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STRUCTURAL CHANGES IN THE RAT TESTES CAUSED BY DIAZINON ADMINISTRATION

MODIFICĂRI STRUCTURALE ÎN TESTICULELE DE ŞOBOLAN INDUSE DE ADMINISTRAREA DIAZINONULUI

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The aim of this study was to find the structural changes in the rat testis after a diazinon administration. The testis structure changes after the diazinon administration of 20 mg/kg b.w. (i.p.) and daily dose of 40 mg/l in drinking water (p.o.) were evaluated in light microscopy. 36 hours after the diazion i.p. administration, the vacuolization of the seminiferous epithelium, evacuation of germ cells into the tubule lumen, epithelium necrosis and interstitium extension were observed. After 90 days of daily oral administration, the changes in the testis were not as visible as in the acute intoxication. The germ cells released from the basal lamina and then were visible in the tubule lumen. Diazinon causes the damage of the germinal epithelium in the testes leading to the spermatogenesis failure. The infertility can then appear.

Keywords: diazinon, testis, histology, morphometry, rat

Introduction

Diazinon is a nonsystemic organophosphate insecticide which has been used since 1956 for the control of soil insects and pests, on ornamental plants, and on fruit and vegetable field crops. Now it is used to control flies around animal facilities, greenhouses, fairgrounds and other businesses and public places where food or animal wastes might accumulate (Dikshith and Diwan, 2003).

Diazinon affects mainly the nervous system regardless of the route of exposure. Some mild signs and symptoms of poisoning include headache, dizziness, weakness, feelings of anxiety, constriction of the pupils, and blurred vision. More severe symptoms include nausea and vomiting, abdominal cramps, slow pulse, diarrhea, pinpoint pupils, difficulty breathing, coma, and possibly death. These effects also occur in animals exposed to high doses of diazinon. There is no evidence that long-term exposure to low levels of diazinon causes harmful effects in people. Diazinon has not been shown to affect fertility in humans (ATSDR, 2008). Limited information is available regarding the reproductive

toxicity of diazinon in orally-exposed laboratory animals. Pina-Guzmán et al. (2005) revealed the high sensitivity of spermatozoa exposed during the late steps of maturation to diazinon. Oral administration of diazinon to male albino rats at dose levels of 1.5 or 3 mg/kg/day for 65 days resulted in significantly decreased reproductive tissue weights, increased percentage of dead and morphologically abnormal spermatozoa, decreased plasma testosterone levels, and decreased fertility as assessed by conception rates of untreated females mated to diazinon-treated males (Abd El-Aziz et al., 1994). Structural changes of the testis of bluegill, *Lepomis macrochirus*, have also been described after the diazinon exposure (Dutta and Meijer, 2003).

The aim of the study was to describe the structural changes of the rat testes after an intreperitoneal and long-term peroral diazinon administration.

Materials and Methods

Thirty males Wistar rats were divided to three groups, diazinon-treated groups (A and B) and control group (C), each containing 10 males. The males were housed individually in plastic cages. The animals had unlimited access to drinking water and feed. Sexually mature male rats of group A were administered a single intraperitoneal dose (20 mg/kg b.w.) of diazinon (Sigma-Aldrich, USA, purity 99%) and young 4 weeks old rats of group B were dosed with a daily diazinon dose of 40 mg/l in drinking water for 90 days. The testis were sampled 36 hours after the diazinon administration (group A) and 90 days after the daily diazinon intake (group B). The samples were weighed, fixed in modified Davidson's solution (Latendresse et al., 2002), stained with hematoxylin-eosine and examined in light microscope Olympus AX 70 Provis (Japan).

