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EFFECT OF DERMAL EXPOSURE OF PREGNANT RATS TO DIAZINON ON THE TESTIS OF MALE OFFSPRING

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ABSTRACT

Diazinon is an insecticide that belongs to a group of chemicals known as organophosphates. It is used in agriculture to control insects on fruit, vegetable, nut and field crops, as well as it is used to make ear tags for cattle. The pure chemical (100% Diazinon) is colorless and practically odorless oil. Diazinon may enter the environment during the manufacturing process, but most environmental contamination comes from agricultural and household application of the chemical to control insects. Diazinon is often sprayed on crops and plants, so small particles of the chemical may be carried away from the field or yard before falling to the ground. **Aim of the study:** To study the histological changes that may occur in offspring rat testis resulting from dermal exposure of the pregnant rats to diazinon solution throughout pregnancy. **Material and method:** The study was performed on 30 pregnant albino rats (Rattus rattus norvegicus albinus). They were divided into two groups (15 in each). The first group was daily immersed in distilled water for 4 minutes throughout their pregnancy until giving birth and their male offspring were considered as control group. The second group was immersed in diazinon solution for the same period as done in the first group and their offspring was considered as experimental group. After birth the male off spring were sacrificed and the testes were taken for histopathological examination. Diameter of seminiferous Tubules and interstitial space were measured in both groups and comparison between the two results was done. **Results:** Significant ($P<0.05$) difference was noticed between the result of seminiferous tubules histological parameters of the experimental and control group as well as significant ($P<0.05$) was found between experimental and control

group. **Conclusion:** An exposure of pregnant rats to diazinon will affect the testicular development of the male offspring.

KEYWORDS: Diazinon, insecticides, organophosphorous, testes.

INTRODUCTION

Diazinon is an organophosphorus insecticide extensively used in agriculture.[1] It is an organophosphate insecticide, chemically related to other common insecticides like malathion and chlorpyrifos.[2] It was first registered in the U.S. in 1956[3] and is sold under a variety of brand names, including DZN[4] and Knox Out 2FM.[5]

The Chemical Abstracts Service (CAS) name for diazinon is O, O-diethyl O-[6- methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate and the International Union of Pure and Applied Chemistry (IUPAC) name is O, O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate.[6] Pure diazinon is acolorless oil. Technical grade diazinon (>90% active ingredient) is an amber to brown liquid.[7] Diazinon is water soluble in a concentration of 0.04 g/L at 20 °C and 30 °C, however other values between 0.054 and 0.069 g/L have been reported in the 20-40 °C temperature range. Diazinon is completely miscible in acetone, benzene, ethanol, toluene, xylene and is soluble in petroleum oils.[8,9,10,11]

Inside living things, diazinon is transformed into a molecule called diazoxon. Diazinon and the more potent diazoxon kill insects by interfering with nervous system function, as do all members of the organophosphate chemical family,[12] Organophosphates attach to acetylcholinesterase (AChE) and prevent it from destroying acetylcholine which responsible for transmitting nerve impuls, causing overstimulation of the nerves.[13]

The general population may be exposed via diazinon-contaminated air, water, or food, but there is little potential for high level exposure because home and garden uses for diazinon have been banned. Significant dermal exposure is likely only near areas where diazinon may be used as a restricted pesticide.[14] Diazinon is low in toxicity following dermal exposure. The acute dermal LD50 is greater than 2020 mg/kg.[15] Researchers found about 2-4% of the applied dose were absorbed by the skin regardless of the site of application or the vehicle used to administer the diazinon.[16]

Farm residents may be exposed to several types of pesticides from various chemical families (e.g., phenoxy acetic acids, triazines, carbamates, and organophosphates) during the course of

a growing season. Several studies have reported positive associations between occupational pesticide exposure and fetal death (spontaneous abortion or stillbirth).[17, 18,19] However, little is known about the human reproductive toxicity of specific pesticide active ingredients and even less about mixtures of pesticides and how they may interact with other risk factors.

In addition to the nature of the chemical and its target, the consequences of exposure to chemical agents depend on the timing of exposure relative to critical windows in development of the fetus or reproductive system.[20, 21] Previous analyses had also discussed the role\ of male pesticide exposure on pregnancy outcomes[22] and time to pregnancy.[23] Other studies have reported that parental exposure to pesticides or application of pesticides in the home is associated with certain birth defects including neural tube and other birth defects.[24]

Studies of the effects of pesticide exposure on children's health have been limited to those of birth defects, childhood cancer, and acute poisonings following ingestion. Several case-control studies have associated parental exposure to pesticides or pesticide use in the home with childhood brain tumors, leukemias and lymphomas, testicular cancers, and other cancers.[25]

MATERIALS AND METHOD

Animals used in the present study were 30 healthy pregnant albino rats (Rattus rattus norvegicus albinus), of approximately 60-80 days age and 20-25 gm weight. The animals were taken from the animal house of the High Institute for Infertility Diagnosis & Assisted Reproductive Technology/ Al Nahrain University. They were maintained under uniform conditions of natural photo period (12 hours light/ dark cycle), and temperature (24-32 C$^{\circ}$). Animals had free access to food and water. These females were divided in to two groups treated group (n=15) and control group (n=15).

