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Spring 2022, Volume 7, Issue 25(30-35)

Evaluation of The Effects of Diazinon Toxin on Some Reproductive Parameters In Male Rats

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DOI: 10.22034/pmj.2022.253550

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Submitted: 2022-04-12
Accepted: 2022-05-18

Keywords:
Organophosphates
Diazinon
Sperm parameters
Oxidative stress

Abstract:

The reproductive system is affected negatively by the organophosphate insecticide diazinon (DZN). Numerous adverse effects on the reproductive system are brought on by it, including testicles degeneration, sex hormone disruption, decreased spermatogenesis, poor sperm quality, and fertility issues. The goal of the current study was to look at how diazinon affected the sperm parameter, sperm viability, and levels of sex hormones in adult male rats. The mature male rat was divided into five groups for this experiment: control (did not receive any substance), Placebo group (only 0.9 percent saline solution was consumed), and the other three groups received DZN (diazinon was administered at doses of 5, 10, and 20 mg / kg for 30 days). Within 30 days after the most recent doses, animals were killed. Radioimmunoassay was used to evaluate the amounts of serum testosterone, LH, and FSH. Sperm parameters such as motility and count were measured by CASA system. Sperm viability was also calculated by eosin-nigrosin staining. Following the injection of DZN, a substantial decrease in testicular weight and sperm concentration was seen. Additionally, DZN caused a significant decrease in serum testosterone concentration as well as sperm viability. Comparing DZN groups to the control and sham groups, LH and FSH levels were higher in the DZN groups. DZN is harmful to the reproductive organs and spermatogenic cells of mammals. Application of DZN should indeed be restricted to a specific framework.

INTRODUCTION

In contrast to the traditional method of treating everyone the same, personalized medicine involves applying specific medical procedures, medications, and/or processes to each patient to prevent, diagnose, and/or treat disease (1). This method is based on detailed knowledge of the individual's specific and explicit characteristics as well as the disease, including genotype, physiology, exposure to environmental, and lifestyle levels, among other factors (2). It is a ground-breaking strategy that incorporates variance into all facets of health care for the prevention and treatment of disease (2). To this end, it is crucial to understand the precise cause of disease and the underlying physiology when attempting to design treatment tools. From this approach, it is anticipated that the treatments will be more effective, cost-effective, and have fewer side effects, which will be beneficial for all patients (3).

Pesticide use in agriculture for crop conservation and pest control has been associated with difficulties with people's health and environmental degradation worldwide (4). Numerous studies have shown that the quality of human sperm has declined over the past few decades, and many have connected this to environmental exposures to pesticides at work (5,6). Although published research have suggested a link between pesticide exposure and male fertility, experts disagree over the precise detrimental effects of pesticides on male reproductive health (7). As a result, the impact of pesticides on human reproductive system is still debatable, and a rising number of researchs have concentrated on the diverse group of pesticides.

Agricultural workers' exposure to organophosphorus pesticides (OP) is a major health concern currently (8). They are extremely poisonous to insects and animals while being quickly digested (8). To control flies, lice, and other insect pests on ornamental plants and food crops, diazinon (DZN), an organophosphorate pesticide (OP), is used extensively in agricultural practices across the world (9). Concerns regarding DZN's impact on humans and laboratory animals have been growing. It prevents the activity of acetylcholinesterase and

other organic processes (10). DZN has been shown in several research to alter male rat blood factors, plasma testosterone, and glucose levels (11). However, little is known about how hazardous DZN is and how it affects male reproductive function. DZN has the potential to interfere with animal reproductive function, according to several research (12). The adverse effects of DZN on human reproductive function, however, have been well-documented. These include decreased libido after percutaneous treatment and notably lower levels of androgenic hormone. We hypothesised in the current investigation that DZN has a harmful impact on sperm parameters and hormone level that might be a risk factor for infertility since the detrimental effect of DZN on male reproductive function is still debatable.

MATERIALS AND METHODS

Animals and material

40 adult male rats weighing between 180 and 200 grams were purchased from the Pastor Institute. The rats were kept in solitary confinement in a room with a 12:12 light/dark cycle and an ambient temperature of 20°C. International Agricultural Chemistry gave diazinon.

Experimental groups

Adult rats were randomly divided into 5 groups, comprising: The control group (n=5) did not receive any substance. Placebo group: Only 0.9% saline solution was ingested. Experimental group 1: received diazinon toxin at a dose of 5 mg / kg orally for 30 days. Experimental group 2 received diazinon toxin at a dose of 10 mg / kg orally for 30 days. Experimental group 3 received diazinon toxin at a dose of 20 mg/kg for 30 days. The amount of LD50 diazinon for laboratory animals was measured orally 50-110 mg/kg orally (13). The Institutional Animal Care and Use Committee approved all animal-related protocols (Kharazmi University, Tehran, Iran).

