Journal of Medicinal Plants and By-products (2015) 1: 39-43

Original Article

Protective Effect of *Salvia officinalis* on Testes Tissue Damages of Rats Intoxicated by Diazinon

Esmail Fattahi[1*] and Mojtaba Vaseghi[2]

[1] *Department of Biology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran*
[2]*Department of Physical Science Education and Sport Science, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran*

Article History: Received: 29 September 2014/Accepted in revised form: 15 February 2015

Abstract

Diazinon is an organophosphate which exerts tissue damages by generating free radicals thereby inducing oxidative stress. The purpose of this study is to determine the protective effect of *Salvia officinalis* L.extract on testes of rats intoxicated by diazinon. In this experimental study, 45 mature male rats were equally divided into three groups of Control (C), Diazinon (DZN) and Diazinon-Extract (DZN-E). The D-E group received a one-time 200 mg/Kg diazinon for the entire period of study and the *S. officinalis* extract for four weeks (5 days a week) via intraperitoneal injection. The animals were sacrificed 24 hours later. Tissue sections were prepared and subjected to microscopic examination. The collected data were statistically analyzed using one way-ANOVA. number of spermatogonia, spermatocytes, spermatids as well as the diameter of seminiferous tubule have been significantly reduced in DZN group compared to those in C group ($P<0.05$). However compared to the DZN group, the DZN-E group showed increase in the number of the cell types under study and the diameter of seminiferous tubule and these increases were statistically significant ($P<0.05$). Our results indicate that *S. officinalis* extract minimizes diazinon-mediated tissue damages on testes probably by scavenging free radicals and so reducing toxicity caused due to oxidative stress.

Key words*:* *salvia officinalis*, Diazinon, Testes, Spermatogonia

Introduction

Exposure to pesticides is considered an important health problem[1]. Organophosphorous pesticides are a group of agricultural toxins capable of inhibiting the cholinesterase enzyme and therefore causing various tissue damages[2]. Such toxins are adsorbed to the body via skin, respiratory or digestive tracts[3] and rapidly converted to active metabolites [4]. Diazinon is organophosphorous pesticides widely utilized to control and eradicate agricultural pests[5]. This toxin is a well-known pollutant of our ecosystem, water and food products. Large quantities of diazinon may remain active for months in water, soil and even in plants and are readily adsorbed upon contact by skin or mucus [6].

One of the unwanted side effects of organophosphorous toxins is on spermatogenesis. Such damage by these toxins clearly can vary in severity depending on the dose and duration of contact with the toxin; however, continuous application of these toxins in residentials and farms could jeopardize the reproductive system in human and animals] 7]. Studies made on laboratory animals indicate that OP toxins can lead to disruption of spermatogenesis] 8]. Even inhalation of these toxins could minimize semen volume, reduce sperm movement, number and structure and

*****Corresponding author: Dept Biology, Ayatollah Amoli Branch, Islamic Azad University, Amol, IRAN, PO BOX 678
E-mail Address: esmail_fattahy@yahoo.com

affect secretion of sex-related hormones[9]. Agriculture toxins can affect testes tissue, degenerate seminiferous tubule and reduce sperm biosynthesis thereby enhancing the chance of infertility[10-12].

Organophosphorous compounds are able to enter reaction with cellular macro- and micromolecules and introduce cellular and genetic damages[13-14]. Some investigators blame lipid peroxidation and production of free radicals such as reactive oxygen species (ROS) following diazinon metabolism and interaction with proteins, nucleic acids and lipids as the main mechanism of diazinon action behind cell damage and death induction in various tissues [15-16]. Due to the damaging consequences of diazinon usage in agriculture, several measures have been proposed to inhibit such damages. The use of natural compounds that bear anti-oxidant properties might be important in reducing oxidative stress and minimize its damages[17].

Since the safety of synthetic anti-oxidants has often been under question, the use of natural resources in particular plants as substitutes draw increasing attention nowadays [18]. *Salvia officinalis* L. which belongs to mint Labiatae is one of these natural resources. This plant has phenolic compounds as the main source of its anti-oxidant properties[19-20]. The anti-oxidant capacity of plant metabolites is determined by the quantity of these compounds[21-22]. Studies indicate that *S. officinalis* scavenges free radical thereby minimizes damaging effects of oxidative stress[23]. Due to the wide application of diazinon and its oxidative damages in various live tissues, on one hand, and the anti-oxidant properties of *S. officinalis*, on the other hand, the current study was designed to determine the protective effect of *S. officinalis* on testes tissue of rats intoxicated by diazinon.