The rats of the control group were hold from their ear by forceps and immersed in distilled water for 4 minutes beginning from their tails. In a same manner the experimental group members were immersed in diazinon solution with a concentration of 0.6 mg of diazinon dissolved in 1 liter of distilled water for the same period of time. This procedure was done daily from beginning of pregnancy until they give birth.

Thirty newborn male rats were separated (fifteen from each group), they were sacrificed by

cervical dislocation; incision of rat abdomen was done, the testis were quickly excised and immersed in few drops of normal saline which was placed in Petri dish to be cleared from surrounding adipose tissue under dissecting microscope using fine surgical scissors, then fixedf in 10% formalin solution.

Histopathological examinations

At the time of tissue collection, the organs were blotted dry from normal saline using filter paper and then weighed with electrical balance, while both right and left testes were fixed with 10% formal saline for a subsequent histological study. For each testis, serial sections (5 µm thick) were made. These sections were stained by hematoxylin-eosin (H & E) and morphometrically examined by light microscopy. For morphometric analysis, the slides images were captured by using Microns Contain TV-Based computer assisted morphometry with a 4X and 10X objectives. The actual measurements were done by using the image analyzer software after accurate calibration using a stage micrometer.

Statistical analysis

Data were represented as means± S.E. The differences were compared for statistical significance by one way ANOVA test. Differences was considered significant at $p<0.05$.

RESULTS

All the pregnant female rats used in this study survived till birth. Many of the offspring obtained from these animals died in the first few days after birth. The animals survived were used for this morphometrical and histological study.

1- Measurement of Diameter of seminiferous tubules (µm) (mean±SD).

The results of this study showed significant ($P<0.05$) difference in the diameter of seminiferous tubules of the experimental group compared to control group as shown in table (1).

Parameter	Control group	Experimental group
diameter of seminiferous tubules (µm) (mean±SD)	**218.51± 16.55**	**182.11+14.24 ***

*Significant ($P<0.05$) difference.

2- Measurement of interstitial space diameter (µm) (mean±SD)

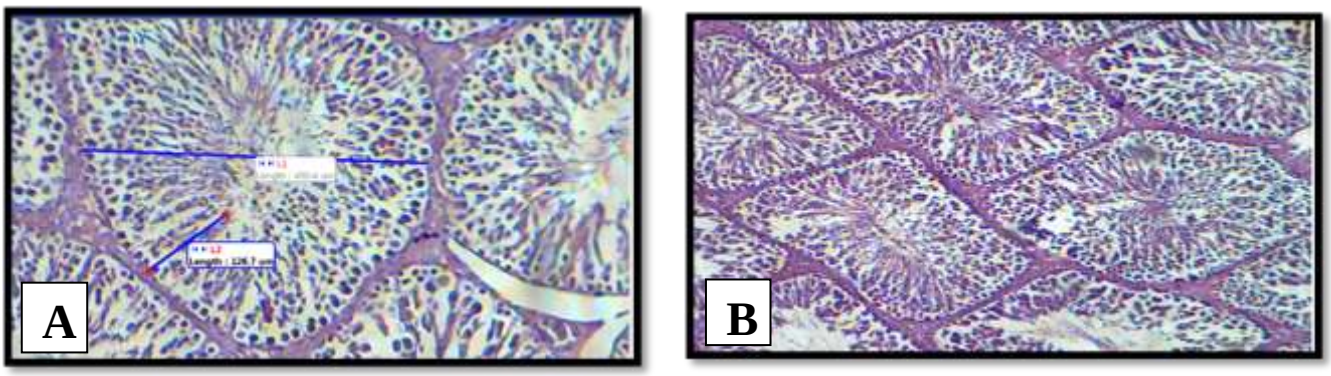
Measurement of interstitial space diameter showed significant (P<0.05) difference between control and experimental groups as shown in table (2).

