \pm 0.23 ^{z,f}
Serum LDL-Cholesterol**	48.1 \pm 1.96	32.9 \pm 1.82 ^z	57.1 \pm 1.83 ^z	66.7 \pm 2.03 ^z	46.6 \pm 2.10 ^b	58.4 \pm 1.09 ^{z,c}

Values are expressed as Mean \pm SEM; n= 6 for each treatment group

* g/dl; ** mg/dl; LD: low dose; HD: high dose.

x $p < 0.05$ in comparison to C

y $p < 0.01$ in comparison to C

z $p < 0.001$ in comparison to C

a $p < 0.05$ in comparison between LD and LD+G

b $p < 0.01$ in comparison between LD and LD+G

c $p < 0.001$ in comparison between LD and LD+G

d $p < 0.05$ in comparison between HD and HD+G

e $p < 0.01$ in comparison between HD and HD+G

f $p < 0.001$ in comparison between HD and HD+G

Table 2: Effects of garlic on AST, ALT, ALP, ACP, and LDH activities in chlorpyrifos-induced oxidative damage in rats.

Parameters (IU/L)	Control	Garlic	Chlorpyrifos		Chlorpyrifos +Garlic	
			LD _(5.4 mg/Kg)	HD _(13.5 mg/Kg)	LD _(5.4 mg/Kg)	HD _(13.5 mg/Kg)
Serum AST	105.9 \pm 4.13	96.3 \pm 2.40 ^y	125.9 \pm 3.69 ^z	138.9 \pm 1.28 ^z	103.9 \pm 6.39 ^c	117.1 \pm 2.91 ^{y,f}
Serum ALT	50.9 \pm 0.65	50.0 \pm 0.57	54.6 \pm 1.20 ^z	58.4 \pm 1.63 ^y	50.5 \pm 0.39 ^b	52.9 \pm 0.84 ^{z,c}
Serum ALP	234.0 \pm 8.43	210.9 \pm 3.54 ^y	266.2 \pm 10.06 ^y	358.7 \pm 9.39 ^z	218.3 \pm 1.53 ^{y,c}	317.4 \pm 7.87 ^{z,f}
Serum ACP	30.3 \pm 0.46	26.9 \pm 1.34 ^y	36.9 \pm 1.99 ^z	49.0 \pm 2.84 ^z	30.8 \pm 0.49 ^b	39.7 \pm 0.63 ^{z,c}
Serum LDH	1.93 \pm 0.04	1.25 \pm 0.02 ^z	2.33 \pm 0.03 ^z	2.97 \pm 0.04 ^z	1.60 \pm 0.07 ^c	1.97 \pm 0.04 ^f

Values are expressed as Mean \pm SEM; n= 6 for each treatment group

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ACP, acid phosphatase; LDH, lactate dehydrogenase; LD: low dose; HD: high dose.

x $p < 0.05$ in comparison to C

y $p < 0.01$ in comparison to C

z $p < 0.001$ in comparison to C

a $p < 0.05$ in comparison between LD and LD+G

b $p < 0.01$ in comparison between LD and LD+G

c $p < 0.001$ in comparison between LD and LD+G

d $p < 0.05$ in comparison between HD and HD+G

e $p < 0.01$ in comparison between HD and HD+G

f $p < 0.001$ in comparison between HD and HD+G

Table 3: Effects of garlic on kidney functions in the blood serum in chlorpyrifos-induced oxidative damage in rats

Parameters (mg/dl)	Control	Garlic	Chlorpyrifos		Chlorpyrifos +Garlic	
			LD _(5.4 mg/Kg)	HD _(13.5 mg/Kg)	LD _(5.4 mg/Kg)	HD _(13.5 mg/Kg)
Serum Urea	46.0± 2.83	34.9± 1.16 ^z	70.5± 1.90 ^z	83.5± 1.78 ^z	50.6± 4.34 ^c	73.4± 0.95 ^{z,f}
Serum Creatinine	1.73± 0.08	1.14± 0.06 ^z	2.33± 0.10 ^y	3.16± 0.13 ^z	1.79± 0.04 ^c	2.45± 0.13 ^{z,f}

Values are expressed as Mean ± SEM; n= 6 for each treatment group.

