Impact of Oxidative Stress and Lipid Peroxidation Induced by Lambda-cyhalothrin on $P_{450}$ in Male Rats: The Ameliorating Effect of Zinc

Moustafa A. Abbassy1, Mamdouh A. Marzouk1, Sameeh A. Mansour2, Hassan A. Shaldam3 and Abdel-Tawab H. Mossa*4

1Department of Plant Protection, Faculty of Agriculture, Damanhour University, Egypt
2Environmental Toxicology Research Unit (ETRU), Pesticide Chemistry Department, National Research Centre (NRC), Tahrir Str., Dokki, Cairo, Egypt
3Center for Environmental Monitoring, Ministry of Health, Egypt

Abstract
The present study was designed to investigate the effect of lambda-cyhalothrin (LCH) on lipid peroxidation, oxidative stress biomarkers and the activity of Cytochrome $P_{450}$ in male rats and the protective role of zinc. LCH was administered orally to rats at dose 2.6 mg/kg b.wt. (Which represents 1/10 LD$_{50}$) with 3 doses per week for 6 weeks (dose period). Rats were divided into four groups, eight rats for, each: (I) control group, (II) zinc group (Zn dose 227 mg/l in drinking water), (III) LCH-treated groups and LCH-Zn group (IV) as in group II and III. The present results demonstrated that LCH induced significant alteration in the activity of antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; glutathiones-s-transferase, GST; glutathione peroxidase, GPx), glutathione reduced (GSH) and lipid peroxidation (LPO) levels and decreased cytochrome $P_{450}$ activity in plasma of male rats. In contrast, zinc-LCH treatment showed insignificant difference, compared to control results, regarding LPO, GST, CAT and $P_{450}$ levels and decreased cytochrome $P_{450}$ activity in the plasma of male rats. The overall results reveal the pronounced ameliorating effect of zinc in LCH-intoxicated rats.

Keywords: Lambda-cyhalothrin; Lipid peroxidation; Antioxidants enzymes; $P_{450}$; Zinc; Rat

Introduction
Synthetic pyrethroids are a diverse class of more than thousand powerful broad spectrum insecticides that are environmentally compatible by virtue of their moderate persistence, low volatility and poor aqueous mobility in soil [1]. They represent approximately one-fourth of the worldwide worldwide insecticides market [2]. Lambda-cyhalothrin (cyano-3-phenoxbenzyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropylcarboxylate) is one of the newer synthetic type II pyrethroid insecticides [3] with effective and persistent activity against a large variety of arthropods harmful both to human and animal health, and to vegetal production [4]. Consistent with its lipophilic nature [5] pyrethroid insecticide such as lambda-cyhalothrin has been found to accumulate in biological membranes leading to oxidative damage. It has been suggested that some effects directly related to pesticide toxicity could be due to changes in membrane fluidity [6,7], in lipid composition [8] and inhibition of enzyme activities [9-11].

In fact, reactive oxygen species (ROS) are produced by univalent reduction of dioxygen to superoxide anion (O$^-$), which in turn disproportionate to H$_2$O$_2$ and O$_2$, spontaneously or through a reaction catalyzed by superoxide dismutase (SOD). Endogenous H$_2$O$_2$ may be converted to H$_2$O either by catalase or glutathione peroxidase (GSH-Px). Otherwise, it may generate a highly reactive free hydroxyl radical (•OH) via a Fenton reaction, which is responsible for oxidative damage. GSH-Px converts H$_2$O$_2$ or other lipid peroxides to water or hydroxyl lipids, and during this process glutathione (GSH) is converted to oxidized glutathione [12]. Antioxidants are defense against free radical and oxidative attacks. They act as free radical scavengers and slow down not only radical oxidation but also the accompanying damaging effects in the body [13]. Previous studies [10,11,14-16] reported that ROS were involved in the toxicity of various pesticides.

