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The Effect of Diazinon Poison on Changes in Testicular Tissue and Testosterone Hormone levels in NMRI Lab Mice

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Abstract

Background and purpose: Diazinon (DZN) is an organ phosphoric pesticide that can cause damage to the cells and tissues while producing oxidative stress. Therefore, this study was conducted to determine the effect of diazinon on testosterone and testicular tissues in mice. **Materials and Methods:** In this in-vitro study, 28 adult male mice with a mean age of 12 weeks were randomly divided into four groups: control, sham and experimental groups 1 and 2. Experimental groups 1 and 2 received diazinon toxin 0.5 and 1.5 mg / kg body weight for gavage for four weeks, respectively. The control group did not receive any substance, and the sham group received the solvent of the poison. The rats were killed seven days later and the testes were examined histologically. Concentration of testosterone was measured by radioimmunoassay method. **Results:** The number of germinal cells and tubes diameter of Seminifer in the experimental group 2 was significantly lower than the control group ($P < 0.05$). **Conclusion:** The results indicate that testicular tissue is susceptible to diazinon and its effect is dependent on dose.

Key words: Diazinon, testicular tissue, Leydig cell, Mouse

Introduction

Pesticides are one of the most important environmental factors that have been widely produced and used in recent years, and have undeniable effects on the environment and human health (Fattahi and et al, 2009;Van der Eerden and et al, 2003). Diazinon is one of the pesticides used today, especially in the northern regions of the country. Organophosphorus compounds are toxic compounds widely used as pesticides in agriculture, industry and gardening. (O, O-diethyl-O- [2-isopropyl-diazinon is the most commonly used -6 methyl-4-pyrimidinyl] phosphoro thioate. It is a pesticide used to control insects and pests in soil, ornamental plants, fruits and vegetables. Takes. This compound can enter the body after contact with the environment, in contact with the body and more through the skin, eyes, respiratory tract and swallow. The mechanism of action of this compound is that with the phosphorylation of amino acid serine in the active site of acetylcholinesterase enzyme, this enzyme inhibits the accumulation of acetylcholine in cholinergic synapses and the occurrence of cholinergic and neurological crises.

Many of the destructive effects of diazinon do not relate to the inhibition of acetylcholinesterase enzyme, but are induced by other cellular mechanisms. One of the mechanisms that is very much considered is the production of free radicals by this compound followed by a change in the antioxidant system of the cell, followed by peroxidation of the cell membrane lipids.

Such poisons can be toxic in animals and will have a negative effect on cellular function in the following doses of direct toxicity to cells and / or impairment of the biochemical process and expression of the gene (Martin and et al, 2003). The severity of the detrimental effect of contacting the toxins depends on the dose, mode, duration of contact and tissue structure in the body (Barrett & Jaward, 2012). Although a more precise mechanism of diazinon is not known, it seems that these toxins inhibit the activity of the cytochrome P450 enzyme. This toxin also changes the expression of the gene and the activity of the metabolic enzymes in the mammalian tissues. For this reason, these toxins are classified in the cell and genetic toxicity group (Yen and et al, 2011; Flaskos, 2012). An increase in the relative weight of testicular and ovary, a decrease in the concentration of 17 beta estradiol, vitellogenin and egg production, the high regulation of key proteins such as CYP19 (aromatase), CYP17 (hydroxylase / lipase), and CYP11A is a negative effect of diazinon (Keramati and et al, 2010). Body tissues show different responses to diazinon. Some tissues are more susceptible to the chronic effects of this toxin.

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Negative effects are not limited to the sex of the female but also affects the male, testicular tissue and its function (Fattahi and et al, 2012; Battaglin and et al, 2011). The metabolic, biochemical, and genomic studies show that diazinon induces oxidative stress in animals through the production of reactive oxygen species and thereby induces oxidative stress on glycolysis, mitochondrial respiratory tract, ATP production, amino acid metabolism, antioxidant defense system and poison Detoxification of the cell (Allen and et al, 2006; Taxvig and et al, 2013). The effects of diazinon in Vitro In and Vivo In are different. Studies have shown that diazinon toxins in Vitro in conditions reduce estradiol, progesterone and testosterone levels, but some of the toxins increase testicular and serum testosterone levels in Vivo in conditions (Barton and et al, 2006; Tully and et al, 2006) (anogenital anomalous distance, relative weight The liver, testicular weight, prostate and liver cell hypertrophy are among the other effects of diazinon (Skolness and et al, 2013). Researchers have shown that the long-term effect of diazinon inhibition inhibits antioxidant activity of catalase and superoxide dismutase and decreases DNA / RNA ratio (Li and et al, 2010). There are various reports of the effects of diazinon on the endocrine and reproductive system. They believe that this compound has no effect on the reproductive system and its function, but some researchers believe that this poison can have a negative effect on the endocrine system and on the growth and reproductive process and reproductive performance (Rockett and et al, 2006). Therefore, according to various reports in Particularly high prevalence of reproductive disorders and also due to the active and highly sensitive structure of reproductive tissues, including testes and reproductive system for external factors, and the widespread use of such pesticides in agriculture, the present study was conducted to investigate the effect of diazinon to testosterone and tissue structure changes The testes have been tested in mice.

