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Mollifying actions of garlic (*Allium sativum*) against Chloropyrifos induced toxicity by using rabbit (*Oryctolagus cuniculus*) as a model subject

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Abstract

Potency of garlic is being acknowledged for years and is frequently used as a remedy for different diseases. In developing countries, the widespread use of pesticides in daily life has caused severe environmental pollution and consequently health hazards, with cases of severe acute and chronic poisoning in organisms. Recently, a growing interest has been shown into utilizing the biological activities of different medicinal herbs against diseases, due to their natural origin, lesser side effects and cost effectiveness. The current study was designed to evaluate the therapeutic role of garlic against the Chloropyrifos (CPF) induced toxicity in male rabbits (*Oryctolagus cuniculus*). For this purpose, male healthy rabbits (N=24) were procured from the local market and were divided into eight groups on the basis of acute and chronic toxicity evaluations. Group "A" and A1 were left untreated and were considered as the control group for acute and chronic toxicity trials respectively. Group "B" and B1 were the Garlic fed group while group "C" and C1 were the Chloropyrifos (CPF) treated group, while group "D" and D1 were garlic co Chloropyrifos (CPF) administered group. Rabbits were orally administered with doses of garlic and CPF for a period of 14 and 60 days on daily basis for evaluating acute and chronic toxicity respectively. At the end of the trials, blood samples were collected in tubes for investigation of the hematological indices. Hematological analyzer (Sysmex KX-21 N, USA made) was used for analysis. The tested parameters showed a variability in values in comparison to the untreated group. In the acute toxicity experiment, a significant percent decrease in the level of Hb (9.83%), RBCs (16.54%), WBCs (20.64%), HCT(12.63%), Plts(5.5%) were observed in pesticide treated group while in garlic fed group the level of Hb (13.44%), RBCs (15.8%) ,WBCs (29.8%), HCT(21.5%), Plts (15%) showed % increase as compare to CPF and Control groups. In CPF co- Garlic administered group, garlic has attenuated the toxic effects of CPF. In conclusion CPF is found to be hematotoxic in nature both in acute and chronic doses and Garlic was found to be protective against its toxicity hence reflecting its importance. Therefore it could be advised as an effective dietary supplement in the developing countries where the use of pesticide is high.

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Introduction

Chlorpyrifos (O, O-diethyl-*O*-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate is an extensively applied insecticide for agricultural and household purposes. It controls fleas, beetles, grubs, fire ants, flies, cutworms, lice and termites and is applied on lawns, cotton, grain, fields, fruits, nuts, vegetable and ornamental plants. The pesticide is available in wettable powder form, granular form, emulsifiable concentrate and dustable powder. Some of the trade names include Brodan®, Dowco 179®, Dursban®, Empire®, Equity®, Eradex®, Lentrek®, Lock-On®, Detmol UA,® Lorsbanv®, Pageant®and Piridane (Tomlin, 2006). Its use was restricted in North America in 2000 because of the concerns over the safety of chlorpyrifos and its metabolites but still, CPF stays in many dwellings despite these constraints (Whyatt *et al.,* 2009). Chloropyrifos being Organophosphorus in nature, are potentially toxic on non-targeted organisms. They bind to the cholinesterase enzyme and inhibits its activity by irreversible phosphorylation leading to increased levels of acetylcholine and stimulating the muscarinic and nicotinic receptors consequently resulting in toxicity (Abou-Donia, 2004). OP insecticides toxicity may occur by inhalation, ingestion or through skin contamination in organisms (Vale, 1998).Oxidative stress caused by CPF is another mechanism of its action in animals (Slotkin *et al*., 2006). It stimulates adverse effects including hemotoxicity (Ambali *et al*., 2010a, 2011b; Uchendu *et al*., 2011a), hepatic dysfunction (Goel *et al*., 2005; Zama *et al*., 2007; Ambali *et al*., 2011a), immunological abnormalities (Blakley *et al.,* 1990; Cox, 1994; Thrasher *et al.,* 1993, 2002), neurochemical and neurobehavioral changes in animals (Dam *et al.,* 1999; Slotkin *et al.,* 2006).

In recent years, preferences have been given to the use of Natural products of animals, plants and microbial sources for the cure of different diseases by man (Parekh and Chanda, 2007). Garlic (*Allium sativum*) is one of the most popular herb, which is used globally to decrease various risk factors associated with several diseases. It belongs to the Liliaceae family (Thomson *et al*., 2007).

Past studies have suggested the potential antilipidemic, antihypertensive, antiglycemic, antithrombotic and antiatherogenic properties of garlic (Bordia *et al*., 1975).

The objective of the current study was to find out the toxicological effect of Chloropyrifos on hematological parameters of animals and the protective role of garlic against it.

Materials and methods

Male rabbits (Oryctolagus cuniculus) were used as a model animal in the trial. The study was conducted in the month of May and june, 2017 at the animal house of Islamia College, Peshawar abiding by the animal ethics. They were acclimatized to the laboratory conditions for fourteen days prior to the experiment and were caged properly with a regular monitoring for cleanliness. It was ensured to provide a period of equal intervals of dark and light photoperiod, proper ventilation, normal temperature of 25 - 30 degree centigrade and food and water supply ad labitum to the animals during the acclimatization and experimental durations.

