

Leydig cell destruction and mast cell accumulation in rat testes after administration of testicular toxicant ethane dimethanesulphonate

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In this study, the effect of the testicular toxicant ethane dimethanesulphonate (EDS) in rat Leydig cells and testis was studied 1,2,3,7,14 and 28 days after EDS injection by light microscopy and enzyme histochemistry. Two days after treatment, Leydig cells showed disorganization, pyknosis and degeneration. The severe toxic effect of EDS on Leydig cells was seen on day 3. These cells were totally disappeared from interstitium 3-days after the treatment. Degeneration of Leydig cells was followed by phagocytosis of macrophages and resulting in their disappearance by day-3. Testis continued to degenerate on day-7. Tubular atrophy, arrest of spermatogenesis and lysis was seen on day-14. Twenty eight days after EDS treatment testes were almost intact but however, Leydig cell number was still low. Increase of fibroblast-like cells and mast cells in the interstitium suggest that there may be an interrelationship between these cells and testes tissue. Results indicate that, the effect of EDS is reversible and after destruction of Leydig cells new Leydig cells appear in the interstitium. [Turk J Med Res 1995; 13(6):165-171]

Key Words: Testis, Ethane dimethanesulphonate, Leydig cell, Mast cells

The methane sulfonic ester of ethylene glycol, Ethane-1,2-dimethanesulphonate (EDS) is a unique testicular toxicant. It has been shown that when injected into adult rats cause temporary infertility. Rapid selective, destruction of mature Leydig cells by a single injection of EDS leads to total depletion within 3-4 days. Approximately after 2-4 weeks of EDS administration new Leydig cells begin to appear in the interstitium and testis has apparently recovered within 4-6 weeks after EDS injection and is capable of maintaining normal spermatogenesis (1-4). Adult and fetal rat Leydig cells respond differently to EDS and fetal Leydig cells are more resistant to the cytotoxic effects of EDS indicating that effects of EDS is age-dependent. Seminiferous cord epithelium also degenerates after EDS treatment. Probably lack of endogenous androgen may cause regression of the spermatogenetic epithelium and/or EDS has toxic effect on Sertoli cells and also germ cells resulting in disturbances in the interaction

between tubules and interstitial tissue (5-9,11). In intact rats, mast cells are seen only beneath the tunica albuginea but when adult rat Leydig cells were destroyed with EDS, high number of mast cells accumulated in the interstitium. In the normal testes their role remains obscure. It is suggested that these cells have paracrine role in testes but they have received little interest (16-18).

In this study, particular emphasis was directed towards testicular cytology and enzyme histochemistry after specific testicular toxicant EDS treatment.

MATERIALS AND METHODS

In the present study, sixty adult Wistar male rats (24 rats for control group, 36 for EDS group) weighted 280±25 g were housed under conditions of 12 h light and dark cycles rat chow and water were provided ad libitum. 75mg/kg BW of EDS is dissolved in dimethylsulphoxide (DMSO) water solution (1/3: v/v) and injected to animals intraperitoneally. Controls received 2 ml/kg (DMSO) vehicle injection only. EDS is not commercially available and was prepared according to the method of Jackson&Jackson (1). EDS and DMSO treated rats were then sacrificed on days 1,2,3,7,14, and 28. For light microscopical examination testes were fixed in Bouin solution then prepared for routine

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histology and 6 μ m thick paraffin sections were stained with hematoxylin and eosin.

Small pieces taken from another testis were fixed in buffered glutaraldehyde (5%) for 5 hours, post fixed with OSO_4 for 2 hours and embedded in araldite. Semi-thin (1 μ m) sections were taken with Nova Ultramicrotome and stained with toluidine blue in order to study the cytoplasmic structure of the cells in more detail. All preparations were examined with Olympus PM-10 ADS photomicroscope.

Enzyme histochemistry

Testis was immediately fixed in cold formol-calcium solution for 24 hours and than 15 nm thick frozen sections were taken. For enzymatic reaction histochemical staining was applied on the frozen sections using with a-naphthyl acetate esterase (ANAE) as a substrate and hexazotized pararosanilin as a coupler for 10 min. at room temperature (pH 6.5) (12).