Material and Methods

Animals: for this study, 45 mature male wistar rats with an average age of 12 weeks and approximate weight of 250±20 were purchased from Iran Pasteur Institute in Amol. The rats were adapted to new environment by keeping them in animal room in Mazandaran University for two weeks. The animal room was set to 25 ^{0}C in temperature with 50-55% humidity. Also, the animals were kept in 12-hour light and 12-hour dark for adaptation purposes. They were then randomly divided to three groups of Control (C), Diazinon (DZN) and Diazinon-Extract (DZN-E).

Preparation of plant extract: *Salvia officinalis* used in this study belonged to species found in Alborz mountains and was supplied by Herbals Reference Store in Karaj, Iran. In order to prepare the hydro-alcoholic extract, 200 g powder made from the plant was added to 70% ethanol in 600 mL. The mix was rotated in a Shaker of KS500 model at 325 rpm for 72 hours. The mix was then filtered through a Wattmann filter paper. The extract solvent was extracted using a rotary device in vacuum and then dried.

Injections: The extract was injected daily and intra-peritoneally to the rats at 100 mg/Kg within 4 weeks (5 days per week). After the last injection, diazinon was injected at the dose of 200 mg/Kg.

Preparation of tissue samples: The animals were anesthetized 24 hours after injection of diazinon, and the testes tissue was collected by seizuring their abdominal cavity. The isolated testes was fixed in 10% formalin and used to prepare 5µm sections. All sections were stained with eosin-hematoxylin. Finally, the number of spermatogenic cells, Leydig cells, Sertoli cells as well as the diameter of seminiferous tubule was counted using a light microscope in which eye piece was equipped with a graticule.

Data analysis: The SPSS version 19 was used to statistically analyze the data. One-way ANOVA was applied to compare differences between controls and experimental groups. Differences between groups were analyzed using Duncan's Multiple Comparisons Test. Differences less than 0.05 ($P<0.05$) were considered significant.

Results

Diameter of seminiferous tubule: The diameter of seminiferous tubule in the DZN group of rats was significantly reduced compared to that in the C group ($P<0.05$). However, this parameter showed significant increase in the DZN-E group compared to the DZN group ($P<0.05$). Also the Diameter of seminiferous tubule in the DZN-E group was significantly smaller than in the C group (Fig. 2; $P<0.05$).

Number of spermatogonia and spermatocytes:

Both spermatogonia and spermatocytes showed reduced number in the DZN group compared to the

C group (P<0.05), but the number of these cells significantly increased in the DZN-E group compared to the DZN group (P<0.05). Cell number in the DZN-E group was reduced compared to the C group but this reduction was insignificant (Table 1 and Fig. 1).

Table 1 mean ± SE of spermatogenic cells in control and experimental groups

Groups Cell	Control	Diazinon	Diazinon-Extract
Spermatogonia	25.33± 4.489 a	18.75± 3.728 b	24.44± 3.502 a
Spermatocyte	35.73± 4.423 a	19.93± 3.449 c	35.22± 4.426 b
Spermatid	37.86± 3.680 a	24.68± 2.522 b	36.94± 3.020 a

Same letters indicate no significant differences between mean groups, and different letters is significant at the 0.05 level.

Table 2 mean ± SE of Leydig/Sertoli cells and diameter of seminiferous tubule in control and experimental groups

Groups Parameters	Control	Diazinon	Diazinon-Extract
Leydig cell	29.06± 3.180 a	18.00± 2.780 b	28.88± 3.944 a
Sertoli cell	4.86± 0.560 a	2.81± 0.647 c	4.55± 0.63 b
Diameter of seminiferous tubule (µm)	62.80± 3.25 a	50.12± 4.39 c	58.30± 3.96 b

Same letters indicate no significant differences between mean groups, and different letters is significant at the 0.05 level.