Experimental design

Twenty four healthy rabbits (*Oryctolagus cuniculus*) weighing I kg each procured from the local animal market and checked for any clinical abnormalities. The healthy rabbits were chosen and acclimatized to the laboratory conditions prior to the experiment for a period of 14 days. Experiment was divided into two section, one for acute toxicity and other for chronic toxicity evaluation of the chloropyrifos. Chloropyrifos was purchased from the local market prepared by Novartis Company with a 46% .A stock solution of chloropyrifos was made and dilutions for 100mg/ml and 20mg/ml were prepared by dilutions formula C1V1=C2V2. Garlic bulbs (30 g) were crushed and added in 60 ml of distilled water to form a solution and squeezed through a double cheesecloth. The aliquots of fresh garlic extract were stored at −20 ◦C till further use during the experiment. One ml of the

extracted aliquot was equivalent to about 500 mg of garlic (Flora *et al.,* 2009).Animals were divided into eight groups, 4 groups in each toxicity evaluation experiment. In acute toxicity experiment Group "A" was kept untreated and called control. Group "B was fed with 95mg/kg body weight of garlic and named garlic group. 100mg/kg body weight chloropyrifos was administered to the group "C" called CPF group. The fourth group (CPF co Garlic administered group) was orally treated with 95mg/kg body weight garlic prior to 100mg kg body weight CPF for a period of 14 days. In chronic toxicity experiment Group "A1" was kept untreated and called control. Group "B1 was fed with 95mg/kg body weight of garlic and named garlic group. 20mg/kg body weight chloropyrifos was administered to the group "C1" called CPF group. The fourth group D1 (CPF co Garlic administered group) was orally treated with 95mg/kg body weight garlic 1 hour prior to 20mg kg body weight CPF for a period of 60 days. The doses were administered according to the pesticide and toxic substance guideline following the limits test procedure for animals.

Blood collection

Blood collection was done at the end of each experiment (i.e. at day 15th from animals of acute toxicity trial and on 61st day of the chronic toxicty trial). The marginal ear vein of the rabbit was chosen for the collection. The hairs over the marginal veins were shaved and were cleaned with 70% alcohol prior to blood collection. A small amount of xylene swabbed was applied on the tip of the ear to increase the blood flow in the vein. Blood was collected with the help of 5cc syringes in EDTA tubes and proper mixing of blood with EDTA was ensured to avoid clotting. Xylene was then washed off with 70% alcohol after the collection in order to minimize skin irritation.

Hematological studies

The USA made Automated Hematological Analyzer Sysmex KX-21 N made was used for analyzing the selected hematological parameters. The parameters selected included red blood corpuscles (RBCs), white blood corpuscle (WBC), hemoglobin (Hb), and packed cell volume (PCV), platelets (PLTs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Statistical analysis

Student "t" test or Analysis of Variance (ANOVA) is applied for data analysis of test and control sample and their mean values were compared by using the software SPSS version (16). Values of $P<0.05$ are significant.

Results

After administering the rabbits with different doses of Chloropyrifos, the animals displayed different clinical signs and symptoms like sneezing, licking of forelimbs, hind limbs and other body parts were observed within 5 - 10-minutes of feeding. Diarrhea, reduced appetite, laziness, jerky movements, muscular spasm, increased salivation, sneezing, coughing, tearing and red eyes In experiment conducted on evaluating chronic toxicity, CPF treated animals presented excessive sneezing and coughing, a loss of appetite with a consequent weight loss, dysuria and in coordination. Dizziness and laziness were also displayed and in coordination was observed in animals after dose administration.

Acute toxic effects of CPF (100mg/kg body weight) on hematological parameters of male in a 14 days trial

Level of RBCs in control group was 7.07±0.21. Its level was 8.2±0.18 with a significant increase of 15.93 % in garlic administered group. The RBCs count in CPF adminstered group was 5.90±0.21 with a significant decrease of 16.54%. In CPF co-garlic administered group, RBCs level was 6.2±0.12 with a significant decrease of 12.30% (Table 1, Fig**.** 1). The WBCs value of control group was 4.07±0.12 while in garlic administered group, it was 5.27±0.15 with a more significant increase of 29.5 %.WBCs count in CPF fed animals was 3.23±0.18 with a significant decrease of 20.64% and 4.1±0.12 with an increase of 0.7 % in CPF co-garlic administered animals. (Table 1, Fig**.** 1).

Table 1. Showing hematological indices of rabbits administered with a dose of 95mg/kg Body weight of garlic and 100 mg/kg body weight of CPF.

