Vol.64: e21210300, 2021
https://doi.org/10.1590/1678-4324-2021210300
ISSN 1678-4324 Online Edition

*Article - Human and Animal Health*

# Protective Role of Ferulic Acid on Testis-Histoarchitecture and Oxidative Damages Induced by Dimethoate in Rats

**Hilmi Uzunbayır**[1]
https://orcid.org/0000-0001-6921-7759

**Fatma Gökçe Apaydın**[1]*
https://orcid.org/0000-0002-2771-7488

[1]Gazi University, Faculty of Science, Department of Biology, Ankara, Turkey.

Editor-in-Chief: Alexandre Rasi Aoki
Associate Editor: Sinvaldo Baglie

Received: 2021.05.09; Accepted: 2021.06.17.

*Correspondence: fguzun@gazi.edu.tr (F.G.A.).

**HIGHLIGHTS**

- Dimethoate caused reproductive toxicity.
- Ferulic acid ameliorated some parameters.
- Histopathology and oxidative stress are very important criters.

**Abstract:** Dimethoate is frequently used all over the world and it caused serious toxicity on target and non-target organisms. In this study, distilled water, ferulic acid, low dose dimetoate, high dose dimetoate, ferulic acid and low dose dimetoate, ferulic acid and high dose dimethoate were given to rats through gavage during the 4-week experiment. For this purpose, the levels of glutathione peroxidase, superoxide dismutase, glutathione-S-transferase, catalase, malondialdehyde and histopathological damages were investigated. After 28 days, no statistically important difference was determined in all investigated parameters in testicular tissues of rats who were administered control and ferulic acid. When the control and ferulic acid groups compare with the low and high dose dimetoate groups, there were statistically significantly changes antioxidant enzymes and malondialdehyde levels. In ferulic acid plus low dose dimethoate treated group and ferulic acid plus high dose dimethoate treatment we demonstrated that the protective role of ferulic acid on examining parameters but not completely. Based on light microscope studies, we can say that both dose dimethoate induced numerous histopathological changes. Less pathological alterations were observed when rats ferulic acid-plus-dimethoate. As a result, it is possible to say that ferulic acid has a partially healing role on the oxidative damage caused by dimethoate.

**Keywords:** Pesticide toxicity; ferulic acid; oxidative stress; histopathology; testicular dysfunction.

## INTRODUCTION

Organophosphorus pesticides (OP) are generally used in agricultural areas as acaricides and insecticides and further in industrial purpose and medicine. Organophosphate pesticides residues have been accumulating in the soil, many vegetables, in certain parts of cereal products and various foods [1, 2]. Accumulation in this way causes toxic ethics in terms of living things' health [3]. Acetylcholinesterase enzyme inhibition is the major way of pesticides [4].

Dimethoate (DMT), (O, O-dimethyl-S-methyl- carbomethyl) is one of the effective organophosphate pesticides (OP) frequently used of worldwide [7]. It is also used to control to pest of apples, pears, fruits [5]. DMT induces number of toxic effects including acetylcholinesterase inhibition [6], hepatotoxicity [6, 7], hematotoxicity [8], cardiotoxicity [9], hepatic dysfunction [10].

Many studies showing the toxic effects of pesticides, flavonoids are used to show a healing effect [11,12]. Since many insecticides are hydrophobic, they attach to the phospholipid layer of membranes especially [13]. Phenolic acids, such as syringic acid, caffeic acid, rosmarinic acid, vanillic acid, ferulic acid, and are commonly found in plants [14]. Ferulic acid (FA) derived from Ferula foetida, a phenolic compound in plant cell walls, and is a source to many aromatic compounds. In addition, FA is known that water soluble antioxidant and it is commonly present in grains, leaves, rice, seeds of coffee, tomato, wheat, oat, flowers, carrot, fruits, bean, peanut, nuts, spinach, avocado, broccoli and pineapple [15, 16].

Formation of free radicals are caused to induce organ injury. It has been shown that dietary antioxidants increase the amount of cellular antioxidants. In addition, antioxidant supplements are used in chelation therapy [17, 18]. Living organisms are always exposed to Reactive oxygen species (ROS), which occur as by-products of anabolism and catabolism reactions, normal cellular respiration, and the autoxidation of xenobiotic, or as a result of oxidative stress seen in many disease states [19].

Oxidative stress is a result of out of balance between reactive oxygen species and antioxidant enzyme defense system, and this balance benefits ROS. This oxidative stress effects a number of cellular functions and caused to various pathological situations in which ROS overwhelm anti-oxidative conservation of the organism, leading to oxidative ruin of the mentioned biological macromolecules, tissue damage, and necrosis as the foundation [20, 21]. In this study we aim examine oxidative stress and pathological changes of the testis of rats after 4–week-subacute exposure to dimethoate and to assess the protective potential of ferulic acid.

## MATERIALS AND METHODS

### Reagents

Ferulic acid (purity>97%), dimethoate (purity>97%), thiobarbituric acid (TBA), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), reduced glutathione, were purchased from Sigma (St. Louis, MO, USA). All other chemical substances used in this study have analytical standards.

### Animals

#### *Ethical approval*

In our study were conducted with the confirmation of Gazi University Animal Experiments Local Ethics Committee (G.U. ET-17.004).

