

Gender-Related Differences in Sensitivity to Diazinon in Gonads of Adult Rats and the Protective Effect of Vitamin E

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Abstract

Objectives: Diazinon (DZN) is an organophosphate pesticide which is known to induce oxidative stress in the target tissues such as the reproductive system. The aim of present study was to compare and evaluate the effects of DZN on Malondialdehyde (MDA) and Glutathione (GSH) levels in testis and ovary of rats and to assess the protective role of vitamin E.

Materials and Methods: This study was conducted on 60 adult Wistar rats including 30 male and 30 female rats. Each of male and female groups included 30 rats and were divided into 5 groups: control (without any intervention), sham group (received only pure olive oil daily), treatment group 1 (received DZN+olive oil daily, 60 mg/kg), treatment group 2 (received DZN, 60 mg/kg + vitamin E, 200 mg/kg daily) and treatment group 3 (received vitamin E daily, 200 mg/kg). MDA and GSH levels were determined in gonads.

Results: In both genders, due to administration of DZN, we found significant reduction in GSH content and increase in MDA level. The use of vitamin E plus DZN increased GSH content while decreased MDA level of ovary and testis compared with DZN treated rats. Gonad of female rats compared with male rats showed more increase in MDA level and further decline in GSH content in treatment groups 1 and 2.

Conclusion: Oxidative stress contributes to DZN-induced sexual organ toxicity. Our results concluded that vitamin E may have a protective role in this toxicity. Female rats are likely more vulnerable to oxidative stress and its damages.

Keywords: Diazinon, Gonads, Oxidative Stress, Vitamin E

Introduction

Pesticides and herbicides are persistent and dangerous chemical compounds, among which organophosphates pesticides are commonly used as insecticides. They are generally the most toxic pesticides for animal species especially vertebrate animals (1). Organophosphorus (OP) compounds have been used in agriculture, industry, medicine, farming, animal keeping and household to destroy insects, worms, nematodes, fungus and weeds for 5 decades (2-5). Unfortunately, the improper use of these products, damage the environment including plants, animals, ground or soil and water, leading to resistance which is an increasing concern in this field (6,7). Residual amounts of organophosphate insecticides are detectable in soil, vegetables, tissue of organisms, grains, and other foods products (7). These pesticides are absorbed by body through the skin and mucous membranes via oral cavity and inhalation (8). It affect various organs of the body including liver (9), kidneys, pancreas (10), immune system (11,12), urinary (13) and reproductive systems (14). Re-

searches have shown that several factors such as duration and concentration of DZN affect its toxicity (7,15).

Diazinon (DZN) (o,o-diethyl-o-[2-isopropyl-6-methyl-4-pyrimidinyl]phosphorothioate) is an OP pesticide which is extensively used to control households insects and vegetable crops (16). DZN inhibit Acetylcholinesterase (AChE) activity in the target tissues, which is the most important action of DZN compound (3). In addition to its inhibitory effect on AChE, oxidative stress is the other mechanism involved in DZN toxicity. This leads to the formation of Reactive Oxygen Species (ROS) and changes the enzymatic activities associated with antioxidant defense mechanisms in the body (17,18).

In normal conditions, there is a fine balance between ROS and antioxidant enzymes in different tissues including the ovary and testis (19). Oxidative Stress (OS) is the result of the imbalance between ROS generation and antioxidants in the body. OS can lead to sperm DNA damage, abnormal sperm morphology and finally male infertility. OS is considered as the major etiological factor leading to

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sperm damage (20).

Until recently, ROS was considered toxic exclusively to human sperm cells. However sufficient evidence exists that suggests small amounts of ROS are necessary for spermatozoa to acquire fertilizing capabilities. Low levels of ROS have been shown to be essential for sperm acrosome reaction, motility, hyperactivation, capacitation and fertilization (21-23). ROS affect multiple physiological procedures from oocyte maturation to fertilization. Although ROS may have a regulatory role in oocyte maturation, folliculogenesis, luteolysis and ovarian steroidogenesis, it can cause tissue damage as lipid peroxidation when it is produced in large amount (24-27).

One of the by-products of lipid peroxidation is Malondialdehyde (MDA). MDA is a major oxidation product of polyunsaturated fatty acids peroxidation and elevated MDA content is a significant indicator of lipid peroxidation (7,28). Thiol groups are sensitive to oxidative damage and normally decrease in response to oxidative stress (29). Glutathione (GSH) is one of the thiol groups, which has an essential role in protecting cells from damage induced by oxidative stress (30).