S.NO	Parameters (units)	Control (n=3)	Garlic (n=3)	CPF(n=3)	CPF+ garlic (n=3)
01	RBC×10^6/µl	7.07±0.21	8.2±0.18*	5.90±0.21*	6.2±0.12*
2	WBC(TLC)×10^3/µl	4.07±0.12	5.27±0.145**	3.23±0.18*	4.1±0.12
3	Hemoglobin g/dl	11.9±0.24	13.5±3.2*	10.73±0.22*	12.13±0.24
4	HCT(PCV) %	37.2±0.44	45.2±0.44***	32.5±0.8**	36.2±1.64
5	Platelet Count×10^3/	3.6E2±4.8	4.14E2±8.11**	3.4E2±5.2*	3.63E2±4.1
6	MCH pg	17±0.6	16.33±0.6	18.33±0.44	17.5±0.3
7	MCHC g/dl	32.2±0.60	30.0±1.0	33.2±1.0	33.12±1.7
8	MCV Fl	54.33±0.44	53.4±.83	57±1.2	55.1±0.7

Values are presented as a mean of three animals in a given group Mean±S.E, students t-test, *Significance ($p<0.05$), **More significance ($p<0.01$), ***High significance ($p<0.001$) in comparison to Control Group.

Level of Hemoglobin (Hb) presented in control group was 11.9±0.24. Hb count was 13.5±3.2 with a significant increase of 13.44% in garlic treated rabbits. In CPF administered group Mean ± S.E value for Hb was 10.73±0.22 with a significant decrease of 9.83% while its value was 12.13±0.24 with an increase of 1.93% in CPF co garlic fed rabbits. (Table 1, Fig. 1).

The value of packed cell volume (PCV)/HCT in control group was 37.2±0.44 and 45.2±0.44 in Garlic administered group with a highly significant increase of 21.50 %. a significant decrease of 12.63% was presented by CPF group while CPF co-garlic administered group showed a decrease of 2.7% with a Mean±S.E value of 36.2±1.64 (Table 1, Fig. 1).

Platelets count was 3.6E2±5 in control group and 3.4E2±5.2 with a significant decrease of 5.5% in CPF administered animals. Animals of Garlic fed group showed a more significant increase of 15% while CPF co-garlic administered animals showed an increase of 0.83% with a Mean±S.E value of 3.63E2±4.1 (Table 1, Fig. 1).

Mean Corpuscular Hemoglobin (MCH) level in control group was 17±0.6 pg. The value was 16.33±0.6 with a decrease of 3.94% in Garlic fed rabbits. in CPF administered group, Mean±S.E value of MCH was 18.33±0.44 with an increase of 7.82 % and 17.5±0.3 with an increase of 2.94% in CPF co garlic fed group. (Table 1, Fig. 1).

In control group, Mean Corpuscular Hemoglobin Concentration (MCHC) was 32.2±0.60 g/dl while 30.0±1.0 with a decrease of 6.83% in garlic treated group. Mean±S.E value of MCHC in the CPF administered animals was 33.2±1.0 with a 3.10% increase while in CPF co- garlic animals MCHC showed an increase of 2.85%. (**Table** 1, Fig. 1).

Mean Corpuscular Volume value was 54.33±0.44fL in control group and 53.4±0.83 with a significant decrease of 1.71% in garlic administered rabbits. in CPF administered group, the value of MCV was 57±1.2 with a 4.91% increase while CPF co garlic fed animals showed an increase of 1.41% (Table 1, Fig. 1).

Chronic toxic effects of CPF (20mg/kg body weight) on hematological parameters of male in a 60 days trial

In evaluating chronic toxicity trials of CPF, a dose of 20mg/kg BW Chloropyrifos was orally administered to rabbits in CPF group for 60 days once a day and 95mg kg BW garlic was fed to rabbits in garlic group. In CPF co- garlic group, 95mg/kg body weight garlic was fed to rabbits 1 hr prior to the 20 mg/kg body weight of CPF. The RBCs level in control group was 6.0±0.57. RBCs count was 7.2±0.2 in garlic administered group with a significant increase of 20 % while 6.45±0.18 with a significant increase of 7.5% in CPF administered animals. In animals of CPF co-garlic administered group, RBC count was 6.8±0.13 with a significant increase of 13.3% (Table 2, Fig. 2).

Table 2. Haematological values of Rabbits in 95mg/kg body weight of garlic fed, 20 mg/kg body weight of CPF fed and a combination of CPF and garlic administered groups for 60 days.

S #	Parameters (Units)	Control (n=3)	Garlic (n=3)	CPF(n=3)	CPF + Garlic (n=3)
01	RBC×10^6/µl	6.0±0.570	7.2±0.2***	6.45±0.18	6.8±0.13**
2	WBC(TLC)×10^3/µl	3.93±0.09	5.93±0.7*	8.53±1.53*	7.3±0.27***
3	Hemoglobin g/dl	12.2±0.17	12.6±0.71	11.53±0.3	11.9±0.61
4	HCT(PCV) %	38.13±0.19	38.4±2.24	35±1.2*	37.5±1.2
5	Platelet Count ×10^3/	3.4E2±6.4	2.12E2±20.3**	1.25E2±6.9***	1.55E2±23.2*
6	MCH pg	20.3±0.4	18±0.53*	19±0.2	18.53±0.4*
7	MCHC g/dl	32±0.32	33±0.15	32.3±0.21	32.1±1.01
8	MCV fL	63±0.62	54.7±1.40**	58.3±0.64**	57.60±0.70**

Above Values are presented as a mean of three animals in a given group Mean±S.E, students t-test, *Significance ($p<0.05$), **More significance ($p<0.01$), ***High significance ($p<0.001$).

