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Research Article

Hepatotoxic effects on subchronic exposure to chlorpyrifos insecticide in Pigeon (*Columba livia domestica*)

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Abstract

Indiscriminate use of pesticides in agriculture sector is getting more attention with regard to its Eco-toxicological impacts. The study was therefore conducted to evaluate the hazardous effects of Chlorpyrifos (CPF) insecticide on the liver of birds. A total of 40 mature and healthy pigeons (*Columba livia domestica)* were used in present study. Birds were divided in four equal groups as A, B, C & D. During acclimatization and treatment periods all birds were supplied equal quantity of food and water on daily basis. Birds in treatment group B, C & D were orally administrated orally 1.3mg, 1.6mg and 2.1 mg/kg body weight/day for conductive 36 days, while birds without CPF treatment kept as control group (A). During treatment period clinical and stress related signs were observed in birds of group (B-D). Highly significant decrease ($P<0.001$) in body weights and significant ($P<0.05$ & $P < 0.01$) decline in feed intake were observed in treated birds of groups (B-D) as compared to birds of control group. CPF induced highly significant ($P< 0.001$) increase in absolute and relative weights of liver and a significant ($P<0.01$) increase in liver enzyme, serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) in birds of group (C-D). Mortality was observed in all treatment groups. Histopathological alterations in liver revealed; enlargement, inflammations and vacuolation of hepatocytes with broaden sinusoidal in treated birds while birds in group D showed major histopathological changes. The results of present study revealed that, the exposure to chlorpyrifos insecticide may induce moderate to severe hepatic alterations in birds. Safe and justified use can minimize such hazardous effect.

Keywords: Biochemical; Chlorpyrifos; Hepatotoxic effects; Histopathological and Pigeon

Introduction

Indiscriminate and widespread use of pesticides to control and eradicate noxious insects/pests in agriculture sector is a more common practice and it is getting more attention with regard to its eco-toxicological impacts. These chemicals may increase yield production while this widespread use of pesticides is causing numerous ecological problems. Pesticides

are also used in livestock and poultry sectors to get rid of ticks, fleas, mosquitoes etc., but raised great concerns about animal's health. [1]. Toxic chemicals induce adverse effects that contribute several physical and biochemical abnormalities in human and animals [2]. Many broad spectrum pesticides are used in domestic and garden application to control and eradication of many species of fruit fly, fleas, ticks, mites and mosquito [3]. The uses of synthetic insecticides are major contributing source to environmental contamination and poses severe threats to public health [4]. Organophosphates (OP) are widely used pesticides in developed and developing countries to control or destroy the noxious pests in agriculture sector [5]. They exhibit significant physiological and cellular changes in exposed organisms by inhibition of cholinesterase and acetylcholinesterase enzymes activity [6]. Long term exposure to (OP) compounds can easily affect the exposed living organisms and cause major health problems as a result reduce their life expectancy [7]. Most of the developing countries, however, lack pesticide related regulations and enforcement. They import and use large number of banned and unregulated organophosphate insecticide [8]. Exposure to neurotoxin pesticides is associated to several diseases, including Alzheimer's, asthma, cancer and others. Living organism can be exposed to pesticides from different sources, including direct or indirect contact with pesticides, contaminated food or water, soil [9]. Organophosphate pesticides (OPs) have huge negative impacts on health especially on various species of mammals and avian [10]. The birds frequently feed on pesticides contaminated insects, grains, fruits and fishes as a result residues of such hazardous chemicals may cause severe alterations in their metabolic organs [11]. Chlorpyrifos (CPF) is a widely used organophosphate insecticide throughout the world to control the noxious pests of agriculture crops, stored food and grains, poultry, livestock and public health sectors. CPF generally metabolized in the liver and its metabolites excrete through kidneys [12]. Long term exposures to (CPF) can pose adverse effects on liver and kidneys of animals and cause severe physiological disorders [13]. Pesticides are toxic substance that may easily dispersed in the environment, where it reacts and rapidly break down in its metabolites that accumulates in the ecosystem and harm the living organisms in it, mainly the predatory avian species. Organophosphate pesticide when it enters into the food chain that damages nervous, metabolic and reproductive systems of exposed organism. CPF is an (OP) insecticide that can enter into the food chain from insects to small birds, and small birds to predatory birds such as Eagles, Hawks and Vultures. Consequently, accumulation of pesticides in birds can damage their health and reduce their population. Therefore, the present study was aimed to evaluate the intensity of biochemical and histopathological effects of chlorpyrifos in major metabolic organ of birds.

