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Histopathological Effects of Dimethoate on Testes of Rats

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Abstract The aim of the current study was to investigate subchronic effect of dimethoate on the testes of rats. The animals of exposed groups were fed with laboratory chow combined with 2, 8 and 20 mg/kg dimethoate for 90 days. When compared to control, there was a statistically significant decrease in relative testis weights of rats treated with 20 mg/kg dimethoate. In light microscopic examinations, histopathological observation of the treated rats revealed that dimethoate caused dose-related testicular damage characterized by moderate to severe seminiferous tubule degeneration as sloughing, atrophy, germ cell degeneration and by partial arrest of spermatogenesis.

Introduction

It is known that exposure to environmental chemicals such as pesticides results in cancers or reduced reproductive functions in humans and animals (Wesseling et al., 1999; Sharpe and Irvine, 2004). Organophosphorus pesticides are widely used in agriculture, industry and for public health purposes. These pesticides are sometimes found to affect non-target organisms including human beings (Chantelli-Forti et al., 1993; Chaudhuri et al., 1999). In mammals, the primary site of action of organophosphorus pesticides is the central and peripheral nervous system by inhibiting acetylcholinesterase, a biochemical event that results in the accumulation of endogenous acetylcholine at the nerve endings (Briggs and Simons, 1986). Several physiological and behavioral dysfunctions occur in animals after exposure to high doses of organophosphorus pesticides (Hall and Clark, 1982; Rattner et al., 1987). Sublethal doses of these pesticides lead to alterations in the reproductive system of mammals (Ray et al., 1991; Chapin et al., 1990). A large number of pesticides were identified as possible or definite endocrine disrupters by the European Commission in September 1999. However, the mechanism of organophosphate-induced gonadal dysfunction remains to be elucidated (Sarkar et al., 2000; Narayana et al., 2006). There are several possible mechanisms for the anti-gonadal actions of organophosphorus pesticides: they may exert a direct inhibitory action on the testis; they may affect the pituitary, causing changes in gonadotrophin concentrations and thus subsequent spermatogenic impairment; or they may change the concentration of the neurotransmitter acetylcholine (Sarkar et al., 2000).

Dimethoate, which is one of the most important organophosphorus insecticides, is frequently used in agriculture. Dimethoate caused testicular damage, damage to sperm production, and reduction in testosterone levels when fed to adult male rats (Afifi et al., 1991). Dimethoate also resulted in decreased thyroxine concentrations in ewes (Rawlings et al., 1998), and affected thyroid metabolism in mice (Maiti and Kar, 1997). It was reported that this insecticide caused a decrease in adrenal and pituitary weights in rats (Shaker et al., 1988).

The purpose of this study was to investigate the effect of dimethoate on the testes of male rats.

Materials and Methods

The protocol was approved by the animal ethical committee of Ege University, Faculty of Medicine (2001–32).

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The studies were conducted on male Wistar albino rats (60–80 g) obtained from the Breeding Center of Experimental Animals at Ege University, Faculty of Medicine. After 15 days of acclimation, the rats were assigned randomly to either the exposure groups (low dose: 2 mg/kg, medium dose: 8 mg/kg, and high dose: 20 mg/kg) or to the control group, each containing 10 rats, which were housed individually in labeled cages (19 × 19 × 12 cm) with solid plastic sides and floors, and stainless-steel grid tops. They were maintained in controlled laboratory conditions with a 12h dark/light cycle, 22 ± 3°C temperature and 35–65% relative humidity. Animals in the control group were orally fed daily with a normal diet of standard laboratory chow, while the animals of the treated groups were fed with laboratory chow combined with dimethoate during 90 consecutive days. A commercial formulation of dimethoate (Korumagor 40 EC, Koruma Agriculture, Turkey) was used in this study. Tap water was also available ad libitum. All animals were weighed weekly throughout the study.

After 90 days of the experiment, all rats in each group were killed humanely by cervical dislocation and examined for gross lesions. During necropsy, their testes were removed and weighed, and subsequently the relative testis weight of each animal was calculated. The results of relative testis weights are presented as mean ± standard deviation (SD). Comparisons were made between the control and treatment groups using one-way analysis of variance (ANOVA). Values of $p \leq 0.05$ were regarded as statistically significant.