WBCs count was 3.93±0.09 in control group and 5.93±0.7 with a significant increase of 50.8 % in garlic fed animals.

In CPF administered animals, WBCs level was 8.53±1.53 with a significant increase of 117.08% while In CPF co-garlic administered group, the value was 7.3±0.27 with a highly significant increase of 85.8% (Table 2, Fig. 2).

Hemoglobin (Hb) level was12.2±0.17g/dl in control group. in garlic administered animals, RBCs count was 12.6±0.71 with an increase of 3.3%. Hb value was 11.53±0.3 in CPF group with a decrease of 5.5 and 11.9±0.61 in CPF co-garlic administered with a decrease of 2.5% (Table 2, Fig. 2).

HCT level in control group was 38.13±0.19% and 38.4±2.24 in garlic administered animals with an increase of 0.3%. PCV value was 35±1.2 with a significant decrease of 8.6% in CPF administered group. in CPF co-garlic administered group, PCV showed value of 37.5±1.2 with a decrease of 2.09% (Table 2, Fig. 2).

Platelet count was 3.4E2±6.4 in control group. it was 2.12E2±20.3 in garlic administered group with a highly significant decrease of 8.8 % and 1.25E2±6.9 with a significant decrease of 63.45% In CPF administered group, Platelet count was 1.55E2±23.2 with a significant decrease of 54.7% in CPF co-garlic administered group (Table 2, Fig. 2).

MCH Value in control group was 20.3±0.4pg while In garlic administered group, it was 18±0.53 with a significant decrease of 11.33 %. In CPF administered animals, observed MCH level was 19±0.2 with a significant decrease of 6.4% and 8.53±0.4 with a significant decrease of 8.7% in CPF co-garlic administered group (Table 2, Fig. 2).

MCHC level in control group was 32±0.32g/dl. in garlic administered group MCHC Value was 33±0.15 with an increase of 3.13 %. MCHC in CPF administered was 32.3±0.21 with an increase of 0.94% while in CPF co-garlic administered group, it was 32.±1.01 with an increase of 0.9% (Table 2, Fig 2).

MCV level was 63±0.62fL in control group. MCV value for garlic administered group was 54.7±1.40 with a significant decrease of 13.2 % while in CPF administered it was 58.3±0.64 with a significant decrease of 0.8%. CPF co-garlic administered group presented an MCV value of 57.60±0.70 with a significant decrease of 0.9% (Table 2, Fig. 2).

Discussion

The effects of CPF on hematological parameters of male rabbits and the possible protective role of garlic is presented in Table 1and Figure 1 for acute toxicity trial and Table 2 and Figure 2 for chronic toxicity

trial. The tables are showing mean standard error values of the indices while the figures are presenting the percent differences. Blood indices significantly change in response to any xenobiotics like pesticide or drugs. It has also been used by researchers for detection of any physiopathological changes might occur due to external stress in form of a pesticide (Modesto and Martinez, 2010; Kumar *et al.,* 2011).

