IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-10, Issue-1, 2019, pp60-66
http://www.bipublication.com

Research Article

Studying the effects of diazinon poison on some reproductive parameters in male rats

Hadi Kord, Mohammad Ghazanfari,

Ali Pirdadehkhani and YounesShakibzadeh*

*Ischemic Disorder Research Center,
Golestan University of Medical Sciences, Gorgan, Iran

[Received: 29/11/2018; Accepted: 02/01/2019; Published: 06/01/2019]

ABSTRACT

Background and Objective: Diazinon has a destructive effect on the reproductive system as an environmental contaminant. The objective of this study was to investigate the effect of this toxin on male reproductive traits.

Analysis Method: In this study, the long-term effect of Diazinon35% Ec on some male reproductive parameters in adult rats was investigated. For this purpose, 40 male rats were divided into five groups. The control group did not receive any substance. The placebo group received 0.9% saline solution and experimental group 1, 2 and 3, respectively, received diazinon with the dose of 20, 10 and 5 kg / mg, every other day over 21 days via gavages. At the end of the experiments, the rats were anesthetized with chloroform, blood samples were taken from the heart and FSH, LH and testosterone hormones were evaluated by standard laboratory methods. Then, the rats were killed and the testicles were removed with epididym and morphological characteristics of the sperms were studied. The results were analyzed by SPSS software and were evaluated by using one-way variance analysis at the level of <0.05.

Results: Following the administration of diazinon, the level of male sex hormones including LH and FSH was significantly increased (P <0.05), while the testosterone level decreased significantly. Reproductive parameters such as number and sperm movement and testosterone weight decreased significantly (P <0.05) compared to the control group.

Conclusion: Diazinon has significant effects on reproductive indices and can cause serious damage. This toxin has adverse effects on the movement and number of sperms and causes infertility by altering hormonal concentrations.

Key words: FSH, LH, Diazinon, Fertility, Testosterone, Sperm Count, Male sex, Rat.

Background and objective

Organophosphates are an important group of pesticides that are used in developing countries to fight pests. These compounds can create a high toxicity for animals and humans (1 and 2). One of the organophosphate pesticides widely used in northern areas of the country is diazinon. Diazinon with chemical name of O-O Diethyl O-2- isopropyl-6-methylpyrimidinyl-4- 1 – g-phosphorothivate), with 0.1 to 0.2% emulsion is usually used in agriculture for controlling and eliminating pests and stem-worm (3).

Such poisons have a high shelf life in the environment and after contact with skin it easily is absorbed by the body and turns into active metabolites in the liver. Most of these metabolites are excreted by the kidney, but the

rest can affect various organs, including the liver, reproductive system, immunity, the nervous system and kidneys, and cause tissue and biochemical changes (4, 5). It should be noted that the complications associated with these compounds depend on the type of toxin, dose, duration of contact and type of tissue. The main mechanism of organophosphates, especially the diazinon, is inhibiting acetylcholine esterase (6). Organophosphates are alkylation agents that react with cellular macromolecules, such as proteins, nucleic acids and lipids, and alter their function (7). Some researchers believe that diazinon induces oxidative stress by producing free radicals and reactive oxygenates, and induces cell death in organisms by increasing lipid peroxidation [8].

The expansion of the use of organophosphates, especially diazinon, and the numerous reports that have been made in recent years on the effects of these toxins on various growth and development processes, have raised concerns about the harmful effects of these pesticides on human health. Organophosphates seems to alter the function of cells, mutation in genes, stopping mitosis, abnormalities in the fetus, stopping DNA synthesis and inducing death. Therefore, according to the reported effects of Toxin Depletion, they classified them in the cytotoxic and genotoxic groups (9-11)

Due to the high use of diazinon by farmers in the country and due to the use of this poison in agricultural products, it seems that this poison causes physiological changes in various parts of the body, including the male reproductive system, due to its presence in high risk toxins. Considering the unwanted side effects and high toxicity, the objective of this study was to investigate the effect of diazinon poison on reproductive physiology in male rats.