Histopathological evaluations are commonly used methods for detecting organ-specific effects related to chemical exposure (Travlos et al., 1996; Crissman et al., 2004). For light microscopic examination, testis samples of the experimental rats were fixed in Bouin's fixative. After routine processing, paraffin-embedded tissue samples were sectioned at 3–5 μm thickness and stained with Mayer's haematoxylin and eosin. Histopathological analyses of testes were based on qualitative and quantitative changes. Quantitative changes were detected by counting 100 tubules in each rat. One hundred tubules per animal were screened and classified as normal, sloughing, atrophy and germ cell degeneration based on the degree of seminiferous tubule degeneration. The tubules with sloughing were those that showed disrupted cell association. Tubules that showed very few or no germ cells were classified as atrophic tubules. Tubules with round spermatids exhibited vacuolated nuclei, round spermatids with a halo appearance and giant cells were classified as tubules with germ cell degeneration. Data are presented as mean ± standard deviation (SD). The differences were compared for statistical significance by one-way ANOVA, which has been carried out using SPSS version 11.0 software. The significance level was set at $p \leq 0.05$.

Results and Discussion

The present study investigated the toxic effects of sublethal dosages of an organophosphorus pesticide, dimethoate, on the testis of rats. The results indicated that dimethoate affects the testicular structure and may cause infertility due to severe atrophy of seminiferous tubules, especially in the high-dose group.

When compared to control animals, there was a statistically significant decrease in the relative testis weights of rats treated with 20 mg/kg dimethoate (Fig. 1). This decrease in testis weights was consistent with severe elimination of germ cells and testicular atrophy. Afifi et al. (1991) also reported that a significant decrease in the testis weights of rats exposed daily to 6.25 and 12.5 mg/kg dimethoate for 65 days. Similarly, other organophosphate insecticides such as methyl parathion and quinalphos decreased testis weights at high dosages (Sarkar et al., 2000; Narayana et al., 2006).

As for the necropsy findings, one testes of two rats in the 20 mg/kg group showed obvious testicular atrophy and asymmetry. For example, while the right testis of one of these rats (total body weight 220 g) was 1.33 g, its atrophic testis was 0.34 g. Histological examinations of testicular sections from both rats revealed that almost all of the seminiferous tubules were atrophic. It was reported that greater than 85% atrophy of the seminiferous tubules caused irreversible infertility (Hess and Nakai, 2000).

In light microscopic examinations, histopathological observation of the treated rats revealed that dimethoate caused dose-related testicular damage characterized by moderate to severe seminiferous tubule degeneration as sloughing, atrophy, germ cell degeneration and by partial arrest of spermatogenesis (Table 1, Fig. 2). The testicular architecture of control animals was normal, characterized by complete spermatogenesis with normal cell association, tubular lumen, and interstitial spaces (Fig. 3). Quantitative findings of histopathological analyses are represented in Table 1 and Fig. 2. When compared to the control group,

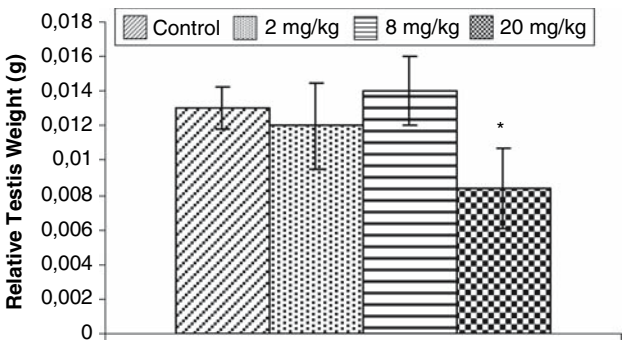

Fig. 1 Relative testes weights of experimental rats. * Statistically significant difference from control ($p \leq 0.05$)

Table 1 Percentage of histopathological classification of the seminiferous tubules in the testes of control and dimethoate-treated groups. Values are given as mean ± SD. * Statistically significant difference from control ($p \leq 0.05$)

Parameters	Control	2 mg/kg	8 mg/kg	20 mg/kg
Normal	98.7 ± 0.68	64.8 ± 5.18*	50.6 ± 4.92*	4.0 ± 0.57*
Sloughing	1.3 ± 0.68	34.3 ± 5.00*	45.3 ± 4.91*	26.8 ± 6.01*
Atrophy	0	0.9 ± 0.34	3.3 ± 1.76	52.5 ± 4.16*
Cell degeneration	0	0	0.7 ± 0.49	16.7 ± 4.25*