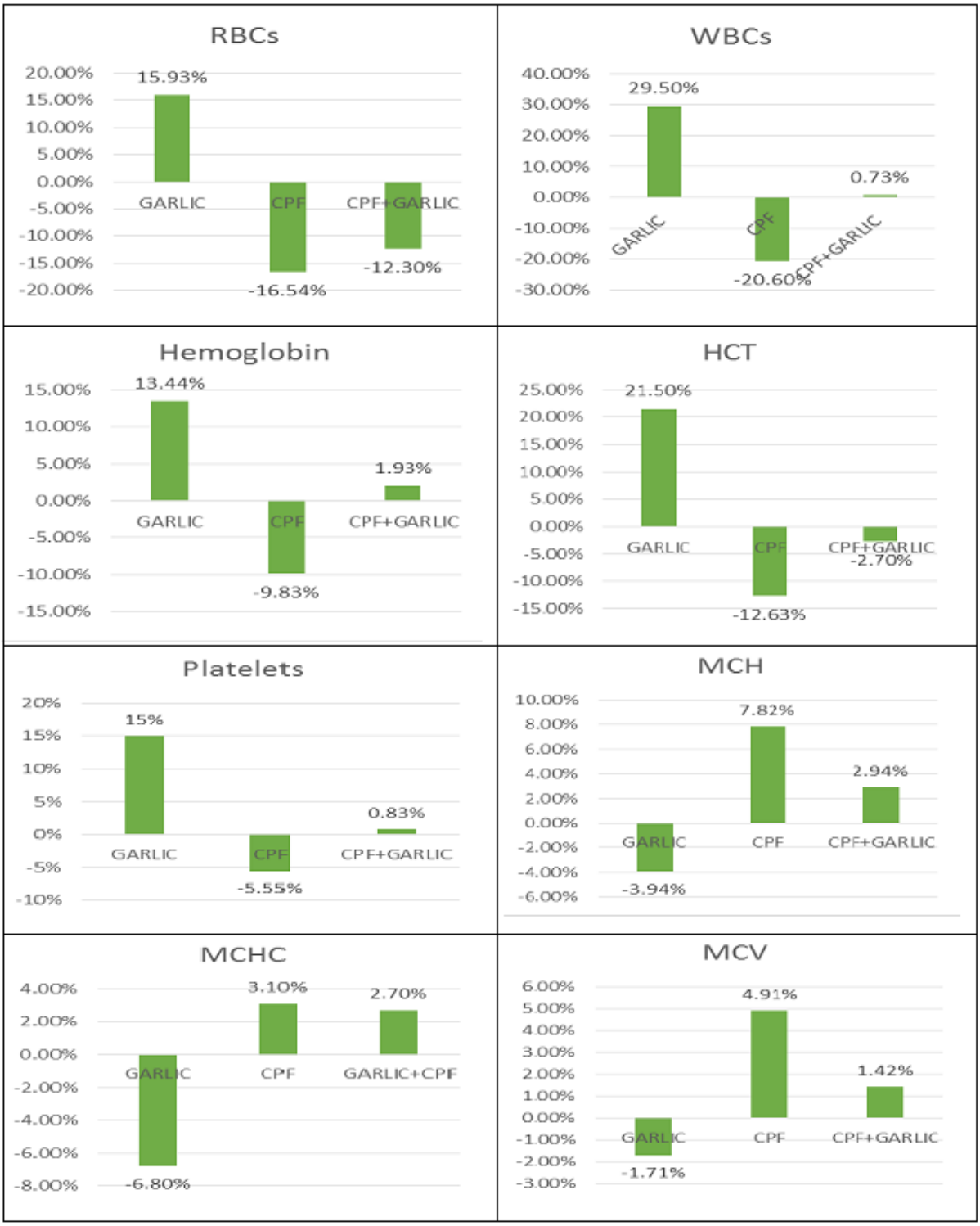

Fig. 1. Haematological indices of male Rabbits fed with the 95mg/kg body weight of garlic fed, 100 mg/kg body weight of CPF and a combination of garlic and CPF in comparison with the control group ***Abbreviations****: red blood cells (RBCs), White blood cells (WBCs), packed cell volume (PCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV).*

In the present study on acute study, Red Blood Cells have shown a significant increase of 15.93% in garlic administered group and a significant decrease of 16.54% in Chloropyrifos treated group of animals. In CPF co garlic administered group, a significant decrease of 12.30% was observed in red blood cells value (Table 1 and Fig. 1). In chronic toxicity trial, red blood cells showed a significant increase of 7.5% in garlic fed animals and 20% in CPF fed rabbits. The RBCs value showed a significant increase of 13.3% In CPF co-garlic administered group (Table 2 and Fig. 2).

A similar result was observed by reporting a decrease in red blood cells in rabbits treated with diazinon and dimethoate insecticides (Salih, 2010). The main mechanism of CPF toxication is by causing lipid peroxidation in a living cell. It is the first step of cellular membrane damage caused by pesticides and any other xenobiotic (Gamble *et al.,* 1995). Red blood cells carry hemoglobin which transport oxygen, hence are susceptible to lipid peroxidation (Hall, 2006). Hydrophobic characteristics of erythrocyte's membrane are decreased by lipid peroxidation consequently changes its affinity and interaction of proteins and lipids. Changes lead to the impairment of the functioning and homeostasis of the membrane of Red blood cells (Dargel, 1992). The toxicants have a hemolyising power on red blood cells membrane and can cause a decrease in RBCs (Ramesh and Saravanan, 2008). Inhibition of Erythropoiesis, haemosynthesis or osmoregulatory dysfunction caused by toxicant can also cause a decrease in the RBC levels. Oxidative denaturation of the globin part of hemoglobin results in the formation of Heinz bodies within red blood cells. These binds to the inner surface of RBC membranes resulting in premature RBCs phagocytosis and the hemolytic anemia. In Garlic treated group, the RBCs level was higher as compared to the control group. In garlic co CPF group, garlic has reduced the toxicity of CPF. Phenolic and flavonoids compounds give garlic its antioxidant properties. It helps in scavenging the free radicals produced as a result of toxic substances and prevent or decrease the lipid peroxidation process (Miller *et al.*, 2000). Past studies have reported the beneficial effects of aged garlic extract against changes in hematological parameters after CPF intoxication. Garlic possesses ingredients that stimulate the erythropoietic system of a living body, resulting in the stimulation of blood cell forming organ and hence results in the increased red blood cells amount (Toghyani *et al.*, 2011).

Hemoglobin level showed an increase in garlic administered group. Hb in Chloropyrifos administered group for toxicity trials showed a decrease in its value as compared to control group. In CPF co -garlic administered group, garlic has attenuated the toxic effects of CPF on hemoglobin level to some extent (Table 1 and Fig. 1).

