Türkiye Tarımsal Araştırmalar Dergisi
dergipark.gov.tr/tutad
Research Article

Turk J Agric Res
2017, 4(3): 275-280
© TÜTAD
ISSN: 2148-2306
e-ISSN: 2528-858X
doi: 10.19159/tutad.310371

The Effects of Fenarimol and Methyl Parathion on Glucose 6-Phosphate Dehydrogenase Enzyme Activity in Rats

Ferda ARI*, Egemen DERE, Hakan TOSUNOĞLU, İmren ALİOĞLU
Uludağ University, Faculty of Science and Art, Department of Biology, Bursa, TURKEY

Received: 03.05.2017 **Accepted:** 26.08.2017

ORCID ID (By author order)
orcid.org/0000-0002-6729-7908 orcid.org/0000-0001-9572-1051 orcid.org/0000-0001-7878-3373 orcid.org/0000-0003-0687-9362
*Corresponding Author: ferdaoz@uludag.edu.tr

Abstract: Fenarimol and methyl parathion are pesticides that have been used in agriculture for several years. These pesticides have significant effects on environmental and human health. Therefore, we investigated the effects of methyl parathion and fenarimol on glucose 6-phosphate dehydrogenase (EC 1.1.1.49) enzyme activity in rats. The glucose 6-phosphate dehydrogenase is the first enzyme of the pentose phosphate pathway and it is important in detoxifying reactions by NADPH generated. In this study, wistar albino rats administrated with methyl parathion (7 mg kg^{-1}) and fenarimol (200 mg kg^{-1}) by intraperitoneally for different periods (2, 4, 8, 16, 32, 64, and 72 h). The glucose 6-phosphate dehydrogenase enzyme activity was assayed in liver, kidney, brain, and small intestine in male and female rats. The exposure of fenarimol and methyl parathion caused increase of glucose 6-phosphate dehydrogenase enzyme activity in rat tissues, especially at last periods. We suggest that this increment of enzyme activity may be the reason of toxic effects of fenarimol and methyl parathion.

Keywords: Glucose 6-phosphate dehydrogenase, fenarimol, methyl parathion, pesticide

1. Introduction

Pesticides are chemical substances, designed for eliminating, preventing, or controlling variety of pests. Pesticides have subgroups such as insecticides, fungicides, acaricides, herbicides according to targets, activity, and structure (Arias-Estévez et al., 2008). Generally they used mixtures and have potential adverse effects on human health and ecosystem (Karadeniz and Yenisoy-Karakaş, 2015). Fenarimol is a pyrimidine-type fungicide used in agriculture, especially for protection from fungal spores or fungi (Paolini et al., 1996; Zhang et al., 2012; Oh et al., 2015). Methyl parathion (MP) is a widely used organophosphate insecticides in agriculture that is used to kill insects on crops (Anonymous, 1998). Fenarimol and MP have potential toxic effects on organisms and environment due to widespread usage. They also affected living organism and metabolic pathways including specific enzymes. Glucose-6-phosphate dehydrogenase is the first enzyme on pentose phosphate pathway (Frederiks and Vreeling-Sindelárová, 2001; Beydemir et al., 2003). This enzyme is able to produce ribose 5-phosphate and NADPH which are essential cellular systems on antioxidant pathway, membrane lipids synthesis, cytochrome p450 system reductive and nucleic acid synthesis (Stanton, 2012).

In this study, we investigated the effects of fenarimol and MP on G6PD enzyme activity in the liver, kidney, brain and small intestine tissues in rats and according to our investigation, there is no information in literature about the effects of fenarimol and MP on G6PD enzyme activity in rat tissues. The present study will provide further insight on toxic mechanism in tissues of rats exposed to fenarimol and MP.