Evaluation of changes in testicular weight and hormone level

Excess and excess lipids around the testicles, the vas deferens, and the epididymis were all removed from the environment in order to study the potential effects of diazinon toxin on testicular weight. Blood sampling was performed five days after the last administration. In order to perform the bleeding operation, the animal was anesthetized with chloroform and then by placing the animal on its back with the fingertips, the location of the heart was determined and blood was taken directly from the animal's heart. Immuno radiometric assay technique was used to measure LH, FSH and testosterone.

Assessment of sperm parameters

By using computer assisted sperm analysis, epididymal sperm parameters were evaluated (CASA). In this procedure total motile and immotile sperm, and concentration of sperm were assessed, along with concentration and sperm motility. In a nutshell, the complete epididymis was taken out and longitudinally sliced to extract sperm from the cauda. After that, the cauda epididymis was transferred to microtubes containing 1 mL of T6 solution and 25 µL BSA (bovine albumin serum), and the microtubes were then put inside an incubator (5 percent CO2 at 37 °C) for 30 minutes, or until spermatozoa formed a sperm suspension.

Assessment of sperm survival

50 µl of the sperm suspension and 50 µl of the 0.5 percent eosin dye were combined in a microtube. After shaking for 30 seconds, 100 µl of nigrosin was added to the initial mixture. A coverslip was put on a slide after a drop of the stained sperm suspension was deposited there. The x400 objective of a light microscope is used for observation. Live spermatozoa had a blue border, whereas dead spermatozoa appeared purple.

Statistical analysis

One-way ANOVA and Tukey post hoc tests were used to examine the data in SPSS version 19. P value of 0.05 above was deemed significant. The mean and standard deviation of all data were displayed (SD).

RESULTS

Diazinon's effects on sexual hormones and testicular weight

The results obtained from testicular weight show that the mean weight of testes in the experimental groups decreased compared to the control group and this reduction was significant only in group 3 (treatment with experimental dose / 20 mg / kg diazinon toxin) Table 1. With increasing doses of diazinon toxin, the mean serum testosterone decreased, and the difference between the means of experimental groups 1 (treatment with an experimental dose of 5 mg/kg diazinon toxin), 2 (treatment with an experimental dose of 10 mg/kg), and 3 (treatment with an experimental dose of 20 mg/kg diazinon toxin) is significantly smaller than that of the control group (Table 1). FSH significantly increased in comparison to control group between experimental groups 2 (treatment with experimental dosage of 10 mg/kg diazinon toxin) and 3 (treatment with experimental dose of 20 mg/kg diazinon toxin). There was no significant alteration between experimental group 1 (treatment with experimental dose of 5 mg / kg diazinon toxin) and placebo group in compared to control. In the study of mean serum LH between experimental groups 3 (treatment with experimental dose of 20 mg / kg diazinon toxin) with the control group, a significant increase was observed. The results are presented in Table 1.

Table 1: Comparison of the mean effect of diazinon toxin on testosterone, FSH, LH, and testicular weight in adult male mice

Group name	Testosterone (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)	Testicular weight
Control	2. 55 ± 0.73	0.9075 ± 0.27	1.1037 ± 0.52	1.430 ± 0.099
Placebo	2.4 ± 0.65	0.9121 ± 0.26	1.109 ± 0.54	1.401 ± 0.091
Group 1 (5 mg/kg)	1.2863 ± 0.49*	0.9563 ± 0.302	1.1612 ± 0.52	1.3473 ± 0.093
Group 2 (10 mg/kg)	1.37 ± 0.43 *	1.5025 ± 0.56 *	1.3850 ± 0.52	1.2976 ± 0.16
Group 3 (20 mg/kg)	0.7613 ± 0.08 **	1.9938 ± 0.33 *	1.8038 ± 0.47 *	1.1743.99

FSH: follicle stimulating hormone, LH: luteinizing hormone. Data represent as mean ± SD * p<0.05.

Evaluation of sperm parameters

The computer-assisted sperm analysis revealed that, in comparison to the control group, the sperm concentration and mobility (progressive, nonprogressive, motile, and immotile) were significantly lower in experimental groups 1 (treatment with experimental dose of 5 mg / kg diazinon toxin), experimental 2 (treatment with experimental dose of 10 mg / kg diazinon toxin), and experimental 3 (treatment with experimental dose of 20 mg/kg diazinon toxin). The fraction of motile sperms significantly decreased in the diazinon groups compared to the control group (p <0.05). Between the control group and placebo group, there was no discernible difference. Additional modifications to sperm properties are listed in Table 2.