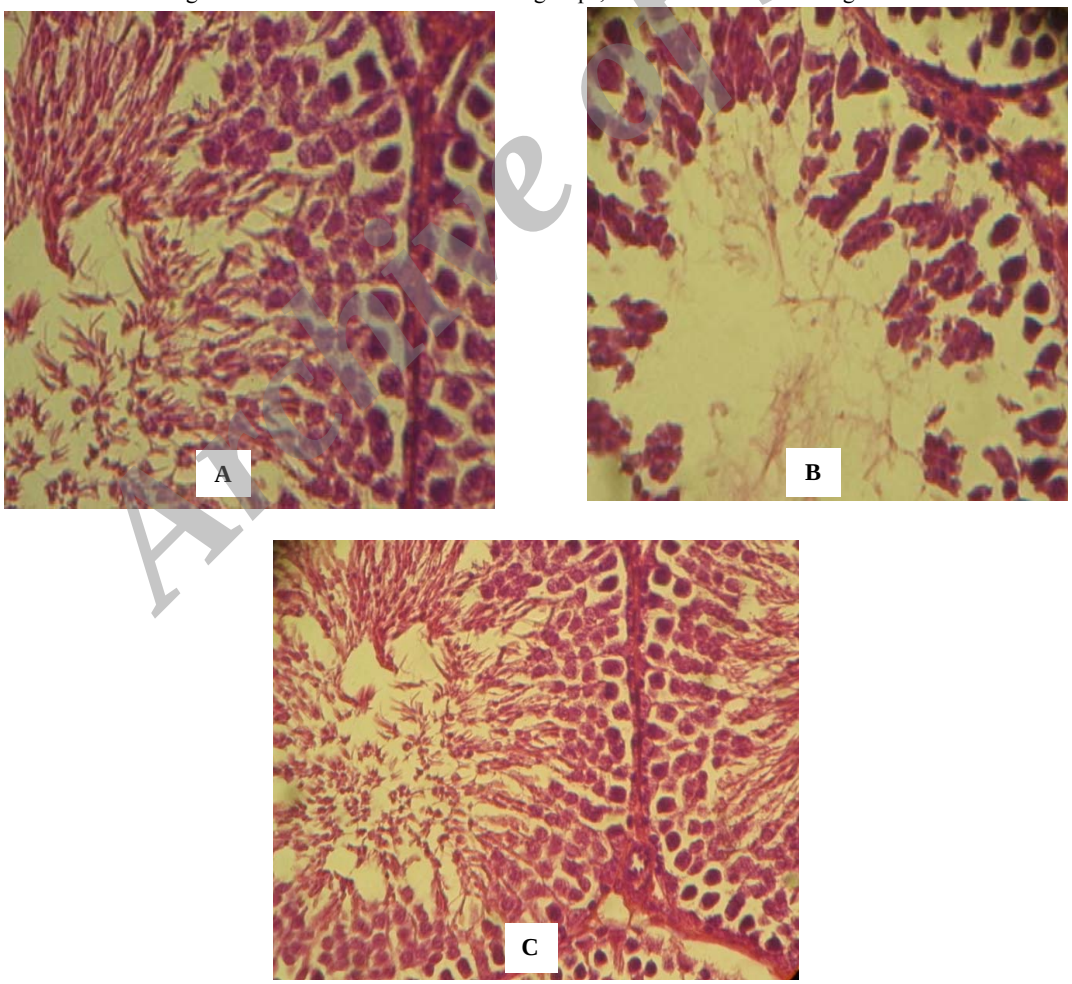

Fig. 1 Micrograph of cross section from Rat testes in Diazinon-Extract (A) , Diazinon (B) and control(C) groups, staining with H&E (400X). Treated rats with diazinon show decrease in the number of spermatogenic, but *Salvia officinalis* L. extract prevents tissue damages induced by diazinon on testes.

Number of spermatids, Leydig cells and Sertoli: Comparison of sections prepared from various animal groups showed that spermatids, Leydig cells and Sertoli in the DZN groups have been reduced in number compared to the C group ($P<0.05$).The number of these cells in the DZN-E group showed significant increase compared to that in the DZN group ($P<0.05$) but insignificant reduction compared to the C group (Tables 1, 2).

Discussion

In this study, significant reduction in the number of spermatogonia, spermatocytes, spermatids, Leydig and Sertoli cells were observed in the diazinon (DZN) group compared to the control (C) group. However in the diazinon+extract (DZN-E) group, the number of cells examined and the diameter of seminiferous tubule showed significant increase compared to the DZN group. These results that reflect the effects of diazinon activity are compatible with those of other studies indicating the efficacy of diazinon and similar toxins. Reports indicate the capacity of organophosphorous toxins in reacting with cellular macro- and micromolecules and damaging consequences of such reactions at cellular and genetic levels [13,14]. Some investigators argue that this group of toxins support lipid peroxidation and induce cell death by increasing free radical levels and inhibiting anti-oxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) that can ultimately result in destruction of various cells and tissues [24,25]. It appears from the results of this study that the reduced number of cells examined and the shrinkage in the diameter of seminiferous tubule in the DZN group correlate with the increased levels of oxidative stress and induced cell death.