2. Materials and Methods

2.1. Animals

Wistar albino rats (*Rattus norvegicus*) divided into control (2 male; 2 female) and experimental groups (4 male; 4 female). All animals (200-250 g) were purchased from Experimental Animals Feeding and Research Centre of Uludağ University. Rats housed in specialized animal room on 12-h light/dark cycle at 21-23 ºC and they were treated with corn oil while experimental groups were injected intraperitoneally with 200 mg kg^{-1} (LD_{50}) dose of fenarimol (Sigma-Aldrich, St. Louis, MO) and 7 mg kg^{-1} (LD_{50}) dose of MP (Sigma-Aldrich, St. Louis, MO). The rats were left without food and water for 24h prior to injection, ensuring the simultaneous initiation of metabolism of animals in both groups at the same time and after the injection phase, food and water were regularly given to the animals until the trial periods were completed. Treated and control rats were kept in plastic metabolic cages. Animals were euthanized via cervical dislocation at each time point (2, 4, 8, 16, 32, 64, and 72h post-injection).

2.2. Determination of glucose 6-phosphate dehydrogenase (G6PD) activity

The liver, kidneys, brain and small intestine were quickly removed and were perfused in ice-cold 0.15 M KCl, immediately after the homogenates were prepared and homogenized at 2000 rpm in a T-line laboratory stirrer type homogenizer. Each homogenate was centrifuged in a Dupont Instruments Sorvall "RC-5 super speed refrigerated centrifuge" at 48000 g for 30 minutes. Protein concentration was determined with the method of Bradford (Bradford, 1976) and bovine serum albumin was used as protein standard. The glucose 6-phosphate dehydrogenase activity was assessed spectrophotometrically via the Bohringer Manheim method (Bohringer, 1973).

2.3. Statistical analysis

Data were analyzed using SPSS 20.0 for windows and independent t-test was applied between data of control and experiment periods. The significance was calculated using one-way analysis of variance (ANOVA) and Student's *t*-test and as a result a value of $p<0.05$ was taken as statistically significant.

3. Results and Discussion

Pesticides are potential chemical pollutants which have increasing usage in agriculture and they have harmful effects on the environment and organisms (Igbedioh, 1991; Aktar et al., 2009). Pesticides change metabolism, especially enzyme activities by activation or inhibition (Beydemir et al., 2003; Ekinci and Beydemir, 2010). In the literature, there are various studies about the effects of pesticides on G6PD activity (Kaur and Sandhu, 2008; Şentürk et al., 2009; Ojha and Srivastava, 2012; Salvo et al., 2012; Topal et al., 2014). However, there is no data about G6PD responses of fenarimol and MP on rat tissues. Thus, it is important to obtain information about the effects of fenarimol and MP on rats.

Glucose 6-Phosphate Dehydrogenase is known as a useful biomarker for antioxidant system and its activity is increased in oxidative stress (Salvemini et al., 1999; Gül et al., 2004). In present study, we investigated the effects of fenarimol and MP on G6PD activities in some tissues. The effect of fenarimol and MP on liver G6PD activity was determined in male and female rats. The G6PD activity did not change after treatment of fenarimol and MP in liver at first hours in experimental periods (2, 4 and 8 hours) while the G6PD activity generally increased at later periods (16, 32 and 64 hours) in rats. Similarly, significant difference at 72 hours was detected in male rats by fenarimol ($p<0.05$). However, the G6PD activation at 32 and 64 hours are significant in female rats by fenarimol ($p<0.05$) (Figure 1).

In kidney, the G6PD activity significantly increased by fenarimol at 16, 32, 64 and 72 hours in male and female rats compared with control group ($p<0.05$). These activations reached maximum level at 16^{th} hour and decreased through the end of the experimental periods (Figure 2a and 2b). However, the G6PD activity did not significantly change by methyl parathion in kidney tissues of rats (Figure 2c and 2d).