Zinc (Zn) is an essential trace element, is relatively nontoxic and is integral to several key functions in human metabolism [17,18]. Not only has Zn been identified as a component of key enzymes and regulatory proteins, it was recently suggested that the preventive effects of zinc may partly be mediated through increase in cytochrome $P_{450}$ enzymes in subjects with alcoholic liver disease [19,20].

However, there is still a clear lack of understanding whether the toxic effects of lambda-cyhalothrin mediated through drug metabolizing enzymes, and further if zinc may have any preventive role in such toxic conditions. Therefore, the present study was designed to evaluate the protective potential effect of zinc on oxidative damage induced by lambda-cyhalothrin in male rats.

Materials and Methods

Chemicals and reagents
Lambda-cyhalothrin (Lambda EG®, EC %) was obtained from Arab Company for Chemical Industries Co., Egypt. Zinc sulfate (ZnSO$_4$·7H$_2$O) was obtained from Merck (Germany). Kits of SOD, CAT, GST, GSH, GR and GPx were obtained from Bio-diagonistic, Dokki, Giza, Egypt. Kit of cytochrome $P_{450}$ was obtained from Boehringer Mannheim GmbH Diagnostics, Germany. All other chemicals were of reagent grades and obtained from the local scientific distributors in Egypt.

*Corresponding author: Abdel-Tawab H. Mossa, Environmental Toxicology Research Unit (ETRU), Pesticide Chemistry Department, National Research Centre (NRC), Tahrir Str., Dokki, Cairo, Egypt, Tel: 202-01005038504; Fax: 202-33370931; E-mail: abdeltawab.mossa@yahoo.com
Moustafa A. Abbassy, Department of Plant Protection, Faculty of Agriculture, Damanhour University. Tel: 0020122404383, Fax: 0020453316535, E-mail: maabbassy@yahoo.com

Received February 20, 2014; Accepted April 16, 2014; Published April 18, 2014
Citation: Abbassy A, Marzouk MA, Mansour SA, Shaldam HA, Mossa ATH (2014) Impact of Oxidative Stress and Lipid Peroxidation Induced by Lambda-cyhalothrin on $P_{450}$ in Male Rats: The Ameliorating Effect of Zinc. J Environ Anal Toxicol 4: 218. doi: 10.4172/2161-0525.1000218
Copyright: © 2014 Abbassy A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Animals

Male albino rats weighing 120 ± 10 g were obtained from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt, and were used in this study. The animals were housed in plastic cages and allowed to adjust to the new environment for a week before starting the experiment. Rats were fed on standard food pellets and tap water ad libitum. The rats were housed at 23 ± 2°C and in daily dark/light cycle. The experimental work on rats was performed with the approval of the Animal Care & Experimental Committee, college of agriculture in Dammanhour, Egypt, and according to the guidance for care and use of laboratory animals [21].

Experimental design

Animals were divided into four groups, eight animals each as the following: Group I: Rats were served as control and given tap water. Group II: Rats were given zinc in drinking water daily at a concentration of 227 mg L−1 as Zn [22]. Group III: Rats were given LCH at a dose 2.6 mg/kg b.w, 1/10 LD₅₀ [23] orally repeated dose day after day over period of 6 weeks (3 doses/week). Group IV: Rats were given LCH and Zn as described in groups II and III. Animals were weighed weekly and the dose was adjusted accordingly.

Blood collection

At the end of exposure period, blood samples were withdrawn from the animals under ether anesthesia by puncturing the retro-orbital venous plexus of the animals with a fine sterilized glass capillary. Blood was collected into heparinized tubes and left for 20 min at room temperature, then centrifuged at 3000 rpm (600 g) for 10 minutes using BOECO model C 28, Germany, to separate the plasma. The plasma was kept in a deep freezer (-20°C) until analyzed within one week.

Oxidative stress biomarkers

Lipid peroxidation (LPO): Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content by a colorimetric method according to Satoh [24]. The MDA values were expressed as nmoles of MDA/ml.

Glutathione reduced (GSH): GSH was assessed spectrophotometrically according to the method of Goldberg and Spooner [25] using Boehringer Mannheim Gmbh Diagonstics kits. The method was based on Glutathione reductase catalysis the reduction of glutathione (GSSG) in the presence of NADPH. The GSH values were expressed as nmoles/ml.

