Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(3): 1573-1578

Received: 05-03-2017
Accepted: 06-04-2017

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Ameliorative effects of garlic (*Allium sativum* L.) against Chlorpyrifos intoxication on lipid profile and liver enzymes of male New Zealand rabbits (*Oryctolagus cuniculus*)

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Abstract
In the present study, protective effect of garlic against Chlorpyrifos -induced toxicity on liver was studied. Adult male rabbits (N=12) weighing 1.3-1.5kg, were used in the study. Animals were divided into four groups with 3 animals each. Group B received 95mg/ kg body weight garlic once a day. Group C was orally administered with 100 mg/kg body weight of CPF. Group D was treated with 95mg/kg body weight garlic followed by 100mg/kg body weight of CPF. All dosages were given daily for a period of 14 days. Results showed a significant increase of 8.76% in the level of LDH and 24.10% significant increase in cholesterol level in CPF administered group. A significant increase of 54% was observed in LDL and 143% highly significant increase in Triglycerides was exhibited by CPF administered group. A significant decrease of 21.73% in HDL was shown by CPF intoxicated group. In garlic treated group, a significant decrease of 21.9% in cholesterol and 18.92% in LDL was observed. In CPF co garlic administered group, garlic mollified the toxicity of CPF significantly in triglycerides to 54.6%. The level of cholesterol, triglycerides and low density lipid was increased in CPF treated group while in garlic treated group the level was decreased. In CPF co garlic administered group, garlic attenuated the toxic effects of CPF. The high density lipid, considered as a good lipid was decreased in CPF group while garlic increased HDL. In group D, garlic reduced the toxicity level of CPF on HDL.

Keywords: Chlorpyrifos, Garlic, Rabbits, liver enzymes, lipid profile

1. Introduction

Misuse of pesticide is the cause of high incidence poisoning in developing countries [1]. Organophosphorus insecticides (OPs) are used on a large scale for a variety of agricultural purposes [2, 3]. It inhibits acetylcholinesterase (AChE) pathway and the consequent accumulation of neurotransmitter acetylcholine (ACh) in synaptic junctions leads to excessive stimulation of postsynaptic cells leading to cholinergic toxicity [4, 5, 6]. Chlorpyrifos (CPF) is one of the organophosphate (OP) insecticides that are broadly used on agricultural food crops and to control public health pests such as mosquitoes and fire ants [7]. The principal action mechanism of CPF is inhibiting acetyl cholinesterase but other mechanisms including induction of oxidative stress, have also been implicated [8]. One of the consequences of uncontrolled oxidative stress is cells, tissues, and organs injury caused by oxidative damage. It has long been proved that high levels of free radicals or reactive oxygen species (ROS) can inflict direct damage to lipid content of the cells. The primary sources of endogenous reactive oxygen species production are the mitochondria, plasma membrane, endoplasmic reticulum, and peroxisomes [9] through a variety of mechanisms including enzymatic reactions and autoxidation of several compounds. Damage to the lipids changes cellular membranes and cellular function. A lot of research has been carried out on antioxidants and their effects on health in past years. The balance between oxidation and antioxidation is critical in maintaining a healthy biological system [10]. Diets rich in fruits and vegetables have been linked with decreased risks of several chronic diseases, such as coronary heart disease and some types of cancers [11]. The protective effects of these fruits and vegetables have been attributed partly to a variety of antioxidant compounds, for example, vitamins C and E, b-carotene and polyphenolics. In the past few years, it has been found that different forms of raw or aged garlic preparations prevented cardiovascular diseases, liver damage and aging which are the diseases to be associated with oxygen radical and lipid peroxidation [12]. Several studies have

also shown that garlic contains active hypocholesterolemic and hypoglycemic components, known as diallyl disulfide and dipropyl disulfide [13]. It has been considered as a therapeutic in nature as it contains many important nutritive and antioxidant constituents as selenium, sulfur compounds, vitamins A, B, C and E [14]. Past studies suggested that consuming garlic extract as a dietary supplement improves the blood lipid profile, strengthen the anti-oxidants capacity. Garlic can reduces [15] the lipid peroxidation a cell might face hence increases antioxidant defense mechanism in animals [16].