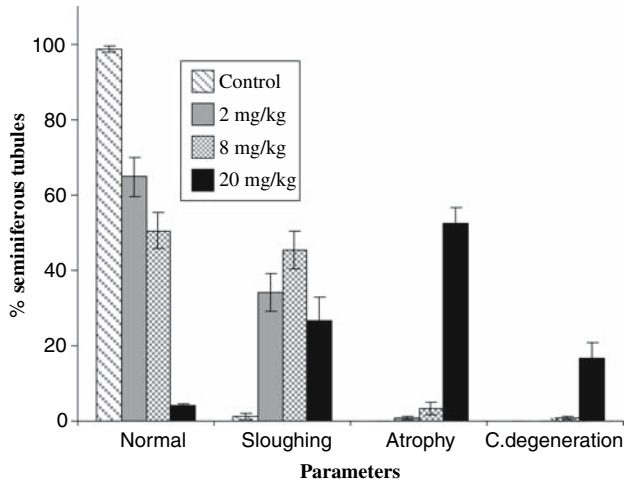

Fig. 2 Classification of seminiferous tubules in the testes of control and dimethoate-treated groups. The seminiferous tubules were classified into normal, sloughing, atrophy, and cell degeneration. Normal tubules showed normal cell association, the tubules with sloughing showed disrupted cell association, the atrophic tubules showed very few or no germ cells, and the tubules with germ cell degeneration showed round spermatids exhibited vacuolated nuclei, round spermatids with a halo appearance and giant cells. Note that atrophy and germ cell degeneration were not observed in the control group and germ cell degeneration was also not observed in the 2 mg/kg dimethoate-treated group. * Statistically significant difference from control ($p \leq 0.05$)

the number of normal tubules significantly decreased in all dimethoate-treated groups due to an increase in degenerative tubules. The incidences of sloughing significantly increased in all treated groups compared to the control. Atrophy and germ cell degeneration were not observed in the control group and germ cell degeneration was also not observed in the 2 mg/kg dimethoate-treated group. The incidences of intensive tubular damage classified as atrophy and germ cell degeneration were significantly different from the control only in the 20 mg/kg dimethoate-treated group. Histopathological changes in seminiferous tubules were stronger in the high-dose (20 mg/kg) group than in the medium (8 mg/kg) and especially the low-dose (2 mg/kg) groups. The affected tubules were lined by very fewer spermatogenic cells (Fig. 4) or only by cell debris, observed mostly in the high-dose group (Fig. 5). 20 mg/kg dimethoate exposure resulted in an apparent loss of all germ cells, including spermatogonia (Fig. 5). The diameter

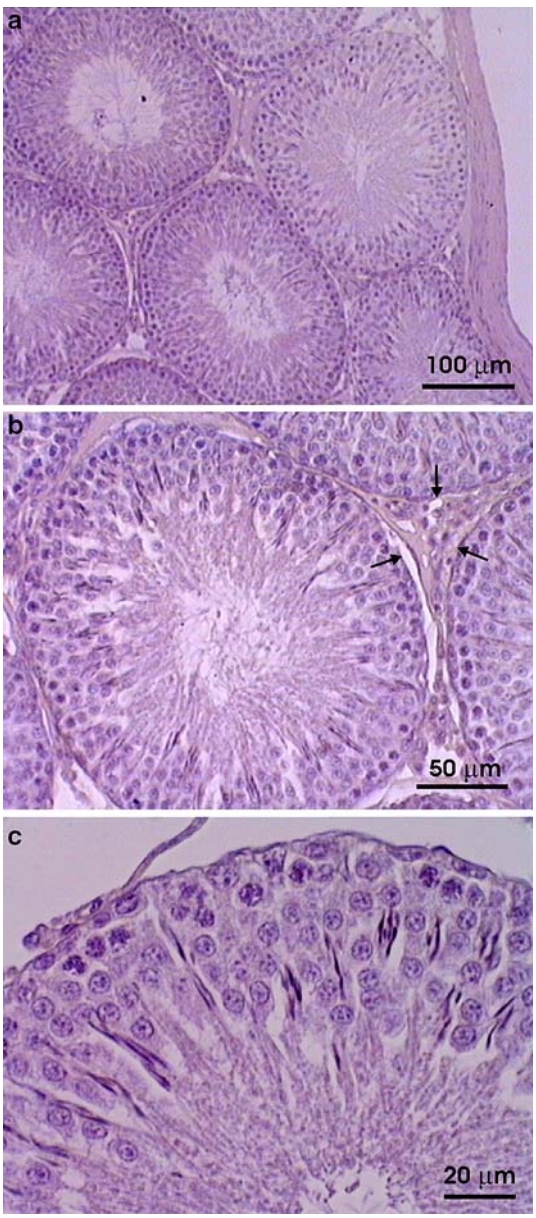

Fig. 3 Testicular sections of control rats which show normal spermatogenesis at three different magnification (a, b and c). Note the normal cell arrangement in the seminiferous tubules. Arrows point to the interstitial space, which appears normal

of the affected tubules was decreased, with the interstitium being more prominent because of the oedema (compare