Parameter	Control group	Experimental group
Interstitial space (µm) (mean±SD)	19.81±2.15	43.09+6.16 *

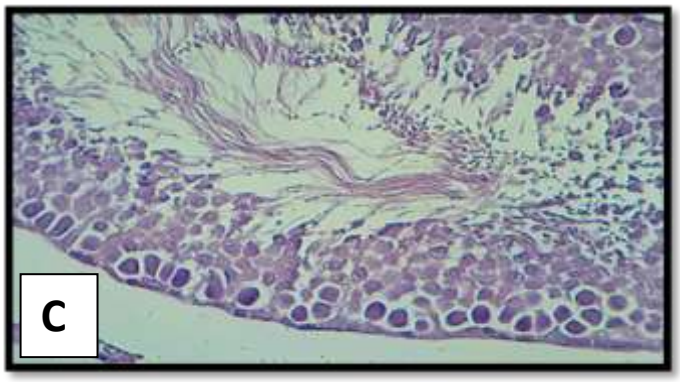
*Significant (P<0.05) difference

3- Histological Observations

The testicular sections of the control group showed seminiferous tubules with normal germ cell population layer thickness with a normal orderly arranged pattern up to mature spermatid. No, malignant or abnormal cell was seen within the germinal epithelium, also no vacuoles is present in the tubules. There were adequate Sertoli cells populations as shown in figure (1)



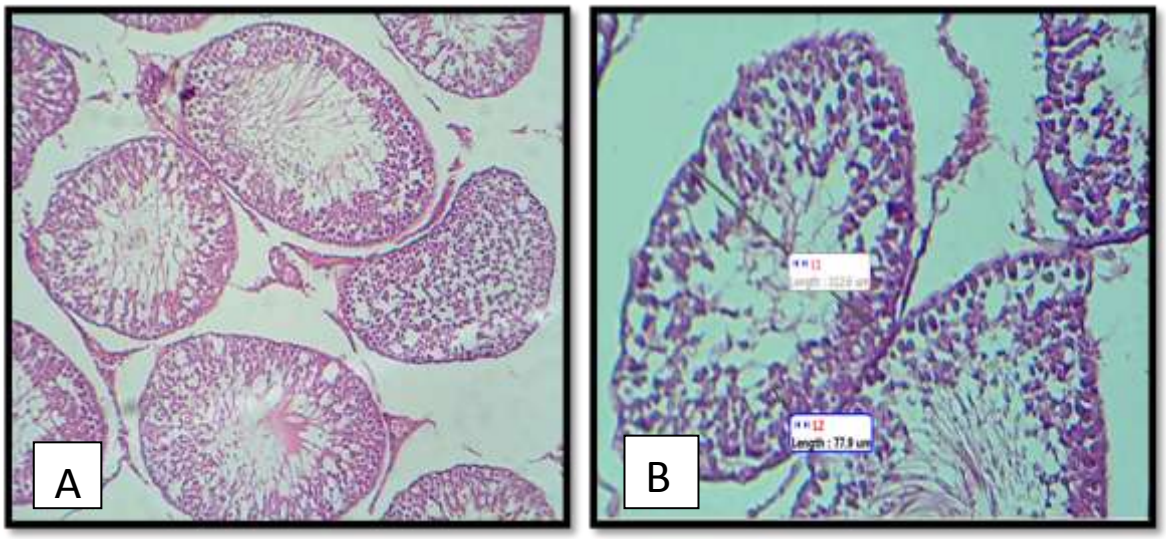
A: (H&E, 10 x) **B: (H&E, 10 x)**



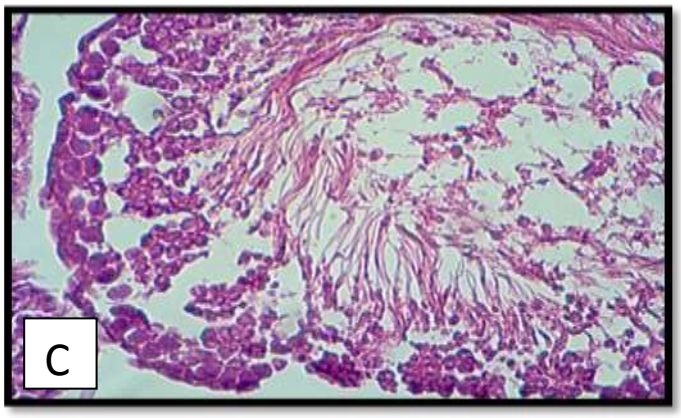
C- (H&E, 40 x)

Figure (1):[A, B & C] Photomicrograph of testes of rat: (control group) showing normal structure of seminiferous tubules. (DST- Diameter of Seminiferous tubules, IS- Interstitial space), (H&E, 10 x & 40x).