Antioxidant enzymes: Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined according to the method of Nishikimi et al. [26]. The method is based on the ability of SOD enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Briefly, 0.05 ml sample was mixed with 1.0 ml buffer (pH 8.5), 0.1 ml nitroblue tetrazolium (NBT) and 0.1 ml NADH. The reaction was initiated by adding 0.01 ml phenazine methosulphate (PMS), and then increased in absorbance was read at 560 nm for five minutes. SOD activity was expressed in µmol/ml.

Catalase (CAT, EC 1.11.1.6) activity was determined according to the method of Abei [27]. The method is based on the decomposition of H₂O₂ by catalase. The sample containing catalase is incubated in the presence of a known concentration of H₂O₂. After incubation for exactly one minute, the reaction is quenched with sodium azide. The amount of H₂O₂ remaining in the reaction mixture is then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminantipyrine, AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) in the presence of H₂O₂ and catalyzed by horseradish peroxidase (HRP). The resulting quinoneimine dye (N-(4-antipyril)-3-chloro-5-sulfonate-p-benzoquinonemonoimine) is measured at 510 nm. The catalase activity was expressed in µmol/ml.

Glutathione peroxidase (GPx; EC 1.11.1.9) was assessed spectrophotometrically according to the method of Paglia and Valentine [28] using Boehringer Mannheim Gmbh Diagonstics kits. The method was based on indirect measure of the activity of c-GPx. Oxidized glutathione (GSSG), produced upon reduction of organic peroxide by c-GPx, and is recycled to its reduced state by the enzyme glutathione reductase (GR). Results were expressed as µmol/ml.

Glutathione-s-transferase (GST; EC 2.5.1.13) activity was assessed spectrophotometrically according to the method of Habig et al. [29]. The method was based on the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced Glutathione (GSH) in a reaction catalyzed by GST. Increase in absorbance was monitored for 3 min at 360 sec intervals at wavelength of 340 nm. Results were expressed as µmol/ml.

Cytochrome P₄₅₀

Cytochrome P₄₅₀ was determined according to the method of Masters et al. [30]. The method was based on the most common reaction catalyzed by cytochromes P₄₅₀ is a monooxygenase reaction, e.g., insertion of one atom of oxygen into an organic substrate (RH) while the other oxygen atom is reduced to water. The function of the cytochrome P₄₅₀ enzymes is to metabolize xenobiotic compounds with which an organism comes into contact. Results were expressed as nmoles/ml.

Spectrophotometric measurements

The spectrophotometric measurements were performed by using JENWAY 6305 UV-Vis spectrophotometer designed and manufactured in the U.K.

Statistical analysis

The results were expressed as mean ± S.D. All data were done with the Statistical Package for Social Sciences (SPSS 17.0 for windows). The results were analyzed using one way analysis of variance (ANOVA) followed by Duncan’s test for comparison between different treatment groups. Statistical significance was set at p ≤ 0.05.

Figure 1: Effect of oral administration of lambda-cyhalothrin (LCH) on lipid peroxidation (LPO) in serum of male rats and the protective role of zinc (Zn). Bars represent the group means ± S.D; a,b,c & d values are not sharing superscripts letters (a,b,c & d) differ significantly at p ≤ 0.05.
Results

Plasma MDA level was markedly increased by LCH administration as compared to control group (Figure 1). The difference between the two groups was statistically significant (2.32 nmoles of MDA/ml vs. 1.35 nmoles of MDA/ml, \( p \leq 0.05 \)). Co-administration of Zn to rats of LCH group alleviated lipid peroxidation induced by LCH in LCH-treatment and modulated significantly (1.92 nmoles of MDA/ml vs. 1.35 nmoles of MDA/ml, \( p \leq 0.05 \)) the levels of MDA in plasma compared to control. Results indicated that treatment with Zn produced a significant reduction in TBARS in LCH-treated rats; however Zn per se did not alter TBARS. As shown in Figure 2, significant decrease in GSH was observed after treatment of rats with LCH compared to control group (50.88 nmoles/ml vs. 85.37 nmoles/ml). Co-administration of Zn with LCH modulated significantly the level of GSH to the normal control value (82.21 nmoles/ml vs. 85.37 nmoles/ml).