The results of our study further indicate that the DZN-E group of animals has increased number of spermatogenic, Leydig and Sertoli cells compared to the DZN group. The histological examinations that we carried out point to the capacity of the *S. officinalis* extract to repair and/ or reconstitute the testes tissue. Two anti-oxidant defense systems occur in organisms that include enzymatic defense system (such as SOD, GPX and CAT) and non-enzymatic system of ascorbic acid, Alpha-tocoferol, Bilirubin, Oric acid, poly-phenols and carotens [23,26]. These compounds inhibit production of free radicals and repair tissues thereby minimizing damages caused by excessive free radicals [27]. Therefore, the use of natural anti-oxidants could be one way to reduce oxidative damages of diazinon toxicity.

Investigations introduce the occurrence of phenolic compounds toyon, syneol and camfer in *S. officinalis* as the main source of the plant's anti-oxidant properties. In 2007, Osava and Kato showed that plant resources can protect tissues from free radical and oxidative damages. Also food supplements such as vitamins and flavonoids used with natural anti-oxidants can minimize oxidative damages[28]. Plant metabolites induce the activity of anti-oxidant enzymes and reduce lipid peroxidation toward minimizing oxidative activities [29-31]. Zupko and colleagues showed that various *S. officinalis* species have inhibitory effects on lipid peroxidation induced by Cu^{2+} and Fe^{2+}-containing compounds that have free radical scavenging activities[30]. Based on the vital role of metal ions transferred during lipid oxidation, the results of our study indicate the capacity of *S. officinalis* in reduction or elimination of free radicals and so inhibition of lipid peroxidation. Therefore, it appears that the *S. officinalis* extract injected to the DZN-E group of rats has been able to neutralize the oxidative toxicity of diazinon leading to protection of cells involved in animals' spermatogenesis.

Conclusion

The results of this study showed that the *S. officinalis* extract induces repair of testes tissue damaged by diazinon. The extract increases cellular anti-oxidant activities and protects testes by scavenging free radicals. Therefore, the compounds present in this plant could form promising resources to supplement natural anti-oxidants.

References

1. Katzung BG. Basic and Clinical pharmacology. 10th. London: Simon & Schuster, 2005, pp. 948
2. Hsieh BH, Deng JF, Ger J, Tsai WJ. Acetylcholinesterase inhibition and the extrapyramidal syndrome: a review of the neurotoxicity of organophosphate. Neurotoxicology. 2001; 22:423-7.
3. Oliveira-Silva JJ, Alves SR, Meyer A, Perez F, Sarcinelli PN, da Costa Mattos RC, Moreira JC. [Influence of socioeconomic factors on the pesticides poisoning, Brazil]. Rev SaudePublica. 2001;35:130-5. (Article in Portuguese)