The objective of this research was to evaluate the effect of Chlorpyrifos on liver enzymes and biochemical parameters and the ameliorating effect of garlic against its toxicity**.**

2. Materials and Methods: The study was carried out at Islamia College University Peshawar in the month of March, 2017. Chlorpyrifos (40% EC) was obtained from the local market of Peshawar. Garlic was obtained from the local vegetable market. Twelve male rabbits (*Oryctolagus cuniculus)* weighing 1.3-1.5kg were procured from the local market and acclimatized for 2 weeks prior to the experiment. They were assigned to four different groups, each with 3 animals and kept in separate cages at room temperature of 26-29 ˚C with a photoperiod of 14hrs light /day. Animals were fed with green fodder and water *ad labitum*. After 14 days of acclimatization to laboratory conditions, the animals were randomly assigned to four groups, each consisting of 3 rabbits. Group A was kept untreated and considered as control group. Animals of group B were orally administered garlic (95mg/kg body weight).Group C (CPF) was treated with 1/10th of LD 50 of CPF (100mg/kg body weight) for acute toxicity.

Group D (CPF+ Garlic) received 100mg/kg body weight of CPF with 60 minutes prior administration of Garlic at 95mg/kg body weight. Dosages of each group were administered on daily basis. Blood samples were collected from marginal ear vein and collected in the gel tubes at the end of the experiment for biochemical analysis of lipid profile and liver enzymes. The collected blood samples were transferred to laboratory. Biochemical analysis was performed through medical biochemical analyzer (MicroLab300, China).

2.1. Statistical Analysis: The results were subsequently analyzed through one way analysis of variance and mean values were compared by using the SPSS version (16).

3. **Results:** Table (1) shows the values of serum AST, ALT, ALP, LDH, LDL, HDL, Cholesterol and Triglycerides in all groups. The results showed a drastic change in the targeted parameters of treated groups. Mean ±S.EM value for AST in control group was 25.00±2.88 U/L while in garlic (+ive control) treated group was 19.67±0.88 U/L with a decrease of 21.31%. CPF (-ive control) treated group showed a value of 31.67±0.58 with 26.68% increase in AST. Mean±S.E.M value of AST was 24.67±2.19 with a decrease of 1.32% in Chlorpyrifos priorily treated Garlic group. (Table 1, Fig. 1.1)**.** Mean ±S.EM value for ALT in control group was 77.8±2.84U/L while garlic treated group showed 67.33±1.33 with a significant decrease of 13.5%. CPF treated group exhibited a value of 86.67±13.00 with an increase of 11.40% and CPF priorily treated garlic group showed a value of 72.00±0.58 with a decrease of 7.46% (Table 1, Fig. 1.2). Mean ±S.EM value for ALP in control group was 2.12E2±4.418 U/L while garlic group showed 1.75E2±13.00 value with 17.54% decrease. CPF treated group showed 2.43E2±14.36 value with a significant increase of 14.62% and CPF co garlic administered group has 2.10E2±20.33 value with a decrease of 0.94% (Table 1, Fig. 1.3). Mean ±S.EM value for LDH in control group was 4.34E2±2.73U/L while in garlic administered group it was 4.22E2±3.94 with a decrease of 2.76.The LDH level in CPF was 4.72E2±21.20 with a significant increase of 8.76% and CPF co garlic administered group it showed 4.50E2±5.79 with an increase of 3.68% (Table 1, Fig. 1.4). Mean ±S.EM value for Low density Lipid (LDL) in control group was 24.67±1.45mg/dl while in garlic treated group the analyzed value was 20.00±0.58 with a significant decrease of 18.92%. The CPF treated group showed a LDL value of 38.0±3.06 with a significant increase of 54% and CPF and garlic co administered group has a LDL value of 24.00±0.58 with a percent decrease of 2.67 (Table 1,Fig 1.5). Mean ±S.EM value for High density Lipid (HDL) in control group was 23.00±1.55mg/dl while in garlic treated group the analyzed value was 24.33±1.20 with an increase of 5.8%.The CPF treated group showed a value of 18.00±0.58 with a significant decrease of 21.37% and CPF and garlic co administered group has a value of 20.67±0.33 with a decrease of 10.13% (Table 1, Fig. 1.6). Mean ±S.EM value of Cholesterol for control group was 74.67±2.72 mg/dl while in garlic administered group, it was 58.33±4.04 with a significant decrease of 21.9%. Cholesterol in CPF group was 92.67±3.93 with a significant increase of 24.10% and in CPF co administered garlic group value was 76.00±5.03 with an increase of 1.78% (Table1, Fig. 1.7)**.** Mean ±S.EM value for TGs in control group was 76.33±2.91mg/dl while in garlic fed group it was 67.33±2.73 with 11.79% decrease. The value in CPF treated group was 1.86E2±21.07 with highly significant increase of 143% and in CPF coadminstered garlic group the value for TG was 1.18E2±4.90 with more significant increase of 54.6% (Table 1, Fig. 1.8).