#### *Animals, treatment and groups*

Adult Wistar rats, weighing 200-250 g. were purchased from Gazi Universitiy Laboratory of Aniımals Raising and Experimental Research Center. The animals had free access to commercial rodent food diet and tap water as also *ad libitum*. All chemicals were applied by oral gavage. Our treatment groups are as follows:

- Group 1: Control: distilled water (n=6)
- Group 2: Ferulic acid (FA): 30 mg/kg bw (n=6)
- Group 3: Low dose-DMT (LDMT): 3mg/kg bw (1/100 $LD_{50}$) (n=6)
- Group 4: High dose-DMT (HDMT): 30 mg/kg bw (1/50 $LD_{50}$) (n=6)
- Group 4: Ferulic acid+Low dose-DMT: 30 mg/kg bw FA and 3mg/kg bw LDMT, respectively (n=6)
- Group 5: Ferulic acid+High dose-DMT: 30 mg/kg bw FA and 30 mg/kg bw HDMT, respectively (n=6)

## Histopathology

After the experimentation to animals is over, the rats were sacrificed and examined for testis tissue pathological abnormalities. After tissues were obtained, they were taken into 10% formalin fixation and dehydrated in alcohol, and finally embedded in paraffin. Then testis sections were cut with 6-7µ thickness. The slides were then dehydrated and stained with hematoxylin and eosin. At least ten slides were observed with microscope (Olympus, Tokyo, Japan). Each tissue slides were investigated and determined for severity of changes using scores on rating chart of none (-), mild (+), moderate (++), and severe (+++) damage (Table 1).

### *Biochemical evaluation for oxidative tissue damages*

After experimental period, animals of different groups were anesthetized using alphamine and alphazine combination. Testis tissues were quickly removed from the rats using standart procedure for biochemical and microscopic studies. For biochemical studies, testicular tissues are carefully separated from auxiliary tissues. and washed with isotonic phosphate-buffered saline solutions. After washing, tissues were frozen in liquid nitrogen and preserved in a -80 freezer until the time of operation. Some of the testicular tissues are homogenized in a Heidolph Silent Crusher M homogenizer and prepared to measure oxidative stress parameters. After that the homogenates were centrifuged at 4 °C. All procedures were carried out at 4 °C. The supernatant was used as a source of MDA content and antioxidant enzyme activities using with spectrophotometer (Shimadzu UV 1700, Kyoto, Japan). The amount of protein in tissues was detected by method of Lowry [22]. Protein determination was made using Bovine serum albumin (BSA).

### *Lipid Peroxidation*

Determination of malondialdehyde (MDA) tissue content was showed using the thiobarbituric acid (TBA) calculation as described by Ohkawa's method [23]. MDA reacts with TBA to form a colored compound. Absorbance was measured at 532 nm to determine the MDA content. The data was expressed as nmol/mg protein.

### *Measurement of superoxide dismutase (SOD)*

We measured the SOD activity according to the method determined by Marklund and Marklund [24] by analyzed the autooxidation of pyrogallol value at 440 nm for 3 min. One unit of total SOD activity was based on the amount of protein that caused 50% pyrogallol inhibition. The total SOD activity was determined as U/mg protein.

### *Measurement of catalase (CAT)*

CAT activity was described by to the method specified by Aebi [25]. We assayed the hydrolysis of $H_2O_2$ and the resulting decline in absorbance at 240nm. CAT activities were defined as nmol/mg protein.

### *Measurement of glutathione-S-transferase (GST)*

GST activity was analyzed by founding the formation of GSH (Glutathione) and the 1-chloro-2,4-dinitrobenzene (CDNB). Increases in absorbance were recorded at 340 nm for 3 min [26]. The specific activity of GST was expressed as nmol/mg protein.

### *Measurement of glutathione peroxidase (GPx)*

GPx activity was detected via $H_2O_2$ as substrate by method described as Paglia and Valentine [27]. We assayed the reaction at 240 nm. GPx activity was described as nmol/mg protein.

### *Statistical analysis*

SPSS 13.0 used in this study for the analyzed of data's. The significance of differences was calculated using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons. $P < 0.05$ was considered statistically significant. Values are mean ±SD of six rats in each group.

## RESULTS

### Histological changes in the testis

In ferulic acid-treated and control group we show that sperm cells at different stages in the seminiferous tubules in normal structure. The seminiferous tubules appeared uniform in size and shape. They were lined by regularly arranges rows of sperm cells at different stages of maturation (Fig 1A-B). After 4 weeks of low dose dimethoate exposure, lesser number of spermatogenic cells, degeneration of seminiferous tubules and edema in interstitial area were observed (Figure 1C). In high dose of dimethoate treated groups we showed that more degeneration of seminiferous tubules, edema in interstitial area, atrophy and decreasing number of spermatogenic cells (Figure1D). After experimental period in ferulic acid+LDMT treated group, spermatogenic cells declined in several seminiferous tubules and less edema in interstitial area (Figure 1E). In ferulic acid plus HDMT we showed that spermatogenic cells decreased in some seminiferous tubules, edema in interstitial area and degeneration in some seminiferous tubules (Figure 1F).