LD: low dose; HD: high dose

x p<0.05 in comparison to C

y p<0.01 in comparison to C

z p<0.001 in comparison to C

a p<0.05 in comparison between LD and LD+G

b p<0.01 in comparison between LD and LD+G

c p<0.001 in comparison between LD and LD+G

d p<0.05 in comparison between HD and HD+G

e p<0.01 in comparison between HD and HD+G

f p<0.001 in comparison between HD and HD+G

Table 4: Effects of garlic on serum TBARS and TAC levels and AChE activity in chlorpyrifos-induced oxidative damage in rats

Parameters	Control	Garlic	Chlorpyrifos		Chlorpyrifos +Garlic	
			LD _(5.4 mg/Kg)	HD _(13.5 mg/Kg)	LD _(5.4 mg/Kg)	HD _(13.5 mg/Kg)
Serum TBARS	2.95± 0.39	1.96± 0.04 ^y	5.95± 0.16 ^z	10.24± 0.52 ^z	4.51± 0.31 ^{y,c}	7.30± 0.21 ^{z,f}
Serum AChE	409.6± 46.8	465.0± 19.6 ^x	199.4± 7.1 ^z	146.1± 11.6 ^z	310.9± 12.8 ^c	190.6± 1.91 ^{y,f}
Serum TAC	1.67± 0.06	1.91± 0.02 ^z	1.23± 0.05 ^z	1.02± 0.04 ^z	1.61± 0.02 ^c	1.31± 0.013 ^{z,f}

Values are expressed as Mean ± SEM; n= 6 for each treatment group

TBARS, thiobarbituric acid reactive substances (μ mole/dl); AChE, acetylcholinesterase (IU/); TAC, Total antioxidant capacity (mMole/L); LD: low dose; HD: high dose

x p<0.05 in comparison to C

y p<0.01 in comparison to C

z p<0.001 in comparison to C

a p<0.05 in comparison between LD and LD+G

b p<0.01 in comparison between LD and LD+G

c p<0.001 in comparison between LD and LD+G

d p<0.05 in comparison between HD and HD+G

e p<0.01 in comparison between HD and HD+G

f p<0.001 in comparison between HD and HD+G

Effect of garlic on kidney function

Biochemical analysis of blood serum showed a significant increase in levels of urea and creatinine in rats treated with CPF. However, pre-treatment of rats with repeated doses of garlic prevented CPF toxicity (Table 3).

Effect of garlic on lipid peroxidation and AChE activity

Administration of CPF to rats caused significant inhibition to AChE in the blood serum (Table 4). Garlic reduced the neurotoxic action of CPF when it was administered in advance. Chlorpyrifos increased lipid peroxidation levels in the blood serum. When rats were given CPF following treatment with garlic, the TBARS level significantly decreased. Low and high dose of CPF significantly decreased TAC, whilst a pre-treatment with garlic caused restoration of TAC level (Table 4).

DISCUSSION

Pesticides, such as organophosphorus and organochlorine compounds commonly used in agriculture for achieving better quality products, are toxic substances

and lead to generation of reactive oxygen species (ROS) which have harmful effects on human health. In this field, CPF, an organophosphorus insecticide is known to cause oxidative stress in different human and animal cells (Jett and Navoa, 2000). Chlorpyrifos is a lipophilic molecule which can easily pass through the cell membrane into the cytoplasm. Once inside the cell, CPF can generate a lot of damages. Oxidative stress caused by various agents (toxins, metals, dioxin and pesticides) is considered as an imminent threat for many organisms since it can lead to death. For these reasons, it is necessary to find solutions against this danger. Within this context, nature can provide us many substances that can attenuate this oxidative stress. Most of these substances are found in our alimentation, as a garlic (Ide et al., 2002), or in some medicinal plants (Dhanabal et al., 2006). In the present study, toxicity of chlorpyrifos on some blood serum parameters and a protective role of garlic were investigated.