Materials and methods

Experimental birds and design

To evaluate the hepatotoxic effects, of chlorpyrifos insecticide a total of 40 healthy and mature pigeons (*Columba livia domestica*) were used in present study. All birds housed in neat and clean wooden wire cages under standard and controlled laboratory condition Pigeons were vaccinated and acclimatized for 15 days before commencement of the experiments. Birds provided standard feed (grains and seeds) and clean drinking water; subsequently birds were randomly divided in to four equal groups (A-D) and housed in separate cages. Their body weights were recorded before start of the experiments. Birds of groups (B, C and D) were assigned as insecticide treatment groups while group A birds kept as control.

Insecticide preparations and study protocol

The (Chlorpyrifos 40EC) insecticide concentration was prepared on **LD_{50}** basis according with toxicology methods [14]. The oral treatment at doses $1/25^{th}$, $1/20^{th}$ and $1/15^{th}$ of LD_{50} (1.3mg, 1.6mg and 2.1 mg/kg body weight/day) by adding in 5ml. distilled water were administrated in each birds of group B, C and D respectively for consecutive 36 days while birds of control group A received same quantity of distilled water. During study all birds of treatment and control groups were provided same quantity of diet (grains and seeds) along with drinking water on daily basis.

During experimental period clinical and stress related signs, symptoms and behavioral changes twice daily were assessed in birds of all groups. These were recorded on the basis of mild, moderate and severe. Mean body weights and feed intake records were compiled in order to compare treatment groups with control.

Biochemical test and Procedure

On day 37 at the end of experimental studies body weight of all birds was recorded. Thereafter 2 ml. blood were drawn from the wing vein of each bird by sterilized syringe and collected in simple vacutainers. Blood samples were centrifuged on 3000 to 4000 rpm and serum was separated for estimations of biochemical tests aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). All biochemical tests were performed through ultraviolet spectrophotometer by using commercially available kits as determined by [1].

Organ weight, morphology and histological procedure

At the end of experiment randomly selected six birds from each group were sacrificed and dissected. After dissections gross examinations, absolute and relative weights of liver were recorded. Retained organs were fixed in Bouin's solution for 24-48 hours for the assessment of histopathological alterations. Graded series of alcohol was used for tissues dehydration and small pieces of liver embedded in paraplast paraffin wax. Thin tissue sections 5 - 6µm were cut on Rotary microtome and stained with Harris's hematoxylin (Gurr, 1956) and Eosin (Putt, 1948). Furthermore, histological examinations and microphotography were performed on BestScope LCD Digital Biological LCD Microscope BLM 260.

Statistical analysis

Data was statistically analyzed by applying factorial analysis of variance (ANOVA). Significance levels were ($P< 0.05$), ($P< 0.01$) and ($P<0.001$) values defined as Mean ± SD. Mean difference between groups were analyzed through Least significant difference(LSD) test by using (Statistics 8.1) program.

Results and discussion

In developing countries like Pakistan mostly farmers are unaware of factual pesticides risks and they are frequently using such hazardous pesticides without following any precautionary measures and guidelines. Furthermore, they dispose used pesticide containers in the surroundings, which ultimately affect non-target vertebrates and invertebrates [15]. Chlorpyrifos (CPF) produces negative impacts on feed intake in birds especially at higher and sub- chronic doses. Their exposures also produce clinical and stress related signs of toxicity such as excessive salivation, gasping, lethargy, convulsions, and frequent defecation in birds. [16]. Rapid absorption and metabolism of chlorpyrifos pose drastic effects on metabolic organs [17]. OPs inhibit activities of cholinesterase enzyme in the avian and mammal species, subsequently it affects central nervous system and clinical manifestations may be observed like diarrhea, ptyalism, tremors, paralysis etc [18].