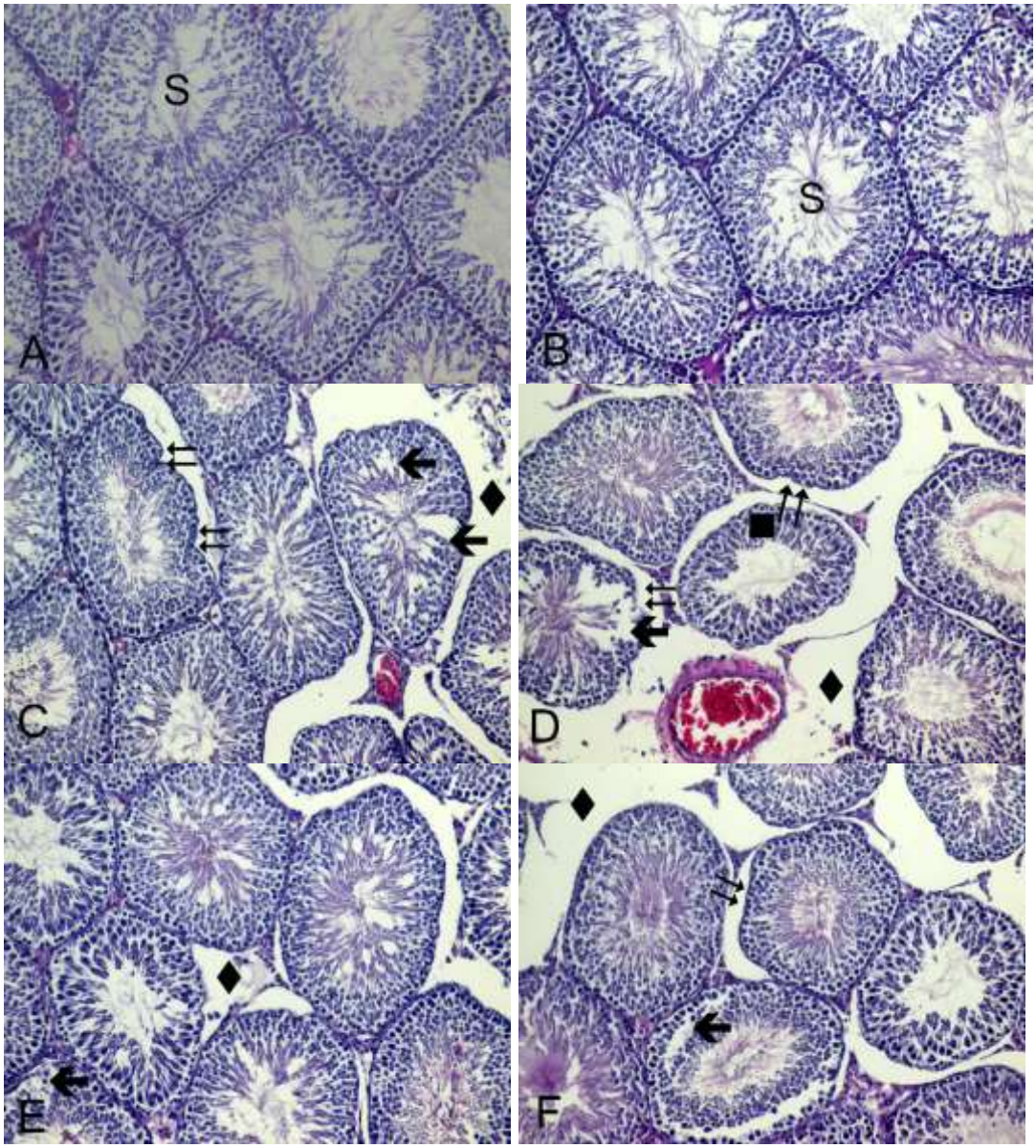


**Figure 1.** (A) Testicular section of control rats showing seminiferous tubules (S) and interstitial tissue X200. (B) Testicular section of FA treated rats showing seminiferous tubules (S) and interstitial tissue X200. (C) Testicular sections of LDMT-treated rats showing edema (♦) in interstitial tissues, decreasing number of spermatogenic cells (🡸) and degenerative chances in seminiferous tubules (⇆) X200. (D) Testicular sections of HDMT-treated rats showing edema (♦) in interstitial tissues, decreasing number of spermatogenic cells (🡸), degenerative chances in seminiferous tubules (⇆) and atrophy (■) X200. (E) Testicular sections of FA+LDMT-treated rats showing edema (♦) in interstitial tissues, decreasing number of spermatogenic cells (🡸) X200. Testicular sections of FA+HDMT-treated rats showing edema (♦) in interstitial tissues, decreasing number of spermatogenic cells (🡸) and degenerative chances in seminiferous tubules (⇆) X200.