Sperm viability after diazinon therapy

Figure 1 depicts the findings of the eosin-negrosin staining, which reveal a substantial difference between the control group and the diazinon groups (5, 10, 20 mg/kg) in terms of sperm viability. After 30 days of oral diazinon therapy (5, 10 and, 20 mg/kg), compared to the control group, the proportion of epididymal sperms that were alive was significantly reduced (50% ± 3.4, 45% ± 5.5 and, 30%± 2.21 respectively) (p<0.05). There was no significant difference between control and placebo groups.

DISCUSSION

An organophosphate chemical called diazinon is often used in domestic and agricultural settings. It has detrimental impacts on the health of living things (14). Pesticide residues in the environment and food items are a concerning indicator that require scientific evaluation at the preclinical and clinical levels (15). Recently, there has been a sharp decline in semen quality. By altering the DNA or proteins bound, DZN can harm testis cells and cause mutations in spermatogonia, which ultimately result in alterations in the sperm. The present study investigated the induced testicular toxicity by DZN on the male adult rat (16). For this purpose, rats were treated with DZN (5, 10 and 20 mg/kg) for 30 days. The results of the current investigation show a considerable decrease in the number and motility of sperm in the rats exposed to diazinon in group 3 (20mg/kg). However, the effect of diazinon on different groups depended on the received dose but a decrease in sperm count was observed in all three groups. Among the 5rat receiving the 5 mg/kg of DZN, sperm count and motility were slightly reduced in all of them. Among the rat receiving a dose of 20 mg/kg of DZN, 3 of them had a significant decrease in sperm count. All assessed motility metrics show a considerable reduction in CASA-based sperm motion analysis in group 3. Also, compared to the control group, sperm movement was reduced in the first and second groups (5, 10 mg/kg). But the highest number of immobile sperms was seen in the group 3 at the dose of 20 mg/kg. Also, our research showed that the weight of the testicles in 3 out of 5 rats receiving a dose of 20 mg of DZN was greatly reduced, while in the other 2 rats, a slight change was observed. The weight of the testicles in the groups receiving the dose of 5 and 10 mg/kg of DZN was associated with a small change.

Table 2. Sperm parameters after being treated with different dose of diazinon toxin.

Parameters	Control	Placebo	Group 1 (5 mg/kg)	Group 2 (10 mg/kg)	Group 3 (20 mg/kg)
Volume (Ml)	80.125 ± 7.73	79.791 ± 7.34	49.25 ± 19.22 *	42.5 ± 10.75 *	35.87 ± 6.08 **
PR (%)	40.34 ± 6.15	35 ± 8.23	22.2 ± 4.29 *	20 ± 5.78 *	15 ± 7.34 **
NP (%)	15.44 ± 7.38	14.58 ± 6.64	13.58 ± 3.96	11 ± 5.64 *	10 ± 5.6 **
IM (%)	30.56 ± 4.15	37.59 ± 8.76	44.62 ± 10.37 *	50 ± 7.5	59 ± 4.78 **
M (%)	57.10 ± 5.15	59.31 ± 10.76***	51 ± 9.26 *	47.62 ± 7.89	41.5 ± 7.9**

PR, progressive, NP, non-progressive, IM, immotile, M, motile. Data represent as mean ± SD * p<0.05.