Antioxidants are scavengers that detoxify excessive ROS and have an important role in maintaining oxidant/antioxidant balance in the body (31). Vitamin E is known as a fat soluble antioxidant which interrupts release of lipid peroxidation in the plasma membrane and thus maintains the integrity of the membrane. Many studies had showed that vitamin E inhibits oxidative stress and lipid peroxidation induced by OP pesticides in experimental animals (32,33) and therefore prevented the adverse effects of active oxygen radicals on spermatogenesis and sperm health (34). Furthermore, antioxidants have substantial role in the female reproductive system (22).

Considering the mentioned evidences and exposure of men and women to OP poisons including DZN, and lack of enough studies evaluating and comparing the oxidative stress induced by DZN in reproductive organs (testis and ovary) of rats, the aim of present study was to evaluate and compare the effects of DZN on MDA and GSH levels in ovary and testis of rats. We also aimed to evaluate the role of vitamin E in protecting rats' reproductive organs (testis and ovary) against oxidative stress induced by DZN and to find the gonad at higher risk, in order to develop proper protective measures.

Materials and Methods

Animals

In this interventional study, 60 adult Wistar rats including 30 male and 30 female rats were purchased from the Animal Center, School of Medicine, Mashhad University of Medical Sciences. The rats were housed in a room under standard laboratory conditions (12 h light, 12 h dark at 22 °C) with free access to water and food. Each of the male and female groups included 30 rats and were divided into 5 groups: control (without any intervention), sham group

(received only pure olive oil daily), treatment group 1 (received DZN+ olive oil daily, 60 mg/kg), treatment group 2 (received DZN, 60 mg/kg + vitamin E, 200 mg/kg daily) and treatment group 3 (received vitamin E daily, 200 mg/kg). In this study, olive oil was used as solvent (14,35-39). DZN and solvent were administrated by intraperitoneal injection and vitamin E was given by gavage. According to the sexual cycle, male animals were sacrificed after 6 weeks and female rats after 2 weeks. MDA as a marker of lipid peroxidation and GSH content were determined in ovary and testis tissues.

Chemicals

For this study, technical DZN was used. DZN was dissolved in olive oil. Malondialdehyde tetrabutylammonium, reduced GSH, DTNB [5, 5' dithiobis-(2-nitrobenzoic acid)] and vitamin E (α -tocopherol acetate) were purchased from Sigma.

Lipid peroxidation test

The amount of lipid peroxidation was assessed through the measurement of MDA levels in ovary and testis tissues. MDA reacts with Thiobarbituric Acid (TBA) and produces a pink colored complex which has the maximum absorbance at 532 nm. To start working, 3 ml phosphoric acid (1%) and 1 ml TBA (0.6%) were added to homogenate 10% tissue in KCl, and then the compound was heated for 45 min in a boiling water bath. After cooling the compound, 4 ml of n-butanol was added to it and vortex-mixed was used for 1 min followed by centrifugation at 3000 g for 10 min. Then the organic layers were removed and transferred to other tubes and absorbance level was read at 532 nm (40). A calibration curve was designed using Malondialdehyde tetrabutylammonium. MDA levels were expressed by nmol/g tissue.

Reduced GSH test

GSH was evaluated in ovary and testis by the method of Moron et al (41). The basis of the work was the formation of yellow color after adding DTNB [5,5' dithiobis-(2-nitrobenzoic acid)] to compounds containing sulfhydryl groups. For this purpose, 300 μ l of homogenates tissue were blended with 300 μ l of 10% Tricolor Acetic Acid (TCA) and vortexed. After centrifugation at 2500 g for 10 min, the upper layers were removed and blended with reaction mixtures containing 2 ml phosphate buffer (pH:8) and 500 μ l DTNB. After 10 min, the absorbance was evaluated at 412 nm using a spectrophotometer (Jenway 6105 uv/vis, UK). At the end, the amount of GSH was determined based on a standard curve drawn with commercially available GSH (Sigma). The GSH Levels were expressed by nmol/g tissue.

Statistical analysis

Results are expressed as mean \pm SD. Statistical analysis was performed with ANOVA followed by Tukey-Kramer test

to compare the differences between groups' means for each gender separately. Independent t-test was used to compare the differences for each index (GSH and MDA) between two genders in different treatments groups. In all analyzes Tukey test was used to compare means. Differences were considered statistically significant when P was less than 0.05.