Materials and Methods

In this experimental study, 28 NMRI male rats weighing from 30 to 35 grams and a mean age of 12 weeks from the Pasteur Institute of the north of Iran were used and used. Mice were kept in separate cages for adaptation to the medium for about one week. Animals were kept under 25 ° C, 50-55% humidity, light periods, 12 hours of light and 12 hours of darkness, and free access to water and food. The ethics of working with laboratory animals was conducted under the auspices of the Laboratory Ethics Committee.

Toxin diazinon was prepared with purity of 98% from Tehran gazal chemical company and then dissolved in distilled water. Animals were randomly divided into four equal groups (n = 7) as follows:

Control group: The mice did not receive any toxic and were kept in optiMum conditions.
Sham group: Mice received distilled water as gavage.
Experimental group 1 received diazinon in a dose of 0.5 mg / kg as gavage.
Experimental group 2 received ketamine diazinon (1.5 mg / kg) for gavage for four weeks (five days a week).

One week after the last injection, the rats were weighed and anesthetized with ether. All blood of the mice was collected from the axillary region and through the opening of the axillary veins, and then the blood serum was isolated by centrifugation for 15 minutes at 3000 rpm. Then testosterone was measured by radioimmunoassay. Then, for testicular studies, the testes were carefully removed and after washing in physiological and drying serum, weighed and placed in formalin 10%. After different stages of tissue and molding, sections were made in 5 microns thick and stained with hematoxylin and eosin. Different cell types including germinal cells, spermatocytes, spermatids, and Leydig and Sertoli cells were counted using a three-piece intermediate eye piece. In each section, two sections were selected from the tissue cut and a total of about 100 fields were evaluated. The diameter of the spermatozoon tubes was measured with a scaled lamellar screen and testes diameter with micrometer. The data were analyzed by ANOVA and $P < 0.05$.

Findings

The results of body weight indicated that there was no significant difference between the experimental groups 1 and 2 with control and sham groups (Table 1)

Relative weight and testes diameter

Relative weight of testes in experimental groups 1 and 2 was 0.027 ± 0.07 and 0.023 ± 0.073, respectively compared to control group (0.066 ± 0.006) and sham group (0.076 ± 0.004) 0) did not show. The testicular diameter in diazinon-treated groups was decreased in comparison to the control group, but it was not statistically significant (Table 1).

Germinal cells and spermatocytes

With the count of germinal cells and spermatocytes per unit area, the mean number of germinal cells in the experimental group was 2.8 ($\pm$ 24.81 $\pm$ 0.9) than the control group ($\pm$ 9.223 $\pm$ 2.33) (0.05) > P), but in experimental group 1 (8.96 $\pm$ 0/678), it was not statistically significant, but statistically significant ($p < 0.05$). The number of germinal cells in the sham group (9.16 $\pm$ 0.56) Compared to the control group, there was no significant difference (Table 1). Also, the number of spermatocytes in the experimental group 1 and 2 (533.0 $\pm$ 58.19 and 90.1 $\pm$ 20.20, respectively) than the control group (20.1 $\pm$ 75.72), but statistically meaningful It is not.

Number of spermatids and diameter of stem cells

The number of spermatids in the experimental groups was lower than the control group, but it was not statistically significant. Also, there was no significant difference between sham and control group. By measuring the diameter of the spermatogonous

tubes, it was found that in the experimental groups, the diameter of the tubes was significantly lower than the control group, but there was no significant difference between the experimental group 1 and the sham group with the control group (Table 1)

Number of Leydig and Sirtouli cells

The number of Leydig cells in the experimental groups was 1.72 ± 4.695 and the experimental group 2 (4.3 ± 0.860) had a significant decrease compared to the control group (± 0.53 ± 231.31). Microscopic examination showed that sertoli cells in the experimental groups did not have a significant difference in comparison with the control group (Table 1)