Analysis method

In this research, adult male rats were used. Samples were divided into five treatment groups. The control group did not receive any substance. The placebo group received 0.9% saline solution and the experimental group 1, 2, and 3 received endosulfan at 5 mg / kg, 10 and 20, respectively, every other day, for 21 days, by oral administration.

Statistical population and study groups:

To conduct the study, 40 adult male rats (about two months old and 200-100 g weight) were purchased from Razi Institute of Clinical Research and divided into 5 groups of 8, including control, placebo (0) and 3 treatment groups (1-2-3).

Control group: did not receive any substance.

Placebo group: Only 0.9% saline solution was given.

Experimental group 1 received diazinon poison at a dose of 5 mg / kg.

Experimental group 2 received diazinon 10 mg / kg dose.

Experimental group received 3 mg of diazinon in a dose of 20 mg / kg.

The LD50 value of diazinon was measured in animals at an oral dose of 50-110 mg / kg (9).

Storage conditions of the samples:

The specimens were kept for two weeks at room temperature at 17 ° C and normal light conditions and free access to water and food (as a cube from the Pars Company). These conditions were also established during the test.

Gastrointestinal feeding method:

Treatments received intravenous injection of different doses of diazinon. The advantages of this method are rapid absorption and entry into the circulatory system. Since the effect of this poison is gastrointestinal and contagious, so the digestive tract was selected. First, rats were weighted accurately and after the necessary calculations, the amount of oral diazinon was determined.

Blood sampling:

Blood collection was performed five days after the last administration. The blood samples were anesthetized with chloroform and then, by placing the animal on the back with the fingers of the heart, and with 5 or 10 ml syringes directly

from the blood of the animal were collected. To test the testosterone, LH, FSH and serum, they were transferred to the laboratory to measure the hormone by making gamma-kits using the immune radiometric assay. After determining each one, the effects of diazinon poison in the experimental and control groups were evaluated statistically.

Study of changes in testicular weight relative to body weight:

In order to investigate the possible effects of diazinon on testicular weight, both testicles of the animal were removed and extra fat and excrement around the testicles and ducts of deaf and epididym were removed and then testicular weight was measured by a digital scale with a sensitivity of 0.001 g (Germany, Bllzos).

Methods for collecting and evaluating sperm:

After blood collection, the rats were anaesthetized in all three experimental, placebo and control groups and after opening the abdominal cavity, the epididym tubes isolated after washing in the physiologic serum and divided into very small parts in a volume of 5 ml of Ringer's solution, and for 5 minutes was shaken smoothly to create a homogeneous solution. From this suspension, one drop was removed and immediately examined by light microscope (Germany, Ziess) and 40x magnification of the percentage of mobile spermatozoids. Sperm microscopic counting was performed using a diluents pipette and neobar lamella.

Statistical analysis method:

SPSS 11 software was used to analyze the data and the results of testosterone, FSH, LH, morphological parameters of sperm and testis weight were calculated and the mean of the groups was evaluated by one way variance and post hoc test. For the experiments, a significant level ($P < 0.05$) was considered to be statistically significant for the presence or absence of significant differences between the samples.

RESULTS

Hormonal Measurement Results:

The mean serum testosterone level was decreased by increasing the dose of diazinon and the mean of experimental group 1 (treated with experimental dose of 5 mg / kg of diazinon) and 2 (treated with experimental dose of 10 mg / kg of diazinon) and 3 (treated with experimental dose of 20 mg kg / Diazinon) with the control group is significantly decreased (Table 1).

