The role of vitamin E in reducing aluminum hydroxide effects on testes of albino rats: a histological and immunohistochemical study

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ABSTRACT: Objective: The objective of this study were to examine the histological changes in the testis after the administration of aluminum hydroxide and the possible protective role of vitamin E. Materials and methods: Thirty adult male albino rats were divided into three equal groups: control group; Aluminum hydroxide treated group received oral aluminum hydroxide at a dose of 30 mg/kg b. wt/day; (protected group) received both aluminum hydroxide (at the same previous dose) and an intraperitoneal injection of vitamin E at 50 IU/kg b. wt. At the end of the experiment (8 weeks), all animals were sacrificed and their testes were excised. Paraffin sections were prepared and stained with H&E, Periodic acid Schiff and immunohistochemical staining for proliferating cell nuclear antigen (PCNA). Morphometric and statistical analyses were carried out. Results: After the administration of aluminum hydroxide, some seminiferous tubules had disturbed basement membrane. The spermatogenic cells decreased in number to few layers and hyaline materials appeared within some tubules. Decreased PAS reaction and PCNA immunostaining and a significant decrease in the tubular parameters were recorded. The concomitant administration of vitamin E with aluminum hydroxide showed noticeable alleviation in histopathological changes induced by aluminum hydroxide in the structures of testis, increased PAS reaction and PCNA immunostaining and a marked increase in tubular parameters. Conclusion, the present study showed that exposure to aluminum hydroxide resulted in marked degenerative effects on the rats’ testis and using vitamin E cannot completely prevent these effects of aluminum hydroxide, but it decreased to some extent the degenerative changes observed in testicular tissues.

Key words: Aluminum hydroxide, Vitamin E, PCNA, Rat, Testicular damage.

INTRODUCTION

Aluminum (Al) is one of the highly abundant elements in the environment and the most common metal and the third most elements in the earth crust (1). The wide distribution of this element ensures the potential for causing human exposure and harm (2). Al compounds are widely used in medicines as antacid, vaccines, antidiarrhoeals, phosphate binders and allergen injections (3), food additives and tooth paste (4), and water purification agents (5). It is still a metal of choice in making various kinds of household cookware and storage utensils.

Al contributes to a variety of cognitive impairments in mice, rabbits, and rat pups (6). Epidemiological studies have indicated a link between Al in drinking water and Alzheimer’s disease and a variety of human and animal studies have implicated learning and memory deficits after Al exposure (7,8). Aluminum chloride is able to generate reactive oxygen species (ROS) (9 ,10). Chinoy et al. (11) reported that Al induced toxicity in epididymis, vas deferens, seminal vesicle, and ventral prostate of mice. AlCl₃-induced free radicals and inhibited antioxidant enzymes in blood and seminal plasma, liver, testes, kidney, lung, and brain of rabbits (9,12).

Oxidative stress has been shown to play an important role in causing male infertility by inducing defects in sperm functions. Excessive production of ROS causes oxidative stress in spermatozoa (13). ROS are central to a host of pathologies, including inflammation, toxicity, and endocrine disruption by environmental chemicals. ROS damage almost all macromolecules of the cell causing impairment of cellular functions.

ROS, such as hydrogen peroxide (H₂O₂), appear to be a key agents causing cytotoxicity in spermatozoa to produce oxidative stress by decreasing the enzymatic defenses (14). ROS are degraded by the organized system of antioxidants. Antioxidants have been described as substances that either directly or indirectly protect cells against adverse effects of xenobiotics, carcinogens, drugs, and toxic agents. Since both spermatogenesis and Leydig steroidogenesis are vulnerable to oxidative stress, the low oxygen tension that
characterizes this issue may be an important component of the mechanisms by which the testis protects itself from free radical mediated damage (15).

The function of Vitamin E (vit. E), has been attributed to its capacity to protect the organism against free-radical attacks by acting as a lipid-based radical chain-breaking molecule. Studies have shown the protective/ameliorative effects of vit. E against the reproductive toxicity of various toxicants. For instance, vit. E treatment is shown to ameliorate aflatoxin-induced changes in the testis of mice (15). In addition, vit. E cotreatment has a protective role against mercury-induced reproductive toxicity in male mice (16). Moreover, vit. E has anti-alkylating properties and protects cells against peroxynitrite-induced lipid oxidation (16). The requirement for vit. E for normal testicular function is well established (17). Vit. E, especially important for normal reproduction, was originally considered a dietary factor for animal nutrition (18).

The present study is planned to evaluate the role of vitamin E as a protective agent against testicular toxicity associated with aluminum hydroxide in male albino rats.

**MATERIALS & METHODS**

**Experimental animals**

Thirty adult male albino rats (*Rattus norvegicus*), each weighing 140 – 160 g, were used in the present study. They were kept under observation for two weeks before the beginning of the experiment to exclude any intercurrent infection. The chosen animals were housed in metal cages at room temperature (25 ± 5 °C) and 12 hr daily normal light periods, under good ventilation and received food and water *ad libitum*. They were divided into three equal groups, each of 10 rats as follows: The 1st group (G1) served as untreated control. The 2nd group (G2) was orally treated with aluminum hydroxide (30 mg/ kg b.wt) according to El-Ashtokhy *et al.* (19), the 3rd group (G3) was orally treated with the same dose of aluminum hydroxide as in the 2nd group and injected intraperitoneally with vitamin E at a dose of 50 IU/kg b. wt. according to Al-Masri (20). At the end of the experiment (8 weeks), ten rats from each of the experimental groups were fasted and sacrificed under anesthesia.