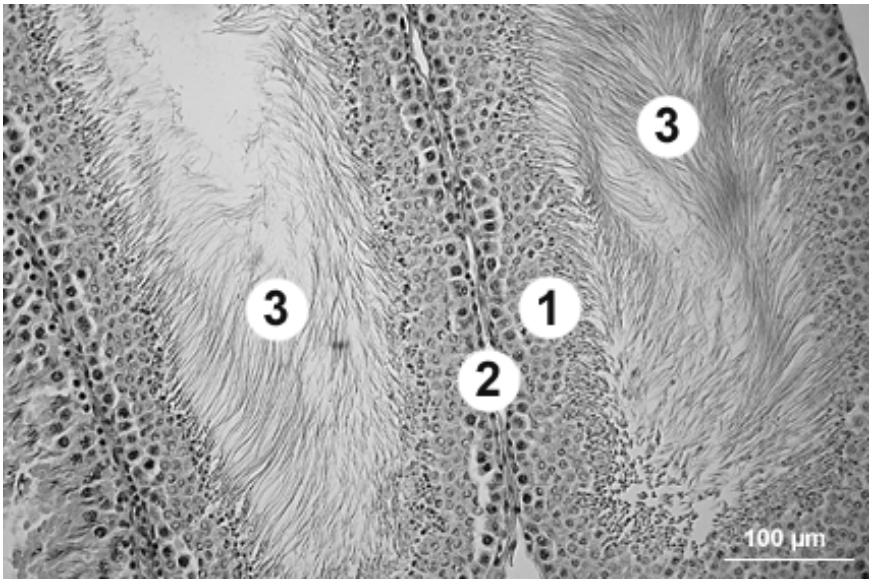
Results and Discussion

The average testis absolute weight did not significantly changed in the diazinon-exposed groups (1.62 ± 0.10 g, group A and 1.42 ± 0.20 g, group B) when compared with that of the control (1.46 ± 0.25 g). Abd El-Aziz et al. (1994) found decrease in the reproductive organs weight after the diazinon dose of 1.5 or 3.0 mg/kg during 65 days. Similar effects have described also El-Hoda and Zidan (2009) in the testis and vesicular gland after the p.o. administration of 50 mg diazinon/kg during 65 days.

Normal testis appearance shows Picture 1. The structure of testis 36 hours after the diazinon intraperitoneal administration looked disrupted. The seminiferous tubules underwent the atrophy and necrosis took place (Pic. 2). The germ cells lost their contact with the basal lamina. Also deluminization of the tubules was seen in the testes. The shrunken tubules were packed with the destroyed cell mixture. The dilation of an interstitial tissue was also observed. Similarly, the testicular atrophy with completely arrested spermatogenesis was observed in male dog of the 10 mg/kg/day group that lost weight and evidenced

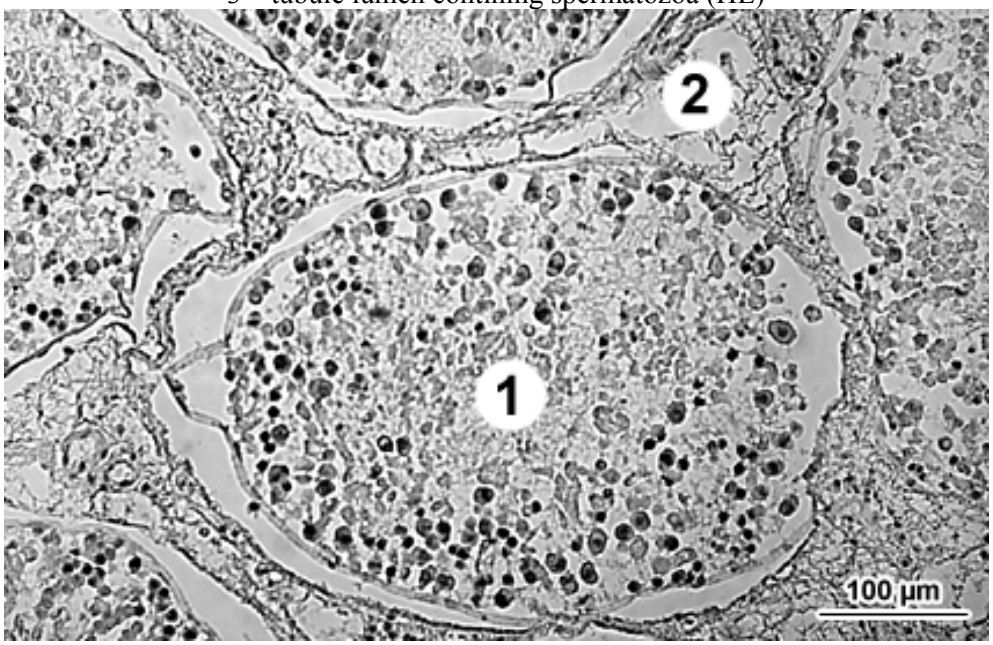
other gross pathological changes. Male Beagle dogs were given daily capsules containing diazinon in corn oil at doses ranging from 2.5 to 20 mg/kg/day for 8 months. Testicular atrophy with completely arrested spermatogenesis was observed in the one male dog of the 10 mg/kg/day group. All three male dogs in the 20 mg/kg/day group suffered similar effects, testicular atrophy observed in 2/3, arrested spermatogenesis observed in the other dog (Earl et al., 1971). Pina-Guzmán et al. (2005) have observed the spermatozoa damage after a single intreperitoneal diazinon administration in mice (8.12 mg/kg). Authors stated that the maturing spermatozoa are possible targets place of diazinon effect in the testis.