**Table1.** Grading of the histopathological changes in the testes sections of dimethoate exposure to rats. Scoring was done as follows: none (-), mild (+), moderate (++) and severe (+++).

| Groups and Pathology | Atrophy | Decreasing number of spermatogenic cells | Disorganization of seminiferous tubules | Edema |
|---|---|---|---|---|
| **Control** | - | - | - | - |
| **FA** | - | - | - | - |
| **LDM** | - | ++ | ++ | ++ |
| **HDM** | + | +++ | ++ | +++ |
| **FA+LDM** | - | + | + | + |
| **FA+HDM** | - | + | + | + |

## Evaluation of MDA levels

As a result of this work, there were not statistically significant changes in MDA levels when the ferulic acid treated rats compared to control rats (Figure 2). There was statistically significant increase in MDA activity in the low and high dose dimethoate-treated group compared to control group. When the ferulic acid plus low dose dimethoate treated groups compared to control group there were statistically significant increase, too. We showed considerably reduced MDA levels significantly in the FA plus LDMT treated group and FA plus HDMT treated group compared to LDMT and HDMT treated groups ($P<0.05$, Figure 2).

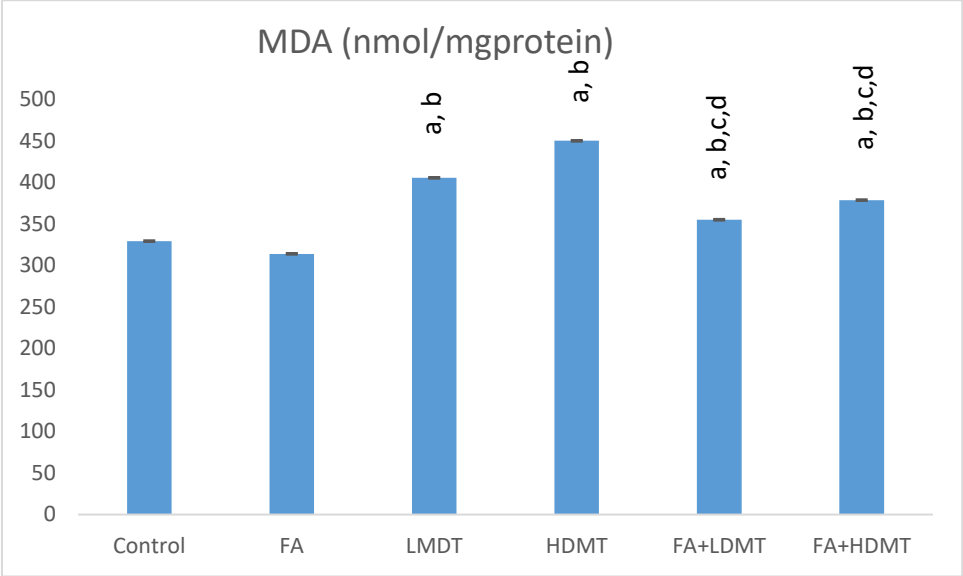


**Figure 2.** Values are mean ± SD of six rats in each group. Significance at $P < 0.05$.
a Comparison of control and other groups.
b Comparison of FA group and other groups.
c Comparison of LDMT group and other groups.
d Comparison of HDMT group and other groups.

## Antioxidant Enzyme Activities

There were not statistically important changes in GST, CAT, and GPx, SOD activities when the ferulic acid treated rats compared to control rats (Figure3-6). A significantly increase was determined in CAT, SOD, GST and GPx activities at the end of the 4th week in low dose and high dose dimethoate groups compared to control group. When feruic acid plus low dose dimethoate and feruic acid plus high dose dimethoate treated groups were compared to control group, SOD, CAT, GST and GPx activities significantly increase at the end of the 4th week. However, all the antioxidant activities were statistically decreased significantly decreased in the FA plus LDMT treated group and FA plus HDMT treated group compared to LDMT and HDMT treated groups ($P<0.05$) (Figure 3-6).

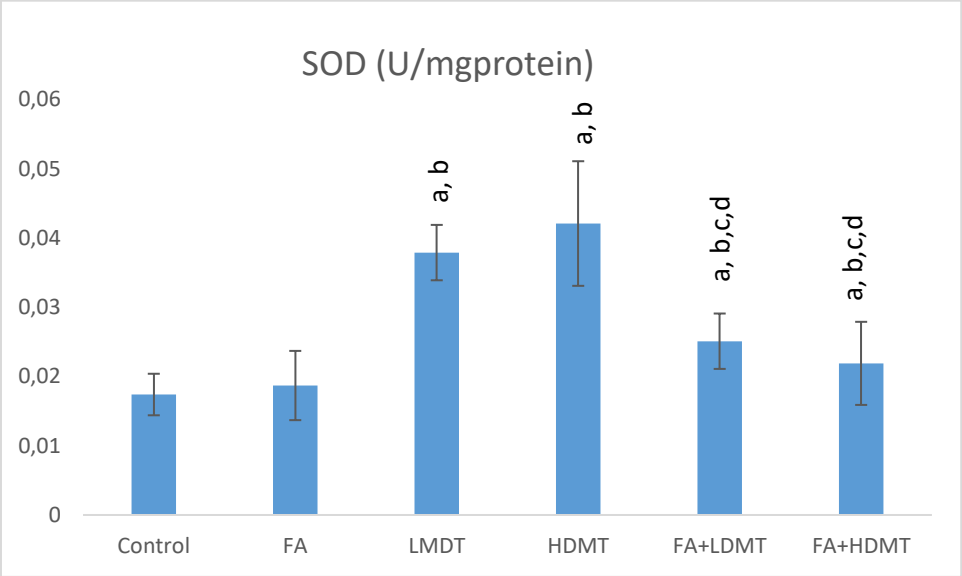


**Figure 3.** Values are mean ± SD of six rats in each group. Significance at P < 0.05.
a Comparison of control and other groups.
b Comparison of FA group and other groups.
c Comparison of LDMT group and other groups.
d Comparison of HDMT group and other groups.