Blood Testosterone Level

The concentration of testosterone was not significantly decreased in test groups 1 and 2 (8.65 ± 4.72 and 8.45 ± 14.22 ± 8.41 ± 8.28 ± 8.8 ± 0.82, respectively), and between groups Sham (8.28 ± 14.22) and control group were not significantly different (Chart 2)

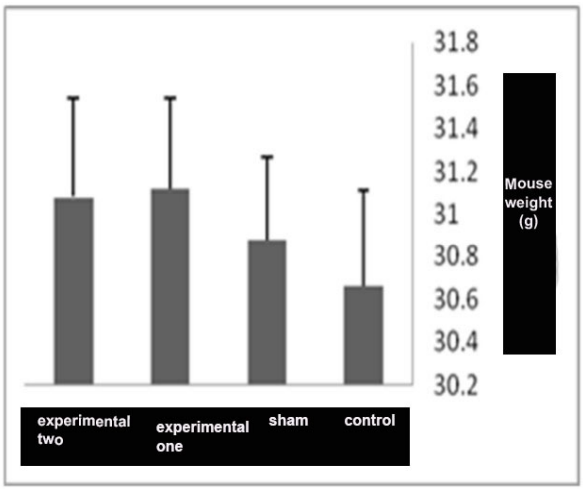

Fig. 1. Compares the mean weight of mice in different groups.

Table 1. The comparison of the mean of the parameters studied in different groups of the same letters indicates that there is no difference in meaning between the groups is

Groups Parameter	group Contro Mean ± SE	group sham Mean ± SE	Experimental group 1 (5/0 mg / kg) Mean ± SE	Experimental group 2 (mg / kg) Mean ± SE
Relative weight of testicle (g)	076/006 ± a 0/0	076/004 ± a 0/0	0/073 ± a 0/001	0.074 ± a 0/003
Testicle diameter (mm)	49/155 ± a 5/0	5/45 ± a 0/3	5/36 ± a 0/1	5/31 ± a 0/659
Germ cell	9/02 ± a 0/233	9/01 ± a 0/56	8/96 ± a 0/678	8/24 ± b 0/120
Spermatocytes	20/72 ± 1/90	20/75 ± 1/72	20/50 ± 0/767	19/58 ± 1/72
Spermathetes	14/678 ± 7/0	7/06 ± 0/25	6/74 ± 0/15	6/66 ± 0/15
Sertoli cells	± 20/114 ± 1/0	20.4 ± 0.1	18/800 ± 1/0	16/509 ± 1/0
Leydig cells	43/231 ± a 5/0	5/35 ± a 0/67	4/72 ± b 0/695	4/42 ± b 0/860
Sperm tube diameter Instrument (square microme er)	133/911 ± a 3	52/231 ± a 131/4	56/128 ± a 128/4	96/245 ± b 123/2

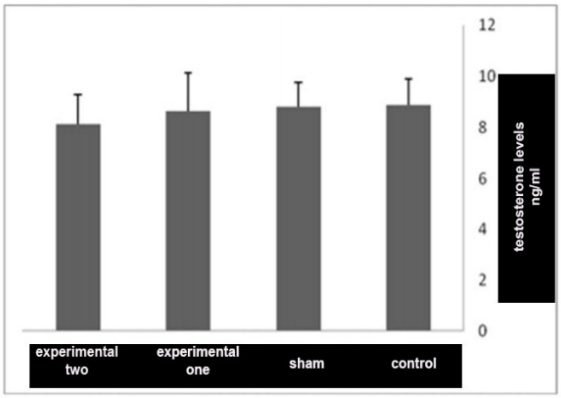

Fig. 2. Comparison of serum testosterone levels in different groups.