In the group of the peroral administration of diazinon, the change were milder than that of the acute exposed rats. In the testes, there were the tubules with continuous spermatogenesis but also degenerative changes appeared. Some of the tubules contained the necrotized epithelium (Pic. 3). On the other hand, no gross or histological evidence of treatment-related damage to reproductive tissues (epididymides, seminal vesicles, testes) was observed in Sprague-Dawley rats exposed to up to 168 mg/kg/day diazinon for 13 weeks via feed (Singh, 1988), or up to 10 mg/kg/day for 98 weeks (Kirchner et al., 1991), or in Beagle dogs exposed to up to 10.9 mg/kg/day for 13 weeks (Barnes, 1988). However, Swan et al. (2003) have noted that diazinon is one of the most important insecticides along with herbicides like alachlor and atrazine that is associated with decreased semen quality in men. Structural changes in the bluegill testis were also described. The changes in the seminiferous tubule diameter, germ cells diameter and connective tissue have been found depending on the time of exposure. The reduction in the tubule, tubule lumen and spermatozoa diameter were highly significant after 2 weeks of exposure to diazinon (Dutta and Meijer, 2003).

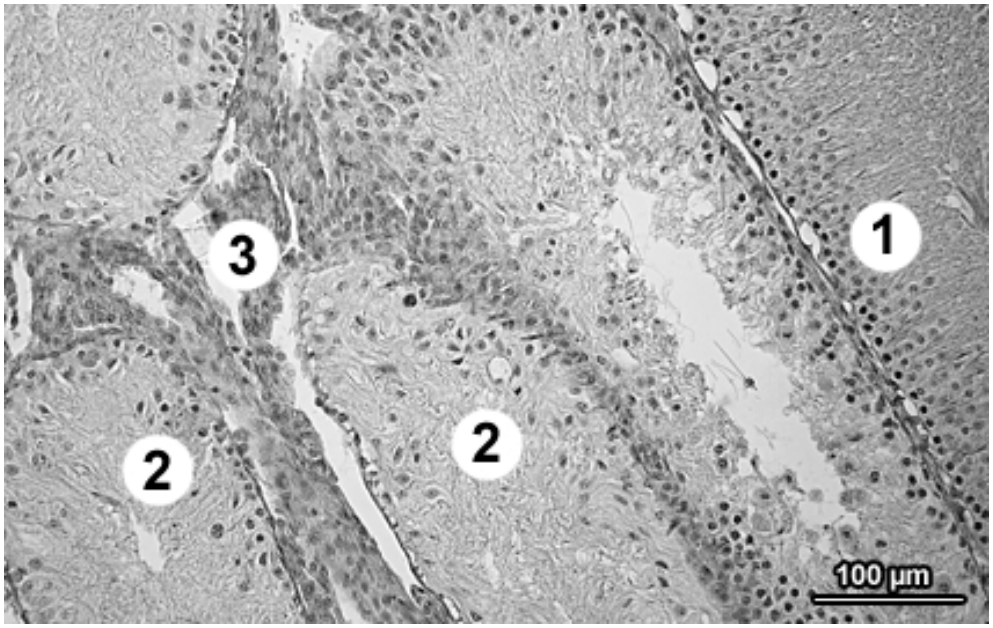


Picture 1 Control rat testis

1 - seminiferous epithelium with continuous spermatogenesis, 2 - interstitium, 3 – tubule lumen contining spermatozoa (HE)



Picture 2 Rat testis 36 hours after the intraperitoneal diazinon administration 1 – shrunken atrophied tubule, 2 – dilated interstitium (HE)



Picture 3 Rat testis after a 90 days of diazinon administration in drinking water 1 – normal seminiferous epithelium, 2 – necrotized epithelium, 3 - interstitium

Conclusions

The results show the significant acute effect of diazinon administration on the testicular structure. The insecticide is able to stop the spermatogenesis and necrotic tissue can be seen in the testis. It is important finding, that also peroral administration of low diazinon doses can damage the spermatogenesis and probably can lead to the fertility failure.