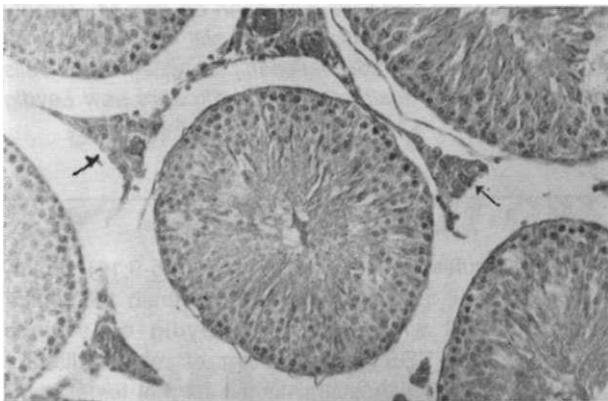


Fig. 1a. Day-1. Control testis (DMSO group). Numerous Leydig cells in the interstitium (arrows). H.E Paraffin Section X64.

Statistics

Testes weights were analyzed by Student's t-test.

RESULTS

Morphologic Findings

One day after DMSO-injection animals displayed normal testes histology and Leydig cells were easily recognized with their deeply stained eosinophilic cytoplasm and prominent nuclei by H.E (fig 1a). When examined the Toluidine blue stained semi-thin Araldite sections, these cells displayed eccentrically located, nuclei, prominent nucleolus and numerous granules in the cytoplasm (Fig 1b). Frozen sections incubated for ANAE enzyme reaction showed positive reaction (Fig 1c). One day after EDS injection seminiferous cords were intact and no degeneration was observed at any stages of spermatogenesis. Histologically normal interstitial tissue contained blood vessels and Leydig cells (Fig 2). However, Leydig cell number

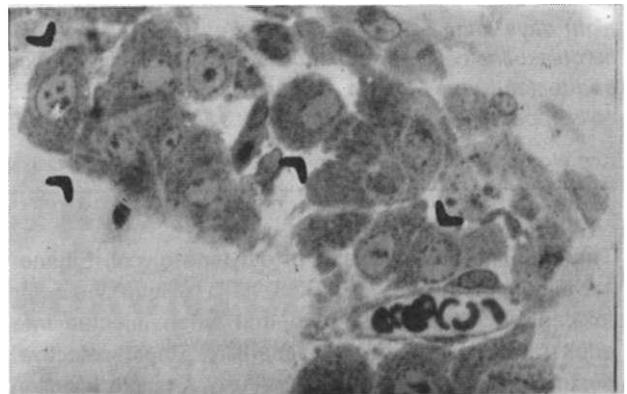


Fig. 1b. Typical Leydig cells in control testes (arrow heads) (Day-1). Araldite section, Toluidine blue X320

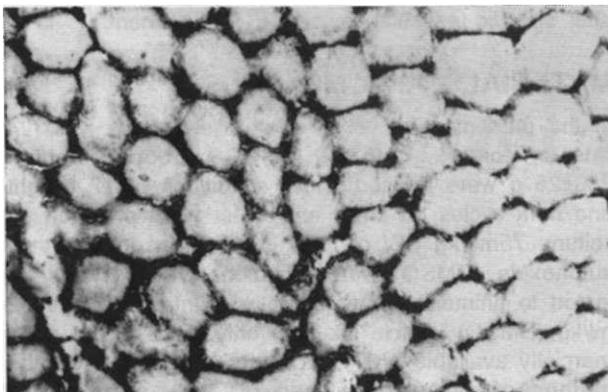


Fig. 1c. Positive ANAE reaction in control group (Day-1). X32.

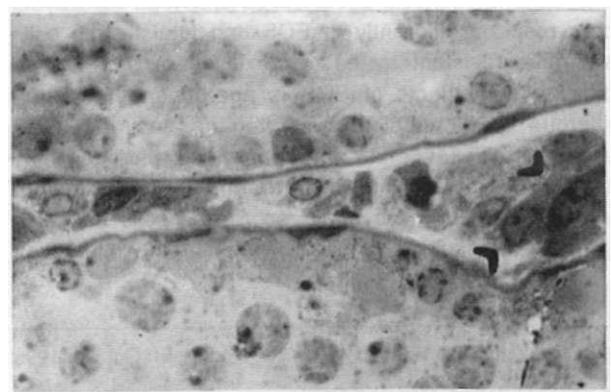


Fig. 2. Testis from 1-day after EDS treatment. Morphologically normal Leydig cells (arrow heads). Araldite section toluidine blue X320