In present study similar type of clinical and stress related signs of toxicity including lethargy, tremors, diarrhea, excessive salivation, seizure, paresis and

drooping of wings were observed in treated birds within one hour of CPF administrations. Birds at higher dose 2.1 mg/kg body weight/day were unable to stand. Finally, six birds of group D four birds of group C and three birds of group B were died during experimental study and no mortality was observed in birds of control group (A). Birds of control group were vigorous and showed normal behavior throughout the study. They were vigilant at the time of feeding and their intake of feed was higher as compared to birds of treatment groups. In birds exposure to Organophosphate insecticides may cause reduction in feed intake and body weight consequently affects vital metabolic organs liver and kidneys [19]. Chlorpyrifos can cause significant reduction in mean body weight in exposed animals together with a significant increase in absolute and relative weights of liver [20, 21]. Overall feed intake in treated birds was significanty lower ($P<0.05$) as compared to control. Feed intake at day 24 and day 36 in groups C&D was reduced at significant ($P<0.01$) level, while group B showed significant ($P < 0.05$) decline in feed intake (Table 1). In present study treated birds of group B-D have shown a highly significant ($P< 0.001$) reduction in their body weights as compared to control group (Figure 1). However, an increase in body weight was recorded in control group birds. Such body weights before and after exposure to insecticides in treatment group are shown in (Figure 2). In accordance with previous finding, results of present study showed a significant increase ($P< 0.05$) in the mean and relative weights of liver in treated birds as compared to control group birds. Birds of group D showed highest increase in absolute and relative mean weights of liver in comparison to birds of group B, C. The enlargement of liver in treated birds was an obvious indication of CPF hepatotoxicity. While birds without treatment of CPF showed normal hepatic mass as compared to treated birds (Table 2). Serum levels of aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) significantly increase in CPF treated chickens; this clearly shows hepatotoxicity in birds [22]. It has been also reported by that, the increase in mean values of ALT, AST and ALP may be due to deterioration of hepatocytes and hepatic necrosis and this indicates organ and muscular damage [23]. The record increase in ALT, AST and ALP enzymes activities may be due to the toxic effect of CPF insecticide on liver [24]. In present study same trends of variations in liver enzymes of treated birds were observed. Such highly significant ($P<0.01$) increase was recorded in levels of serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) in birds of group B-D as compared to control group (Table 3). Hepatotoxicity is a common pathological feature of may result from long term accumulation of toxic chemical substances. Necroscopic observations of liver in birds shows paleness, swollen and crumbled consistency on exposure to organophosphate insecticides while untreated birds have dark brown coloration and normal consistency [25]. Chlorpyrifos produces mild to moderate hepatic lesions together with proliferation of major hepatic duct [26]. In addition CPF induces hyperplasia, congestion vacuolar disintegrations, dilation of sinusoids space and pyknoticnuclei [27]. Same numerous numbers of histological changes was observed in treated birds. The microscopic examinations revealed inflammation of hepatocytes in birds of group B (Figure 3b) whereas, group C birds showed vacuolatios and degenerative changes in hepatocytes (Figure 3c). In contrast to group B and C, birds of group D exhibited severe alterations like deteriorated hepatocytes, hepatic hypertrophy, necrosis, pyknotic nuclei and proliferation central and bile ducts as shown in (Figure 3d). The histological sections of liver from

control group birds appeared with normal structure of hepatocytes, central vein and normal sinusoidal space (Figure 3a).

Table 1. Indicates significant (P<0.05) decline of feed intake in Chlorpyrifos treated birds of groups C & D, at day 12. Also significant (P<0.01) decrease on day 24 & 36 in treated birds of groups C and D and (P<0.05) in group B and compared to the control group. Mean values not sharing same letters are indicating significantly different with each other

No of Days	GROUP A (Control)	GROUP B	GROUP C	GROUP D
DAY 12	58.57±4.5^{a}	53.5±3.5^{a}	48.28±4.28^{b}*	41.21±2.88^{c}*
DAY 24	62.583±3.95^{a}	51.64±3.1^{b}*	45.5±2.85^{c}**	40.35±3.5^{c}**
DAY 36	65.33±4.1^{a}	49.5±3.57^{b}*	43.07±3.10^{c}**	38.12±3.08^{d}**

