Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

The Comparison of the Effect of Diazinon and Paraoxon on Biomarkers of Oxidative Stress in Rat Serum

Maryam Salehi,[1] Mahvash Jafari,*[2] Masoud Saleh-Moqadam,[1] Alireza Asgari[3]

1. Department of Biochemistry, Payame Noor University, Mashhad, Iran
2. Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Exercise Physiology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Article information

Article history:
Received: 5 January 2011
Accepted: 14 July 2011
Available online:17 August 2011

Keywords:
Diazinon
Paraxaon
Oxidative stress
Rat
Serum

*Corresponding author at:
Department of Biochemistry,
Faculty of Medicine,
Baqiyatallah University of
Medical Sciences, Tehran, Iran.
E-mail:
jafari@bmsu.ac.ir

Abstract

Background: Diazinon and paraoxon are a group of pesticide organophosphates which are widely used in agriculture. Some organophosphates increase the production of free radicals. This study aims to compare the effects of diazinon and paraoxon on biomarkers of oxidative stress in rat serum.

Materials and Methods : This experimental study was performed in 2010 in Baqiyatallah (a.s) University of Medical Sciences. 49 male Wistar rats were randomly divided into 7 groups: the control group was given corn oil as a solvent of diazinon and paraoxon and the other six groups were given diazinon at doses 30, 50 and 100 mg/kg and paraoxon at doses 0.3, 0.7 and 1 mg/kg intraperitoneally. 24 hours after the injection, blood was collected by cardiac puncture and serum was prepared. Then, the activities of butrylcholinesterase enzyme (BChE), superoxide dismutase (SOD), catalase (CAT) and lactate dehydrogenase (LDH) and glutathione level (GSH) were measured by chemical methods.

Results: The decreased SOD and CAT activities and GSH level after administration of Diazinon (more than 30 mg/kg) and paraoxon (more than 0.3 mg/kg) is significant compared to control group, whereas the activity of LDH enzyme significantly increases. The decreased BChE activity is not significant compared to control group.

Conclusion: Diazinon and paraoxon induce the production of free radicals and oxidative stress in a dose-dependent manner. The decreased activity of antioxidant enzymes and depleted GSH content probably represent the antioxidant defense system failure against the toxic actions of free radicals and oxidative tissue damage. Paraoxon has more severe effects than diazinon.

Introduction

Organophosphate compounds are widely used to control insects and ectoparasites. In addition, these compounds including tabon, sarin and malathion have been used in several chemical attacks by Iraqi forces during Iran-Iraq war. Due to the easy access and high toxicity of these compounds, the incidence of accidental poisoning and suicide is extensive and it is responsible for about one hundred thousand annual poisoning in the world. In Iran, these compounds are one of the reasons for deaths from poisoning [1, 2]. Diazinon (DZN) and paraoxon (POX) are of main organophosphate pesticides which are used for the control of various insects in crops, ornamental plants, fruit and vegetables. These compounds with amino acid serine phosphorylation in the active site of acetylcholinesterase enzyme leads to the inhibition of the enzyme and accumulation of acetylcholine in cholinergic synapses and cholinergic crisis, seizures and in acute cases, brain injury and death [2, 3]. Being under acute and sub-acute exposure of some organophosphates inhibits cholinesterase and increases the production of free radicals and oxidative stress.[2] The reaction of free radicals with lipids in cell membranes causes lipid peroxidation and destruction of membrane structure. Harmful effects of free radicals is controlled by the cellular antioxidant defense system includes antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT). In addition, glutathione (GSH), as an antioxidant, increases the solubility and excretion of toxins through the kidneys. The imbalance between production of free radicals and antioxidant defense systems in the body is called oxidative stress, which causes many diseases [4, 5]. Studies showed that after administration of oral malathion to rats, changes of activity of antioxidant enzymes and increase of lipid peroxidation are observed in erythrocyte, saliva and plasma [2, 6]. On the other hand, by examination of the effect of chlorpyrifos and cypermethrin on liver

rat increased serum concentrations of liver function indices and changes in antioxidant parameters have been reported [7]. Due to the variation of substitutions in chemical structure of organophosphates and their different effects on different tissues, further studies are necessary to understand the mechanism of action of these compounds. Very few studies are conducted on the effects of diazinon on in vivo antioxidant system. In the present study, the effects of two toxins of DZN and POX on some indices of oxidative stress in rat serum have been compared.