ETHANE DIMETHANESULPHONATE AND RAT TESTES

Table 1. Paired Testis Weights

Control group	mg	SD	EDS	mg	SD
1.gün	2397	±78.9	1.gün	2375	±110.8*
2.gun	2432.5	±78.9	2.gün	2360	±58.31*
3.gun	2407.5	±94.03	3.gün	2301	±26.4*
7.gün	2455	±42.3	7.gün	2035	±37.82***
14.gün	2487	±103.08	14.gun	1380	±48.99***
28.gun	2501.25	±43.66	28.gün	2045	±39.37**

Significant difference compared to controls (**P<0.001), (*P>0.05 NS)

was slightly reduced on day-1 when compared to vehicle injected group. Testis weights were similar in DMSO and EDS injected groups (Table 1).

Two days after EDS treatment Leydig cell number continued to reduce and interstitium contained degenerated Leydig cells with darkly-stained cytoplasm and pyknotic nucleus. Morphologically normal Leydig cells were also present on day-2. Seminiferous cords displayed normal histology but slightly increased lipid granules were present between spermatogonia and spermatocytes. Weaker ANAE reactivity was detected on day-2 testes tissue. No differences were observed in DMSO injected group (Fig 3).

Three days after EDS administration, Leydig cell population was almost absent from the interstitium. Remaining Leydig cells showed degenerative changes ranging from lipid vacuolization disintegration and pyknosis. Cell infiltration and macrophages were seen in the interstitium. Occurrences of phagocytic macrophages in the interstitium were a typical observation on day-3 and were identified as pale-staining cells showing cytoplasmic vesicles and granules. After EDS treatment degenerated Leydig cells were phagocytosed by these cells and therefore macrophages become enlarged and vacuolated. This feature was not observed in any of the control animals examined. Similar histol-

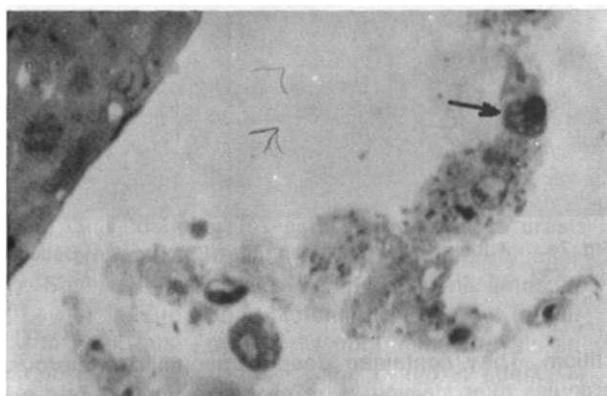


Fig. 3. Testis from 2-day after EDS treatment Pyknotic Leydig cell (arrow). Araldite Section Toluidine blue X320 Positive ANAE reaction in control group (Day-1). X32.

ogy with only notable change being a highly reduced number of Leydig cell population was observed in testes 3-days after EDS treatment. Moderately degenerated seminiferous tubules with lipid vacuoles were present. No significant loss of testes weights was observed in this group (Fig 4).

On day-7 after EDS treatment, Leydig cells were still absent from the interstitium. Tubules continued to degenerate. Interstitial cell infiltration was still present (Fig 5a-b). Residual bodies, degenerated spermatids with lipid granules were observed in the seminiferous cord epithelium. However, normal spermatogenesis was still present in intact tubules. In this group testes, ANAE enzyme reaction was negative (Fig 5c) Testicular weight was reduced when compared to first 3 groups after EDS treatment. Vehicle-injected animals showed normal testis histology.

The most severe toxic effect of EDS was seen on day-14. Cord epithelium was disorganized and spermatogenesis was completely arrested (Fig 6a). Tubules contained numerous degenerated cells and dense eosinophilic bodies in their lumens and some tubules showed lysis (Fig 6b). Fibroblasts were increased in number but in contrast, fewer mast cells were observed in the interstitium. Testicular degeneration and atrophy was more severe on day-14 when compared to previous groups. Degenerations were more prominent in central parts of the organ than the periphery. Significant reduction of testis weight was also observed on day-14 (Table 1).