References

1. Abd El-Aziz, M.I., Sahlab, A.M., Abd El-Khalik, M., 1994, Influence of diazinon and deltamethrine on reproductive organs and fertility of male rats, *Dtsch Tierarztl Wochenschr* **101**, 230-232.

2. ATSDR, 2008, Toxicological profile for diazinon, ATSDR, USA, 298 pg.

3. Barnes, T.B., 1988, *90-Day oral toxicity study in dogs*. EPA guidelines no. 82-1. Laboratory study number 882012. *Ciba-Geigy Corporation. U.S. Environmental Protection Agency.*MRID40815004.

4. Dikshith, T.S.S., Diwan, P.V., 2003, Industrial guide to chemical and drug safety, *A Wiley-IEEE*. 629 pg.

5. Dutta, H.M., Meijer, H.J., 2003, Sublethal effects of diazinon on the structure of the testis of bluegill, Lepomis macrochirus: a microscopic analysis, *Environ Pollut* **125**, 355-360.

6. Earl, F.L., Melveger, B.E., Reinwall, J.E. et al., 1971, Diazinon toxicity-comparative studies in dogs and miniature swine, *Toxicol Appl Pharmacol* **18**, 285-295.

7. El-Hoda, N., Zidan, A., 2009, Evaluation of the reproductive toxicity of chlorpyrifos methyl, diazinon and profenofos pesticides in male rat, *Int J Pharm* **5**, 51-57.

8. Kirchner, F.R., McCormick, G.C., Arthur, A.T., 1991, One/two year oral toxicity study in rats. *Ciba-Geigy Corporation. U.S. Environmental Protection Agency*. MRID41942002.

9. Latendresse, J.R., Warbrittion, A.R., Jonassen, H., Creasy, D.M., 2002, Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid, *Toxicol Pathol* **30**, 524-533.

10. Piña-Guzmán, B., Solís-Heredia, M.J., Quintanilla-Vega, B., 2005, Diazinon alters sperm chromatin structure in mice by phosphorylating nuclear protamines, *Toxicol Appl Pharmacol* **202**, 189-198.

11. Singh, A.R., 1988, *90-Day oral toxicity study in rats.* EPA guidelines no. 82-1. Laboratory study number 882011. *Ciba-Geigy Corporation. U.S. Environmental Protection Agency.* MRID40815003.

12. Swan, S.H., Kruse, R.L., Liu, F. et al., 2003, Semen quality in relation to biomarkers of pesticide exposure, *Environ Health Perspect* **111**, 1478-1484.