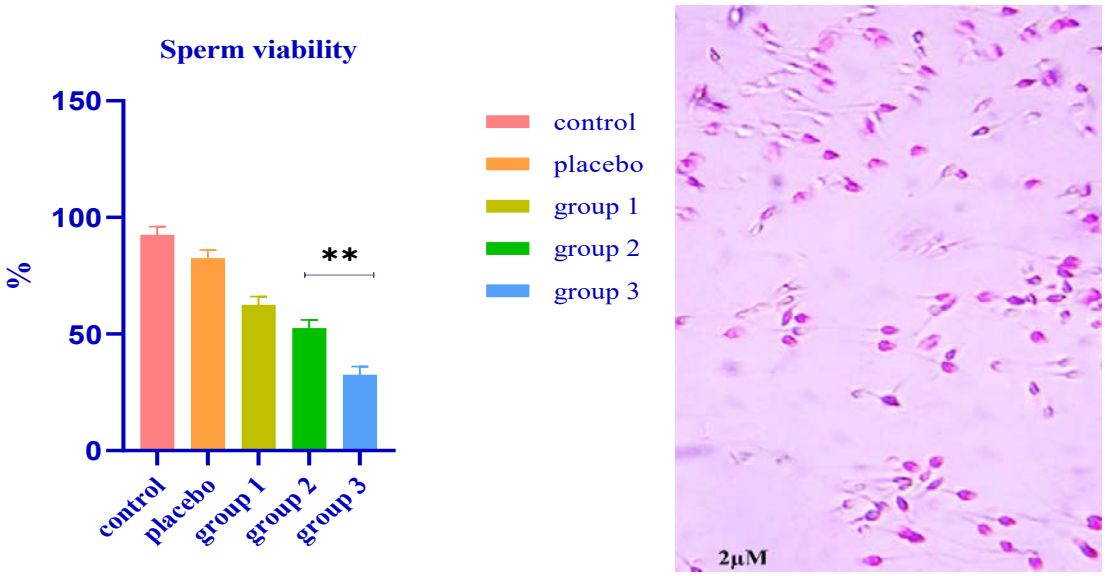

Figure 1. Assessment of sperm viability by eosin-nigrosin staining. The results showed a significant decrease of sperm viability in the group 3 (treatment with experimental dose of 20 mg/kg diazinon toxin). Live sperm look white, whereas only dead sperm are stained with eosin, making them a dark pink color.

Our results show that the effects of DZN on testis weight, sperm count and motility may be different among different rats.

Additionally, a higher percentage of sperm with pink heads and a higher percentage of dead sperm dramatically increased in diazinon administration groups. Our results showed that the highest amount of dead sperms was in the group receiving a dose of 20 mg/kg of DZN. Also, our microscopic evaluation showed that among 5 mice receiving 20 mg/kg dose of DZN, 3 of them had higher percentage of dead sperms. These results concur with those from human and animal research studies that have been published. In this investigation, rats given oral doses of 5, 10, and 20 mg/kg diazinon for 30 days showed decreased sperm concentration and motility, decreased testicular weight, and an increase in dead sperm. Numerous processes might account for the decreased sperm quality brought on by DZN (17). Reduced sperm quantity, movement, and shape, as well as other reproductive abnormalities, may be caused by the modification in sperm parameters, which has a direct impact on testicular tissue (18). According to certain research, DZN can cause biochemical abnormalities in the ovaries and testes as well as morphological and functional alterations (19). Based on particular research, a low testicular Leydig cell count is linked to low testosterone synthesis, which might lead to spermatogenic deficits (20). Our findings show that sublethal doses of diazinon administration can negatively affect reproductive function by reducing testicular bulk and blood levels of testosterone while raising circulating gonadotropin concentrations.

The loss of all types of seminiferous tubules and spermatogenesis may be caused by diazinon therapy, according to several histological investigations conducted on animals (21). Since maintaining LH serum levels is crucial for starting and supporting spermatogenesis, excessive levels of circulating LH may contribute to Sertoli cell and germinal cell degeneration (22). Among the different groups, serum LH and FSH levels were associated with a significant increase in the group treated with a dose of 20 mg/kg of DZN. Serum LH and FSH levels were increased in 4 out of 5 rats in this group as expected. Also, serum testosterone among different mice of this group was reduced with a small difference. Studies demonstrate that OP disrupts the epithelium's microtubules, which ultimately results in tubular atrophy. Additionally, interruption of spermatogenesis may result from elevated serum LH levels, which are harmful to germinal cells (19). Also, the fact that severe gonadotoxicity might be observed with extended exposure to DZN was demonstrated by a decrease in testis weight. OPs have a history of disrupting reproduction. In rats exposed to OP pesticides, broken sperms, cytoplasmic vesicles, and impaired sperm movement are the main indicators of lower quality (23). Reduced sperm counts were discovered in Chinese pesticide manufacturing employees by Pedungtod et al. in 2007. According to observations, OPs can cause oxidative stress by changing the activity of the free radical scavenging enzymes (24). The toxicity of different pesticides may be influenced by the reactive oxygen species (ROS) that OP causes. The damaging effects of ROS on reproductive tissue may increase as a result of decreased antioxidant effectiveness. DZN administration increased lipid peroxidation (LPO)

in rat erythrocytes, as demonstrated by Sutcu et al. A causal connection is thought to exist between the high levels of polyunsaturated fatty acids (PUFA) found in spermatozoa's plasma membranes and the low levels of scavenging antioxidants found in their cytoplasm. As a result, it is speculated that one of these pathways that warrants further research may be the oxidative damage caused by DZN.

CONCLUSION

Diazinon has significantly impacted the reproductive system in male rat, in conclusion. The current findings show that DZN exposure directly affects rat testicles, and an imbalance in the levels of circulating testosterone and gonadotropins may lower fertility. Couples trying to get pregnant should get individualized advice addressing any potentially detrimental occupational exposures, notwithstanding the fact that further study is needed to explain and enhance this finding in relation to human reproduction. Medical research still has opportunity for advancement in the area of male infertility. In order to obtain the best diagnostic and therapeutic advancements for each individual, personalized medicine and its possible focused treatment of male infertility were introduced in this study.

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