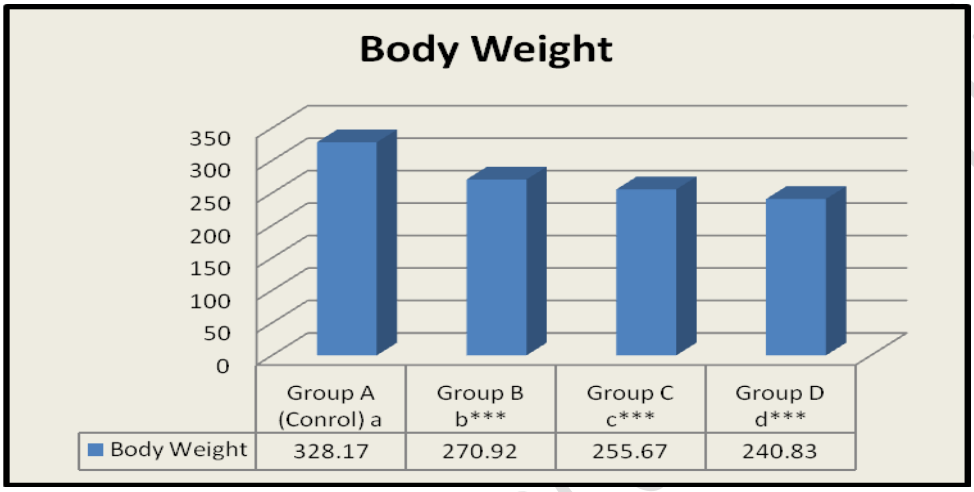

Figure 1. Showing Body weights in treated birds of groups B, C & D with highly significant decline (P < 0.001) * as compared to control group at the end of experiment day. Mean values not sharing same letters are significantly different with each other**

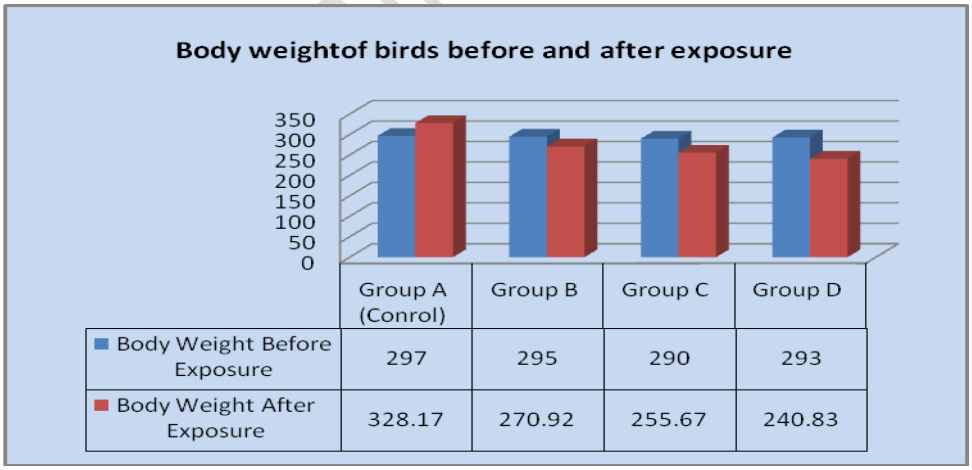

Figure 2. Birds of group B, C & D are showing highly significant (P < 0.001) * decline in their body weights after exposure to insecticide as compared before exposure. While Control group birds at the end of experiment day showing highly significant (P < 0.001) *** increase in their mean body weight as compared to day before commencement of experiment on day 01**

Table 2. Liver weight (Absolute) in chlorpyrifos treated birds of treated group B-D with significant increase (P < 0.001) * as compared to group A. Mean Values not sharing same letters are significantly different with each other**

Groups	liver Weight (Absolute)	Relative Weight%
Group A (Control) d	6.49±0.08 ^{d}	1.97±0.02 ^{d}
Group B c***	6.97±0.01^{c***}	2.57±0.04^{c***}
Group C b***	7.25±0.09^{b***}	2.85±0.07^{b***}
Group D a***	7.75±0.06^{a***}	3.22±0.04^{a***}

Table 3. Serum biochemical parameters in chlorpyrifos treated birds of Group B-D with significant (P < 0.01) ** increased mean values as compared to Control. Values sharing not same letters are significantly different with each other. Serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP)

Parameters	Group A (Control) Mean± SD U/L	Group B Mean± SD U/L	Group C Mean± SD U/L	Group D Mean± SD U/L
ALP	293±21.88^{a}	397±24.75^{b**}	640±23.33^{c**}	843±28.45^{d**}
AST	168 ±6.44^{a}	304.5±13.23^{b**}	424±15.33^{c**}	497±17.88^{d**}
ALT	14.8±2.33^{a}	24.7±3.44^{b**}	31.6±3.88^{c**}	39±4.10^{d**}