Results

Effects of DZN and vitamin E on GSH and MDA in male rats

The means of GSH and MDA levels in different groups of adult male rats are demonstrated in Table 1. Comparison of means of GSH content in different groups had shown no significant differences between control, sham and treatment group 3, but GSH content in these groups were significantly more than treatment groups 1 and 2. The highest GSH content was measured in control group and the lowest content was in treatment group 1.

Comparison of means of MDA level in different treatment groups had shown no significant differences between control, sham and treatment group 3, but MDA level in these groups were significantly lower than treatment groups 1 and 2. Highest MDA level was in treatment group 1.

Effects of DZN and vitamin E on GSH and MDA in female rats

The means of glutathione and MDA levels in different groups of adult female rats are demonstrated in Table 2. Comparison of means of GSH content in different treatment groups had shown no significant differences between control, sham and treatment group 3, but GSH content in these groups were significantly more than

treatment groups 1 and 2. The highest GSH content was measured in control group and the lowest content was in treatment group 1.

Comparison of means of MDA level in different treatment groups of female rats had shown no significant differences between control, sham and treatment group 3, but MDA level in these groups were significantly lower than treatment groups 1 and 2. Highest MDA level was in treatment group 1.

Effects of DZN and vitamin E on GSH in male and female rats

The means of GSH content in different groups of male and female rats are presented in Table 3. According to the obtained results, GSH content in all groups showed significant difference between the two genders ($P < 0.05$). So in all the groups, GSH content in male was significantly higher than GSH content in female. Also according to the results obtained from comparison of cases with the control group, we found that GSH content decreased in male treatment group 1 by 49%, and in female treatment group 1 by 65%. We also found 35% decrease in GSH contents in male treatment group 2 and 51% decrease in female treatment group 2.

Effects of DZN and vitamin E on MDA in male and female rats

The means of MDA level in different groups of male and female rats are presented in Table 4. According to the obtained results, MDA level in all groups showed significant difference between the two genders ($P < 0.05$). So in all the groups, MDA level in male was significantly higher than MDA level in female.

Table 1. Mean of GSH and MDA among various groups in male rats

Treatments	Glutathione	Malondialdehyde
Control	*8951.285±28/21 ^c	112.64±3/6 ^b
Sham (Olive oil 60 mg/kg)	8389.241±17/07 ^c	114.90±2/8 ^b
T1 (DZN 60 mg/kg+Olive oil)	4543.679±15/97 ^a	173.82±4.6 ^d
T2 (DZN 60 mg/kg+Vit E 200 mg/kg)	5756.510±17/33 ^b	140.78±1/7 ^c
T3(Vit E 200 mg/kg)	8906.913±22/18 ^c	112.07±3/5 ^b

*Values with small different letters in columns are significantly different and similar letters are not significantly different ($P < 0.05$) in various groups. Values expressed are mean±SD of triplicate measurement.

Table 2. Mean of GSH and MDA among various groups in female rats

Treatments	Glutathione	Malondialdehyde
Control	*4908.515±35/55 ^c	55.23±3 ^a
Sham (Olive oil 60 mg/kg)	4663.089±17/07 ^c	45.49±3 ^a
T1 (DZN 60 mg/kg+Olive oil)	1733.461±15/97 ^a	95.41±1.8 ^d
T2 (DZN 60 mg/kg+Vit E 200 mg/kg)	2394.594±42/98 ^b	78.54±2/7 ^c
T3(Vit E 200 mg/kg)	4859.429±90/37 ^c	56.6±1/7 ^a

*Values with small different letters in columns are significantly different and similar letters are not significantly different ($P < 0.05$) in various groups. Values expressed are mean±SD of triplicate measurement.

Table 3. Mean of GSH among various groups in female and male rats

Treatments	Male (testicle)	% Decreasing in male vs. control	Female (ovary)	% Decreasing in female vs. control
Control	*8951.285±28/21 ^b	-	4908.515±35/55 ^a	-
Sham (Olive oil 60 mg/kg)	8389.241±17/07 ^b	6	4663.089±17/07 ^a	5
T1 (DZN 60 mg/kg + Olive oil)	4543.679±15/97 ^b	49	1733.461±15/97 ^a	65
T2 (DZN 60 mg/kg + Vit E 200 mg/kg)	5756.510±17/33 ^b	35	2394.594±42/98 ^a	51
T3 (Vit E 200 mg/kg)	8906.913±22/18 ^b	0.5	4859.429±90/37 ^a	1

*Values with small different letters in rows are significantly different and similar letters are not significantly different ($P < 0.05$) in various groups. Values expressed are mean±SD of triplicate measurement.