It is found that G6PD activity is increased in liver, and kidneys treated with fenarimol and MP, especially in male rats. These increments may be responsible to get rid of the toxic effects of MP and fenarimol, since G6PD plays an important role in detoxification pathway. Similarly, Rodriguez-Ariza et al. (1993) found that G6PD activity was increased after pesticide toxicity in gray mullet (*Mugil* sp.). In another study, it was shown that G6PD activity was increased in gill of fresh water mussel, *Lamellidens marginalis* by MP (Moorthy et al., 1985).

Effects of fenarimol and MP on G6PD activity in brain tissues of rats are shown in Figure 3. According to the results, fenarimol exhibited increased G6PD activity at last experimental periods (8, 16, 32 and 64 hours) compared with the controls in male and female rats. These increases are significant ($p<0.05$) at 8, 32 and 64 hours in

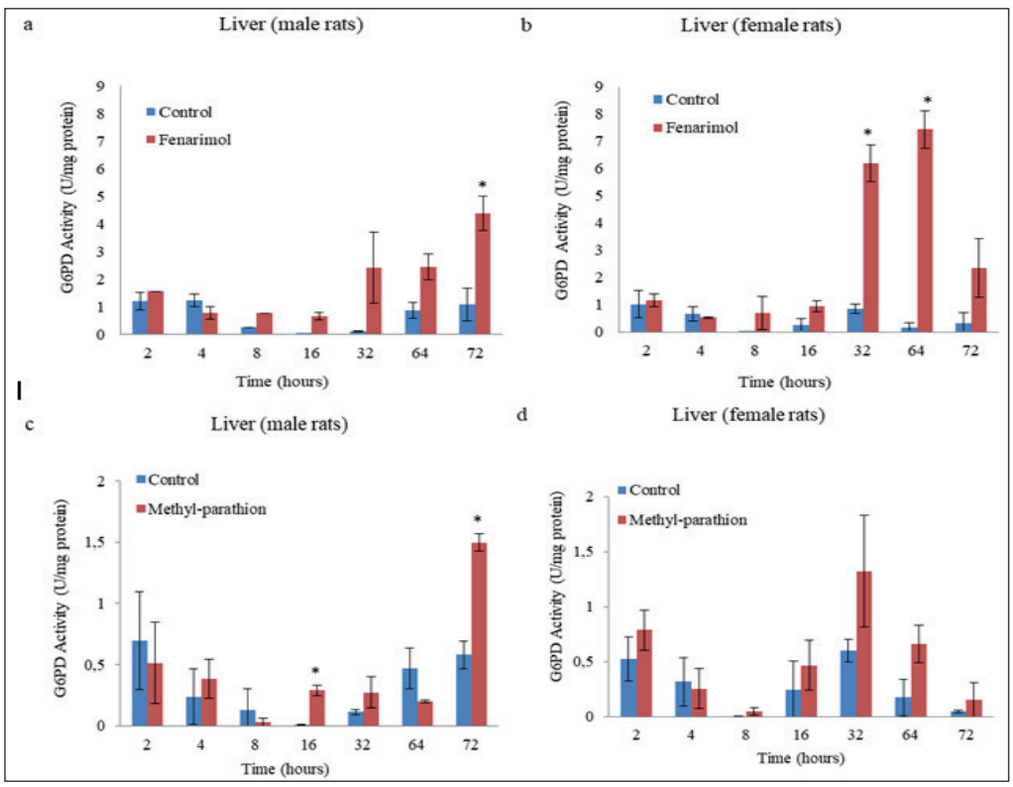

Figure 1. Effects of fenarimol (a, b) and methyl-parathion (c, d) on glucose 6-phosphate dehydrogenase (G6PD) activity (U/mg protein) in liver of rats

*: Denotes statically significant differences in comparison with control ($p<0.05$)