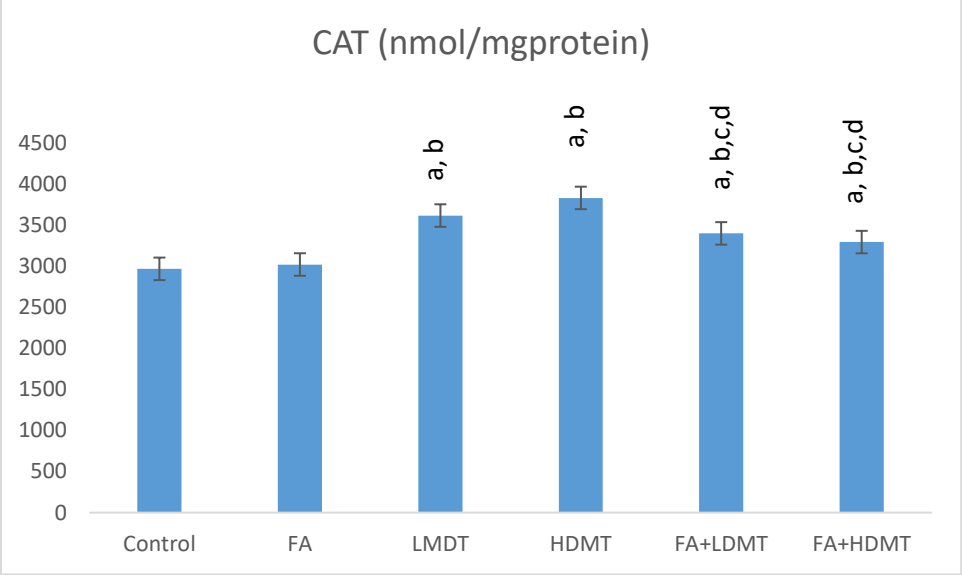


**Figure 4.** Values are mean ± SD of six rats in each group. Significance at P < 0.05.
a Comparison of control and other groups.
b Comparison of FA group and other groups.
c Comparison of LDMT group and other groups.
d Comparison of HDMT group and other groups.

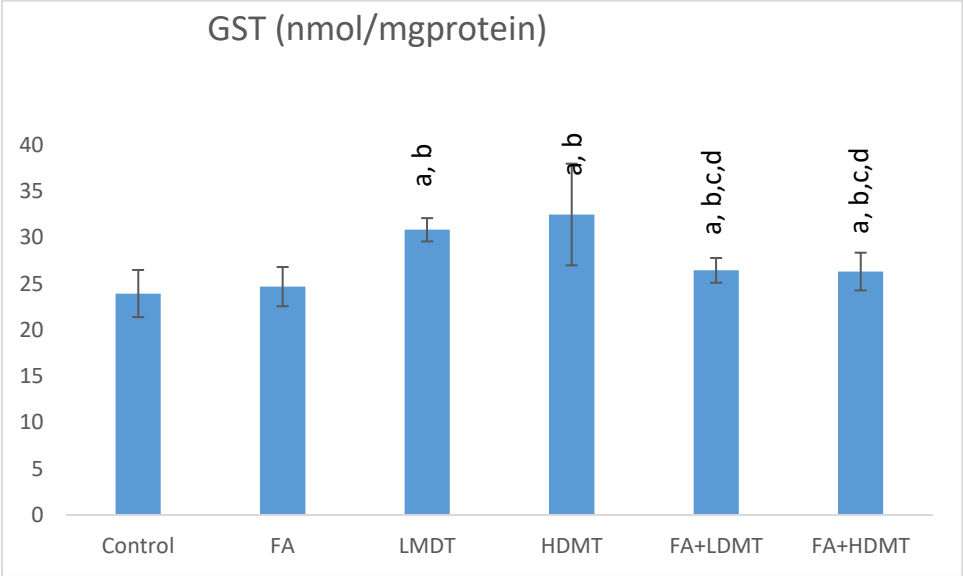


**Figure 5.** Values are mean ± SD of six rats in each group. Significance at P < 0.05.
a Comparison of control and other groups.
b Comparison of FA group and other groups.
c Comparison of LDMT group and other groups.
d Comparison of HDMT group and other groups.

