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Morphometric analysis of rat testes after intraperitoneal administration of single dose of cadmium and diazinon

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ABSTRACT

The present study was conducted to evaluate the reproductive risk associated with exposure of adult male rats to ubiquitous environmental toxicants cadmium and diazinon. A total of 40 Wistar rats were randomized into 4 groups of 10 animals each and intraperitoneally injected with physiological solution (control group), cadmium (2 mg/kg body wt), diazinon (20 mg/kg body wt) separately and in combination. 36 hours after administration, significant alterations in testis weight in diazinon and cadmium-diazinon animals while only slight decline in testicular weight in cadmium treated group were observed. The morphometric data supported histopathological disorders in seminiferous tubules resulting in tubular degeneration and spermatogenic damage with significant reduction in seminiferous epithelial volume and significant enlargement of interstitial volume in all experimental groups. A significantly diminished diameter of seminiferous tubules and significantly decreased tubule lumen filled with detached germ cells from disarranged epithelial layers confirmed tubule constrictions. Experimental exposure caused oedema and haemorrhage in interstitium as a consequence of blood vessels damage and dilatation. Results did not indicate synergistic or additional effect of simultaneous administration of cadmium and diazinon to the rat testis. Further research would be necessary to clarify the effects of cadmium-diazinon interactions on male reproduction health.

KEYWORDS: cadmium, diazinon, testicular histopathology, morphometry, rat

INTRODUCTION

Cadmium is one of the most toxic metals, with no known beneficial physiological role [1], occurring in the environment naturally and as a pollutant emanating from industrial and agricultural sources [2]. Cadmium is a contaminant found in most human foodstuffs, which renders diet a primary source of exposure among nonsmoking, non occupationally exposed populations [3]. Major accumulation occurs in various tissues and may produce serious effects and cause histological and biochemical alterations in organs such as the kidneys, liver, lungs, cardiovascular, immune and reproductive systems [2, 4]. Cadmium induction of reactive oxygen species disrupts the balance between free radicals and the cells antioxidant defense system leading to increased oxidative stress [5]. Numerous studies [6, 7, 8] have demonstrated that the testis is exceedingly sensitive to cadmium toxicity, and have also associated cadmium with recent declining male fertility [9].

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Diazinon is a thionophosphorus organophosphate pesticide (O,O-diethyl O-2-isopropyl-6-methyl-pyrimidin-4-yl phosphorothioate) entering the environment from agricultural and household application of the chemical to control pest insects. The ubiquitous nature of organophosphate pesticides leads to a significant organophosphate exposure involving multiple routes [10]. Despite its low persistence in the environment, it is a nonspecific insecticide and highly toxic to animals and humans [11]. Like other organophosphate pesticides, the main toxic action of diazinon is disruption of the cholinergic pathway in nerve transmissions by inhibition of acetylcholinesterase activity [12, 13, 14]. Besides neurotoxic effects, diazinon also induces oxidative stress and causes various pathological changes in tissues and organs [14, 15, 16]. In addition to these effects that may follow acute exposure to diazinon, there is an increasing interest in reproductive toxicity of organophosphates, particularly on the male reproductive system [17, 18, 19].

Humans and animals are exposed simultaneously to various environmental contaminants in air, water and food with potential effects on reproductive health [20]. In most natural situations, exposures do not involve only a single chemical but a multitude of agents. In these situations, chemicals that produce similar end results may act in a cumulative manner either synergistically or additively [21] or unrecognized interactions could result [14].

The weight and the volume of reproductive organs and histopathology of the testes are sensitive endpoints for detecting adverse effects of chemicals on male reproduction in animal species [22, 23]. Among animal models, rats are the preferred species for reproductive toxicity testing because of their convenient size and well-characterized reproductive processes [24].

Purpose of study

The aim of this study was to clarify the impacts of environmental contaminants, cadmium and diazinon on testicular structure in male adult Wistar rats. The evaluation was based on the macroscopic observations and analysis of histological sections using quantitative morphometric analysis.

MATERIALS AND METHODS

Animals

Forty mature, 4 months old male rats of the Wistar strain (weighing approximately 410 g) were randomly assigned into four groups of ten animals. The males were housed individually in plastic cages under constant temperature (20-22°C), humidity (55 ± 10%), and 12/12 h cycle of light and darkness with access to food (feed mixture M3, Machal, Czech Republic) and drinking water *ad libitum*. All experiments were conducted in accordance with accepted standards of animal care in accredited laboratory (SK PC 50004, SUA Nitra).