Table 1. Comparison of the average effect of diazinon on testosterone hormones FSH, LH, number and percentage of sperm motility and testicular weight in adult male rats

Groups	Testosterone	FSH	LH	Sperm numbers	Sperm mobility percentage	Relative weight of testicles to body weight
Control	± 0/73095 2/55	± 0/27936 0/9075	± 0/529 1/1037	± 7/98995 80/125	57/375 ± 5/31675	1/4304 ± 0/09931
Placebo	2/4± 0/6541	± 0/2654 0/9121	± 0/54 1/109	79/791 ±7/341	57/1 ± 5/2514	1/401 ± 0/09146
Group1	±0/4917 *1/2863	± 30232 0/9563	± 0/52051 1/1612	± 19/2261 *49/25	51/875 ± 4/61171	1/3473 ± 0/09321
Group2	± 0/43395 *1/37	± 0/56454 *1/5025	± 0/41376 1/3850	± 10/75706 *42/5	*47/625 ± 8/94327	1/2976 ± 0/16696
Group3	± 0/0802 *0/7613	± 0/33441 *1/9938	± 0/4728* 1/8038	± 6/08129 *35/875	*41/5 ± 7/81939	*1/1743 ± 0/9931

Between the experimental groups 2 (treating with 10 mg / kg dose of diazinon) and 3 (experimental with 20 mg / kg diazinon FSH) with the control group increased significantly. However, there was no significant difference between experimental group 1 (experimental dose 5mg / kg doses of diazinon) and placebo group with control group.

In evaluating the mean of serum LH, there was a significant increase in the serum LH between the experimental groups (treating with experimental dose of 20 mg / kg of diazinon) with the control group. However, there was no significant difference between the experimental groups (treating with experimental dose of 5 kg / mg of diazinon) and 2 (treating with experimental dose of 10 mg / kg of diazinon) and placebo group in control group (Table 1).

The effects of Diazinon on Sperm

The effect of Diazinon of the number of Sperm:

The mean number of sperms between experimental groups 1 (treating with experimental dose of 5 mg / kg of diazinon) and experimental 2 (treating with experimental dose of 10 mg / kg of diazinon) and experimental (3) (treating with experimental dose of 20 mg / kg of diazinon) decreased with control group Significant decrease is seen.

Mobility: As shown in Table 1, the average percentage of moving sperm between the experimental groups 2 (treating with experimental dose of 10 mg / kg of diazinon) and 3 (treating with experimental dose of 20 mg / kg of diazinon) with the control group was significantly decreased. However, there is no significant difference between the experimental groups (treating with experimental dose of kg / 5 mg diazinon) and the placebo group with the control group.

Testicide to body weight ratio:

The results of testicular weighing show that the mean testicular weight in the experimental groups was lower than that of the control group and this decrease was significant only in group 3 (treating with experimental dose / kg 20mg of diazinon) (Table 1).

DISCUSSION

Due to the high volume of used pesticides, human exposure to such pesticides is almost unavoidable and can occur unintentionally and accidentally and consequently through the use of pesticides and their remaining in the environment. The diazinon has good insecticidal properties and has no evaporative properties and is completely stable against light, and is dangerous to humans and animals. (10) Diazinon is a toxic substance and, if inhaled, it can be fatal or absorbed through the skin. Oral consumption has a greater effect on skin contact (11). The amount of absorption and toxicity increases in the presence of solutions such as alcohol and artematic solvents (9).

Diazinon cannot inhibit the biosynthesis of testicular androgens in experimental animals and cause obvious damage to the kidneys and testicles (1), so that diazinon can affect the rat's reproductive system (10). Behavioral and neurological changes have also been considered (11). The damage to the thyroid follicles is also seen in rats (11). Diazinon is also known to damage the endocrine system (6).

The results of this study showed that the action of chemical pesticides depends on their dose level, with increasing the dose of toxin, the severity of its effects increases. Therefore, it seems that if these pesticides enter the body for a long time, they may, in addition to damage to various organs of the body, the reproductive system is also affected.

This study showed that oral administration of diazinon in rat is effective on the concentration of sex hormones and the spermatogenesis process reducing spermatogenic cells. This result emphasizes the effect of diazinonon the complications and the use of 20 mg / kg dose in

this study caused a lot of changes in many testicular parameters. Diazinon seems to reduce the number and mobility of sperm with its toxic effect.