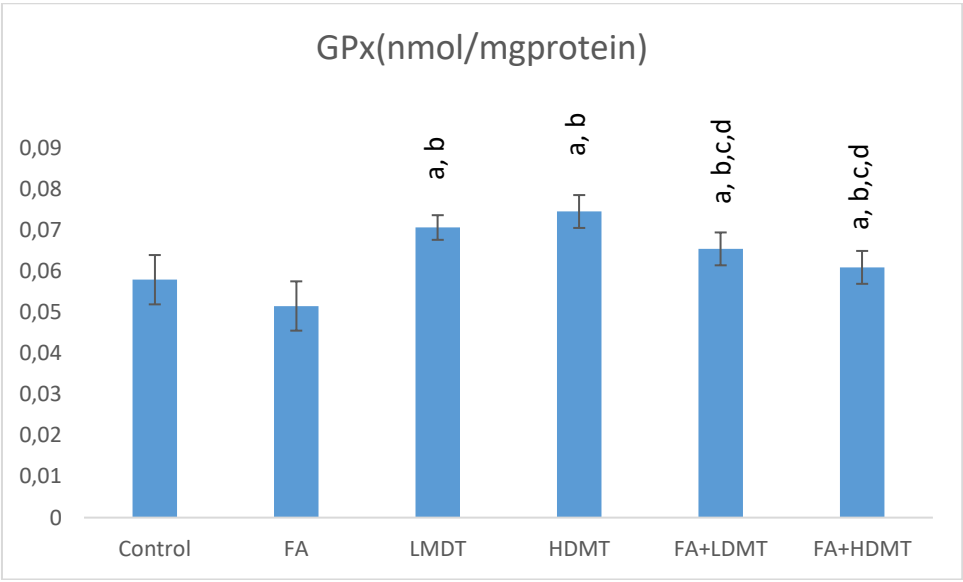


**Figure 6.** Values are mean ± SD of six rats in each group. Significance at P < 0.05.
a Comparison of control and other groups.
b Comparison of FA group and other groups.
c Comparison of LDMT group and other groups.
d Comparison of HDMT group and other groups.

## DISCUSSION

The oral dose LD50 of dimethoate is 300 mg/kg for male experimental rats [10]. In the present study, DMT was given at 1/10 and 1/100 LD50 different oral dose, depending on dose we determined several histopathological changes and oxidative damage in rat testes; however, no rats died during 28 days of experimental protocols. It has been known that pesticides cause various pathological changes on cell and tissue in the experimental male animals' reproduction system [28,29]. Dimethoate is a widely used pesticide affecting different organs. Acute and chronic studies of dimethoate have shown that this pesticide is very toxic effects to mammals [7].

OP insecticide such as Malathion, caused decline of sperm motility in the testes [28]. In the present study, different doses of dimethoate caused pathological effetcs in the spermatojenic cells after subacute exposure.

Low and especially high dose of DMT caused interstitial edema and degenerations in the seminiferous tubules. These effects caused by the toxic effect of the DMT on tissues.

OPs adversely affect the male reproductive system in several ways such as changing antioxidant enzyme activities and causing lipid peroxidation [11]. Reactive oxygen species cause damage to sperm and other organelle membrane structures through peroxidation of macromolecules, thereby altering sperm motility. With the increased amount of ROS, spermatozoa are damaged and lipid peroxidation, which is polyunsaturated oil peroxidation, occurs. Reactive oxygen species cause problems in sperm production in the male reproductive system [30].

An imbalance of pro-oxidant and antioxidant value amount in tissue and primarily macromelucules is known to cause important damage to cell membranes, such as proteins, carbohydrates, DNA, and finally injure the tissues and all systems [31,32]. Therefore, exogenous antioxidant supplementation would have a significant mission on the cell's antioxidant defenses to deactivate dimethoate intoxication. Lipid peroxidation is known to oxidative destruction of lipid in being of oxidative free radicals [33, 34]. In cellular antioxidant enzymes like SOD, metabolizes the alteration of O2- to less-reactive species O2 and to the H2O2 [35]. SOD works with other enzymes inside the cell like GST, GPx and CAT because of conjunction with to eliminate H2O2 [36]. CAT play a key role, which catalyses the decomposition of H2O2 to H2O and O2 [37]. GST, GPx, and GR work together with glutathione in the decomposition of H2O2 and other organic hydroperoxides [37]. GSH is a multitasking primarily intracellular non-enzymatic antioxidant. It acts as thiol disulfide buffer in the cell. GSH is one of the enzymes that protect cells from reactive oxygen species by being converted into its oxidized form, GSSG [38]. So the level of these enzymes gives information about cellular toxicity.

## CONCLUSION

In conclusion, when we look at the differences in testicular histology of DMT-intoxicated rats showed many histopathological damages as compared to normal rats. Additionally, administration of ferulic acid could preserved the general histological features of the tissue. Oxidative stress is major mechanism has been proposed involved in DMT toxicity. Exposure to DMT at low and high doses disrupted the oxidative balance in the testes and histopathological damages, which is a critical organ. and tissues could be overcome through simultaneous treatment with ferulic acid. It could be suggested that the supplementation with antioxidants may be a potential therapy in the treatment of subchronic DMT intoxication.

Antioxidants are of biological importance for the normal functioning of the immune system as well as the elimination of many carcinogenic compounds [39]. Taken together, ferulic acid may be regarded as a promising dietary supplement for treatment of testes diseases due to its well-known beneficial biological properties, availability, and nutritional value.

Testes are important targets of xenobiotic, and per oxidative damage which is most important cause distribution of testicular function. Also, taking phenolic compounds with food causes protective effects in testicular dysfunction [40-42]. It is important to note that many xenobiotic especially insecticides accumulate in the primarily fatty tissues and may have accumulates in organs that lead to reproductive disorders.