The effects of LCH treatment on the activities of SOD, CAT, GPx and GST in plasma are shown in Figure 3. Activities of SOD (88.08 μmol/ml vs. 104.87 μmol/ml), GPx (0.76 μmol/ml vs. 0.87 μmol/ml) and GST (0.80 μmol/ml vs. 1.07 μmol/ml) in plasma were significantly decreased (\( p \leq 0.05 \)), while CAT activity (0.69 μmol/ml vs. 0.48 μmol/ml) was significantly increased compared to control group. Co-administration of Zn with LCH alleviated lipid peroxidation and induced the activity of SOD and GPx in plasma compared with control values. The activity of SOD and GPx was returned to their control values in LCH+Zn-treated rats, while the decrease of GST and increase of CAT were significant (\( p \leq 0.05 \)) compared with LCH+Zn-treated group (Figure 3).

Plasma \( P_{\text{450}} \) activity was markedly decreased by LCH administration as compared to control group (Figure 4). The difference between the two groups was statistically significant (0.035 nmoles/ml vs. 0.136 nmoles/ml). Zn administered to rats of LCH+Zn group alleviated \( P_{\text{450}} \) activity induced by LCH treatment and modulated significantly (0.115 nmoles/ml vs.0.136 nmoles/ml, \( p \leq 0.05 \)) the activity of \( P_{\text{450}} \) in plasma compared to control. Results indicated that treatment with Zn produced a significant increase in \( P_{\text{450}} \) in LCH-treated rats.

Discussion

Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics (e.g. pesticides) and some of these free radicals interact with various tissue components, resulting in dysfunction [10,14]. In fact, oxidative damage due to excessive production of reactive oxygen species (ROS) has been associated with defective organs dysfunction [14,31] and the inhibition of enzymes involved in free radical removal led to the accumulation of \( \text{H}_2\text{O}_2 \), which promoted lipid peroxidation and modulation of DNA, altered gene expression and cell death [32].

SOD, CAT and GPx are known to play an important role in

![Figure 2: Effect of oral administration of lambda-cyhalothrin (LCH) on glutathione reduced(GSH) level in serum of male rats and the protective role of zinc (Zn). Bars represent the group means ± S.D.; \( a, b, c, \) & \( d \) values are not sharing superscripts letters \((a, b, c, d)\) differ significantly at \( p \leq 0.05 \).](image)

![Figure 3: Effect of oral administration of lambda-cyhalothrin (LCH) on SOD, CAT,GPx and GST activity in serum of male rats and the protective role of zinc (Zn). Bars represent the group means ± S.D.; \( a, b, c, d \) values are not sharing superscripts letters \((a, b, c, d)\) differ significantly at \( p \leq 0.05 \).](image)
Effect of oral administration of lambda-cyhalothrin (LCH) on P450 in male rats. The ameliorating effect of zinc. J Environ Anal Toxicol 4: 218. doi: 10.4172/2161-0525.1000218

Figure 4: Effect of oral administration of lambda-cyhalothrin (LCH) on P450 activity in serum of male rats and the protective role of zinc (Zn). Bars represent the group means ± S.D. a, b, c, d values are not sharing superscripts letters (a, b, c & d) differ significantly at p ≤ 0.05.