4. Discussion: Liver is the main organ involved in metabolism and detoxification of xenobiotic agents and is exposed on maximum to the toxic effects of these substances and their byproducts [17], hence finding the serum biochemical changes of liver related parameters and liver enzymes give the most precise result of the toxicity of these element. Serum liver enzymes including Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) are mainly used as an indicator in the evaluation of hepatic damage. In Table (1), results revealed that there was an increase in all enzymes activity in the CPF group as compared to Garlic treated group while in CPF co administered garlic group, garlic mitigated the toxicity of CPF. The increase in AST, ALT, ALP and LDH in CPF treated group is in fact an indicator of liver injury. Such elevations in liver enzymes after CPF intoxication was documented by other researchers as well [18, 19]. An increase in these enzymes was exhibited by CPF fed male rats and vitamin C mollified the toxicity of CPF on liver enzymes [20]. In a study on biochemical parameters of CPF intoxicated rats, the level of AST and ALT showed an obvious increase [17]. Results of a study on untreated rats and rats with either Chlorpyrifos or Profenofos for 60 days in their recommended doses elicited a marked elevation in serum LDH activity after the end of the study when compared with normal control group [21]. Elevation of serum AST and ALT enzymes in blood is an indication of liver damage in an

organism [22]. When the cell membrane of liver cell gets damaged, different enzymes normally a part of cytosol are released into the blood stream. Therefore, the increase in these enzymes may be due to liver dysfunctioning and a possible disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane takes place [23, 24]. In present study the + ive control group fed with garlic showed a decrease in liver enzymes as compared to the control group values while in CPF co garlic administered group, garlic attenuated the toxic effect of CPF and showed a decrease in level of all enzymes as compared to the CPF group. A study showed that use of any synthetic chemical generates free radicals in body and causes oxidative stress and cellular injury [25]. Free radicals produced by pesticides play an important role in its toxicity. To neutralize free radicles and counteract its adverse effects, cells have an array of antioxidant enzymes [26]. Studies carried out on garlic composition showed that it contains two main classes of antioxidant i.e. flavonoids and diallyl sulphide, trisulphide and allyle-cysteine [27, 28]. Garlic prevents oxidative stress by scavenging radicals [29] which is the reason that it has mitigated the toxicity of CPF in present study and lowered the enzymes level in a garlic administered group by scavenging the free radicals produced by a cell in a normal conditions.