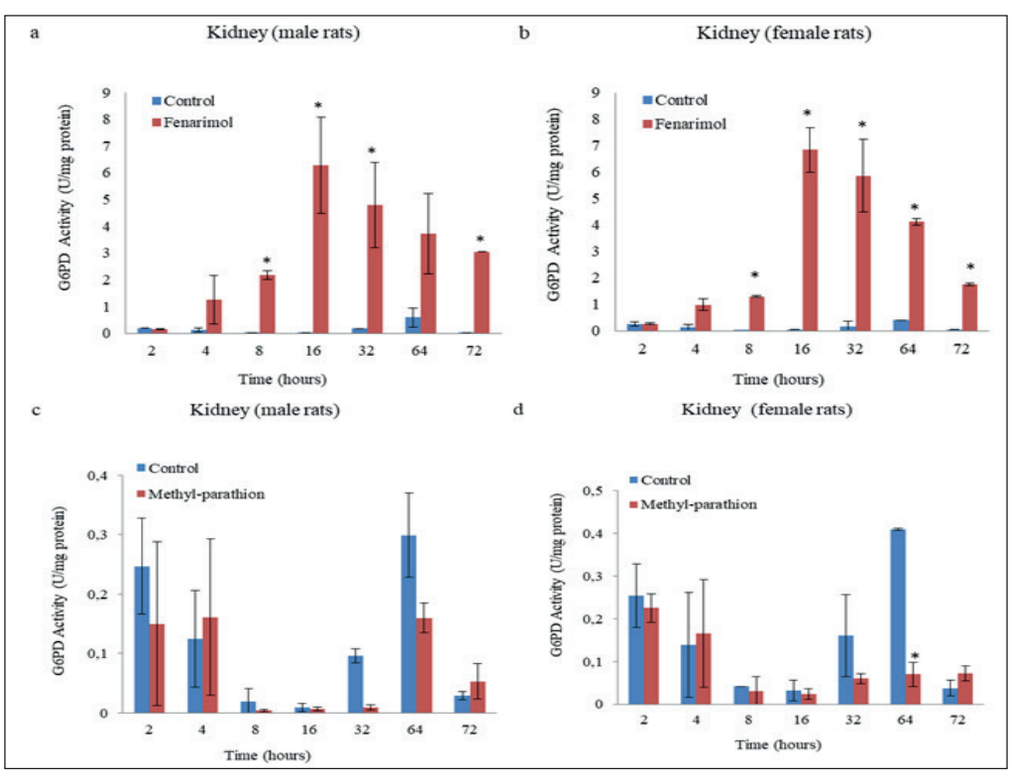

Figure 2. Effects of fenarimol (a, b) and methyl-parathion (c, d) on glucose 6-phosphate dehydrogenase (G6PD) activity (U/mg protein) in kidney of rats

*: Denotes statically significant differences in comparison with control ($p<0.05$)

brain tissues of female rats (Figure 3a and 3b). Although, the G6PD activity was decreased at 16 and 32 hours in male rats by methyl parathion (Figure 3c and 3d). In the study of Abdel-Mobdy et al. (2017), it was demonstrated that the G6PD elevates the ratio of carbofuran in liver kidney and brain of rats.

The G6PD activity of small intestine tissue increased at last experimental periods (32, 64 and 72 hours) by fenarimol in male and female rats compared with the control group. These results were found to be significant at 32 and 64 hours in male and at 64 hours in female rats ($p<0.05$) (Figure 4a and 4b). The G6PD activity was significantly increased at 16 hours in male and at 32 hours in female rats by methyl parathion (Figure 4c and 4d). In a study, rats were given hexachlorobenzene (environmental pollutants) and measured G6PD activity in tissues (intestine, liver, kidney etc.). It was found that G6PD activity decreased by hexachlorobenzene in all tissues (Khan et al., 2017).