Twenty-eight days after EDS injection, seminiferous cords were generally regenerated and spermatogenesis was almost actively exhibited (Fig 7a). Although low number of Leydig cells present in this group, their population was slightly increased the major phenomenon on day-28 was the appearance of new Leydig cells with increased lipid granules in their cytoplasm located close to tubular membrane (Fig 7b). ANAE enzyme reaction applied on frozen sections was



Fig. 4. Day-3 Testes illustrating phagocytic macrophage (arrow). Interstitium contained no Leydig cells. Araldite Section, Toluidine blue X320.

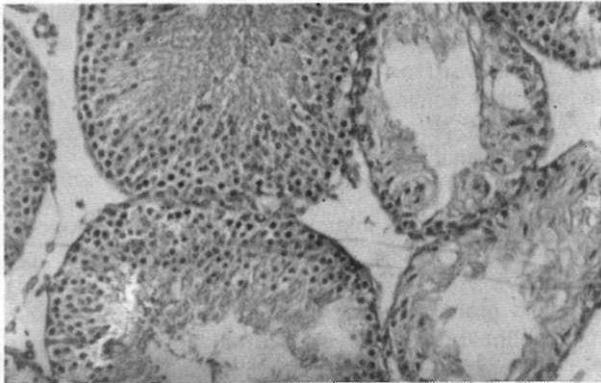


Fig. 5a. Degenerated tubules at 7-days after EDS treatment. No Leydig cell present in the interstitium. Paraffin Section H.E X64.

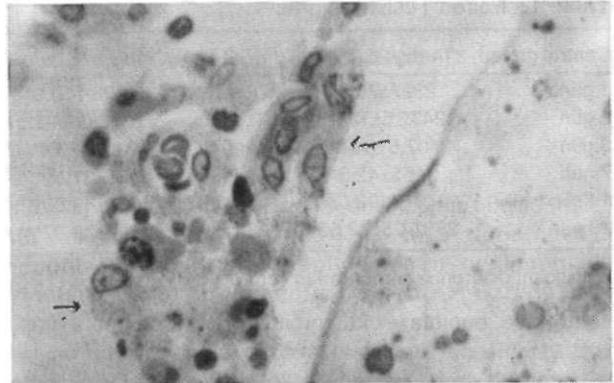


Fig. 5b. Cell infiltration in the interstitium (arrows) (day-7). Araldite Section, Toluidine blue X320.

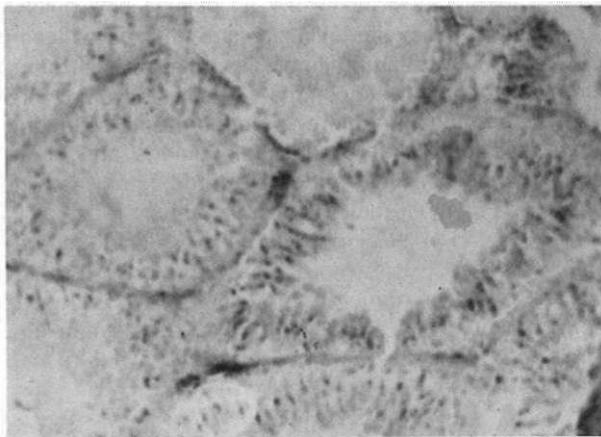


Fig. 5c. Negative ANAE reaction on day-7 X64.

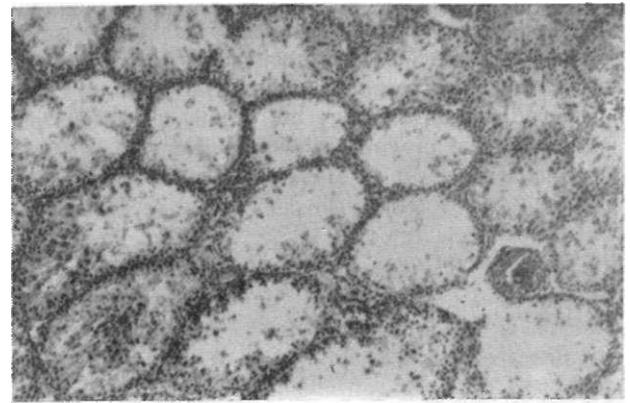


Fig. 6a. a-b Highly degenerated tubules 14-Days after EDS exposure a:Paraffin Section HE X32