Diazinon with its toxic effects increases the amount of LH and FSH, and decreases testosterone, the number and sperm movement, and the weight of the testicles compared to the control group.

Testosterone lowering in experimental groups can be based on the following hypotheses: 1) Diazinon causes atrophy and decreases the number of testosterone producing lydic cells. The long-term effects of this toxin can damage testicular tissue and reduce testosterone levels (10).

2. Diazinon directly affects the tissues and sex cells and causes various damages to testicular structure and various cell types in the process of spermatogenesis and hormonal disorders.

3. Vittozzi et al. (2001) evaluated the effect of pesticide survival on farmers' health and blood levels, and found that long-term toxic effects on the liver, kidney and testicles (9) and a significant reduction in testosterone levels (3,5).

One of the reasons for increasing LH is that diazinon control the negative feedback of LH and by preventing reversible inhibition, increases the LH hormone. On the other hand, reducing testosterone levels increases LH. As the LH increases, the FSH hormone also increases (2). The researchers showed that the diazinon biosynthesis insecticide restricted ovary androgens in adult rats. In examining the effect of testosterone and plasma gonadotropins and testosterone on the testicular and serum levels reduced serum testosterone levels (2) and these results are not consistent with the results of the present study.

Organ phosphoric insecticides, including diazinon, have different endocrine-receptor binding tendencies, which are very similar to estrogen receptors, react with the endocrine system and cause the system to fail (11.5). In another study on the rat, it was found that the increase in the removal of androgens is associated with a decrease in serum testosterone levels which is itself due to contamination with diazinon. (12,7) According to studies, other pesticides such as Lindane, Randap, with protein star distortion failure (estrogenic regulation), steroidogenesis is limited (4). The protein has a role in transport cholesterol in the inner mitochondrial membrane where cytochrome p450 enzyme starts all of the steroid hormones(1).

One of the reasons for reducing the weight of the testicles is the decrease in the number of different classes of spermatogenic cells and the diameter of the spermatogenic tubes, which leads to a decrease in the testicular weight. This decrease can be due to a decrease in the cell population in the testis spermatozoon tubes or in the reduction of the interstitial cells of the spermatozoa tubes (5, 3). It seems that this poison, by stopping the mitosis and induction of cell death, leads to a decrease in germinal cells, which are essential in the spermatogenesis process, and the reduction of these cells necessarily affects the cell lines, such as spermatocytes, which ultimately will reduce sperm.

CONCLUSION

The results of this study confirm the previous studies suggesting that dose-dependent effects of diazinon may have significant adverse effects on reproductive indices and altered the level of male sex hormones and spermatogenesis process, which led to the reduction of spermatogenic cells and, at the same time, the percentage of moving spermatozoa is reduced. All of these can cause serious damage to the reproductive system and cause infertility.