We observed higher G6PD activity in the liver and kidney tissues than others. The liver is the main organ for the detoxification of xenobiotic, antioxidants and toxic materials, that are metabolized in the liver and ejaculated through the kidney, so we found the G6PD activity was higher in liver and kidney through fenarimol and MP toxicity. It was observed that MP and fenarimol have genotoxic effects on rats as toxicity (De Castro et al., 2005; Ojha and Gupta, 2015). In addition, we observed that G6PD activities in tissues are higher in male rats than female rats. Differences may occur by means of sex hormones being affected by fenarimol and MP. Ventura et al. (2016) showed that chlorpyrifos, which is a commonly used organophosphate pesticide changed hormonal balance in female rats. In another study, it was observed that atrazine has endocrine disrupter and inhibited testosterone production in male rats (Friedmann, 2002). A recent work has shown that exposure to propiconazole fungicide have effects on reproductive parameters by endocrine distribution in male rats (Costa et al., 2015). Another study has shown that combination of cypermethrin and MP have changed endocrine hormone levels, and immune functions in rats (Liu et al., 2006). In addition, MP has harmful effects on the reproductive system of male rats (Joshi et al., 2003).

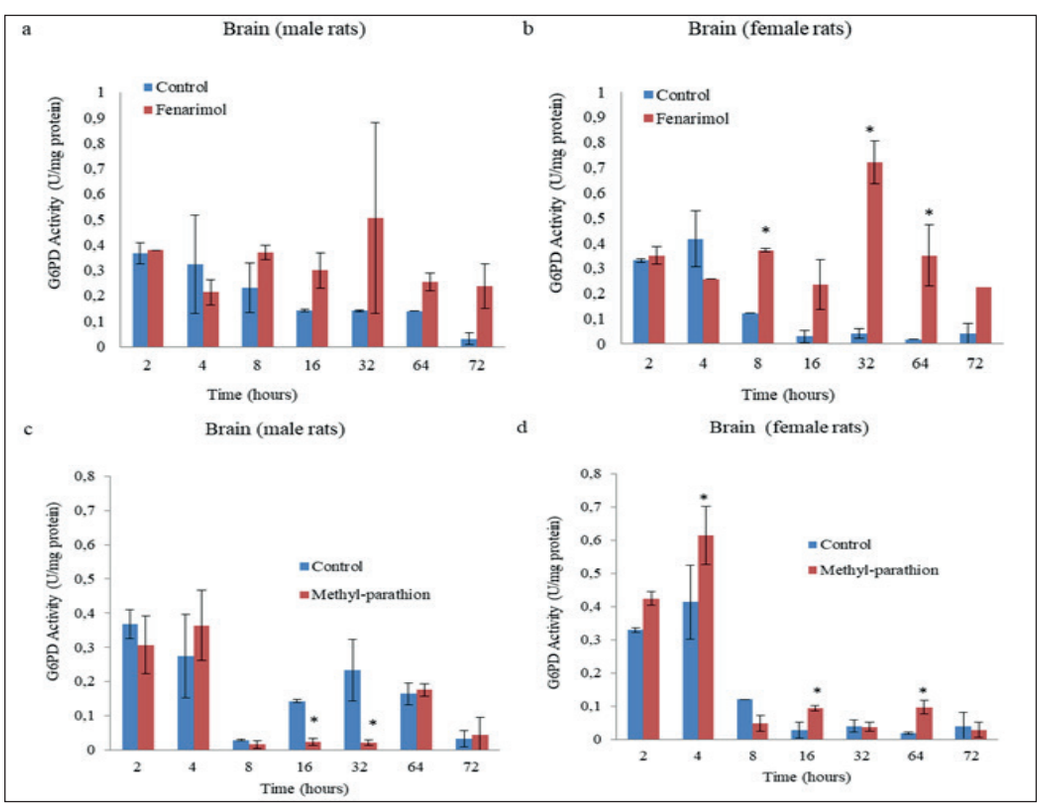

Figure 3. Effects of fenarimol (a, b) and methyl-parathion (c, d) on glucose 6-phosphate dehydrogenase (G6PD) activity (U/mg protein) in brain of rats