Materials and Methods

Chemicals: Nitroblue tetrazolium (NBT), dithiobis 2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA) and other chemicals with high purity were purchased from Merck and Sigma Companies of Germany. Diazinons (purity of 100%) from Supelco-USA and ethyl paraoxon (purity of 99%) from Sigma Company were purchased. Stock solutions of DZN with concentration of 400 mg/ml and POX with concentration of 4 mg/ml were prepared freshly in corn oil.

This experimental study was performed in 2010 in Baqiyatallah (a.s) University of Medical Sciences. 49 male Wistar rats weighing 200-250 g were kept under natural conditions of light, darkness, food and water for a week to get accustomed to the environment in animal house of Baqiyatallah University of Medical Sciences. The animals were then randomly divided into 7 groups: the control group was given corn oil as a solvent and the other six groups were given DZN (at doses 30, 50 and 100 mg/kg) and POX (at doses 0.3, 0.7 and 1 mg/kg) intraperitoneally. 24 hours after the injection, blood was collected by cardiac puncture and serum was prepared and stored at −70ºC until biochemical analysis.

Butrylcholinesterase activity measurement: Ellman method was used to measure the activity of cholinesterase [8]. 10 µl of serum was added to the cuvet containing 0.425 mM DTNB dissolved in 0.1 M sodium phosphate buffer pH 7.5 with 490 µl of distilled water. Then, 0.5 ml of butryltiocholine iodide of 20mM as substrate was added to the cuvet. It was incubated at 37°C for 5 minutes. Then, 0.5 ml Quinidine sulfate was added to stop the reaction. The absorbance was read at wavelength of 412 nm for 5 minutes. Specific activity was calculated based on the unit/ mg protein.

Measurement of SOD activity: SOD enzyme activity was measured using Winterbourn method [9]. 0.1 M EDTA in 0.3 mM sodium cyanide and 1.5 mM NBT were added to an appropriate volume of serum in a cuvet and after mixing, it was incubated at 37ºC for 5 minutes. Then, 0.12 mM riboflavin was added to 0.067 M potassium phosphate buffer with pH=7.8 and it was placed at room temperature for 10 minutes. The absorbance was read at a wavelength of 560 nm for 5 minutes and the specific activity was calculated by the unit per mg protein.

Measurement of CAT activity: The activity of CAT enzyme was measured by Aebi method [10]. The reaction was started by adding 30 mM H_2O_2 to an appropriate volume of serum in 50 mM sodium phosphate buffer with pH=7. Then, the absorbance was read at a wavelength of 240 nm within 3 minutes. Specific activity was calculated based on the unit/mg protein.

Measurement of LDH activity: LDH enzyme activity was measured using Parsazmun Company kit of Iran. Sample absorbance at 340 nm was read within 3 minutes, and the specific activity was expressed by the unit per mg protein.

Determination of GSH concentration: Thietz method was used to determine GSH level [11]. An appropriate volume of serum was mixed with 5% Sulfosalicylic acid. The sample was centrifuged at 2000 ×g for 10 min at 4ºC. 100 µl of supernatant was added to 810 µl of 0.3 M disodium phosphate. Then the reaction was started by adding 90 µl of 0.04% DTNB in 0.1% sodium citrate. The absorbance changes at 412 nm were read within 5 minutes. Standard curves were plotted and glutathione concentration was calculated using 1mg/ml glutathione solution.

Protein assay: Bradford method was used to determine protein concentration [12]. Appropriate volume of serum reached the volume of 1ml to which 3ml of Bradford solution was added and was incubated for 10 minutes. Then, absorbance was read at 595 nm wavelength. Protein concentration was calculated using standard plotting using 1mg/ml bovine serum albumin solution (BSA).

Statistical analysis: Data analysis was performed using INSTAT software as one-way ANOVA followed by Tukey post-hoc multiple comparison test. *p*-values less than 0.05 were considered statistically significant. Data were expressed as Mean ± SD.

Results

The results of the effect of different doses of DZN and POX on the activity of serum butrylcholinesterase enzyme in Fig. 1 shows that the change of butyrylcholinesterase enzyme activity is not significant compared to the control group.

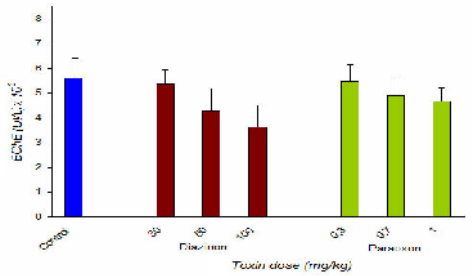

Figure 1. The activity of serum butrylcholinesterase enzyme of rats treated with various doses of diazinon and paraoxon after 24 hours.