REFERENCES

1. Shah MD, Iqbal M. Diazinon-induced oxidative stress and renal dysfunction in

rats. Food ChemToxicol. 2010;48(12):3345-53.
2. Anbarkeh FR, Nikravesh MR, Jalali M, Sadeghnia HR, Sargazi Z, Mohammdzadeh L. Single dose effect of diazinon on biochemical parameters in testis tissue of adult rats and the protective effectofvitE. IJRM. 2014;12(11):731-6.
3. Fattahi E, Parivar K, Jorsaraei SGA, Moghadamnia AA. The effect of diazinon in leydig cells and sexual hormones in mice. BabolUni Med Sci J. 2007;9 (4): 15- 22. [In Persian]
4. Fattahi E, Jorsaraei SGA, Parivar K, Moghaddamnia AA. The Effects of a Single Dosage of Diazinon and Hinosan on the Structure of Testis Tissue and Sexual Hormones in Mice. Yakhteh Med J. 2010; 12(3): 405- 410. [In Persian]
5. Yilmaz N, Yilmaz M, Altuntas I. Diazinon induced brain toxicity and protection by vitamins E plus C. ToxicolInd Health. 2011;28(1):51-7.
6. MalijiGh, JorsaraeiSGh, Zabihi E, Fattahi E, Rezaie E, SohanFaraji A. Diazinon alters sex hormones, Interferon-gamma, Interleukin-4 and 10 in male Wistar rats. GorganUni Med Sci J. 2014;16:22-28[In Persian].
7. Fattahi E, Jorsaraei SGA, Parivar K, Moghadamnia AA. Influence of diazinon on spermatogenesis in mice. Semnan Uni Med Sci J. 2007; 9(1): 75-82. [In Persian]
8. Sargazi Z, Nikravesh MR, Jalali M, Sadeghnia HR, RahimiAnbarkeh F. Apoptotic Effect of Organophosphorus Insecticide Diazinon on Rat Ovary and Protective Effect of Vitamin E. IJT. 2016;10(2): 37- 44.
9. Slotkin TA, Tate CA, Ryde IT, Levin ED, Seidler FJ. Organophosphate insecticides target the serotonergic system in developing rat brain regions: disparate effects of diazinon and parathion at doses spanning the threshold for cholinesterase inhibition. Environ Health Perspect. 2006; 114(10): 1542-6.
10. Gaido K, Dohme L, Wang F, Chen I, BlankvoortB, Ramamoorthy K, et al. Comparative estrogenicactivity of wine extracts and organochlorine pesticideresidues in food. Environ Health Perspect.1998 ;106 Suppl 6:1347-51.
11. Boereboom FT, van Dijk A, van Zoonen P, MeulenbeltJ. Nonaccidentalendosulfan intoxication: acase report with toxicokinetic calculations and tissueconcentrations. J ToxicolClinToxicol. 1998;36 (4):345-52.
12. Oliveira-Silva JJ, Alves SR, Meyer A, Perez F,Sarcinelli PN, da Costa Mattos RC, et al. [Influenceof socioeconomic factors on the pesticides poisoning,Brazil]. Rev SaudePublica. 2001;35(2):130-5.Portuguese.
13. Hela DG, Lambropoulou DA, Konstantinou IK, AlbanisTA. Environmental monitoring and ecologicalrisk assessment for pesticide contamination andeffects in Lake Pamvotis, northwestern Greece.Environ Toxicol Chem. 2005;24(6):1548-56.
14. Betancourt M, Reséndiz A, Fierro EC. Effect of twoinsecticides and two herbicides on the porcine spermmotility patterns using computer-assisted semenanalysis (CASA) in vitro. ReprodToxicol. 2006;22(3):508-12.
15. Soto AM, Sonnenschein C, Chung KL, FernandezMF, Olea N, Serrano FO. The E-SCREEN assay asa tool to identify estrogens: an update on estrogenicenvironmental pollutants. Environ Health Perspect.1995 ;103 Suppl 7:113-22.
16. Jamil K, Shaik AP, Mahboob M, Krishna D. Effectof organophosphorus anorganochlorine pesticides(monochrotophos, chlorpyriphos, dimethoate, andendosulfan) on human lymphocytes in-vitro. DrugChemToxicol. 2004;27(2):133-44.

17. Sinha N, Adhikari N, K Saxena D. Effect of endosulfanduring fetal gonadal differentiation on spermatogenesisin rats. Environ ToxicolPharmacol. 2001;10 (1-2):29-32.
18. Grünfeld HT, Bonefeld-Jorgensen EC. Effect of invitro estrogenic pesticides on human oestrogen receptoralpha and beta mRNA levels. Toxicol Lett.2004;151(3):467-80.
19. Walsh LP, Webster DR, Stocco DM. Dimethoate inhibitssteroidogenesis by disrupting transcription ofthe steroidogenic acute regulatory (StAR) gene. JEndocrinol. 2000;167(2):253-63.
20. Vittozzi L, Fabrizi L, Di Consiglio E, Testai E.Mechanistic aspects oforganophosphorothionate toxicity in fish and humans. Environ Int. 2001;26(3):125-9.