Experimental design

Rats of the group A were injected a single intraperitoneal dose (2 mg/kg body wt) of cadmium in the form of $CdCl_2$ (Reachem, Slovak Republic, purity 96%) in physiological solution, rats in the group B were injected with a single dose (20 mg/kg body wt) of diazinon (Sigma-Aldrich, USA, purity 99%) intraperitoneally in physiological solution, and rats in the C group were given a simultaneous dose of cadmium (2 mg/kg body wt) and diazinon (20 mg/kg body wt) intraperitoneally in physiological solution. The fourth group served as a control, received only physiological solution.

Tissue preparation and histopathological examinations

Animals were anaesthetized with ether and sacrificed 36 h following an experimental administration. In each case, right and left testes were immediately removed and weighed. Testes were fixed in modified Davidson's solution [25]. After processing, tissues were embedded in paraffin and cut into 5 µm sections and stained with haematoxylin-eosine for morphometric measurements.

Morphometric analysis

Testicular weight (g), mean seminiferous tubule diameter (µm), relative volume of testicular structures (seminiferous epithelium, intraepithelial empty spaces, tubule lumen, interstitial tissue, blood vessels) (%), and absolute volume of testicular structures (μm^3) were determined using

modified quantitative morphometric methods [26] with PC morphometric software M. I. S. Quick Photo and using light microscope Olympus AX 70 Provis (Japan).

Statistical analysis

Comparisons between the groups were assessed by one-way analysis of variance (ANOVA) and *post hoc* Scheffe test using the Statgraphics Centurion XV software. All values were presented as mean ± standard deviation, and statistically significant differences ($P<0.05$) and extremely significant differences ($P<0.01$; $P<0.001$).

RESULTS AND DISCUSSION

Macroscopically, the control testes appeared normal, pinkish in color, but after a cadmium administration, the testes were dark red looking, as compared with control. Disruption of the tight junctions in the microvessels led to the leakage of blood cells, most notably erythrocytes, into the interstitial space, causing haemorrhage and oedema [9]. No changes in macroscopic appearance were noted in the diazinon-exposed rats. No significant alterations in gross testicular morphology observed in the co-treatment treatment group seem to be due to the competitive effects of cadmium and diazinon with each other for entry through the vascular system, which is a critical target of cadmium toxicity [27]. Mean testicular weight of rats in diazinon treated group was found to be significantly higher ($P<0.01$) from that of the control (1.45 ± 0.25g) while in group C with cadmium and diazinon administration a significant testicular weight reduction occurred ($P<0.05$). The decrease in testicular weight appears to be related to reduced tubular size and decrease in the volume of seminiferous epithelium with suppressed spermatogenesis [28], whereas the observed increase in weight of testes could be attributed to the oedematous effects of organophosphates [29]. This observation is in contradiction with several other studies [17, 30] showing that testis weight depends on the route, dose and duration of exposure to organophosphates [23, 31]. An interesting finding was that the rat testicular weight from group injected only with cadmium showed no significant difference in comparison with control, although decreased testes mass associated with cadmium toxicity have been observed [9, 32, 33]. Our findings support the hypothesis stated in recent publications [34, 35], which suggests that exposure to cadmium had no significant effect on testicular weight. However, it has been found that testicular mass is not as sensitive indicator of testicular toxicity as histologic variables, such as seminiferous tubule diameter. The reproductive organs of rats are large enough that sufficient tissue is available to examine biochemically or morphologically even after a 50 to 75% reduction in weight [24] and morphometrical methods represent very sensitive instruments to evaluate structural changes, which could not be confirmed merely by the observation of testis morphology [8].

The morphometric analysis revealed highly significant decrease ($P<0.001$) in mean of the seminiferous tubule diameter (μm) in all experimental groups in comparison to the control (244.72 ± 42.15 μm). The tubule diameter reduction was stronger in cadmium group than in diazinon and cadmium-diazinon group. The decrease in diameter of the seminiferous tubule was the consequence of the germ cell layers disarrangement and decrease in the number by sloughing of germinal cells [17, 31]. The data obtained from the mean testicular weights and the diameters of seminiferous tubules are given in Table 1.