*: Denotes statically significant differences in comparison with control ($p<0.05$)

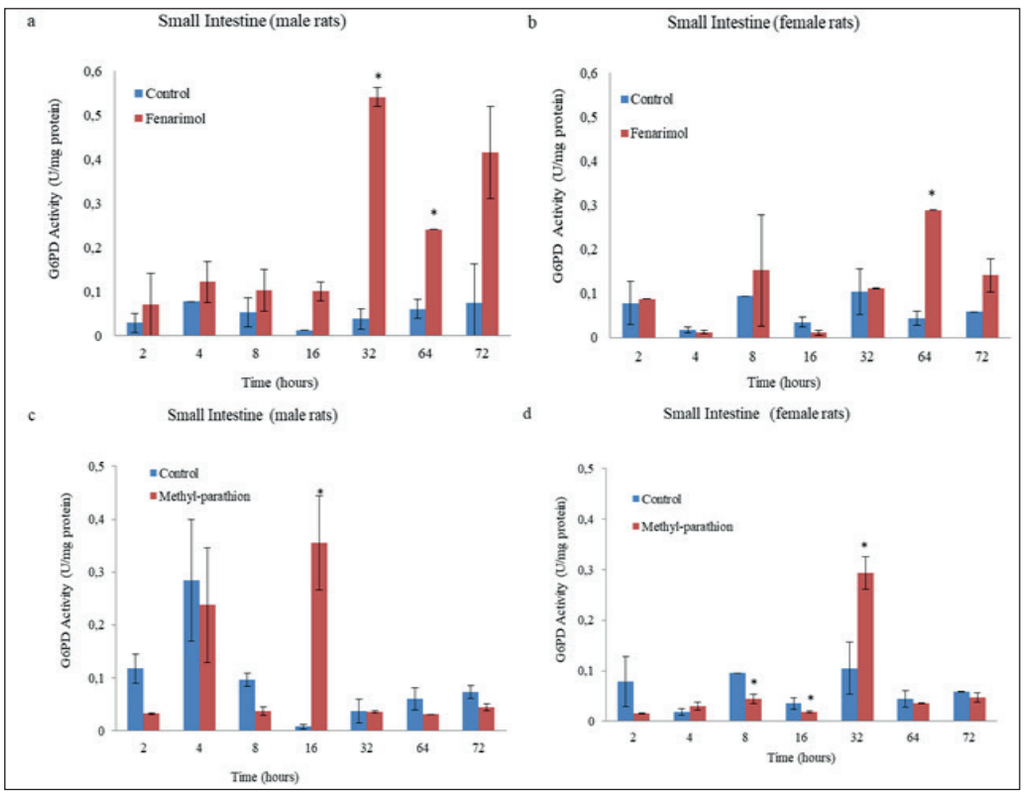

Figure 4. Effects of fenarimol (a, b) and methyl-parathion (c, d) on glucose 6-phosphate dehydrogenase (G6PD) activity (U/mg protein) in small intestine of rats

*: Denotes statically significant differences in comparison with control ($p<0.05$)

4. Conclusion

In conclusion, according to the results from the experiments, we can suggest that G6PD can be a useful biomarker in field monitoring of the effects of pesticide exposure on wildlife. Also, fenarimol and MP, widely used in agriculture, directly or indirectly affect the environment and the living organisms. Since its critically important to public health, usage of such chemicals should be careful and selective, and consumers and producers should be informed for possible and real damages.

References

Abdel-Mobdy, Y.E., El-Beltagi, H.S., Abdel-Mobdy, A.E., 2017. Alleviation of carbofuran toxicity effect by parsley on lipids profile of male albino rats. *Fresenius Environmental Bulletin*, 26(7): 4764-4773.

Aktar, M.W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology*, 2(1): 1-12.

Anonymous, 1998. US EPA Methyl Parathion Hazard Characterization. United States Environmental Protection Agency (US EPA), Washington, DC, 20460, USA.