The results of the effect of different doses of DZN and POX on the activity of SOD of serum in Fig. 2 shows that the decrease in SOD activity at doses higher than 30 mg/kg DZN and 0.3 mg/kg POX is significant compared to the control group. In addition, decreased activity of SOD at dose 100 mg/kg is significant compared to dose 30 mg/kg of DZN (p<0.01).

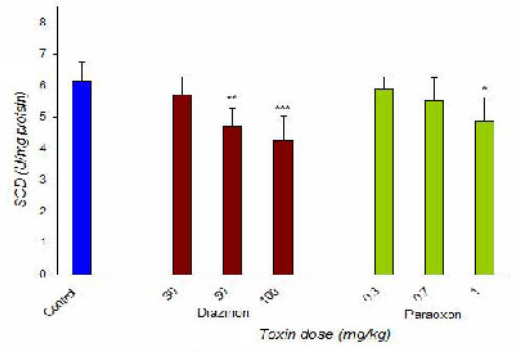

Figure 2. The activity of superoxide dismutase in serum after 24-hour at presence of different doses of diazinon and paraoxon. $^{*}p<0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. control.

The results of the effect of different doses of DZN and POX on the activity of CAT of serum in Fig. 3 shows that the decrease in CAT activity at doses higher than 30 mg/kg DZN and 0.3 mg/kg POX is significant compared to the control group. In addition, CAT activity at dose 1mg/kg significantly decreases compared to dose 0.3 mg/kg of POX ($p < 0.05$).

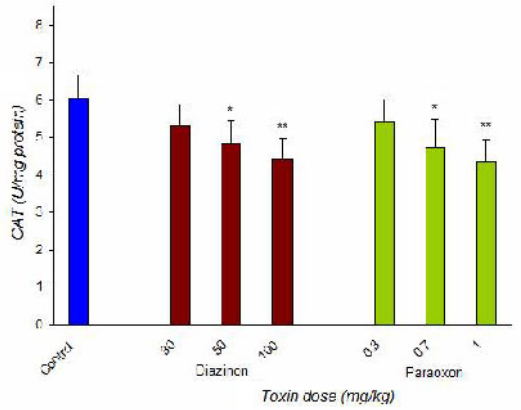

Figure 3. Serum catalase activity enzyme of rats treated with various doses of diazinon and paraoxon 24 hours after exposure. $^{*}p<0.05$ and $^{**}p<0.01$ vs. control.

The results of the effect of different doses of DZN and POX on the activity of LDH in serum in figure 4 shows that the increase in LDH activity at doses higher than 30 mg/kg DZN and 0.7 mg/kg POX is significant compared to the control group. The LDH enzyme activity in dose 1 mg/kg significantly increases compared to dose 0.3 mg/kg of POX (p<0.01).

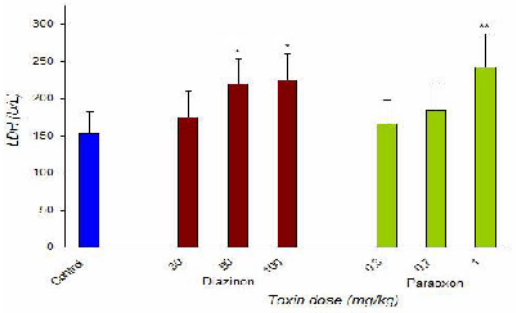

Figure 4. Effects of various doses of diazinon and paraoxon on activity of serum LDH enzyme after 24. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. control.

The results of the effect of different doses of DZN and POX on GSH concentration of serum in Fig. 5 shows that decrease of GSH concentration at doses higher than 30 mg/kg DZN and 0.3mg/kg POX is significant compared to the control group. In addition, GSH concentration in dose 100 mg/kg significantly decreases compared to dose 30 mg/kg of DZN (p< 0.01) and dose 1mg/kg significantly decreases compared to dose 0.3 mg/kg of POX ($p < 0.01$).