**Conflicts of interests:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## REFERENCES

1. International Agency for Research on Cancer. Miscellaneous pesticides. IARC Monogr Eval Carcinog Risk Chem Hum 1983; 30.
2. Poet TS, Kousba AA, Dennison SL, Timchalk C. Physiologically Based Pharmacokinetic/ Pharmacodynamic Model for the Organophosphorus Pesticide Diazinon. NeuroToxicology. 2004;25:1013–30. https://doi.org/10.1016/j.neuro.2004.03.002.
3. Aroonvilairat S, Tangjarukij S, Sornprachum T, Chaisuriya P, Siwadune T, Ratanabanangkoon K. Effects of topical exposure to a mixture of chlorpyrifos, cypermethrin and captan on the hematological and immunological systems in male Wistar rats. Environ Toxicol Pharm. 2018;59:53–60. https://doi.org/10.1016/j.etap.2018.02.010.
4. Tripathi S, Srivastav, AK. Liver profile of rats after long-term ingestion of different doses of chlorpyrifos. Pestic Biochem Physiol. 2010; 97: 60–65. https://doi.org/10.1016/j.pestbp.2009.12.005
5. Sayım F. Dimethoate-induced biochemical and histopathological changes in the liver of rats. Exp Toxicol Pathol. 2007; 59: 237–43. https://doi.org/10.1016/j.etp.2007.05.008.
6. Eken A. Dimethoate organophosphate insecticide toxicity and the role of oxidative stress. Toxicology. 2021; 59-68. https://doi.org/10.1016/B978-0-12-819092-0.00007-8.

7. Amara IB, Soudani N, Troudi A, Bouaziz H, Boudawara T, Zeghal N. Antioxidant effect of vitamin E and selenium on hepatotoxicity induced by dimethoate in female adult rats. Ecotoxicol Environ Saf. 2011; 74: 811–9. https://doi.org/10.1016/j.pestbp.2011.08.012.
8. Undeger U, Institoris L, Siroki O, Nehez M, Desi I. Simultaneous geno- and immunotoxicological investigations for early detection of organophosphate toxicity in rats. Ecotoxicol Environ Saf. 2000; 45: 43–8. https://doi.org/10.1006/eesa.1999.1844.
9. Amara IB, Soudani N, Hakim A, Troudi A, Zeghal KM, Boudawara T, Zeghal N. Protective Effects of Vitamin E and Selenium Against Dimethoate-Induced Cardiotoxicity in Vivo: Biochemical and Histological Studies. Environ Toxicol 2013; 28: 630–43. https://doi.org/10.1002/tox.20759.
10. Sharma Y, Bashir S, Irshad M, Nag TC, Dogra TD. Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. Toxicology. 2005; 215: 173–81. https://doi.org/10.1016/j.tox.2005.06.029.
11. Kalender Y, Kaya S, Pandir D, Apaydın FG, Demir F. Protective effects of catechin and quercetin on antioxidant status lipid peroxidation and testis histoarchitecture induced by chlorpyrifos in male rats. Environ Toxicol Pharmacol. 2012; 33:141-8. https://doi.org/10.1016/j.etap.2011.12.008.
12. Zeng X, Du Z, Ding X, Jiang W. Protective effects of dietary flavonoids against pesticide-induced toxicity: A review. Trends Food Sci Technol. 2021; 109: 271-9. https://doi.org/10.1016/j.tifs.2021.01.046.
13. Lee A, East J, Balgaug P. Interactions of insecticides with biological membranes. Pestic Sci 1991;32:317-27.
14. Ozturk Sarıkaya SB, Gulcin I, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of human erythrocyte isozymes I and II with a series of phenolic acids. Chem Biol Drug Des. 2010;75:515–20.
15. Ghosh S, Chowdhury S, Sarkar P, Sil PC. Ameliorative role of ferulic acid against diabetes associated oxidative stress induced spleen damage. Food Chem Toxicol 2018;118:272-86. https://doi.org/10.1016/j.fct.2018.05.029.
16. Alazzouni AS, Dkhil MA, Gadelmawl MHA, Gabri MS, Farag AH, Hassan BN. Ferulic acid as anticarcinogenic agent against 1,2-dimethylhydrazine induced colon cancer in rats. J King Saud Univ Sci. 2021;33:101354. https://doi.org/10.1016/j.jksus.2021.101354.
17. Prior RL, Cao G. Antioxidant phytochemicals in fruits and vegetables; diet and health implications. Hortic Sci. 2000; 35: 588–592.
18. Kiefer I, Prock P, Lawrence C, Wise J, Bieger W, Bayer P, Rathmanner T, Kunze M, Rieder A. Supplementation with mixed fruit and vegetable juice concentrates increased serum anti-oxidants and folate in healthy adults. J Am Coll Nutr. 2004;23:205–11. https://doi.org/10.1080/07315724.2004.10719362
19. Anraku M, Gebicki JM, Iohara D, Tomida H, Uekama K, Maruyama T, Hirayama F, Otagiri M. Antioxidant activities of chitosans and its derivatives in in vitro and in vivo studies. Carbohyd Polym. 2018;199:141–9. https://doi.org/10.1016/j.carbpol.2018.07.016.
20. Gulcin I. Antioxidants and antioxidant methods: an updated overview. Arch Toxicol. 2020;94:651–715. https://doi.org/10.1007/s00204-020-02689-3.
21. Apaydın FG, Bas H, Kalender Y. Lead and Cadmium Induced Oxidative Stress in the Epididymis and Spleen of Rats: Effects of Sesamol. Comm J Biol, 2021; 5: 13-7. https://doi.org/10.31594/commagene.797945.
22. Lowry OH, Rosebrough NJ, Farr Randall AL. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;19:265.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-8. doi:10.1016/0003-2697(79)90738-3.
24. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47:469-74. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x.
25. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-6. https://doi.org/10.1016/S0076-6879(84)05016-3.
26. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249:7130-9. https://doi.org/10.1016/S0021-9258(19)42083-8.
27. Paglia DE, Valentine WN. Studies on the quantative and qualitative characterization of glutathione peroxidase. J Lab Clin Med. 1987;70:158-65.
28. Uzun FG, Kalender S, Pandır D, Demir F, Kalender Y. Malathion induced testicular toxicity in male rats and the protective effect of vitamins C and E. Food Chem Toxicol. 2009;47:1903-8. https://doi.org/10.1016/j.fct.2009.05.001
29. Ravula AR, Yenugu S. Effect of oral administration of a mixture of pyrethroids at doses relevant to human exposure on the general and male reproductive physiology in the rat. Ecotoxicol Environ Safe. 2021; 208: 111714. https://doi.org/10.1016/j.ecoenv.2020.111714.
30. Latchoumycandane C, Chitra KC, Mathur PP. The effect of methoxychlor on the epididymal antioxidant system of adult rats. Reprod Toxicol. 2002;16:161-72. https://doi.org/10.1016/S0890-6238(02)00002-3.

31. Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. Toxicology. 2002;180:33–44. https://doi.org/10.1016/S0300-483X(02)00380-3.
32. Nikkhah E, Shirani K, Rezaee R, Karimi G. Protective Effects of Taurine against Hepatotoxicity Induced by Pharmaceuticals and Environmental Chemicals. Toxicol Environ Chem. 2021,103:56-84. https://doi.org/10.1080/02772248.2021.1892113.
33. Kamalakkanan N, Prince PSM. Antidiabetic and anti-oxidant activity of Aegle marmelos extract in streptozotocin-induced diabetic rats. Pharm Biol. 2004;42:125–30. https://doi.org/10.1080/13880200490510937.
34. Apaydın FG, Aslanturk A, Uzunhisarcikli M, Bas H, Kalender S, Kalender Y. Histopathological and biochemical studies on the effect of curcumin and taurine against bisphenol A toxicity in male rats. Environ Sci Poll Res. 2019;26:12302–10. https://doi.org/10.1007/s11356-019-04578-4.
35. Dixit AK, Bhatnagar D, Kumar V, Chawla D, Fakhruddin K, Bhatnagar D. Antioxidant potential and radioprotective effect of soy isoflavone against gamma irradiation induced oxidative stress. J Funct Foods. 2012;4:197–206. https://doi.org/10.1016/j.jff.2011.10.005.
36. Rames T. Oxidative stress and hepatocellular mitochondrial dysfunction attenuated by asiatic acid in streptozotocin-induced diabetic rats. J King Saud Univ Sci. 2021;33:101369. https://doi.org/10.1016/j.jksus.2021.101369.
37. Dewanjee S, Sah R, Karmakar S, Gangopadhyay M. Toxic effects of lead exposure in Wistar rats: Involvement of oxidative stress and the beneficial role of edible jute (*Corchorus olitorius*) leaves. Food Chem Toxicol. 2013;55:78-91. https://doi.org/10.1016/j.fct.2012.12.040.
38. Jones DP. Redox potential of GSH/GSSG couple: assay and biological significance. Methods Enzymol. 2002;348:93–112. https://doi.org/10.1016/S0076-6879(02)48630-2.
39. Verma RS, Mehta A, Srivastava N. Attenuation by antioxidant vitamins. Pestic Biochem Physiol. 2007;88:191-6. https://doi.org/10.1016/j.pestbp.2006.11.002.
40. Merra E, Calzaretti G, Bobba A, Storelli M, Casalino E. Antioxidant role of hydroxytyrosol on oxidative stress in cadmium-intoxicated rats: different effect in spleen and testes. Drug Chem Toxicol. 2014;37(4):420-6. https://doi.org/10.3109/01480545.2013.878950.
41. Kalender S, Apaydın FG, Kalender Y. Testicular toxicity of orally administrated bisphenol A in rats and protective role of taurine and curcumin. Pak J Pharm Sci. 2019;3:1043-7.
42. Salau VF, Erukainure OL, Olofinsan KA, Islam, MS. Vanillin exerts therapeutic effects against hyperglycemia-altered glucose metabolism and purinergic activities in testicular tissues of diabetic rats. Reprod Toxicol. 2021;102: 24-34. https://doi.org/10.1016/j.reprotox.2021.03.007.