scavenging ROS. SOD catalyzes the destruction of the superoxide radicals to H2O2, while CAT together with GPx reduces the H2O2 into water and oxygen to prevent oxidative stress and in maintaining cell homeostasis. Also, GST is play essential role in the detoxification process. In the present study LCH induced significant decrease in the activity SOD, GPx and GST and increase in CAT activity in plasma of treated rats. So, the change in SOD, GPx, GST and CAT might be in response to increased oxidative stress. When a condition of antioxidant stress strongly establishes, the defense capacities against ROS becomes insufficient [32], in turn ROS also affects the antioxidant defense mechanisms, reduces the intracellular concentration of GSH, lipid peroxidation and alter the activity of antioxidant enzymes e.g., SOD, CAT, GPx and GST. The changes in these oxidative stress biomarkers have been reported to be an indicator of tissue’s ability to cope with oxidative stress [10,14,33]. ROS has also been known to decrease the detoxification system produced by GST [34]. Considering that GSTs are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms [35], the significant decrease of GST activity in plasma of male rats after LCH administration may indicate insufficient detoxification of LCH in rat. Also, an important function of GST in response to oxidative stress is its ability to conjugate GSH with lipid peroxidation products [36]. Previous studies demonstrated that pyrethroids exposure altered antioxidant defense mechanisms and enhanced lipid peroxidation in rat liver [14,37-39], erythrocytes [37] and in fis [40]. Exposure of rats to a single dose of the pyrethroids, cypermethrin (25 μg kg⁻¹) and fenvalerate (4.5 μg kg⁻¹), lowered the activities of the antioxidant enzymes SOD and CAT, resulting in both lipid peroxidation and decreased levels of GSH in erythrocytes [41].

Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity [10,11,14]. Malondialdehyde (MDA) level in LCH treatment was significantly higher than that in control. These indirectly suggest an increased production of oxygen free radicals in rats. Highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce malondialdehyde, a lipid peroxidation product. Previous studies indicate that insecticides in both in vivo [10,11,42] and in vitro tests [43] alter the enzyme activities associated with antioxidant defense mechanisms.

Our results revealed that co-administration of zinc with LCH to treated animals retained the level of GSH and the activity SOD and GPx at the normal values. Catalase, CAT, GST activity and LOP level were improved, and such alterations were still significant in zinc-LCH-treated rats. The observed normalization trend of GSH, SOD and GPx following zinc treatment could possibly due to dismutation of O2•- to H2O2 which is catalyzed by SOD. Zinc is known to induce the production of metallothionine, which is very rich in cysteine, and is an excellent scavenger of ·OH [44,45]. Also, the NADPH oxidases are a group of plasma membrane associated enzymes, which catalize the production of O2•- from oxygen by using NADPH as the electron donor. Zinc is an inhibitor of this enzyme [46].

Cytochrome P450 enzymes are essential for the metabolism and detoxification of many xenobiotics (e.g. pesticides). It has been reported that many chemicals (e.g. pesticides, drug) interactions are the result of an alteration of CYP expression and metabolism [47]. In the current study, LCH decreased cytochrome P450 activity in LCH-treated rat. This may be due to the inhibition of heme synthesis and destruction of cytochrome P450 [48]. Previous studies showed that many pesticides have been reported to inhibit the activity and alteration in the expression of various cytochrome P450 isforms (e.g. parathion, methomyl). These changes may increase the sensitivity of cells against reactive endogenous metabolites or other xenobiotics [49-51]. Co-administration of zinc to LCH-treated animals improved the activity of cytochrome P450 compared to LCH-treated rat. This change may due to the antioxidant role of zinc and alter the enzyme activities associated with antioxidant defense mechanisms.

Conclusion

The results of the present study demonstrated that exposure to LCH induced oxidative stress; lipid peroxidation and reduced P450 activity in the plasma of LCH-treated male rats. The overall results reveal the pronounced ameliorating effect of zinc in LCH-intoxicated rats.