Table 4. Mean of MDA among various groups in female and male rats

Treatments	Male (testicle)	% Increasing in male vs. control	Female (ovary)	% Increasing in female vs. control
Control	*112.64±3/6 ^b		55.23±3 ^a	
Sham (Olive oil 60 mg/kg)	114.90±2/8 ^b	2	53.49±3 ^a	-3
T1 (DZN 60 mg/kg+Olive oil)	173.82±4.6 ^b	54	95.41±1.8 ^a	72
T2 (DZN 60 mg/kg+Vit E 200 mg/kg)	140.78±1/7 ^b	24	78.54±2/7 ^a	42
T3 (Vit E 200 mg/kg)	112.07±3/5 ^b	-0.5	56.6±1/7 ^a	2

*Values with small different letters in rows are significantly different and similar letters are not significantly different ($P < 0.05$) in various groups. Values expressed are mean ±SD of triplicate measurement.

The obtained results also showed that MDA level increased in both male and female treatment group 1 by 54 and 72%, respectively, compared with control group. We also found 24% increase in MDA levels in male treatment group 2 and 42% increase in female treatment group 2.

Discussion

DZN is used in pest control of fruits and plants. It is also used as ectoparasiticide for cattle in veterinary applications (42). DZN is absorbed from the gastrointestinal tract and rapidly metabolized (9). OP insecticides lead to biochemical and histopathological changes in several organs such as liver, kidney, immune system, pancreas, cardiac and vascular wall and reproductive system (43,44). Ovary which has a main role in the reproductive function by synthesizing hormones and producing oocyte, is one of the target organs (45,46). Also DZN by making changes in the DNA or in proteins bound can damage testis tissue and create mutations in spermatogonia which eventually lead to changes in the sperm morphology and function (47-49). Based on the above evidences, the aim of the study was to investigate and compare the DZN-induced oxidative stress in sexual organs (testis and ovary) and to assess the protective role of vitamin E. One of the mechanisms of toxicity of DZN is increasing oxidative stress by the generation of free radicals and tissue lipid peroxidation. To assess this toxic effect, MDA and GSH contents were determined in reproductive tissues (testis and ovary). The intensity of lipid peroxidation was assessed through measurement of MDA levels in testis and ovary; increased MDA levels are a significant indicator of lipid peroxidation.

Comparison of means of MDA levels in different groups

for each gender have shown no significant difference between control, sham and treatments group 3, but the MDA levels were significantly lower in mentioned groups compared with treatment groups 1 and 2.

One of the mechanisms of DZN toxicity is inducing oxidative stress and producing tissue lipid peroxidation. Since DZN was solely used in treatment group 1, we observed the highest MDA level and increasing tissue lipid peroxidation in treatment group 1.

Koc et al. confirmed that endosulfan and malathion exposure increase MDA levels in ovarian tissues of female rats which is an indicator of free radical formation and lipid peroxidation during metabolism of these insecticides (50). Jahromi et al. found that malathion which is one of the OP insecticides, also may increase MDA level in ovary (4).

In this study in both genders, due to administration of DZN, we found significant reduction in GSH content in treatment group 1. Razavi et al. reported that exposure to DZN significantly increased MDA level and decreased GSH contents in heart tissue (51). Shah et al. reported that DZN administration induces lipid peroxidation in a dose-dependent style in rats. They also demonstrated that DZN treatment decreased renal GSH, reduced the activities of antioxidant enzymes including the enzymes involved in GSH metabolism and large production of oxidants which is associated with renal damage. All of them are involved in cascade of events leading to DZN-mediated renal oxidative stress and toxicity (1). Ahmadi et al. showed that DZN increased GST enzyme activity in spleen tissue and increased consumption of GSH, which indicate the increase of body's defense against toxin, and

its rapid excretion (52). The reduced GSH reservoir in the body and reduced antioxidant activity in many tissues following the use of OPs were observed (53).

Based on above reported results, DZN induces peroxidation of lipid and increases formation of free radicals in rats' reproductive tissues which cause oxidative stress. Therefore gonads of both genders are vulnerable to oxidative stress induced by DZN and related damages. The damages induced by oxidative stress in male include lipid peroxidation of sperm membrane; decreased sperm motility and damage to DNA, sperm nucleus and protein, which may change the structure and function of sperm and play a role in infertility (54,55). Oxidative stress in female can damage female fertility in different ways by affecting ovulation, fertility, embryo development and implantation. So it is considered as a main infertility factor in female (56).