In experimental group, sections showed different degrees of degenerative changes in testes were observed. There was a decrease in thickness of germ cell layer, widening of the central seminiferous tubules lumen and prominent germ cell population necrosis. Multiple vacuoles were seen within the tubules. Sertoli cells were abnormal in number and shape as compared to control group. A peritubular fibrotic change had been seen also in the testicular sections. The results also showed a highly significant decrease in diameters of seminiferous tubules, primary spermatocytes, spermatids and increase in interstitial space when exposed to diazinon solution compared with control group as shown in figure (2 a ,b & c).



A: (H&E, 10 x) **B: (H&E, 10 x)**



C :(H&E, 40 x)

Figure (2): Photomicrograph of testes of rat: A, B& C: (experimental group) showing changes in the structure of seminiferous tubules. (DST- Diameter of Seminiferous tubules, IS- Interstitial space), (H&E, 10x) & (H&E, 40x).

DISCUSSION

There is widespread concern that reproductive function is currently subject to the deleterious effects of chemical agents in the environment. According to the endocrine disruption hypothesis, exposure to chemicals, including pesticides, results in a range of reproductive disturbances in humans and in various wildlife species, some of which may be used as sentinel species indicating an increase in the threat to human reproduction and health.[26]

Some studies have showed that DZN was capable of inducing structural and functional changes[27] and some biochemical alterations in the ovaries and testes[28], delays in sexual development, stillbirths, death of newborn offspring, and birth defects.[29]

The consequences of exposure to chemical agents depend on the nature of the chemical and its target, the timing of exposure in relation to the critical windows in development of the fetus or reproductive system.[30]

On the other hand, an association between late abortions and postconception exposure may suggest that postconception exposure to specific pesticides tends to damage the fetus or fetus–placenta complex rather than cause chromosomal anomalies.[31]

In the present study the results showed that the exposure of pregnant rats to diazinon caused significant difference in the diameter of seminiferous tubules and interstitial space. These results may be caused by altering the activity and reactivity of the adenylyl cyclase signaling cascade, which would disrupt cell development in all areas of the body, not only those cholinergically regulated.[32]

The histological sections of testes showed degenerative changes of the testicular tissue. The above mentioned effects of organophosphorus pesticides could be due to their ability to form free radicals.[33] The results of previous work on Methidathion showed that the using of antioxidants vitamins A, C, and E, reduces the toxicity of Methidathion.[34]

REFERENCES

1. Alahyary P. Ilkhani M. and Fathy F. The potential toxicity of Diazinon on physiological factors in male rabbits. Pak. J. Biol. Sci., 2008; 11(1): 127-130.
2. Reigart, J.R. and J.R. Roberts. 1999. Recognition and management of pesticide poisoning. Fifth edition. Washington, D.C. U. S. EPA., 1999; 34-38.

3. U.S. EPA. Office of Pesticides and Toxic Substances. Guidance for the registration of pesticide products containing diazinon as the active ingredient. Washington, DC, Dec. 1988; 6.
4. Novartis Crop Protection, Inc. DZN Diazinon 50W material safety data sheet. Greensboro NC, Feb. 1999; 24. www.cdms.net.
5. Elf Atochem North America, Inc. Knox out 2FM material safety data sheet. Philadelphia PA, Mar. 2000; 22. www.cdms.net.
6. Tomlin, C. D. S. The Pesticide Manual: A World Compendium, 14th ed.; British Crop Protection Council: Hampshire, UK, 2006.
7. Reregistration Eligibility Decision (RED) Diazinon; EPA 738-R-04-006; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, 2006.
8. Howard, P. E. Handbook of Environmental Fate and Exposure Data for Organic Chemicals; Lewis Publishers, Inc.: Chelsea, MI, 1991; Vol. III: 209-221.
9. Hornsby, A. G.; Wauchope, R. D.; Herner A.E. Pesticide Properties in the Environment; Springer-Verlag: New York, 1996; 83-84.
10. Risks of Diazinon Use to the Federally Listed Endangered Barton Springs Salamander (Eurycea sosorum); U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, 2007.
11. Ney, R. E. Fate and Transport of Organic Chemicals in the Environment, 2nd ed.; Government Institutes, Inc.: Rockville, MD, 1995; 9-24.
12. U.S. Dept. of Health and Human Services. Public Health Service. Agency for Toxic Substances and Disease Registry. Toxicological profile for diazinon. Atlanta, GA, Aug. 1996; 82.
13. Ware, G.W. The pesticide book. Fresno, CA: Thomson Publications, 2000; 181.
14. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Diazinon. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services., 2008.
15. Diazinon: Revised HED Human Health Risk Assessment for the Reregistration Eligibility Decision (RED); U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, 2000.