Concerning albumin and cholesterol values, their quantification shows a variation after chlorpyrifos administration. Albumin is the most abundant blood serum protein and is produced in the liver; after CPF

administration its level decreased, what agrees with the report of Peebles et al. (2005). Normally, the reduction of albumin level indicates a liver disease. This reduction could be attributed to changes in the protein and free amino acid metabolism and their synthesis in the liver (Ncibi et al., 2008). In the same field, Li et al. (2007) suggested that albumin could be used as a biomarker of chlorpyrifos toxicity. The level of cholesterol increased in animals treated with CPF, the same trend was seen with other organophosphate insecticides (Kalender et al., 2005). As observed in the present study, garlic pretreatment can decrease blood cholesterol level and can improve blood lipid profile to a significant extent. These results show that garlic extracts exert considerable antioxidant potency in vivo as well, and protect cellular structures against peroxidation. The high antioxidant potential of garlic may be a result of its high content of sulfur compounds (Prasad et al., 1995). Oxidation of cholesterol fractions (in particular, LDL) has been accepted as playing an important role in the atherosclerotic process (Liu et al., 1992), and because lipid peroxidation is a radical process implicated in this formation (Wen et al., 1996), it has been proposed, that garlic extract rich in an antioxidant content may confer beneficial effects in this regard. With respect to the cholesterol lowering property of garlic, it has been suggested that some constituents of garlic may act as inhibitors for some enzymes, such as hydroxyl methyl glutaryl CoA reductase, which participate in the cholesterol synthesis (Gebhardt, 1991; Gebhardt and Beck, 1996).

The organophosphorus insecticides induce an obvious increase in AST, ALT, ALP, ACP and LDH activities for both LD and HD treated groups. This fact is a conventional indicator of liver injury (Rao, 2006). When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream. Elevation of AST and ALT indicates the utilization of amino acids for the oxidation or for glucogenesis and is used to determine liver damage (Philip et al., 1995). Also, the elevation in ALP level suggests an increase in lysosomal mobilization and cell necrosis due to pesticide toxicity. In this field, Kalender et al. (2005) and Etim et al. (2006) assumed an increase of ALP level after diazinon and lindane induced hepatotoxicity. The increase of LDH activity is considered as an indicator of liver damage. Chen et al. (2000) observed a significant rise in serum LDH activity after liver affection. They referred the augmentation due to muscle-LDH release into the blood circulation.

Urea and creatinine are waste products of protein metabolism that need to be excreted by the kidney, therefore a marked increase in serum urea and creatinine, as noticed in this study, confirms an indication of functional damage to the kidney (Garba et al., 2007). Urea level can be increased by many other factors such as

dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney, since kidney damage is the only significant factor that increases the serum creatinine level (Nwanjo et al., 2005). Therefore, significant increases in urea and creatinine levels, noticed in this study, are a classical sign that the kidney was adversely affected by CPF administration.

Chlorpyrifos, like other OPs, is an anticholinesterase compound which covalently modifies AChE thus inhibiting its activity (Verma et al., 2007). The protection of AChE activity in rat serum is offered by the pre-treatment with garlic. Generation of oxidative stress and consequent lipid peroxidation by pesticides is reported in many species. Due to the high concentration of polyunsaturated fatty acids in cells, lipid peroxidation is a major outcome of the free radical-mediated injury (Verma et al., 2007). Sheweita et al. (2001) have reported that pre-treatment of rats with vitamin E, vitamin C or garlic, as single and repeated doses prior to the administration of carbon tetrachloride (CCl₄), reduced levels of TBARS caused by CCl₄. Gultekin et al. (2001) have shown that pre-treatment of rats with repeated doses of melatonin or a combination of vitamins E and C prior to the administration of chlorpyrifos-ethyl reduced lipid peroxidation caused by CPF.

Results of the present study clearly indicate that previous administration of the garlic combats oxidative stress induced by chlorpyrifos-ethyl in the rat blood serum. Medicinal plants serve as therapeutic alternative, safer choices, or in some cases, as the only effective treatment.

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