Similar pattern of variations was observed in animals of chronic toxicity trial. Hb level presented a decrease of 5.5% in CPF fed animals and an increase of 3.3% in garlic fed animals. In CPF co Garlic fed group, Hb exhibited a decrease of 2.5% (Table 2 and Fig. 2). Similar pattern with a decrease in Hb was reported in rabbits, orally treated with Malathion and Cypermethrin for a duration of 30 and 60 days (Riaz, 2017). The present result of an increase Hb in garlic treated animals is in agreement with the significant increase observed in Hb level in rabbits fed with 100mg/kg body weight garlic (Oluwole, 2001).

The lowering of hemoglobin level after CPF application in the present study indicates that it has caused erythropenia and acute anemia as reported in the past studies (Auta, 2001; Svoboda *et al.,* 2001). The decrease in HB level due to CPF administration might have occurred because of the release of oxygen radicals due to the toxic stress of CPF (Muttappa, 2015). The decrease in Hb content of the CPF treated animals, may be attributed to decreased synthesis of Hemoglobin, an increase in the rate of its destruction and slower activities of hemopoietic organs (Yousafzai, 2004). An increase in Hb level was found in garlic fed group while a mollifying action of garlic against CPF was observed in CPF co Garlic fed the group.

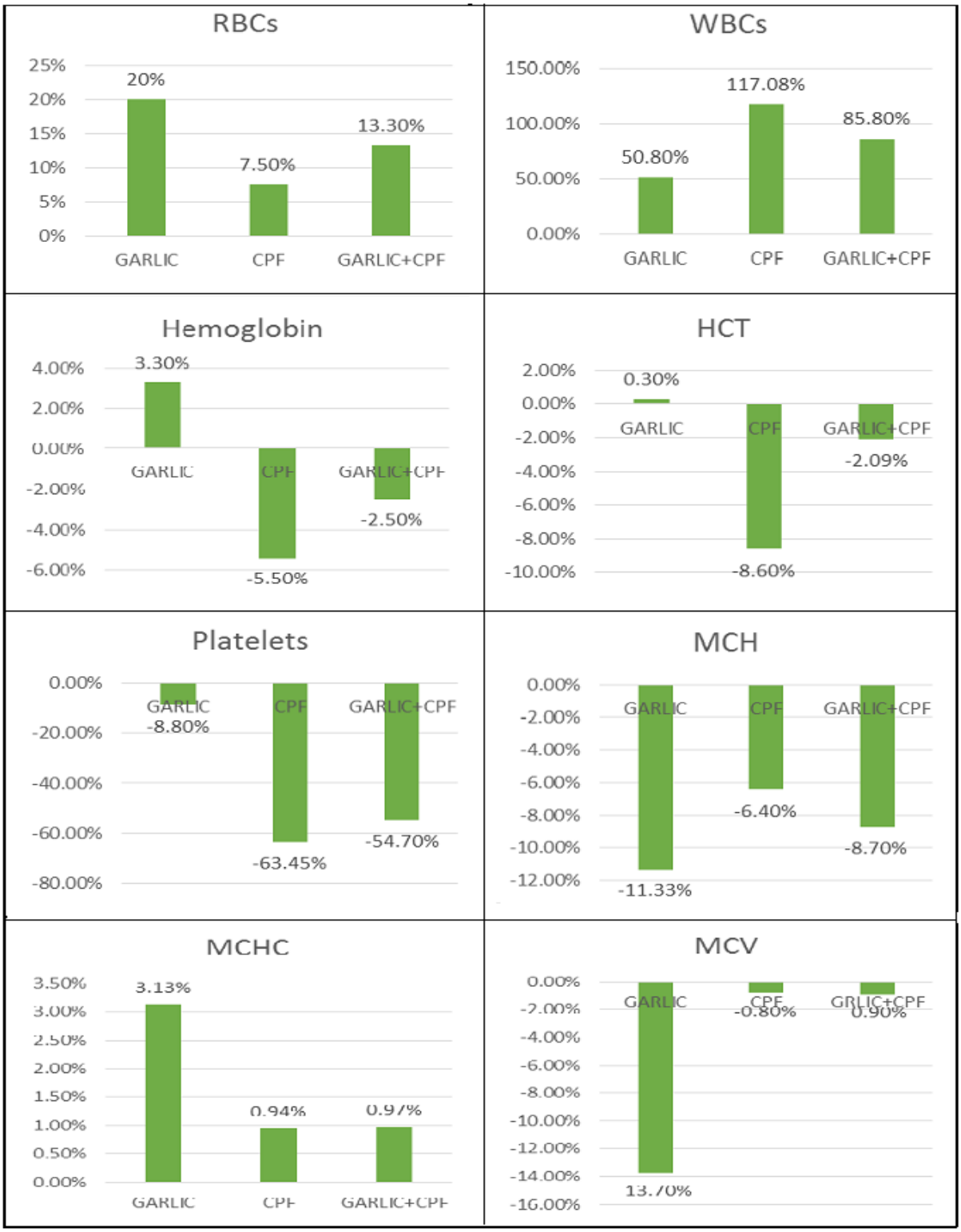

Fig. 2. Showing effects CPF and Garlic in 60 days trial on Haematological values of blood of different groups.