Testes are the most sensitive organs to acute cadmium-induced damage with irreversible necrosis [36] and haemorrhagic injury [27]. The testes first become swollen, followed by congestion and oedema, and extensive haemorrhage and necrosis occur within 24 hr after cadmium injection [37]. Cross-sections of testes from rats treated with cadmium chloride at single dose of 2 mg/kg body wt showed varying degrees of testicular destruction. Degeneration and disorganization in seminiferous epithelium, desquamation and necrosis in germinal cells, irregularity and atrophy in seminiferous tubules were observed in histological structure. These alterations resulted in significant reduction in germinal epithelium volume ($P<0.001$) and decrease in volume of the tubular lumen ($P<0.001$). The occurrence of increased intraepithelial empty spaces ($P<0.001$) in epithelium was caused by detached germ cells filling the tubular lumen.

Table 1. Morphometric evaluation of testicular weights and diameter of seminiferous tubules.

Group	Testicular weight (g)	Seminiferous tubule diameter (μm)
Control	1.45 ± 0.25	244.72 ± 42.15
A	1.49 ± 0.32	190.67 ± 28.06***
B	1.62 ± 0.10**	214.39 ± 42.52***
C	1.29 ± 0.16*	214.96 ± 35.65***

The values are expressed as means ± standard deviation; *P<0.05; **P<0.01; ***P<0.001.

In parallel with the volume change in seminiferous tubules, a significant increase in volume of the interstitial connective tissue (P<0.001) with significant dilatation of blood vessels (P<0.001) and massive haemorrhagic necrosis were also detected (Figure 2). The histomorphometric results are in accordance with previous reports [8, 9, 38] confirming that cadmium-induced testicular necrosis occurs after ischaemia due to rupture of the microvasculature [27, 36].

Diazinon exposure at dose of 20 mg/kg body wt resulted in significant morphological distortions in the testicular tissue of adult male rat testes. The morphometric results supported significant histopathological disorders in seminiferous tubules resulting in tubular degeneration and spermatogenic inhibition. The shrunken tubules were surrounded by highly undulating basal laminae (Figure 3). Histopathological studies in animals have shown that diazinon treatment causes disorganization of seminiferous epithelium, with discontinuous germ cell layers, as well as an extensive sloughing of germ cells releasing into the lumen resulting in decreases in the number of spermatocytes and spermatids, which would eventually result in a decrease of spermatozoa number and inhibition of spermatogenesis [17]. Compared with controls (Figure 1), seminiferous tubules underwent the atrophy and marked reduction in size and volume of the seminiferous epithelium (P<0.001) appeared. The decrease in seminiferous epithelium volume can be attributed to a combination of reduced proliferation and apoptosis in the male germ after an acute exposure to diazinon [16, 39]. Sloughing of germ cells from disintegrated epithelium into the lumen caused a significant increase of intraepithelial empty spaces (P<0.001) and significant deluminization of tubules (P<0.001). The extension (P<0.001) and fibrotisation of interstitium with dilated blood vessels (P<0.001), necrosis of Leydig cell and presence of oedema were observed. Histomorphometry confirmed the effect of organophosphates on testicular tissue [17, 28, 39] and suggest that the disruption of tissues around seminiferous tubules with reduction in size of seminiferous tubules and lumens may affect male fertility [31].

The histological lesions of testes from rats intraperitoneally exposed to cadmium (2 mg/kg body wt) and diazinon (20 mg/kg body wt) simultaneously exhibited significant decrease (P<0.001) in the relative volume and total area of germinal epithelium and tubule lumen compared to the control although found changes in testicular structure were less expressive than in group with a single administration of cadmium. An increase in capillary size (P<0.001) led to the development of interstitial oedema. Vacuole formation in the seminiferous epithelium and volume of interstitial tissue were significantly increased (P<0.001) only to the levels found in diazinon treated group (Figure 4). It is an interesting finding that cadmium in combination with diazinon was not able to damage the testis as extensively as in a single dose. A synergistic or additional effect of acute coexposure to cadmium and diazinon was supposed, but the results did not confirm these assumptions. These data provide a novel insight into the toxicology of cadmium and diazinon interactions on male reproduction. The results of the effects of intraperitoneal administration of cadmium, diazinon and cadmium-diazinon coexposure on testicular tissue in male adult rats are given in Tables 2 and 3.