4. Schroeder JC. Metabolic susceptibility to agricultural pesticides and non-Hodgkin's lymphoma.Scand J Work Environ Health.2005;31:26-32.
5. Kamrin MA. Pesticide profiles: toxicity, environmental impact and fate. 7th. Boca Raton: Lewis Publishers. 1997, pp. 157-9.
6. Dahlgren JG, Takhar HS, Ruffalo CA, Zwass M. Health effects of diazinon on a family. J Toxicol. Clin. Toxicol. 2004;42:579- 91.
7. Fattahi E, Parivar K, JorsaraeiSGhA, Moghadamnia AA. The effects of diazinon on testosterone, FSH and LH levels and testicular tissue in mice. Iran J Reprod Med. 2009;7:59-64.
8. Blanco-Muǒz J, Morales MM, Lacasaå M, Aguilar-Garduǒ C, Bassol S, CebriↃn ME. Exposure to organophosphate pesticides and male hormone profile in floriculturist of the state of Morelos, Mexico. Hum Reprod. 2010;25:1787-95.
9. Yucra S, Rubio J, Gasco M, Gonzales C, Steenland K, Gonzales GF. Semen quality and reproductive sex hormone levels in Peruvian pesticide sprayers.Int J Occup Environ Health. 2006;12:355-61.
10. Fattahi E, Jorsaraei SGA, Gardaneh M. The effects of carbaryl on the pituitary-gonad axis in male rats. Iran J Reprod Med. 2012;10:419-424.
11. Fattahy E, Jorsaraei SGA, Parivar K, Moghaddamnia AA. Influence of Diazinon on spermatogenesis in mice. Koomesh J. 2007; 9:75-82. (in Persian)
12. Fattahi E, Jorsaraei SGA, Parivar K, Moghaddamnia AA. The effects of a single dosage of Diazinon and Hinosan on the structureof testis tissue and sexual hormones in Mice. Cell J. 2010;12:405-10. (in Persian)
13. Kurodak K, Yamaguchy Y, Endo G. Mitotic toxicity,sister chromatid exchange, and rec assay of pesticides. Arch Environ ContamToxicol. 1992;23:13-18.
14. Evenson D, Jost L. Sperm chromatin structure assay for fertility assessment. CurrProtocCytom. chapter : Unit. 2001; pp:7.13.
15. Rush T, Liu XQ, Hjelmhaug J, Lobner D. Mechanisms of chlorpyrifos and diazinon induced neurotoxicity in cortical culture. Neuroscience. 2010; 166:899-906.
16. Yu F, Wang Z, Ju B, Wang Y, Wang J, Bai D. Apoptotic effect of organophosphorus insecticide chlorpyrifos on mouse retina in vivo via oxidative stress and protection of combination of vitamins C and E. ExpToxicolPathol. 2008 ;59:415-23.
17. Fallah-mohammadi Z, Hajizadeh-Moghaddam A, Aghasi M, Esmaeili AH. Neuroprotective effects of voluntary exercise and hydroalcoholic extraction of Eriobotrya Japonica on dopamine and tyrosine hydroxylase in the striatum of parkinsonian rats. Koomesh J. 2013;15:31-38(in Persian)
18. Osawa T, Kato Y. Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. Ann. NY. Acad. Sci. 2007;1043:440-451.
19. Durling NE, Catchpole OJ, Grey JB, Webby RF, Mitchell KA, Foo LY, *et al*. Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol-water mixtures. Food Chem. 2007;101:1417-24.
20. Yinrong L, Yeap F. Antioxidant activities of polyphenols from sage (Salvia officinalis). Food Chem 2001;75:197-202.
21. Ames BM, Shigena MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA 1993;90:7915-7922.
22. Stadtman ER. Protein oxidation and aging. Science. 1992;57:1220-1224.
23- Esmaeili MA, Sonboli A, Kanani MR, Sadeghi H. Salvia sahendica prevents tissue damages induced by alcohol in oxidative stress conditions: Effect on liver and kidney oxidative parameters. J. Med. Plant Res. 2009; 3:276-283.
24. Contreras HR, Bustos Obregon E. Morphological alterations in mouse testis by single dose of malathion. J ExpZool. 1999; 284:355-9.
25-Altuntas I, Kilinc I, Orhan H, Demirel R, Koylu H, Delibas N. The effects of diazinon on lipid peroxidation and antioxidant enzymes in erythrocytes in vitro. Hum Exp Toxicol. 2004; 23:9-13.
26- Siesh H, Stanl W. Vitamin E and C.Betacarotene and other Carotenoids as antioxidants. Am J Clin Nutr. 1995; 82:1315-1321.
27. Halliwell B, Gutteridge JM. Role of free-radicals and catalitic metal ions in human disease: an overview. Methods Enzymol. 1990; 186: 1-85.
28. Osawa T, Kato Y. Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. Ann N Y Acad Sci. 2007;1043:440-451.
29. Hohmann J, Zupkó I, Rédei D, Csányi M, Falkay G, Máthé I, Janicsák G. Protective effects of the aerial parts of *Salvia officinalis*, *Melissa Officinalis* and *Lavandula angustifolia* and their constituents against enzyme-dependent and enzyme-independent lipid peroxidation. Planta Med. 1999; 656:576-8.
30. Zupkó I, Hohmann J, Rédei D, Falkay G, Janicsák G, Máthé I. Antioxidant activity of leaves of Salvia species in enzyme-dependent and enzyme-independent systems of lipid peroxidation and their phenolic constituents. Planta Med. 2001;674:366-8.
31. Miroslav R, Jana T, KlaudiaČ, Iveta P, KatarínaV, Štefan F. Effect of dietary *Salvia officinalis* essential oil and sodium selenite supplementation on antioxidative status and blood phagocytic activity in broiler chickens. ACTA VET. BRNO. 2013;82:043-048.