References

1. Erstedt KM (1999) Environmental fate of synthetic pyrethroids during spray drifts and field runoff treatments in aquatic microcosms. Chemosphere 39: 1737-1769.
2. Casida JE, Quiatad GB (1998) Golden age of insecticide research: past, present, or future? Annu Rev Entomol 43: 1-16.
3. Meister RT (1992) editor Farm chemicals handbook 92. Willough- by, OH: Meister Publishing Co.
4. WHO (1999) Cyhalothrin, environmental health criteria, 99, Geneva, Switzerland.
5. Michelangeli F, Robson MJ, East JM, Lee AG (1990) The conformation of pyrethroids bound to lipid layers. Biochimica et Biophysica Acta 1028: 49-57.
6. Sarkar SN, Balasubramanian SV, Sidkar SK (1993) Effect of Fenvalerate, a pyrethroid insecticide on membrane fluidity. Biochem Biophys Acta 1147: 137-142.
7. Antunes-Madeira MC, Videira RA, Madeira VM (1994) Effects of parathion on membrane organization and its implications for the mechanisms of toxicity. Biochim Biophys Acta 1190: 149-154.
8. Perez-Albarsanz MA, Lopez-Aparicio P, Senar S, Recó MN (1991) Effects of lindane on fluidity and lipid composition in rat renal cortexmembranes. Biochim Biophys Acta 1066: 124-130.
9. Jones GT, Lee AG (1986) Effects of pyrethroids on the activity of a purified Cat++ Mg++ ATPase. Pestic Biochem Physiol25: 420-430.
10. Mansour SA, Mossa AH (2009) Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. Pest Biochem Physiol 93: 34-39.
11. Mossa AH, Heikal TM, Omara EA (2012) Physiological and histopathological changes in the liver of male rats exposed to paracetamol and diazinon. Asian Pacific J. Trop Biomed 12: S1683-S1690.
12. Bachowshi S, Kolaja KL, Xu Y, Ketcham CA, Stevenson DE, et al. (1997) Role of oxidative stress in the mechanism of dieldrin's hepatotoxicity. Ann Clin Lab Sci 27: 196-209.

13. Nice D (1997) Antioxidant based nutraceuticals. In: Yalpani(MEdn), New Technologies for Healthy Foods and Nutraceuticals. Science Publishers, Shrewsbury105-123.

14. Mossa AT, Refaie AA, Ramadan A, Bouajila J (2013) Amelioration of prallethrin-induced oxidative stress and hepatotoxicity in rat by the administration of Origanummajoranaessential oil. Biomed Res Int 2013: 1-11.

15. Marzouk MA, Abbassy MA, Mansour SA, Mossa AH, Elsayed SR (2011) Effect of dimethoate, dicofol and voltaren on oxidant/antioxidant status in male rats: Role of selenium. J Agric Sci Camb Univ Egypt 19: 40-60.

16. Divwedi PD, Das D, Khanna SK (1998)Role of cytochrome P450 in quinolipophoxicity: effect on hepatic and brain antioxidant enzymes in rats. Food Chem Toxicol 36: 437-444.

17. Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. Nutrition 18: 872-879.

18. Daniel H, Tom Dieck H (2004) Nutrient-gene interactions: a single nutrient and hundreds of target genes. Biol Chem 385: 571-583.

19. Zhou Z, Wang L, Song Z, Saari JT, McClain CJ, et al. (2005) Zinc supplementation prevents alcoholic liver injury in mice through attenuation of oxidative stress. Am J Pathol 166: 1681-1690.

20. Kang YJ, Zhou Z (2005) Zinc prevention and treatment of alcoholic liver disease. Mol Aspects Med 26: 391-404.

21. NRC (1996) Guide for the Care and Use of Laboratory Animals, NationalAman Research Council, Acad. Press, Washington, DC.

22. 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.

23. Marzouk MA, Abbassy MA, Mansour SA, Shaldam HA (2012) Liver function alterations induced by lambada-cyhalothrin in male albino Norway rats Rattusnorvegicus: ameliorative effect of zinc. Egypt J Agric Res 90: 263-285.

24. Satoh K (1978) Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta15: 37-43.

25. Goldberg DM, Spooner RJ (1983) In Methods of Enzymatic Analysis (Bergmeyen, H.V.Ed.) 3rd edition, VerlagChemie, Deerfield beach, F13: 258-265.

26. Nishikimi M, Roa NA, Yogi K (1972)The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Common 46: 849-854.

27. Aebi H (1984) Catalase in vitro. Method Enzymol 105: 121-126.

28. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70: 158-189.