Considering the importance of comparing the vulnerability rate of male and female rats' gonads to DZN-induced oxidative stress and the lack of proper studies in this field, this study was performed to evaluate and compare the effects of DZN on MDA and GSH levels and oxidative stress in reproductive organs of rats and to find the more vulnerable gonad, in order to help with the development of proper protective measures.

Many authors have studied gender-related differences in pesticide toxicity in rats. Gaines et al. (57) study showed that most of the tested pesticides were more toxic to female rats. Edson and Noakes showed that male rat was more susceptible to DZN (58). Donald B. Davies demonstrated that the female rat was more sensitive to the toxicity of dietary DZN as compared to the male (59). This study however was under taken to determine whether a difference in sensitivity to DZN-induced gonads toxicity exists between males and females.

According to the obtained results, MDA levels in all groups showed significant difference between two genders ($P < 0.05$). However, MDA levels in gonad of male rats were significantly higher than gonad of female rats in all groups. Since MDA levels increased in treatment group 1 in comparison with the control group in both genders (54% increase in male and 72% increase in female), we conclude that DZN-exposed sexual organ of female rats (ovary) have likely more tissue lipid peroxidation compared with sexual organ of male rats (testis).

Furthermore considering the increase in MDA level in treatment group 2 compared with the control group (24% increase in male and 42% increase in female), it indicated that despite the use of vitamin E besides DZN in these treatment groups and decrease in MDA level elevation compared with treatment groups 1, but again more tissue lipid peroxidation was observed in sexual organ of female rats compared with male rats and vitamin E cannot protect completely from sexual organ in both genders.

According to obtained results, GSH contents in all groups had significant difference ($P < 0.05$) between the two genders. GSH content in male was significantly more than the

female group in all groups. Considering the decrease in GSH content in treatment group 1 in comparison with the control group (49% decrease in male and 65% decrease in female), we found that the DZN-exposed sexual organ of female rats, demonstrated more decline in GSH content compared with sexual organ of male. According to the substantial role of GSH in protecting cells from damages induced by oxidative stress, we can say that female rats are more prone to the adverse effects of oxidative stress. Overall, the gonad of female rats compared with male gonads showed more increase in MDA level and further decline in GSH content. It can be concluded that gonad of female rats are likely more vulnerable to oxidative stress induced by DZN and its consequences.

Cells have several ways to reduce the effects of oxidative stress: with repairing damage or reducing oxidative damage by enzymatic and non-enzymatic antioxidants (7). Vitamin E as non-enzymatic antioxidant is lipid-soluble vitamin present in biological membrane (60,61). Antioxidants such as vitamin E, protect sperm from ROS producing abnormal sperm, increase sperm motility, scavenge ROS, prevent DNA fragmentation and stimulate spermatozoa (20).

Studies have shown that antioxidants such as vitamin E have a substantial role in the female reproductive system (36).

In present study treatment group 2 received antioxidant plus DZN. We observed considerable decrease in MDA level and increase in GSH content of ovary and testis compared with treatment groups 1 (DZN treated rats). This is due to the inhibitory effects of vitamin E on oxidative stress and tissue lipid peroxidation.

Sutcu et al. had also showed that DZN increased lipid peroxidation in erythrocytes of rat and combinations of vitamin C and E reduced lipid peroxidation (62). Yilmaz et al. reported that oxidative stress contributes to DZN-induced brain toxicity and vitamins E plus C combination may have a protective effect on this toxicity (63). Kalender et al. showed endosulphan administration increased MDA level in rats and vitamin E protected cells and cellular structures from oxidative damage by reducing MDA level (64). Another study had reported that vitamin C and E prevent hepatotoxicity induced by methyl parathion in rats (61). Previous studies had shown that vitamin E reduced MDA level in DZN-induced heart tissue toxicity but does not prevent toxicity completely (7).

Conclusion

DZN induced lipid peroxidation and increased oxygen free radicals in the reproductive tissues in both gender. So gonads in male and female rats are vulnerable to oxidative stress and its damages including infertility. Vitamin E reduced oxidative stress and lipid peroxidation induced by OP pesticides in gonads. In addition, the sexual organs of female rats compared with male rats showed more increase in MDA level and further decline in GSH content.

Considering the effect of oxidative stress in the multiple physiological processes from oocyte maturation to fertilization, embryo development and pathophysiology of infertility, it can be concluded that female rats are more vulnerable to oxidative stress and its consequences including infertility. Therefore, it is necessary to prevent further toxin entry into the body which causes gonadal dysfunction.

Ethical issues

All the experimental protocols were approved by the Ethical Committee of Mashhad University of Medical Science.

Conflict of interests

Authors declare that there is no any conflict of interests.

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