The results in Table.1 showed an increase in serum cholesterol and Triglycerides (TG) in CPF intoxicated rabbits as compared to the garlic and CPF co garlic administered groups, which agrees with the previous studies. The High Density Lipids (HDL) level was decreased in CPF intoxicated group while garlic increased HDL and played an ameliorative effect in CPF co administered garlic group by keeping the HDL value high as compared to CPF group. The same trend was reported in other organophosphorus pesticide [30]. Previous studies demonstrated an increase in the conc. of TGs in the animals treated with different insecticides, including the organophosphate, dichlorvos [31] and diazinon [32]. An increase in the serum total cholesterol and TG in CPF intoxicated rabbits as compared with C and VC group was reported in previous studies. Although, serum total cholesterol and TGs concentration in the (VC+CPF) group decreased compared to that recorded in the CPF group, but it was still high in comparison to either C or VC group [17]. This result also agrees with past study that has shown the ability of vitamin C to mitigate toxicity induced by CPF. A significant decrease in HDL levels were recorded in all treated groups. Moreover, HDL concentrations in rats exposed to lead co administered Vitamin C or Vitamin E groups were significantly higher than those of Pb group. The simultaneous supplementation with Vitamin C + Vitamin E to Pb treated rats led to an even higher HDL level compared to the administration of either of the vitamins alone [33]. A significant increase in lipid peroxidation and a decrease in endogenous antioxidants levels were exhibited in the liver and kidney of lead exposed animals [34]. Research suggests that the formation of free radicals is a major way of pesticides toxicity [35]. Organophosphates have been reported to produce oxidative stress in a cell [36]. A previous study on rats intoxicated with CPF showed oxidative stress in a living cell and results in the accumulation of lipid peroxidation products in different organs [37]. Cells undergoes lipid peroxidation and follow different methods to reduce oxidative stress and repair damaged molecules. The primary defense mechanism is fighting with enzymatic and non-enzymatic antioxidants which have been proved to scavenge free radicals and reactive oxygen species. The antioxidant enzymes, superoxide dismutase, catalase, and glutathione peroxidase have been significantly affected by pesticides including CPF [38], resulting in loss of defensive mechanism and consequent increase in LDL, TG, Cholesterol and a decrease in HDL. The elevation of serum or plasma TGs and total cholesterol has been attributed to inhibition of the lipase enzyme activity of both the hepatic TGs and plasma lipoproteins [39]. As observed in the present study in CPF pretreated garlic group, garlic can decrease blood cholesterol level and improve lipid profile. These results showed that garlic extract put a considerable antioxidant potency and protect cellular structures against lipid per oxidation. The high antioxidant potential of garlic may be due to high content of sulfur compounds [40]. With respect to the cholesterol lowering property of garlic, it has been suggested that some constituents of garlic as inhibitors may act for some enzymes, such as hydroxyl methyl glutaryl CoA reductase, which participate in the cholesterol synthesis [41, 42]. It has been proposed, that garlic extract rich in an antioxidant content, may confers beneficial effects in this regard.