Arias-Estevez, M., Lo´pez-Periago, E., Marti´nez-Carballo, E., Simal-Ga´ndara, J., Mejuto Juan, C., Garci´a-Ri´o, L., 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agriculture, Ecosystems & Environment*, 123(4): 247-260.

Beydemir, S., Gülçin, I., Kufrevioğlu, O.I., Çiftçi, M., 2003. Glucose 6-phosphate dehydrogenase: In vitro and in vivo effects of dantrolene sodium. *Polish Journal of Pharmacology*, 55(5): 787-792.

Bohringer, M., 1973. GmbH., G6PD: Glucose 6-Phosphate Dehydrogenase. *Biochemica Information I*, pp. 99.

Bradford, M.M., 1976. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-254.

Costa, N.O., Vieira, M.L., Sgarioni, V., Pereira, M.R., Montagnini, B.G., Mesquita Sde, F., Gerardin, D.C., 2015. Evaluation of the reproductive toxicity of fungicide propiconazole in male rats. *Toxicology*, 335: 55-61.

De Castro, V.L., De Mello, M.A., Poli, P., Zucchi, T.M., 2005. Prenatal and perinatal fenarimol-induced genotoxicity in leukocytes of in vivo treated rats. *Mutation Research*, 583(1): 95-104.

Ekinci, D., Beydemir, S., 2010. Risk assessment of pesticides and fungicides for acid–base regulation

and salttransportin rainbow trouttissues. *Pesticide Biochemistry and Physiology*, 97(1): 66-70.

Frederiks, W.M., Vreeling-Sindelárová, H., 2001. Localization of glucose-6- phosphate dehydrogenase activity on ribosomes of granular endoplasmic reticulum, in peroxisomes and peripheral cytoplasm of rat liver parenchymal cells. *The Histochemical Journal*, 33(6): 345-353.

Friedmann, A.S., 2002. Atrazineinhibition of testosterone production in rat males following peripubertal exposure. *Reproductive Toxicology*, 16(3): 275-279.

Gül, S., Belge Kurutas, E., Yıldız, E., Sahan, A., Doran, F., 2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan Dam Lake, Turkey. *Environment International*, 30(5): 605-609.

Igbedioh, S.O., 1991. Effects of agricultural pesticides on humans, animals and higher plants in developing countries. *Archives of Environmental Health*, 46(4): 218.

Joshi, S.C., Mathur, R., Gajraj, A., Sharma, T., 2003. İnfluence of methyl parathiom on reproductive parameters in male rats. *Environmental Toxicology and Pharmacology*, 14(3): 91-98.

Karadeniz, H., Yenisoy Karakaş, S., 2015. Spatial distributions and seasonal variations of organochlorine pesticidesin waterandsoil samplesinBolu, Turkey. *Environmental Monitoring and Assessment*, 187(3): 94.

Kaur, R., Sandhu, H.S., 2008. In vivo changes in antioxidant system and protective role of selenium in chlorpyrifos-induced subchronic toxicity in Bubalus bubalis. *Environmental Toxicology and Pharmacology*, 26(1): 45-48.

Khan, S., Priyamvada, S., Khan, S.A., Khan, W., Yusufi, A.N.K., 2017. Studies on Hexachlorobenzene (HCB) Induced Toxicity and Oxidative Damage in the Kidney and Other Rat Tissues. *International Journal of Drug Metabolism & Toxicology*, 1(1): 1-9.

Liu, P., Song, X.X., Wen, W.H., Yuan, W.H., Chen, X.M., 2006. Effects of mixed cypermethrin and methylparathion on endocrine hormone levels and immune functions in rats: II. Interaction. *Wei Sheng Yan Jiu= Journal of hygiene research*, 35(5): 531-533.