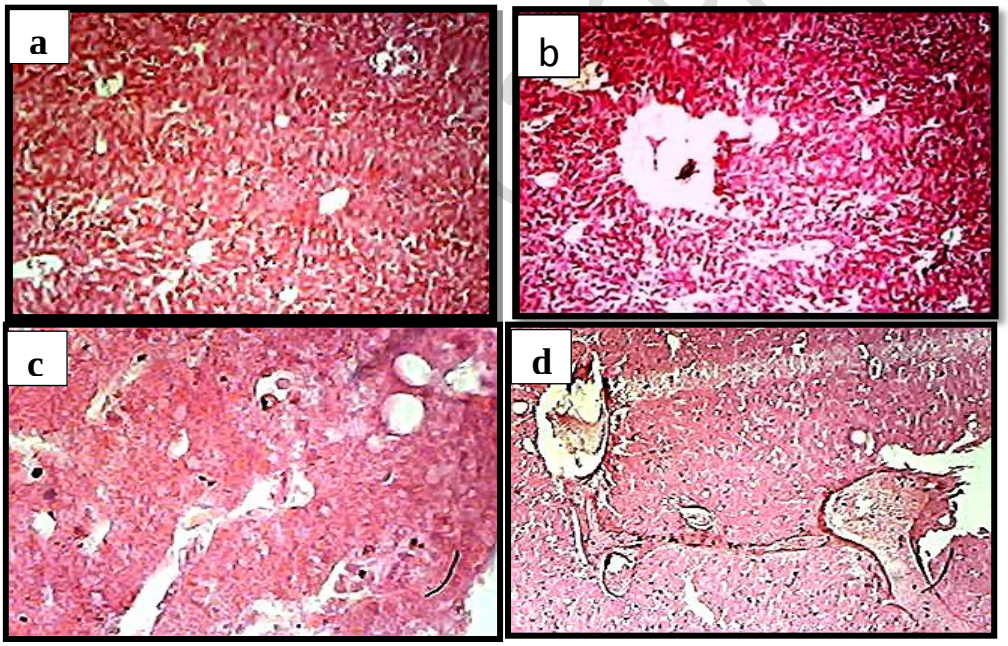

Figure 3 (a-d). (a) Liver section of control group birds presenting normal hepatic cells organization with appropriate sinusoid space having Kupfer cells, normal diameter of hepatic and bile ducts (H & E, x10).
(b) Histopathological section of liver of chlorpyrifos treatment group B is showing inflammation of hepatocytes, (H & E, x10)
(c) vacuolation and congested ducts and inflammatory changes in chlorpyrifos treated pigeons group C, (H & E, x10).
(d) Section of liver showing hypertrophy, deterioration of the hepatocytes and increased diameter of central duct in chlorpyrifos treatment group D, (H & E, x10)

Conclusion

Therefore, it is concluded that the hepatotoxicity could be attribute to the adverse effects of chlorpyrifos in birds. Safe use and efficient pesticide related regulations and enforcement can minimize such undesirable effects.

Authors' contributions

Conceived and designed the experiments: SA Memon, N Memon, Performed the experiments: SA Memon, Analyzed the data: SA Memon, SA Shaikh & N Sheikh, Contributed reagents/ materials/ analysis tools: SA Memon, SA Shaikh, Wrote the paper: SA Memon & N Memon.