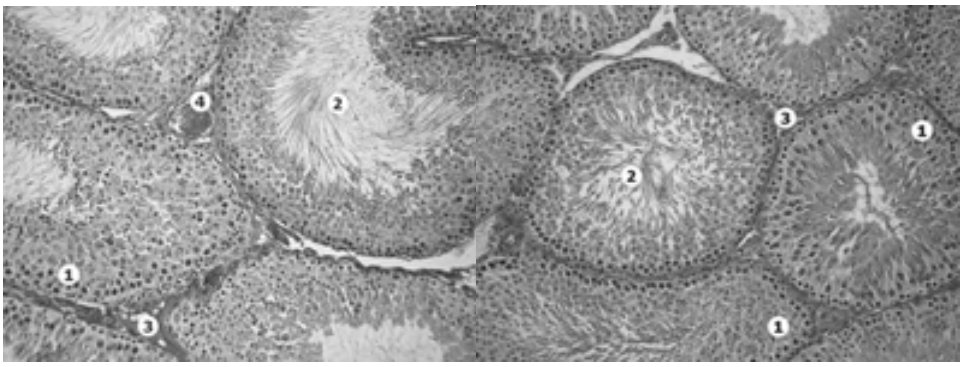

Figure 1. Control rat testis (200x, HE). **1** - tubules containing germinal gells at various stages of spermatogenesis surrounded by the Sertoli cells are predominant; the germ cells are organized in concentric layers, **2** - tubule lumen with released spermatozoa showing the presence of active spermatogenesis, **3** - interstitial connective tissue with Leydig cells, **4** - intact blood vessels filled with erythrocytes.

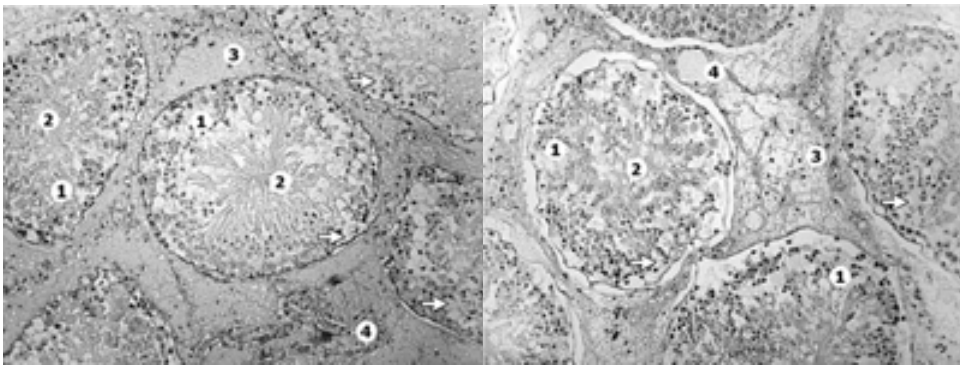

Figure 2. Rat testis 36 hours after i.p. cadmium administration (200x, HE). **1** - seminiferous tubules with undulated and thickened basal membrane; disintegration of seminiferous epithelium, **2** - deluminization of the tubules, **3** - ischaemia and interstitial oedema, **4** - dilated blood vessel, arrows - intraepithelial spaces as a result of released germ cells from epithelium.

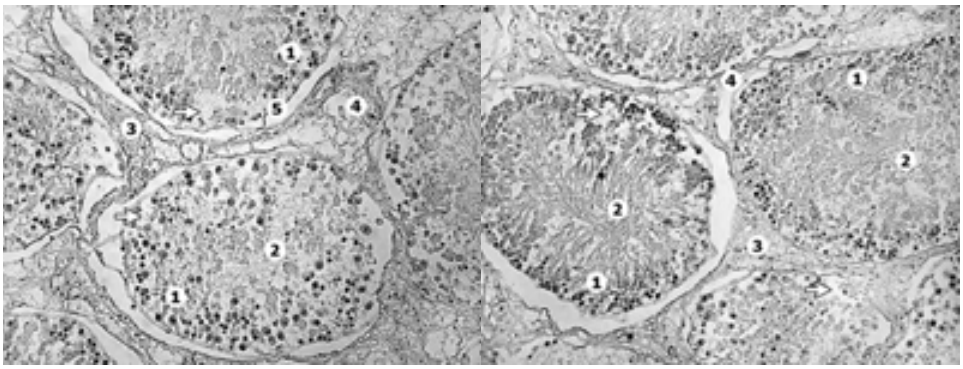

Figure 3. Rat testis 36 hours after i.p. diazinon administration (200x, HE). **1** - shrunken atrophied and irregularly outlined tubule with seminiferous epithelium destruction, **2** - lumen filled by degenerated germinal cells, **3** - extended interstitial spaces, **4** - dilated blood vessel, **5** - undulated basal lamina, arrows - the vacuolization of the seminiferous epithelium.