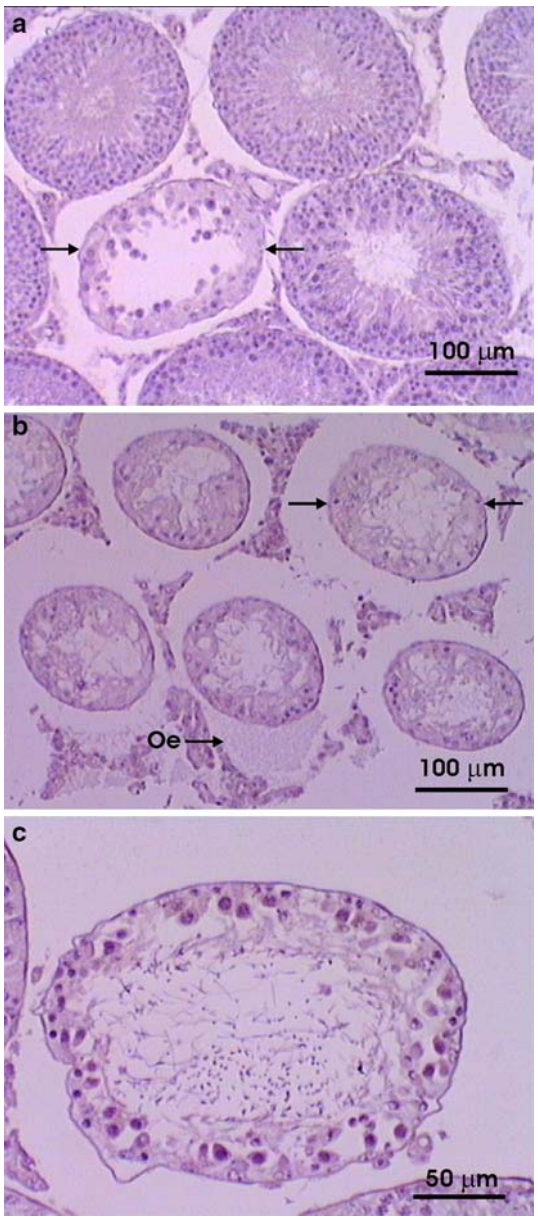

Fig. 4 Testicular sections of rats treated with 2 (a) and 8 mg/kg dimethoate (b and c) showing seminiferous tubule degeneration. Note that the interstitial spaces are enlarged due to tubular atropy and oedema. Arrows point to the atrophic tubules (a and b) and oedema (Oe) (b)