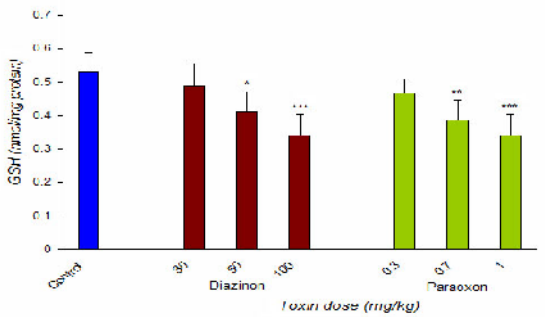

Figure 5: The glutathione concentration in serum after 24-hour at presence of different doses of diazinon and paraoxon. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ vs. control

Discussion

The results of this study indicate that the activity of enzymes SOD and CAT decreases at doses higher than 30 mg/kg DZN and 0.3 mg/kg POX. Serum contains little CAT, SOD, GPx and GSH; its antioxidant properties are dependent on small molecules such as ascorbic acid, bilirubin, uric acid, ferritin and transferring [13]. SOD is the first line of defense against superoxide anion radicals, which converts it into H_2O_2 and O_2. H_2O_2 is converted into H_2O and O_2 through CAT enzyme. The decrease in SOD activity probably increases superoxide radicals. This increase had an inhibitory effect on CAT activity and decrease CAT activity, which can lead to tissue damage and oxidative stress [4]. This study results are consistent with results of studies showing that

organophosphate of phosphamidon and DZN inhibit the activity of SOD [14, 15]. Khan et al with study on the effect of chlorpyrifos and cypermethrin (administration for 2 weeks) on liver rats reported that the activity of SOD and CAT were decreased.[7] On the other hand, in some studies increased activity of antioxidant enzymes has been reported after prescription of the pesticide compounds. Sharma et al showed that administration of oral dimethoate to rats causes a dose-dependent increase in SOD and CAT activity in brain and liver [16].

The studies of Altuntas showed the increase of SOD activity during DZN and Fenton poisoning [17].The studies of Monterio et al showed that methyl parathion or folisuper leads to the production of free radical and increased activity of SOD and CAT enzyme in liver and muscle of fish [18]. The studies of Akturk et al showed that DZN increased the activity of SOD and CAT in rat heart and RBC [19]. The studies of Ghani et al showed that intraperitoneal administration of POX for 4 hours in rats increased the activity of enzymes SOD and CAT in the brain tissue [20]. The studies of Isik et al showed that consumption of DZN and methyl parathion to fish for 24 hours increases the activity of SOD in liver and lungs [21]. This difference of results in different studies is caused by the animal type, breed and species, tissue type, dose, exposure time and administration rout of toxin.

LDH is a cytoplasmic enzyme which is used to evaluate cellular damage as a marker to examine toxicity of a chemical substance and cell lysis. The activity of this enzyme is directly correlated with the extent of cell death [22]. In this study, the effect of DZN and POX on serum LDH activity shows that LDH activity increases at doses higher than 30 mg/kg DZN and 1 mg/kg POX. The increase of this enzyme may be due to the increased tissue damage and its leak into the serum. The studies of Gokcimen et al showed that DZN administration to rats for 24 hours increased the activity of LDH enzyme in the liver and pancreas [23]. In animal studies, Manna et al also reported the increased activity of serum LDH after administration of some compounds such as alpha-cypermethrin [24]. Mishra et al showed that consumption of endosulfan to fish decreased LDH activity in the liver and skeletal muscle [25]. The studies of Ghani et al showed that administration of POX to rats for 4 hours decreased LDH of liver [20]. Different results could be due to differences in administration type, the concentration of the toxin used, breed and species of animal, tissue type and even specific LDH isoenzymes.

GSH is a peptide containing thiol, one of main cellular antioxidant which is synthesized in the liver cytoplasm and then distributed to the other tissues through blood. GSH plays an important role in scavengering ROS and detoxification of drugs and chemicals [26]. GSH is essential for conversion of dehydroascorbic into ascorbate which acts as antioxidant to neutralize superoxide [27]. In the present study, the concentration of serum GSH decreases at doses higher than 30 mg/kg DZN and 0.3 mg/kg POX. The decreased GSH may be due to increased utilization of GSH for conjugation and/or participation of GSH as an antioxidant in neutralizing free radicals [6].

In addition, decreased GSH of serum is also a reflection of hepatic GSH synthesis reduction which decreases GSH of extrahepatic tissues and disturbs GSH homeostasis of the whole-body [4, 5]. The studies of Buyukokuroglu et al showed that, Fenthion consumption decreased GSH level of human muscle after 24 hour [28].