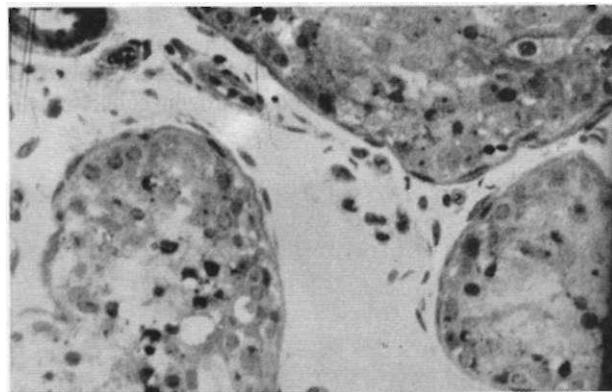


Fig. 6b. Araldite, Toluidine blue X320

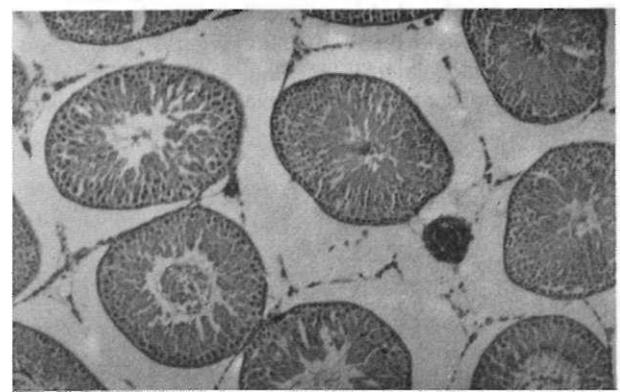


Fig. 7a. Tubular regeneration on day-28. Paraffin Section H.E. X32.

moderately positive for Leydig cells Another significant feature on day-28 was the accumulation of the mast cells in the mast cells in the interstitium. In the previous groups, mast cells were always localized beneath the tunica albuginea but not in the inter-

stitium. They contained deeply basophilic numerous granules after staining with Toluidine blue (Fig 7c-d-e). In vehicle injected group, mast cell accumulation was not seen in the interstitium except few located beneath the tunica albuginea. Testes from vehicle-injected

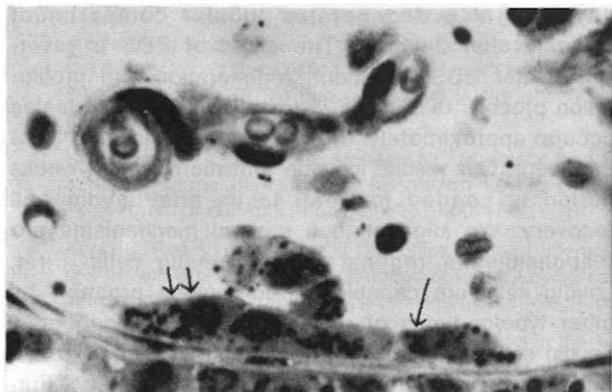


Fig. 7b. New Leydig cells with lipid inclusions (arrows) (Day-28). araldite section Toluidine blue X32

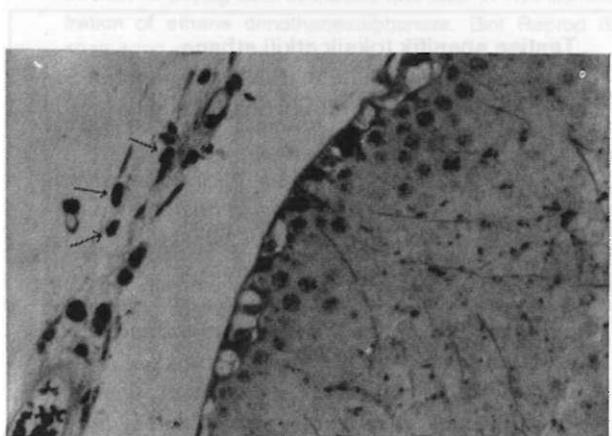


Fig. 7d. Mast cells (arrow) in the interstitium. araldite section Toluidine blue X64