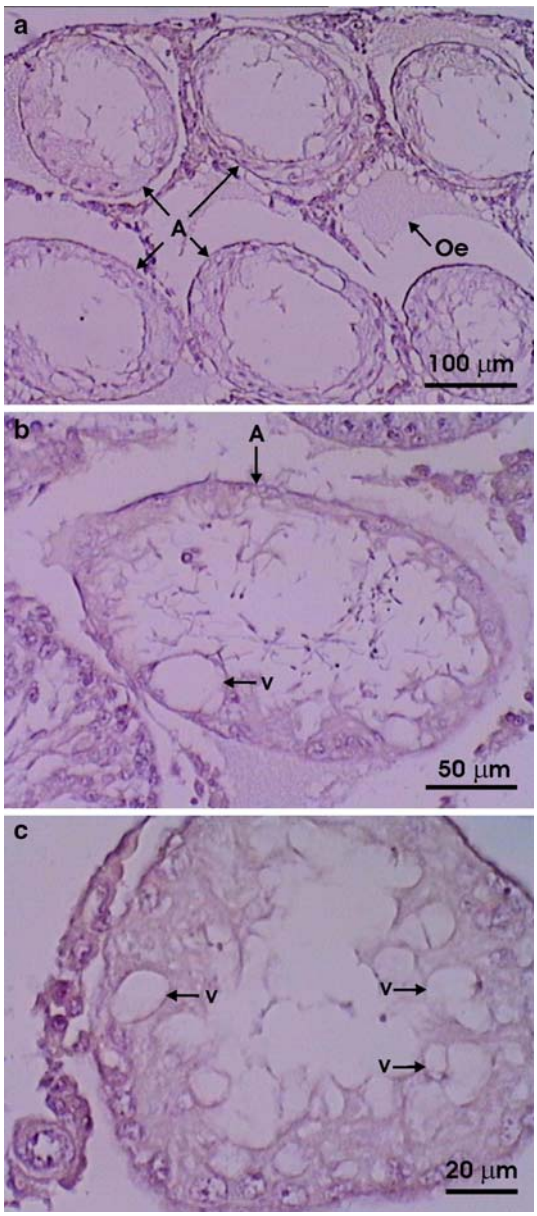

Fig. 5 Testicular sections of rats treated with 20 mg/kg dimethoate, which show further seminiferous tubule degeneration characterized by severe atrophy of tubules and vacuolation in Sertoli cells. Note the loss of all germ cells and, the enlargement of interstitial spaces due to tubular atropy and oedema. Atrophic tubules (A), oedema (Oe), vacuole (V)

Figs. 4b and 5a with Fig. 1a), and so the interstitial space was enlarged due to oedematous fluid and tubular atrophy, especially in the high-dose group (Figs. 4 and 5). Oedema and haemorrhage were reported in the interstitial tissue of the testes of rats exposed to dimethoate (Afifi et al., 1991) and to two or three pesticide combinations of dimethoate, endosulfan, and carbaryl (Selmanoğlu and Akay, 2000). Increased interstitial space due to tubular atrophy was also reported in rats exposed to carbendazim and methyl parathion (Hess and Nakai, 2000; Narayana et al., 2006).

Vacuolation was observed in Sertoli cells of rats exposed to 20 mg/kg dimethoate (Fig. 5). Creasy (2001) reported that one of the most common morphological responses of Sertoli cells to injury is vacuolation and subsequent to the vacuolation; germ cell degeneration, disorganization or exfoliation is generally seen. Similarly, the seminiferous tubules also showed irregular arrangement of spermatogenic cells and dimethoate caused germ cell degeneration and depletion, also sloughing of germ cells into the tubular lumen. Sloughing of germ cells was observed clearly in the

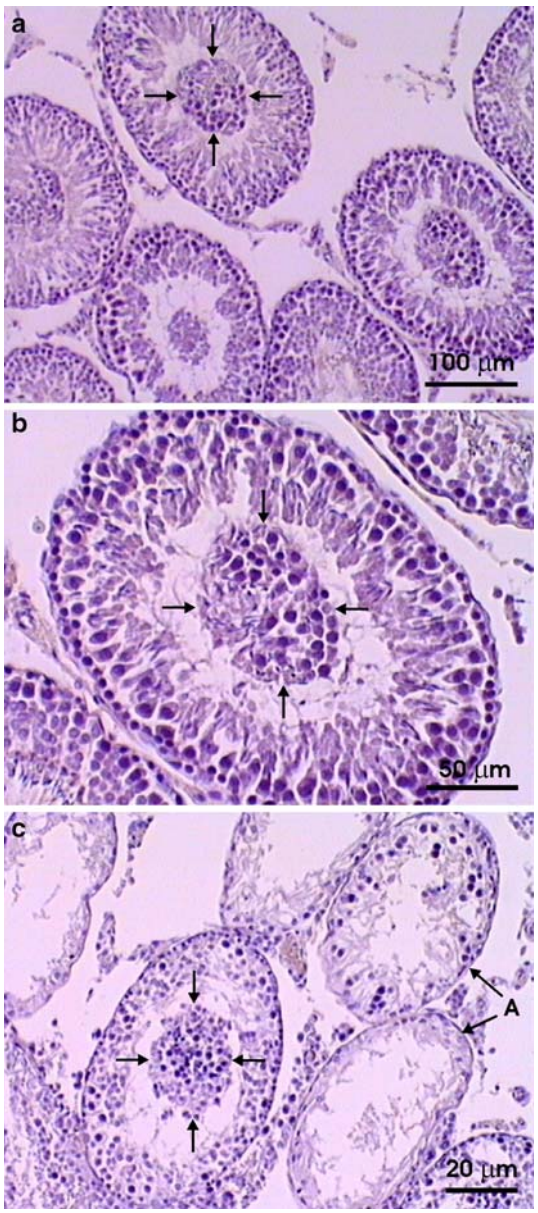

Fig. 6 Testicular sections of rats treated with 8 (a and b) and 20 mg/kg dimethoate (c) showing seminiferous tubule degeneration. Note the sloughing of germ cells into the tubular lumen. Arrows point to the sloughing, Atrophic tubules (A)