The initial step of heme synthesis (iron part of Hb) occurring in mitochondria, requires vitamin B6 (pyridoxine). The process is catalyzed by an enzyme δ - aminolevulinic acid (ALA) synthase. The increase in Hb in garlic group is most probably due to the fact that fresh garlic products after crushing are a source of many vitamins, minerals, trace elements and Sulphur compounds that help in its synthesis.

In garlic metabolism, the end product Allicin, can also stimulate kidney for the production and secretion of erythropoietin (Toghyani *et al.,* 2011).

The level of white blood cells showed a significant increase of 29.5% in garlic treated group and a significant decrease of 20.6% in CPF administered animals. In CPF co-garlic administered group, garlic has kept the WBC level closer to the reference ranges by attenuating the toxicity of CPF (Table 1 and Fig. 1).

In chronic toxicity evaluation of CPF, the WBCs showed a significant increase of 117.08% in CPF fed group while a significant increase of 50.8% in garlic treated animals. In CPF co Garlic fed group, animals exhibited a significant increase of 85.8% in WBCs level (Table 2 and Fig. 2).

In a past study, a similar significant decrease was found in total leukocyte count in Cypermethrin fed male rabbits (Ahmad *et al.*, 2009). The decreased level of WBC is attributed to the immunosuppressive effects of the organophosphorus pesticide (Ahlam, 2009). The leukopenia observed in the experiment may have occurred due to neutropenia and lymphocytopenia in animals (Ambali *et al.*, 2007). A slower rate of WBCs production or their inhibited release in the circulatory system can be a reason for the decrease in white blood cells (Goel *et al.,* 2006a). The immunotoxic potency of CPF may be attributed to the decrease in WBC level. Garlic *(Allium sativum)* has important constituents i.e. selenium, vitamins, Sulphur which play an important role in the stimulation of immune system and functioning of the organs related to blood cells formation such as thymus, spleen and bone marrow (Jeorg and Lee, 1998). A little elevation in leukocytes count in garlic administered groups was observed, which may be due to immunostimulatory effects of garlic (Parsad and Virmani, 2009).

The present findings are in accordance with the observation made by Ramesh and Saravanan, (2008), in which a significant increase in total leucocyte count in *Cyprinus carpio* was observed after CPF administration. This increase in leukocytes might be due to activation of the immune system of the animals treated with any contaminant i.e. a pesticide (Modesto and Martinez, 2010).

In the present study on the acute toxicity of CPF, PCV exhibited a significant increase of 21.50% in garlic fed group while a significant decrease of 12.6% was observed in the CPF administered group. In CPC co-garlic administered group, garlic tends to keep the PCV level closer to the control group by mollifying the toxicity of CPF with a decrease of 2.7% (Table 1 and Fig. 1). In a chronic toxicity trial, PCV showed an increase in garlic and a decrease in CPF administered group. In garlic co-administered group, garlic mollified the toxic effects of CPF (Table 2 and Fig. 2).

The present results agree with the past reports. A significant decrease in PCV parameter was observed in female rabbits exposed to Malathion (an organophosphate insecticide (Shaheen, 2017). Similar findings of decrease in PCV was observed in male rabbits treated with Carbofuran and Cypermethrin with high and medium doses (Iqbal, 2012). The decrease in hematocrit (PCV) level might happen either an animal loses its appetite in diseased or is poisoned with pesticides (Gill and Pant, 1985). Alteration in the metabolic rate of an organism can also lead to low HCT values. The low rate of erythropoiesis or hemolytic activities in an organism can also lead to low HCT values (Larson, 1975). The decrease in packed cell volume may also have occurred because of non-regenerative anemia due to direct injury to the hematopoietic organs in animals (Das, 2007). Garlic has ingredients that stimulate the erythropo**ie**tic system to produce more RBCs and increased value of packed cell volume. The Increase in PCV level in the garlic group can be attributed to the antioxidant activities of *Allium sativum* (Toghyani *et al.,* 2011).

The platelet count in the present study showed a significant increase of 15% in garlic treated group and a decrease of 5.55% was observed in the CPF treated group. Garlic reduced the toxic effects of CPF in CPF co Garlic administered group (Table 1 and Fig. 1). The significant decrease in platelet in CPF intoxicated animals is an indication of thrombocytopenia (Ambali, 2010) that might have happened because of the oxidative damage caused by CPF to platelet

membranes. A direct relationship between oxidative stress and thrombocytopenia in patients treated with malarial parasites were observed (Araujo, 2008). Rats treated with vitamin A and E showed a significant improvement in the level of platelets suggesting the leading role of oxidative stress in CPF-induced thrombocytopenia in living cells (Ambali, 2010). The present increase in platelets count in garlic treated group of rabbits can be attributed to the stimulation of bone marrow activity by Allium species (garlic).