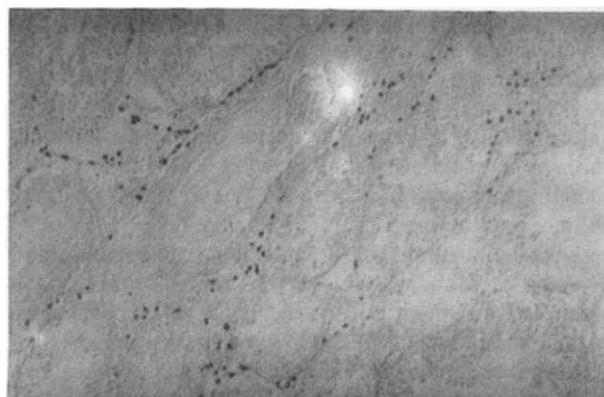


Fig. 7c. Mast cells in the interstitium (Day-28) Paraffin section $\bullet >n$ Toluidine blue X32

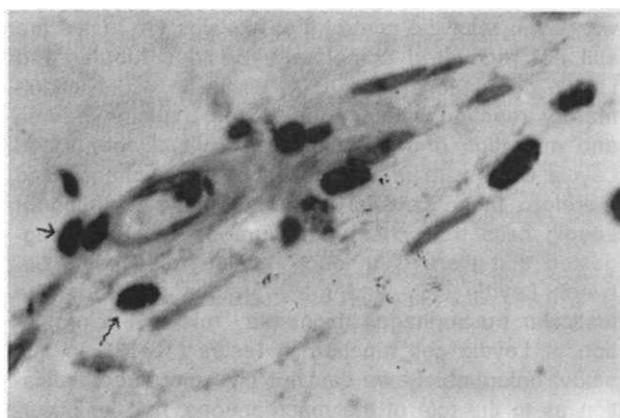


Fig. 7e. Higher magnification of interstitial mast cells (arrow), araldite section, Toluidine blue X320.

animals were exhibited normal testicular morphology and contained no mast cells in the interstitium. Lowered testes weights were measured on day-28 (Table 1).

DISCUSSION

In the present study, toxic effect of EDS on Leydig cells, testes morphology and mast cell accumulation has been examined in particular. EDS is a known alkylating agent that alkylates specific Leydig cell proteins and thus interferes with metabolic function of the cell. When injected into adult rats, EDS destroys Leydig cells, degenerates seminiferous cords, arrests spermatogenesis and finally causes temporary infertility. Severs' reports indicate that adult and immature rat Leydig cells respond differently to EDS and in contrast to adult rats, Leydig cells from immature rats appear to be more resistant to the cytotoxic effects of EDS. Therefore it is suggested that the effect of EDS is age dependent (2,4-7,10). The question here was whether the differential effects of EDS on adult and immature rat Leydig cells are mediated by extrates-

ticular factors or intratesticular factors or intrinsic to Leydig cell itself. To resolve this issue the effect of EDS was studied in mature rats in the absence of other organ systems (i.e isolated-Perfused testes) and in the absence of other testicular cell types (i.e, purified Leydig cells) (9). Results have shown that responses to EDS of Leydig cells are intrinsic (9). Although the nature of proliferating factor/or factors are also not yet known, Leydig cell precursors can be stimulated both by paracrine and endocrine factors. After elimination of Leydig cells from testis, EDS elevates LH and FSH secretion due to absence of *l*he negative feedback control of testosterone that stimulates the proliferation of Leydig cell precursors and thus newly formed Leydig cells can occur after high level of gonadotrophic stimulation and /or local factors may stimulate the Leydig cell precursors other than LH (1,3,8). To ... knowledge, may be the most interesting aspect of the EDS model is the observation that regeneration of Leydig cells begins with in 2-4 weeks after treatment (6,8).