The studies of Monteiro et al also showed that the consumption of methyl parathion (MP) by fish decreased GSH level in tissues of liver, white muscle and gills [18]. The studies of Khan et al showed that consumption of cypermethrin and malathion by rats reduces GSH level in the liver [7].

Butrylcholinesterase is one of the target enzymes of organophosphates (diazinon and paraoxon) whose toxic effects are created through cholinesterase inhibition, which is necessary for nervous system function. This enzyme exists in serum and liver and is particularly important in terms of pharmacology and toxicology and quickly reveals the acute effects of organophosphates [2, 29].

Measuring the activity of this enzyme has high value in the diagnosis of poisoning with insecticides. In this study, serum BChE activity did not change significantly due to the administration of DZN and POX. This result is probably due to the improvement of serum BChE activity after 24 hours and performance of other body systems such as antioxidant defense system. In addition, the effect of organophosphates on BChE activity is dependent on the consumed dose, contact duration, and type of tissue and animal. The studies of Sun et al showed that BChE enzyme activity makes no changes in the blood sample after prescription of human BChE and then, administration of soman after 14 months [30].The studies of Sayim et al showed that BChE enzyme activity was decreased after consumption of dimethoate to rats for 90 consecutive days [31].

The studies of Nikookar et al showed that after consumption of malathion for 14 days to rats reduces the activity of BChE in the liver and serum [32].Comparison of the effect of DZN and POX on the parameters of serum oxidative stress shows that both toxins act similarly. However, the POX dose used, which is much less than DZN, indicates its greater toxicity. P=S bond exists in diazinon structure, which turns into P=O bond in paraoxon structure, This change leads to more severe activity of this oganophosphate [33, 34].

In summary, the results suggest that the effects of DZN and POX on serum antioxidant defense system are in a dose-dependent manner. The decreased activity of antioxidant enzymes and depleted GSH

content probably represents the antioxidant defense system failure against the toxic actions of free radicals, which may lead to oxidative stress. POX has much more severe effects.

Acknowledgements

This work was supported by a grant from Chemical Injuries Research Center and Neuroscience Research Center of Baqiyatallah University of Medical Sciences.