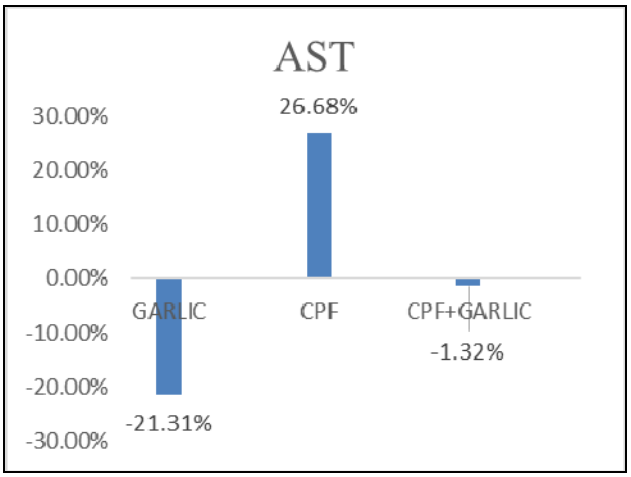

Fig 1.1: AST level in treated group

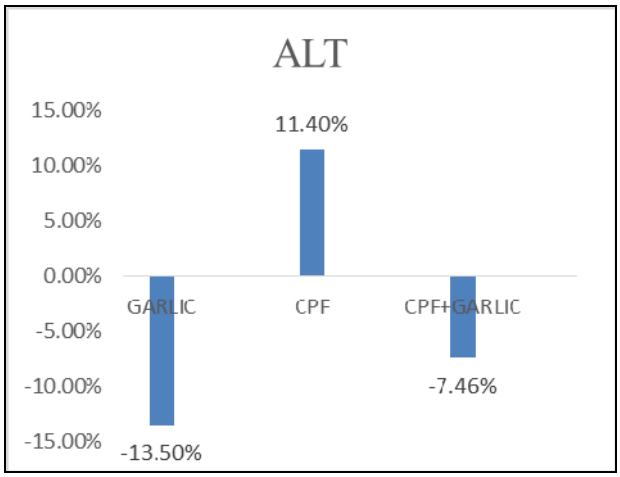

Fig 1.2: ALT level in treated group

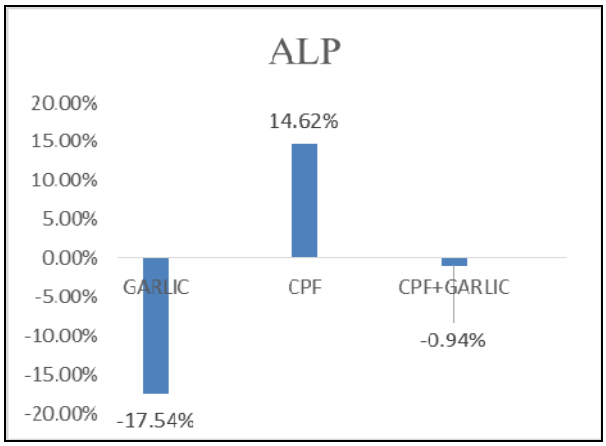

Fig 1.3: ALP level in treated group

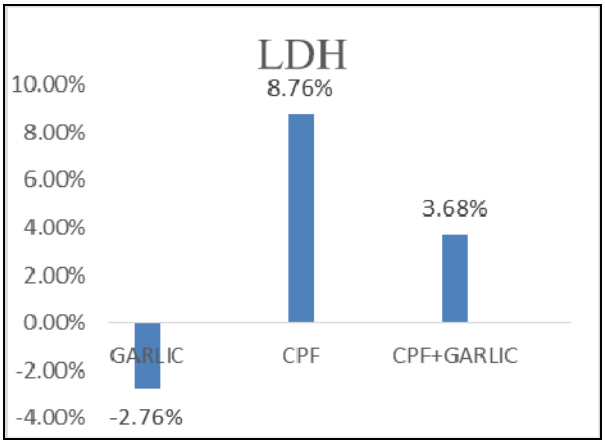

Fig 1.4: LDH level in treated group

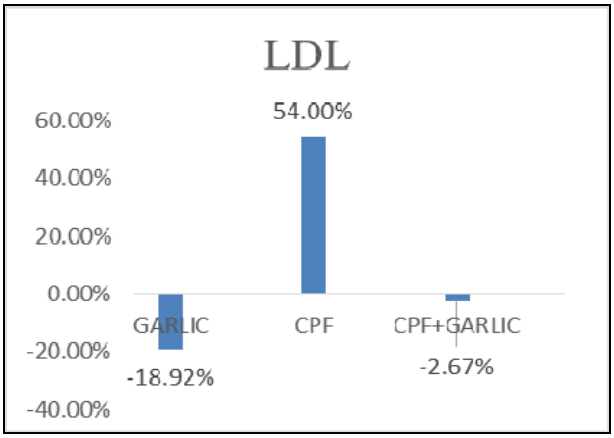

Fig 1.5: LDL level in treated group

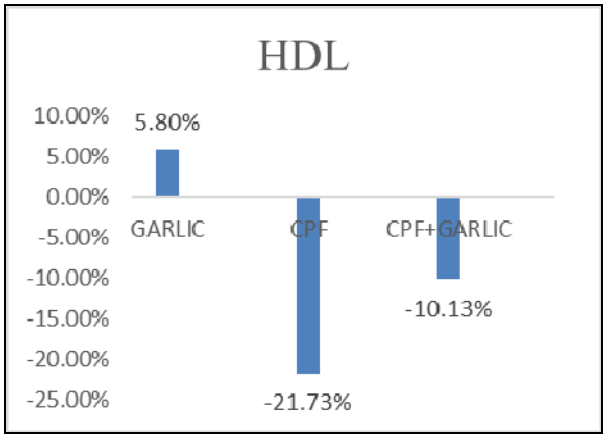

Fig 1.6: HDL level in treated group

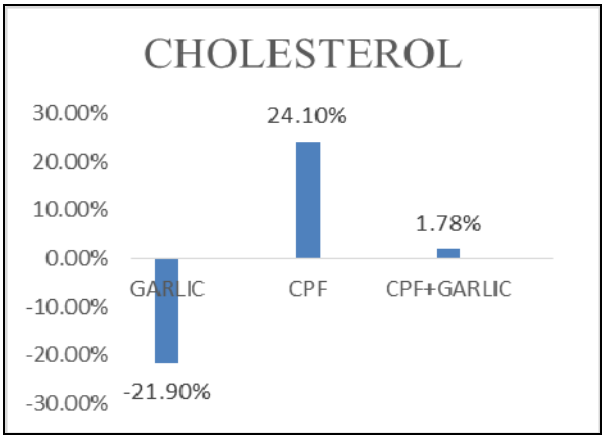

Fig 1.7: Cholesterol level in treated group

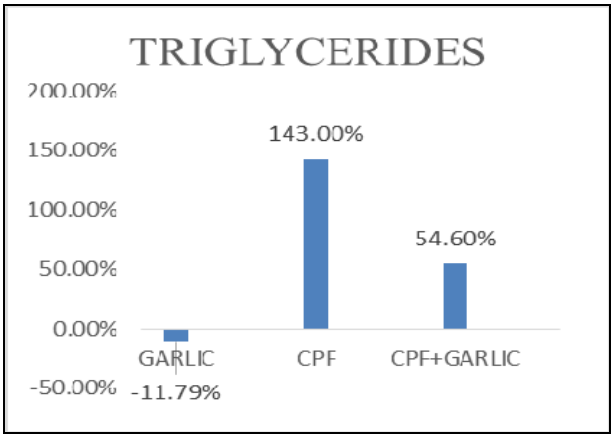

Fig 1.8: Triglycerides level in treated group

Fig 1: Liver enzymes and lipid profile after 14 days of oral administration of CPF, Garlic and CPF coadminstered Garlic. Abbreviation used: ALT (Alanine Transaminase), AST (Aspartate aminotransferase, ALP (Alkaline phosphatase), LDH (Lactate dehydrogenase), LDL (Low density Lipids), HDL (high density lipids)

Table 1: Biochemical parameters exhibited by different groups after 14 days treatment

S#	Parameters	Control	Garlic	CPF	CPF+GARLIC
01	AST (U/L)	25.00±2.88	19.67±0.88	31.67±0.58	24.67±2.19
02	ALT(U/L)	77.8±2.84	67.33±1.33*	86.67±13.00	72.00±0.58