Moorthy, K.S., Kasi Reddy, B., Swami, K.S., Chetty, C.S., 1985. Glucose metabolism in hepatopancreas and gill of Lamellidens marginallis during methyl parathion toxicity. *Pesticide Biochemistry and Physiology*, 24(1): 40-44.

Oh, K., Matsumoto, T., Yamagami, A., Hoshi, T., Nakano, T., Yoshizawa, Y., 2015, Fenarimol, a Pyrimidine-Type Fungicide, İnhibits Brassinosteroid Biosynthesis. *İnternational Journal of Molecular Science*, 16(8): 17273-17288.

Ojha, A., Srivastava, N., 2012. Redox imbalance in rat tissues exposed with organophosphate pesticides and therapeutic potential of antioxidant vitamins. *Ecotoxicology and Environmental Safety*, 75(1): 230-241.

Ojha, A., Gupta, Y.K., 2015. Evaluation of genotoxic potential of commonly used organophosphate pesticides in peripheral blood lymphocytes of rats. *Human & Experimental Toxicology*, 34(4): 390-400.

Paolini, M., Mesirca, R., Pozzetti, L., Sapone, A., Cantelli-Forti, G., 1996. Molecular non-genetic biomarkers related to Fenarimol cocarcinogenesis: organ- and sex-specific CYP induction in rat. *Cancer Letters*, 101(2): 171-178.

Rodriguez-Ariza, A., Peinado, J., Pueyo, C., López-Barea, J., 1993. Biochemical indicators of oxidative stress in fish from polluted littoral areas. *Canadian Journal of Fisheries and Aquatic Sciences*, 50(12): 2568-2573.

Salvemini, F., Franzé, A., Iervolino, A., Filosa, S., Salzano, S., Ursini, M.V., 1999. Enhanced glutathione levels and oxidoresistance me diated by increased glucose-6 phosphate dehydrogenase expression. *The Journal of Biological Chemistry*, 274(5): 2750-2757.

Salvo, L.M., Bainy, A.C., Ventura, E.C., Marques, M.R., Silva, J.R., Klemz, C., Silva De Assis, H.C., 2012. Assessment of the sublethal toxicity of organochlorine pesticide endosulfan in juvenile common carp (*Cyprinus carpio*). *Journal of Environmental Science and Health, Part A, Toxic/Hazardous Substances & Environmental Engineering*, 47(11): 1652-1658.

Stanton, R.C., 2012, Glucose-6-phosphate dehydrogenase, NADPH, and cell survival. *İnternational Union of Biochemistry and Molecular Biology life Life*, 64(5): 362-369.

Şentürk, M., Ceyhun, S.B., Erdoğan, O., Küfrevioğlu, Ö.İ., 2009. *In vitro* and *in vivo* effects of some pesticides on glucose-6-phosphate dehydrogenase enzyme activity from rainbow trout (*Oncorhynchus mykiss*) erythrocytes. *Pesticide Biochemistry and Physiology*, 95(2): 95-99.

Topal, A., Atamanalp, M., Oruç, E., Kırıcı, M., Kocaman, E.M., 2014. Apoptotic effects and glucose-6-phosphate dehydrogenase responses in liver and gill tissues of rainbow trputtreated with chlorpyrifos. *Tissue & Cell*, 46(6): 490-496.

Ventura, C., Nieto, M.R., Bourguignon, N., Lux-Lantos, V., Rodriguez, H., Cao, G., Randi, A., Cocca, C., Núñez, M., 2016. Pesticide chlorpyrifos acts as an endocrine disruptor in adult rats causing changes in mammary gland and hormonal balance. *The Journal of Steroid Biochemistry and Molecular Biology*, 156: 1-9.

Zhang, H., Wang, X., Zhuang, S., Qian, M., Jiang, K., Wang, X., Xu, H., Qi, P., Wang, Q., 2012. Enantioselective separation and simultaneous determination of fenarimol and nuarimol in fruits, vegetables, and soil by liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 404(6-7): 1983-1991.