References

1. Storm JE, Rozman KK, Doull J. Occupational exposure limits for 30 organophosphate pesticides based on inhibition of red blood cell acetylcholinesterase. Toxicology 2000; 150: 1-29.
2. Abdollahi M, Mostafalou S, Pournourmohammadi S, et al. Oxidative stress and cholinesterase inhibition in salvia and plasma of rats following subchronic exposure to malathion. Comp Biochem Physiol 2004; 137: 29-34.
3. Kappers WA, Edwards RJ, Murray S and Boobis AR. Diazinon is activated by CYP2C19 in human liver. Toxicol Appl Pharmacol 2001; 177: 68-76.
4. Limon-Pacheco J, Gonsebatt ME. The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutation Res Gen Toxicol Environ Mut 2009; 674: 137-147.
5. Sleven H, Gibbs JE, Heales S, et al. Depletion of reduced glutathione precedes inactivation of mitochondrial enzymes following limbic status epilepticus in the rat hippocampus. Neurochemistry International 2006; 48: 75-82.
6. John S, Kale M, Rathore N and Bhatnagar D. Protective effect of vitamine E in dimethoate and malathion induced oxidative stress in rat erythrocytes. J Nut Biochem 2001; 12: 500-4.
7. Khan SM, Sobti RC, Katarial A. Pesticide-induced alteration in mice hepato-oxidative status and protective effects of black tea extract. Clinica Chim Acta 2005; 358: 131-138.
8. Ellman GL, Kourtney KD, Andres VJR, et al. A new and rapid colorimetric determination of acethylcholinesterase activity. Biochem Pharmacol 1961; 7: 88-95.
9. Winterbourn C, Hawkins R, Brian M and Carrell R. The estimation of red cell superoxide dismutase activity. J Lab Clin Med 1975; 85: 337.
10. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-126.
11. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. Anal Biochem 1969; 27: 502-22.
12. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the priniple of protein-dye binding. Anal Biochem 1976; 72: 248-254.
13. Naghii MR. Sulfur mustard intoxication, oxidative stress, and antioxidants. Military Med 2002; 167: 573-575.
14. Naqvi SM, Hasan M. Acetylhomocystein thiolactone protection against phosphamidon-induced alteration of regional superoxide dismutase activity in the central nervous system and its correlation with altered lipid peroxidation. Indian J Exper Biol 1992; 30: 850-852.
15. Abdou HM, El Mzoudy RH. Oxidative damage, hyperlipidemia and histological alterations of cardiac and skeletal muscles induced by different doses of diazinon in female rats, J. Hazardous Materials 2010; 182: 273–278.
16. Sharma Y, Basher S, Irshad M, et al. Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. Toxicology 2005; 215: 173-181.
17. Altuntas L, Kilinc L, Orhan O and Demiral R. The effect of diazinon on lipid peroxidation and antioxidant enzymes in erythrocytes in vitro. Hum Exper Toxicol 2004; 23: 9-13.
18. Monterio DM, Rantin FT, Kalinin AL. The effects of selenium on oxidative stress biomarkers in the freshwater characid fish matrinxa, brycon cephalus exposed to organophosphate insecticide folisuper 600 br® (methyl parathion). Comp Biochem Physiol 2009; 149: 40-49.
19. Akturk O, Demirin H, Sutcu R, et al. The effects of diazinon on lipid peroxidation and antioxidant enzymes in rat heart and ameliorating role of vitamin E and vitamin C. Cell Biol Toxicol 2006; 6: 455-461.
20. Ghani E, Mohmmadi M, Jafari M, et al. [Evaluation of oxidative stress index in brain tissue of rats after expose to paraoxon] Persian. Kowsar Med J 2008; 1: 1-8.
21. Isik I, Celik I. Acute effects of methyl parathion and diazinon as inducers for oxidative stress on certain biomarkers in various tissues of rainbowtrout (Oncorhynchus mykiss). Pest Biochem Physiol 2008; 92: 38-42.
22. El-Demerdash FM. Lipid peroxidation, oxidative stress and acetylcholinesterase in rat brain exposed to organophosphate and pyrethroid insecticides. Food Chem Toxicol 2011; 49: 1346–1352.
23. Gokcimen A, Gulle K, Demirin H, et al. Effects of diazinon at different doses on rat liver and pancreas tissues. Pest Biochem Physiol 2007; 87: 103-108.
24. Manna S, Bhattacharyya D, Mandal TK and Das S. Repeated dose toxicity of alfa-cypermethrin in rats. J Vet Sci 2004; 5: 241-245.
25. Mishra R, Shukla SP. Endosulfan mediated effects on lactate dehydrogenase from the catfish. Toxicol Lett 1998; 95: 145.
26. Meister A, Anderson ME. Glutathione. Ann Rev Biochem; 52: 711-760.
27. Winkler BS, Orselli SM, Rex TS. The redox couple between glutathione, ascorbic acid: A chemical and physiological perspective. Free Radic Biol Med 1994; 17: 333-349.
28. Buyukokuroglu ME, Cemek M, Tosun M, et al. Dantrolen may prevent organophosphate-induced oxidative stress and muscle injury. Pest Biochem Physiol 2008; 92: 156-163.

29. Mishra H. Simplified procedures for purification and stabilization of human plasma butylcholinesterase. Process Biochem 2004; 39: 877-882.
30. Sun W, Bhupendra P, Lenz DE and Saxena A. Long-term effects of human butyrylcholinesterase pretreatment followed by acute soman challenge in cynomolgus monkeys. Chem Biol Int; 175: 2008: 428-430.
31. Sayim AF. Dimethoate-induced biochemical and histopathological changes in the liver of rats. Exp Toxicol Pathol 2007; 59: 237-243.
32. Nikokar N. [Study of correlation between rat liver and serum carboxylesterase and cholinesterase exposed to malathion] Persian [dissertation]. Tehran: Tehran University.
33. Mutch E, Blain PG, Williams FM. The role of methabolism in determining susceptibility to parathion toxicity in man. Toxicol Lett 1999; 107: 177-187.
34. Arufe MI, Romero JL, Gamero JJ and Mereno MJ. Oxidation of cholinesterase-inhibiting pesticides: A simple experiment to illustrate the role of bioactivation in the toxicity of chemicals. Biochem Educat 2000; 28: 174-177.

Please cite this article as: Salehi M, Jafari M, Saleh-Moqadam M, Asgari A. The comparison of the effect of diazinon and paraoxon on biomarkers of oxidative stress in rat serum. Zahedan J Res Med Sci (ZJRMS) 2012; 14(3): 18-23.