03	ALP(U/L)	2.12E2±4.41	1.75E2±13.00	2.43E2±14.36	2.10E2±20.33
04	LDH(U/L)	4.34E2±2.73	4.22E2±3.94	4.72E2±21.20*	4.50E2±5.79.
05	LDL mg/dl	24.67±1.45	20.00±0.58*	38.0±3.06*	24.00±0.58
06	HDL mg/dl	23.00±1.55	24.33±1.20	18.00±0.58*	20.67±0.33
07	CHOL mg/dl	74.67±2.72	58.33±4.05*	92.67±3.93*	76.00±5.03
08	TGs mg/dl	76.33±2.91	67.33±2.73	1.86E2±21.07**	1.18E2±4.90**

Table (1) shows the biochemical parameters exhibited by different groups

Values are expressed as mean of 3 animals Mean ±SEM, Student t- test *Significance (p<0.05), **More significance (p<0.01), ***High significance (<<0.001). Abbreviation used: ALT (Alanine Transaminase), AST (aspartate Transaminase), ALP (Alkaline phosphatase), LDH (Lactate dehydrogenase), LDL (Low density Lipids), HDL (high density lipids), CHOL (Cholesterol), TGs (Triglycerides)

5. Conclusion

The results of present study demonstrated that Chlorpyrifos caused oxidative stress by increasing lipid per oxidation in rabbits. Garlic being an antioxidant played ameliorative role against CPF toxicity by scavenging free radicals resulting in a decreased lipid per oxidation.

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