References

1. Hussain RF, Mahmood MZ, Khan A & Muhammad F (2011). Pathological and genotoxic effects of atrazine in Japanese quail (*Coturnix japonica*) *Ecotoxicology* (20): 1-8.
2. Ahmad L, Khan A & Khan MZ (2012). Pyrethroid induced reproductive toxico-pathology in non target species. *Pakistan Vet J* (32): 1-9.
3. Gervais JA, Luukinen B, Buhl K & Stone D (2009). Malathion Technical Fact Sheet; National Pesticide Information Center, Oregon State +University Extension Services.
4. Saxena P & Saxena AK (2010). Cypermethrin induced biochemical alterations in the blood of albino rats. *Jordan J Biol Sci* (3): 111-114
5. Donaldson D, Kiely T & Grube A (2002). Pesticide Industry Sales and Usage: 1998 and 1999 Market Estimates. EPA-733-R- 02-001. Washington, DC: U.S. Environmental Protection Agency
6. Moye JK & Pritsos CA (2011). Effects of chlorpyrifos and aldicarb on flight activity and related cholinesterase inhibition in homing pigeons, *Columba livia* potential for migration effects. *Bull Environ Cont Toxicol. (*84): 677–681.
7. Muhammad F, Javed I, Akhtar M, Rahman ZU, Awais MM, Saleemi MK & Anwar MI (2012). Quantitative structure activity relationship and risk analysis of some pesticides in the cattle milk. *Pakistan Vet J* (32): 589-592
8. De Silva HJ, Samarawickrema NA & Wickremasinghe AR (2006). Toxicity due to organophosphorus compounds: what about chronic exposure? *Trans R Soc Trop Med Hyg* (100): 803–6
9. Edwards FL, Yedjou CG & Tchounwou PB (2013). Involvement of oxidative stress in methyl parathion andparathion-induced toxicity and genotoxicity to human liver carcinoma (Hepg2) cells. *Environ Toxicol* (28): 342–348.
10. Iqbal Z, Babar W, Sindhu, ZUD, Abbas RZ & Sajid MS (2012). Evaluation of anthelmintic activity of different fractions of *Azadirachta indica* A. Juss seed extract. *Pak Vet J* (32): 579-583.
11. Memon SA, Memon N, Shaikh SA, Butt Z & Mal B (2015). Patho-biochemical biomarkers of hepatotoxicity on exposure to bifenthrin insecticide in birds (Columba livia) *Pure App Biol.* 4(4): 597-604
12. Barr D, Allen R, Olsson AO, Bravo R, Caltabiano RM, Montesano A, Nguyen J, Udunka S, Walden D & Walker RD (2005). Concentrations of selective metabolites of organophosphorus pesticides in the United States population. *Environ. Res.* (99): 314–326.
13. Mehta AR, Verma S & Srivastava N (2009). Chlorpyrifos induced alterations in the levels of hydrogen peroxide, nitrate and nitrite in rat brain and liver. *Pest Biochem Physiol* (94): 55-59
14. Weil CS (1952). Tables for convenient calculation of median effective dose (LD 50 or ED 50) and instructions in their use. *Biometrics* (8): 249.

15. Moore P, Yedjou CG & Tchounwou PB (2009). Malathion-induced oxidative stress, cytotoxicity and genotoxicity in human liver (HepG2) cells. *Envir. Toxic* (25): 221-226
16. Kousba AA, Poet TS & Timchalk C (2007). Age-related brain cholinesterase inhibition kinetics following invitro incubation with chlorpyrifos-oxon and diazinon oxon. *Toxicol Sci* (95): 147–155.
17. Mohammad FK, Al-Badrany YM & Al-Jobory MM (2008). Acute toxicity and cholinesterase inhibition in chicks dosed orally with organophosphate insecticides, *Arch Hyg Rada Toxicol* (59): 145-151
18. Aly N, El-Gendy K, Mahmoud F & El-Sebae AK (2010). Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. *Pest Biochem Physiol* (97): 7–12.
19. Uzun FG, Kalender S, Durak D, Demir F & Kalender Y (2009). Malathion-induced testicular toxicity in male rats and the protective effect of vitamins C and E. *Food Chem Toxicol* (47): 1903–1908.
20. Heikal TM, El-Sherbiny M, Hassan SA, Arafa A & Ghanem HZ (2012). Antioxidant Effect of selenium on hepatotoxicity induced by chlorpyrifos in male rats. *Inter J Pharm Sci* (94): 603-609
21. Mansour SA, Heikal TM, Refaie AA & Mossa AH (2011) Antihepatotoxic activity of fennel (Foeniculum vulgare Mill.) essential oil against chlorpyrifos-induced liver injury in rats. *Global J Environ Sci. Tech* (1): 1-10.
22. Tuzmen N, Candan N, Kaya E & Demiryas N (2008). Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidative defense mechanisms and lipid peroxidation in rat liver. *Cell Biochem Func* (26): 119–124.
23. Mehta A, Verma RS & Srivastava N (2008). Chlorpyrifos-Induced DNA damage in rat liver and brain. *Environ Mol Mut* (49): 426-433.
24. Kammon AM, Brar RS, Banga HS & Sodhi S (20100. Patho-biochemical studies on hepatotoxicity andnephrotoxicity on exposure to chlorpyrifos and imidacloprid in layer chickens. *Vet Arch* (80): 663-672.
25. Verma RS, Mnugya A & Srivastava N (2007). In vivo chlorpyrifos induced oxidative stress: attenuation by antioxidant vitamins, *Pest Biochem Physiol* (88): 191–196.
26. Mehta AR, Verma S & Srivastava N (2009). Chlorpyrifos induced alterations in the levels of hydrogen peroxide, nitrate and nitrite in rat brain and liver. *Pest Biochem Physiol* (940): 55-59.
27. Goel A, Dani V & Dhawan DK (2005). Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. *Chem Biol Interact* (156): 131-140.