In this study, we did not observed newly appeared Leydig cells in first 2-weeks. However, new

population of Leydig cells were located beneath the tunica albuginea in week-4. It is possible that, the differentiation process is disturbed by EDS in first 2-weeks because the half-life of EDS is 12 days in plasma or the first 2-week is used for maturation. In addition, the presence of fibroblast like cells in the interstitium also strongly suggest that these cells were may be the part of precursors of Leydig cells. EDS also causes testis atrophy and thus loss of testis weight. Change of testis weight has been reported 2-days after EDS treatment but we did not find any difference in this study on day-2 (6). However at the end of day-3, testes weights were slightly reduced but this was not statistically different from control. The significant (42%) reduction of testes weight seen on day-14 was a normal result, because severe tubular degeneration was observed in this group. After 4-week, testis weight was lower than the control that explains the testes are still not recovered completely. Besides tubular and Leydig cell degeneration, EDS elicits an acute inflammatory reaction within adult testes, causing edema and migration of mononucleated cells. Degenerated Leydig cells were phagocytosed by macrophages and therefore these cells give rise to the elimination of Leydig cells from the interstitium. It has been suggested that there is a functional interrelationship between Leydig cells and macrophages and therefore testicular macrophages might play role in the regulation of Leydig cell function in testes (13-15). In this study, unfortunately we can not give any direct indication as to the role of the macrophages, but the occurrence of macrophages probably was a result of testicular damage. Another striking feature seen in this study was, accumulation of interstitial mast cells 4-weeks after EDS treatment. Several authors have reported that, EDS is ineffective at 30 days of age in inducing mast cells and therefore effect of EDS seems to be related to age as it seen in immature Leydig cell. In intact rats, Leydig cells can inhibit mast cell proliferation by blocking stimulatory factors. However, after destruction of Leydig cells by EDS, high numbers of mast cells can accumulate in the interstitium.

These results suggest that, mast cell accumulation related to the absence of these possible stimulatory and /or inhibitory factors secreted by Leydig cells or, degeneration of testis tissue may cause this accumulation (16,17,18). It has also been reported that Leydig cells are not able to act on mature mast cells (16). This probably explains the presence of high number of mast cells seen in this study on day-28. Therefore we suggest that, in degenerated testes mast cells may play role in regeneration and/or in regulation or there may be some other factors present in testes which have a stimulatory and/or inhibitory effects on these cells. In summary, the present data demonstrates that single injection of EDS destroys Leydig cells totally within 3-

days but also degenerates tubular compartment severely after 2-weeks. The effect of EDS is reversible. After EDS treatment, regeneration and proliferation process take place in testes. Recovery of testes occurs approximately after 4-weeks following EDS treatment. Our results indicate that more than 4-weeks period is needed for total testis and Leydig cell recovery. We suggest that, several mechanisms are responsible for regeneration of Leydig cells. First; Leydig cells are capable to reorganized probably by other type/or types of cells, or differentiating of new Leydig cell population from precursors is related to LH. Second; mast cells may play role in regeneration. Further work is needed to explain these mechanisms.

Testise spesifik toksik etkili ethane dimethanesulphonate'ın (EDS) sıçan testisi leydig hücrelerinde yaptığı bozukluklar ve mast hücresi çoğalması

Bu çalışmada, testise toksik etkili ethanedimethane sulphonat'm (EDS) Leydig hücreleri ve testis dokusuna yaptığı etkiler EDS enjeksiyonundan 1,2,3,7,14,ve 28 sonra ışık mikroskopi ve histokimya metoduyla incelendi. EDS uygulamasından 2 gün sonra Leydig hücrelerinde organizasyon bozukluğu, piknoz ve dejenerasyon görüldü. EDS'nin bu hücrelerdeki en şiddetli etkisi ise 3. günde belirlendi. Leydig hücreleri 3. günde intertisyumdan tamamen yok olmuşlardı. Leydig hücrelerindeki bozulma makrofajların fagositozuyla devam ederek sonuçta bunların ortadan kalmalarına neden oluyordu. 7. günde testis bozulmaya devam ediyordu. 14. günde ise tubullerde atrofi, spermatogenezin durduğu ve lizis görüldü. 28 gün sonra ise testisin tamamına yakını düzelmisti ancak Leydig hücreleri az sayıda görüldüler, intertisyumda yeralan fibroblast ve mast hücrelerinin sayıca artışı, bunların hem birbirleriyle ve hem de testis dokusuyla ilişkisi olduğunu düşündürdü. Sonuç olarak, EDS'nin testisteki etkisi geriye dönüşlüdür. Leydig hücrelerinin bozulmasından sonra dokuda yeni Leydig hücreleri ortaya çıkmaktadır. [Türk J MedRes 1995; 13(6): 165-171]

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