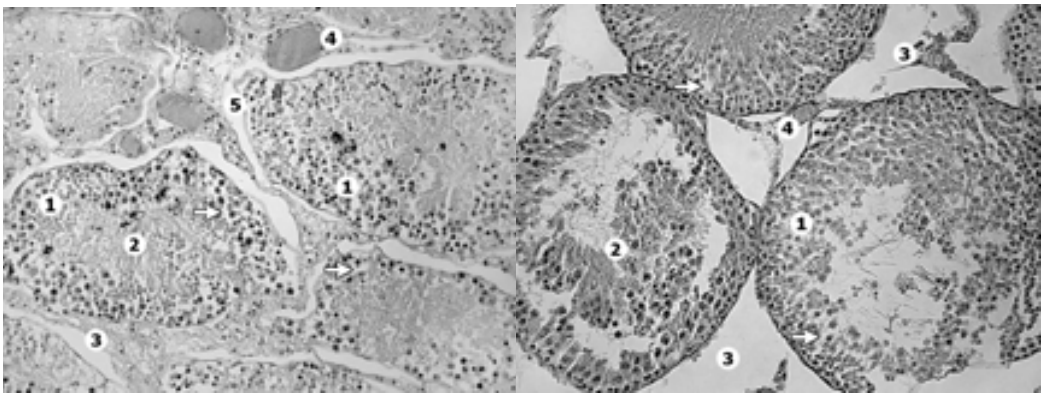

Figure 4. Rat testis 36 hours after i.p. cadmium-diazinon administration (200x, HE). **1** - vacuolization and necrosis of seminiferous epithelium, **2** - lumen filled by damaged germinal cells, **3** - interstitium volume extension, **4** - dilated blood vessel, **5** - undulated basal lamina, arrows - intraepithelial empty spaces due to lack of germ cells in epithelium.

Table 2. Relative volume of testicular structures (%).

Group	Seminiferous epithelium	Intraepithelial spaces	Tubule lumen	Interstitial tissue	Blood vessels
Control	64.82 ± 7.24	0.38 ± 1.02	23.58 ± 8.91	10.93 ± 4.31	0.29 ± 0.53
A	44.05 ± 8.10***	11.22 ± 4.81***	8.99 ± 6.27***	33.23 ± 10.56***	2.52 ± 4.54***
B	55.16 ± 8.90***	6.90 ± 5.55***	7.14 ± 8.29***	29.87 ± 9.45***	0.94 ± 1.45***
C	49.29 ± 9.74***	6.76 ± 5.78***	13.28 ± 7.72***	29.41 ± 11.51***	1.27 ± 2.58***

The values are expressed as means ± standard deviation; ***P<0.001.

Table 3. Absolute volume of testicular structures (μm^3).

Group	Seminiferous epithelium	Intraepithelial spaces	Tubule lumen	Interstitial tissue	Blood vessels
Control	93.34 ± 10.43	0.55 ± 1.47	33.96 ± 12.83	15.73 ± 6.20	0.42 ± 0.76
A	63.43 ± 11.66***	16.15 ± 6.92***	12.94 ± 9.02***	47.85 ± 15.21***	3.62 ± 6.53***
B	79.42 ± 12.82***	9.93 ± 7.99***	10.28 ± 11.93***	43.02 ± 13.60***	1.35 ± 2.09***
C	70.97 ± 14.03***	9.73 ± 8.32***	19.12 ± 11.12***	42.35 ± 16.58***	1.82 ± 3.71***

The values are expressed as means ± standard deviation; ***P<0.001.

CONCLUSIONS

The present study was carried out to observe the acute toxic effects of cadmium and diazinon in single doses on the morphology and histology of testis in the albino Wistar rats. Testicular histology and morphometry can support the detection of environmental toxins reproductive effects in males. The research has shown that acute intraperitoneal cadmium and diazinon intoxication induces morphological distortions at tubular and interstitial levels in the testicular tissue of adult rats. Supporting the morphometric results, animal study has demonstrated that cadmium and diazinon act as testicular toxicants disrupting reproductive system and exposure to these compounds may lead to fertility failure. A synergistic or additional effect of acute coexposure to cadmium and diazinon in male rats was supposed, but the results of this study did not confirm these assumptions, although the testis of cadmium and diazinon treated group had gone the most obvious reduction in size. Further studies are

necessary to assess and adequately elucidate the effects of environmental risk factors on both animal and human male reproductive health.

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