29. Habig WH, Pabst MJ, Jakoby WB (1974)Glutathione transferase: A first enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130-7139.

30. Masters BSS,Williams CH, Kamin H (1967) Methods in Enzymology X: 565-573.

31. Murphy PM (2009) How mitochondria produce reactive oxygen species. Biochem J 417: 1–13.

32. Halliwell B, Gutteridge JM (2000) Free radicals in Biology and medicine, Oxford University Press 148-149.

33. Mimić-Oka J, Simić T, Djukanović L, Rejić Z, Davicević Z (1999) Alteration in plasma antioxidant capacity in various degrees of chronic renal failure. Clin Nephrol. 51: 233-241.

34. Yamamoto Y, Yamashita S (1999) Plasma ubiquinone to ubiquinol ratio in patients with hepatitis, cirrhosis, and hepatoma, and in patients treated with percutaneous transluminal coronary reperfusion. Bio Factors 9: 241-246.

35. Hayes JD, Flanagan JU, Jowsey IR (2005) Glutathione transferases. Annu Rev Pharmacol Toxicol 45: 51-88.

36. Rao AV, Shaha C (2000) Role of glutathione S-transferases in oxidative stress-induced male germ cell apoptosis. Free Radic Biol Med 15:1015-1027.

37. Prasanthi K, Muralidhara PS, Rajini K (2005) Fenvalerate-induced oxidative damage in rat tissues and its attenuation by dietary sesame oil. Food Chem Toxicol 43: 299-306.

38. Youssef MI, Awad TI, Mohamed EH (2006) Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. Toxicology 227: 240-247.

39. Tuzmen N, Candan N, Kaya E, Demiray N (2008) Biochemical effects of chlorpyrifos and deltamethrin on altered anti-oxidative defense mechanisms and lipid peroxidation in rat liver. Cell Biochem Funct 26: 119-124.

40. Ghosh TK (1989) Influence of cypermethrin on the oxidative metabolism of fababeerohana.Proc Indian NatnSci Acad 115-120.

41. Kale M, Rathore N, John S, Bhatnagar D (1999) Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. ToxicolLett105: 197-205.

42. Ogutcu A, Uzunhisarcikili M, Kalender S, Durak D, Bayrakdar F, et al. (2006) The effects of organophosphate insecticide diazinon on malondialdehyde levels and myocardial cells in rat heart tissue and protective role of vitamin E. Pest Bioch Physio86: 93-98.

43. Mansour SA, Mossa AH, Heikal TM (2009) Effect of methomyl on lipid peroxidation and antioxidant enzymes in rat erythrocytes: in vitro studies. Toxicol Indus Health 25: 557-563.

44. Seagrave J, Tobey RA, Hildebrand CE (1983) Zinc effects on glutathione metabolism relationship to zinc-induced protection from alkylating agents. Biochem Pharmacol 32: 3017-3021.

45. Prasad AS (1993) Zinc and enzymes. In: A.S. Prasad (Ed.), Biochemistry of Zinc, Plenum Press, New York.

46. Prasad AS (2008) Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. Exp Gerontol43: 370-377.

47. Michalet EL (1998) Update: clinically significant cytochrome P-450 drug interactions. Pharmacotherapy 18: 84-112.

48. Slaughter RL, Edwards DJ (1995) Recent advances: the cytochrome P450 system: studies of the covalent binding of the sulfur atom. Mol Pharma72: 933-944.

49. Kamataki T, Neal RA (1976) Metabolism of diethyl p-nitrophenylphospho rothionate (parathion) by a reconstituted mixed-function oxidase enzyme system: studies of the covalent binding of the sulfur atom. Mol Pharma col12: 933-944.

50. Fossi MC, Leonzio C, Massi A, Lari L, Casini S (1992) Serum esterase and carbamate contamination. Arch Environ Cont Toxicol 23: 99-104.

51. Timbrell J (2000) Principles of biochemical toxicology 3rd ed. London:Taylor and Francis.