16. Draft Toxicological Profile for Diazinon; U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry: Atlanta, 2006.
17. Taha TE, Gray RH. Agricultural pesticide exposure and peri-natal mortality in central Sudan. Bull WHO., 1993; 71: 317–321.
18. Restrepo M, Muñoz N, Day NE, Parra JE, de Romero L, Nguyen-Dinh X. Prevalence of adverse reproductive outcomes in a population occupationally exposed to pesticides in Colombia. Scand J Work Environ Health., 1990; 16: 232–238.
19. Pastore LM, Hertz-Picciotto I, Beaumont JJ. Risk of stillbirth from occupational and residential exposures. Occup Environ Med., 1997; 54: 511–518.
20. Pryor JL, Hughes C, Foster W, Hales BF, Robaire B. Critical windows of exposure for children's health: the reproductive system in animals and humans. Environ Health Perspect., 2000; 108(3): 491–503.
21. Lemasters GK, Perreault SD, Hales BF, Hatch M, Hirshfield AN, Hughes CL, Kimmel GL, Lamb JC, Pryor JL, Rubin C, et al. Workshop to identify critical windows ofexposure for children's health: reproductive health in children and adolescents work group summary. Environ Health Perspect., 2000; 108(3): 505–509.
22. Savitz DA, Arbuckle T, Kaczor D, Curtis KM. Male pesticide exposure and pregnancy outcome. Am J Epidemiol., 1997; 146: 1025–1036.
23. Curtis KM, Savitz DA, Weinberg CR, Arbuckle TE. The effect of pesticide exposure on time to pregnancy. Epidemiology., 1999; 10: 112–117.
24. Shaw GM, Wasserman CR, O'Malley CD. Maternal pesticide exposures as risk factors for orofacial clefts and neural tube defects. Am J Epidemiol., 1995; 141(11): S3.
25. Buckley JD, Robison LL, Swotinsky R, Garabrant DH, LeBeau M, Manchester P, Nesbit ME, Odom L, Peters JM, Woods WG. Occupational exposures of parents of children with acute nonlymphocytic leukemia: a report from the Childrens Cancer Study Group. Cancer Res., 1989; 49: 4030-4037.
26. Toppari J, Larsen J, Christiansen P, Giwercman A, Grandjean P, Guillette L, Jégou B, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer O, Müller J, Rajpert-De Meyts E, Scheike T, Sharpe R, Sumpter J, Skakkebaek NE: Male reproductive health and environmental xenoestrogens. Environ Health Perspect., 1996; 104: 741-803.
27. Wesseling C, Antich D, Hogstedt C, Rodríguez AC, Ahlbom A. Geographical differences of cancer incidence in Costa Rica in relation to environmental and occupational pesticide exposure. Int J Epidemiol., 1999; 28: 365-374.

28. Dutta HM, Meijer HJ. Sublethal effects of diazinon on the structure of the testis of bluegill, Lepomis macrochirus: amicroscopic analysis. Enviromental pollution., 2003; 125: 355-360.
29. Caroline Cox, Insecticide facts sheet Diazinon. Toxicology- Journal of Pesticide Reform/ summer., 2000; 20(2).
30. Pryor JL, Hughes C, Foster W, Hales BF, Robaire B. Critical windows of exposure for children's health: the reproductive system in animals and humans. Environ Health Perspect., 2000; 108(3): 491–503.
31. Tye E. Arbuckle, Zhiqiu Lin, and Leslie S. Mery An Exploratory Analysis of the Effect of Pesticide Exposure on the Risk of Spontaneous Abortion in an Ontario Farm Population- Children's health articles. Perspect., 2001; 109: 851–857. http://ehpnet1.niehs.nih.gov/docs/2001/109p851-857arbuckle/abstract.html
32. Song X, Seidler F, Saleh J, Zhang J, and Padilla S, Slotkin T. Cellular mechanisms for developmental toxicity of chlorpyrifos: targeting the adenylyl cyclase signaling cascade. Toxicol AppI Pharmacol., 1997; 145: 158-174.
33. Vidyasagar J. Karunakar N. Reddy M.S. Rajnarayana K.Surender T.and Krishna D. Oxidative stress and antioxidant status in acute organophosphorus insecticide poisoning. indi. J. Pharmacol., 2004; 36: 76-79.
34. Elias M.A. and Saif M.A. The protection effect of vitamins A, C, and E, against the potential toxicity of Methidathion on blood factors in male rabbits. Yem. J. Biol. Sci. 2009; 5(1): 133-136.