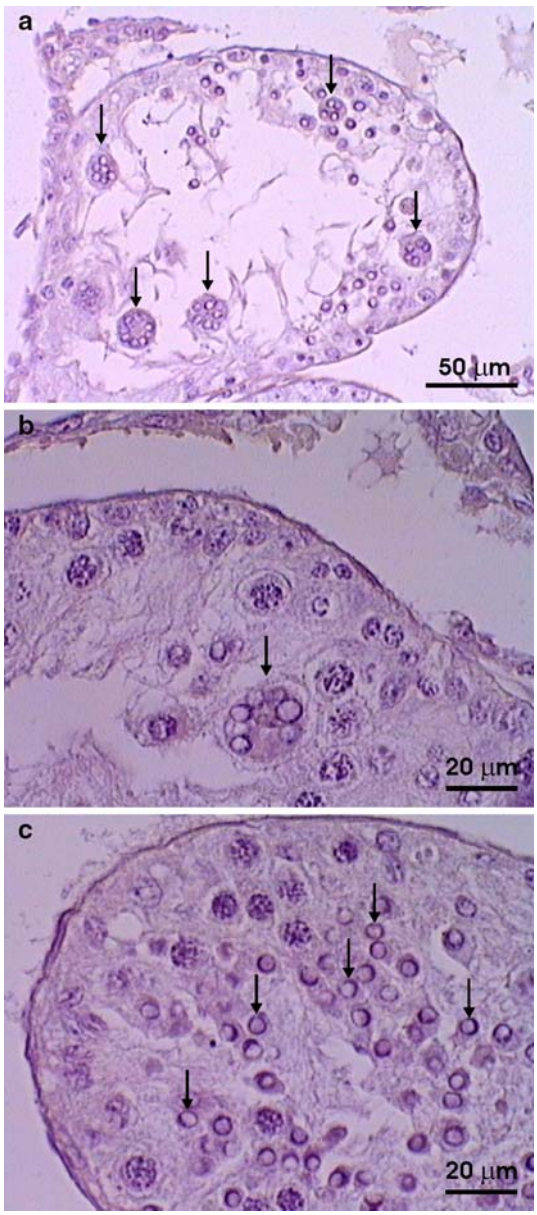

Fig. 7 Testicular sections of rats treated with 20 mg/kg dimethoate showing progressive germ cell degeneration. Note the multinucleated giant cells showing a halo appearance in the round spermatid nuclei. Arrows indicate the multinucleated giant cells (a and b) and the round spermatids with a halo appearance (c)

rats of medium and high dose groups (Fig. 6). According to Hess and Nakai (2000), sloughing is caused by the effects of the chemical on microtubules and intermediate filaments of the Sertoli cells, and these effects spread to dividing germ cells and naturally lead to tubular atrophy. Testicular sections from rats exposed to 20 mg/kg dimethoate also showed that round spermatids exhibited vacuolated nuclei, round spermatids with a halo appearance, and these spermatids were observed forming round multinucleated clusters called giant cells (Fig. 7). The halo appearance of round spermatid nuclei and the formation of giant cells are common deformities caused by several chemicals (Hess et al., 1988). The multinucleated giant cells observed in the present study and other studies (Hess et al., 1988; Sarkar et al., 2000; Narayana et al., 2006) may be the end result of germ cell degeneration and widening of intercellular bridges (Hess et al., 1988; Creasy, 2001). The results of the present study were similar to the defects of organophosphate insecticides such as methyl parathion and quinalphos on the testes of rats, which were sloughing, multinucleated

giant cells, necrosis of seminiferous epithelium, cellular degeneration, and tubular atrophy (Sarkar et al., 2000; Narayana et al., 2006). Another organophosphorus insecticide, dimethyl-methyl-phosphate, has been reported to produce similar histologic abnormalities in the testes of rats characterized by lack of spermatogenesis and by degeneration, vacuolization and necrosis of spermatogenic cells (Dunnick et al., 1984).

In conclusion, it was obvious from the present study that dimethoate caused dose-related and severe degenerative damages on the testes of rats.

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