In trial of chronic toxicity of CPF, the platelets showed a different pattern of variations in different groups. In CPF fed animals, platelets showed a significant decrease of 63.5%. A decrease of 8.8% and 54.7% was observed in Garlic and Garlic co CPF groups respectively (Table 2 and Fig**.** 2). In a study on insecticide phostoxin, platelet count presented a significantly lower value in rabbits exposed to said insecticide (Okolie *et al.,* 2004). Decrease in platelet indices was observed in the group of animals received 1g/kg and 3.5g/kg body weight of garlic extract for a period of 21 days on daily basis (Olaniyan, 2013). The significant decrease in platelets in animals that have been exposed to CPF is an indication of that exposing animals to chronic CPF caused thrombocytopenia (low platelets level) (Ambali, 2010). This decrease might be attributed to the oxidative damage caused by CPF to platelet membranes. A direct relationship between oxidative stress and thrombocytopenia in patients treated with malarial parasites was suggested by Araujo, (2008). The decrease in platelets count was observed in animals exposed to chronic garlic consumption might have happened due to the pro-oxidant activities of Allium species (Banerjee and Maulik, 2002). A significant decrease in platelets was observed in case of garlic treatment alone, which may be due to dual properties of garlic i.e. antioxidant as well as pro-oxidant activities. It is also known that a sulfur compound present in garlic significantly prolongs the bleeding time and thrombin time (Chan, 2007).

The garlic fed group presented a decrease in MCH, MCHC and MCV values and an increase in MCH, MCHC and MCV were found in CPF treated group in the acute study (Table 1 and Fig**.** 1). An increase in all these indices was presented by CPF co Garlic group. A decrease in MCH, MCHC, and MCV in the blood of *C.carpio* after 0.04mg/L CPF intoxication (Ural, 2013). A significant increase was observed in MCHC value in male rabbits treated with 5% garlic powder while no significant difference in 1% garlic fed a group of animals was seen as compared to control group (Al-Joweri, 2014). In CPF treated albino rats, a decrease in MCHC was observed with single, double and multiple doses (Savithri, 2010). The decrease in the MCH, MCHC and MCV level occurs due to RBC destruction and a decrease in Hemoglobin synthesis. The low level of hemoglobin content in red blood cells indicates microcytic hypo chromic anemia (Savithri, 2010). Iron deficiency, decreased Hb production, or sometimes vitamin B6 deficiency can also be the cause of low MCH level. Lipemia (turbidity caused by the accumulation of lipoprotein molecules) in a cell and hemolysis are the causes of increased MCH (Latimer, 2003). The reduction in blood parameters in intoxicated rabbits might have occurred due to malfunctioning of hematopoietic systems (Banaee *et al.*, 2008, 2011).

In trial on chronic toxicity of CPF, the level of MCH, MCV showed a decrease in all three groups while MCHC showed an increase in CPF, Garlic and CPF co Garlic fed groups (Table 2 and Fig**.** 2). Similar results with a Significant increase in MCV and MCH was also reported by Ambali *et al., (*2011) in Wistar rats exposed to chloropyrifos while MCHC did not alter significantly. The decrease in the indices might be due to due to Red Blood Cells destruction and a decrease in Hemoglobin synthesis and hemoglobin content in RBCs indicating the symptoms of microcytic hypo chromic anemia (Savithri, 2010). Banaee *et al.,* (2008, 2011) presented a reduction in blood parameters in Malathion intoxicated fishes and attributed it to the malfunctioning of hematopoietic system.

Abbreviations used

Red blood cells RBCs, White blood cells WBC, Packed

cell volume PCV, Mean Corpuscular Hemoglobin MCH, Mean Corpuscular Hemoglobin Concentration MCHC, Mean Corpuscular Volume MCV.

Conclusion

The current study suggests that Chlorpyrifos has affected hematological parameters and proven to be heamatotoxic in nature. Tremors and dizziness in animals in acute and chronic exposures to CPF represent nervous incoordination, reflecting damage to the nervous system.

The Hemotoxicity in response to CPF administration for acute and chronic studies, could have happened primarily by the generation of reactive oxygen species (ROS), which cause damage to the membranous components of the cell in an organism. CPF has caused a decrease in RBCs in acute and an increase in chronic studies while a decrease in Hb in both trials, which represents its hematotoxic nature. Garlic feeding caused an increase in RBC and Hb both in acute and chronic trials indicating its erythropoeitic activity. Increase in Hb in garlic fed animals is most probably due to Fresh garlic being a source of many vitamins, Sulphur compounds and minerals, and trace elements. CPF caused a fall in WBCs in acute and an increase in chronic trials, which showed its immunotoxic activities while an increase in WBC with Garlic feeding reflects its immunostimulatory effects. CPF might have caused an oxidative stress, as per the symptoms and results in hematological parameters observed.

In vitro exposure to the CPF has been reported to affect the activities of antioxidant enzymes present in a living cell. Garlic, being an antioxidant might have played a role in protection against the toxicity of CPF in the present study while the toxoicty of garlic can be attributed to its prooxidant nature.

Supervised use of the pesticide is recommended to minimize the toxic effects of pesticides on non target organisms and intake of Garlic in daily use is advised to add up its antioxidative properties along with body antioxidant enzyme to combat oxidative stress.

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