Discussion

In this study, the number of germinal cells, spermatocytes, spermatids, sertoli and leydig cells, diameter of stem cells and serum testosterone levels decreased. However, the reduction in germinal cells, leydig cells and diameter of the spermatozoa tubes was significantly higher in the experimental groups than in the control group. These results are consistent with studies by some researchers, in which the intake of diazinon toxins directly damages testicular cells or indirect damage and causes a reduction in hormone deficiency in spermatogens. These disorders are associated with reduced sperm production, defective sperm production, and impairment of androgen production (Nesnow and et al, 2011; Bruno and et al, 2009). In this study, the number of spermatogenic cells and sertoli and leydig cells has decreased. The results of many studies on the effect of diazinon on On the reproductive system, it has been shown that there is a significant effect of these chemicals on long-term and long-term doses on sperm and its parameters (Bruno and et al, 2009; Keramati and et al, 2010). It should be noted that the sperm parameters are not limited to the number or movement of the sperm. Morphology of the sperm and its chromosome structure are of particular importance. Dicks have reported in their studies that toxins affect DNA, RNA, and protein levels, causing genetic, cellular, and mitotic stasis (Goetz and et al, 2009). Some also believe that these toxins Oxidative-antioxidant balance in the body, and may produce reactive oxygenates and induce oxidative stress and inhibit antioxidant enzymes such as catalase and superoxide dismutase through the production of radicals, which ultimately reduce ATP production and induced cell death (Allen and et al, 2006;Hester and et al, 2012; Skolness and et al, 2013). The decrease in cells in this study seems to follow this mechanism. However, the destructive effects of pesticides are

different, but in most cases it determines the dose and exposure time of the toxic effect of the toxin (Goetz & Dix 2009). In this study, diazinon in higher concentrations had a more negative effect on the factors involved, indicating that the negative effects of toxin are dependent on the dose.

Some tissues and cells are more susceptible to toxins, and in this study, the number of germinal cells is more affected by venom than other spermatozoa cells and shows a significant decrease. In our study during the microscopic examination of testicular tissue, the number of spermatid cells in both experimental groups was reduced compared to the control group. Since the number of spermatids is dependent on spermatogonial cells and spermatocytes, and according to the results of this study, which indicates a decrease in the number of spermatogenic cells, it can ultimately affect the number of spermatids and reduce their number. It seems, however, that this decrease, although it may be seen in the affected area, is in general indicative of the reduction of spermatid cells in the entire testicular tissue. Therefore, it is inferred that diazinon, by its various mechanisms, which in some cases results in the destruction of sperm proteins, anomalies, or damage to the chromosome, ultimately leads to a reduction in sperm. Although this reduction may not have a significant negative effect on the first days and months, especially in individuals with genetically and inherited high quality sperm, but certainly for those groups of low quality parameters Spermatozoa, which in quality and quantity are on the line of fertility or infertility, will be very dangerous. The results of this study indicate that diazinon toxicant reduces leydig cells and diameter of spermatozoa tubes. Research has shown that toxins with an effect on the mitochondria that the cell's power plant is considered to reduce or stop energy production and thereby cause cell death. It also increases the production of reactive oxygen species. It is noteworthy that this toxin, by inhibiting cell growth and DNA replication, ultimately results in a decrease in the leydig cells and diameters of the spermatozoa by negatively affecting the cell cycle and cell division. (Li and et al, 2010).

Other possible factors that reduce the diameter of the sperm tubes can be the reduction of spermatogenic cell lines in these tubes, which is the result of this study. The results of this study are consistent with Fatahi et al. (2.1). The effect of toxins on changes in leydig cells and diameter of stemming tubes is different. Some of the compounds of this category increase and some of them have a reverse effect and reduce it (20.1). Also, blood testosterone levels have been reduced in this study. This decrease was more pronounced in the experimental group 2, which received a higher dose of diazinon. These results are consistent with the findings of Taxvig et al. (Barrett & Jaward, 2012). In a research conducted by the researcher that the poison was taken alone and combined with other toxins on the mouse, the hormonal results showed that the progesterone levels increased in all experimental groups, but the testosterone levels decreased significantly. Also, in this study, the number of leydig cells decreased. Therefore, after lowering the Leydig cells that are responsible for the secretion of testosterone in the male, it is expected that the amount of this hormone will also decrease in the serum. In fact, after the reduction of testosterone, the first issue that comes to mind is changes in leydig cells. Since the number of leydig cells has decreased in this study, the production of this hormone has also decreased. Given the role of testosterone in sexual differentiation, it probably will create a secondary infertility.

By examining different aspects, it seems that factors such as inhibition of steroid biosynthesis, androgen deficiency and / or degradation of leydig cells play a role in reducing testosterone levels. But what can be inferred from the results of this study is that lowering testosterone is associated with the reduction and degeneration of the number of Leydig cells. It seems that the effects of diazinon poisoning on the tissues of the body have been proven to be very different, but different in different people may differ according to the required conditions. Although all researchers have not achieved a single result, they all agree that diazinon affects fertility, fertility characteristics, and the process of spermatogenesis, and the present study has achieved similar results. This, of course, does not mean that infertility will be created immediately. But engagement of the community towards an unwanted infertility, especially if it occurs in adolescence and adolescence, may have negative effects in the long run.

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