**Chemicals and drugs**

Aluminum hydroxide was purchased from Kahira Pharma and Chem. InD Co., Cairo, Egypt. It was prepared immediately before use by dissolving it in distilled water. Vitamin E was purchased from El-Gomhoria Company (Cairo, Egypt). It was given intraperitoneally and daily.

**Histological preparations**

**Preparation of paraffin sections**

For the histological preparations, animals were anaesthetized under light diethyl ether and dissected to remove the left testis at the end of the 8th week of treatment. Testicular tissues were cut into small pieces and then fixed in 10% neutral buffered formalin for 24 hours. The tissue was routinely processed and sectioned at 4 to 5 μm thickness with a microtome and stained with haematoxylin and eosin for histopathological studies and Periodic Acid Schiff's technique for carbohydrates distribution (21).

**Proliferating Cell Nuclear Antigen**

Immunohistochemical locations of proliferating cell nuclear antigen (PCNA) were performed in paraffin sections (4 μm thick) to determine proliferating cells. For this, representative slices were deparaffinized, rehydrated and immunostained by the peroxidase anti-peroxidase method. High temperature antigen unmasking technique was employed in 0.01M citrate buffer pH 6.0 in microwave oven, twice for 5 min each. Blocking of non-specific reaction was performed with 1% normal goat serum and 3% non-fat milk, and PCNA mouse monoclonal antibody (Novo Castra NCL-PCNA) (1:100). After rinsing in phosphate buffered saline (0.01mol/l PBS, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS and incubated in ABC (avidine and biotine complex) reagents ABC-kit-Vector) and incubated in peroxidase reaction (3,3'-diaminobenzidine tetrahydrochloride, Sigma) containing 0.01% H₂O₂ in PBS buffer. For the control reaction, some slides were processed omitting the primary antibody and other slides omitting the primary and secondary antibodies (22). Proliferating cell nuclear antigen (PCNA) appeared as brown nuclear staining.

**Morphometric Analysis**

The diagonal diameter of seminiferous tubules, the thickness of testicular epithelium, perimeter of the nuclei, the number of interstitial cells and the area% of proliferating cell nuclear antigen reaction were estimated. The data were obtained using a Leica Qwin 500 Image Analyzer Computer System (Cambridge, England, UK) at the Histology Department, Faculty of Medicine, Ain Shams University. The procedure was performed using H&E and immunohistochemical stained sections. Measurements were performed with 10 non overlapping fields for each rat of four randomly chosen rats of each group at X 200 magnification.
Statistical analysis

The statistical analysis of the measured data was carried out using the Statistical Package for Social Sciences (SPSS) for Windows version 12.0 software. All data were represented as mean ± standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA) followed by LSD (least significant difference) for comparison between groups. P values less than 0.05 (P < 0.05) were considered to be statistically significant (23).

RESULTS

Histopathological study

Testes of control rats showed normal features of testicular tissue as illustrated in figures (1a &b). The testicular parynchyma is formed of seminiferous tubules lined by stratified germinal epithelium and covered with thin basal laminae. They are separated by interstitium containing clusters of interstitial cells and blood vessels (Fig.1a). Each tubule possesses epithelial cells involved of Sertoli cells and the germ cells of various stages, covering the complete process of spermatogenesis (Fig. 1a&b). Sertoli cells exhibit typical irregular nuclei and well-defined cytoplasm. Spermatogonia are oval in shape and rest upon the basal lamina of the seminiferous tubule. Immediately above them are spherical primary spermatocytes, recognized by their copious cytoplasm and large nuclei containing coarse clumps of chromatin. Secondary spermatocytes are not seen in these sections due to rapid division processes. Therefore, above the primary spermatocytes, there are large clusters of small rounded spermatids with rounded nuclei devoid of coarse clumps of heterochromatin, followed by elongated spermatids which undergo dramatic changes, forming spermatozoa. The seminiferous tubules showed normal features with successive stages of transformation of spermatogonia into spermatozoa (Figs. 1a&b).

In aluminum hydroxide treated rats, testicular sections revealed loss of histological architecture of seminiferous tubules. Some seminiferous tubules had disturbed basement membrane, disorganized germinal epithelium and interstitial vacuolation (Fig. 1c& d). Most of the spermatogenic cells were undifferentiated and separated from the basement membrane with small size, deeply stained nuclei and multiple intercellular spaces were also observed (Figs. 1c & d). In other tubules, the spermatogenic cells decreased in number to few layers, consequently the lumen of the tubules appeared wide and contained desquamated epithelial cells and no sperms were observed (Fig. 1e). Hyaline materials appeared within some tubules and thickened blood vessels were also observed (Fig.1f).

Sections of testicular tissues obtained from adult rat treated with aluminum hydroxide and vitamin E revealed better histological structure than those of aluminum hydroxide treated rats (Figs. 1g & h). The seminiferous tubules were separated by large amount of hyaline material (Fig.1g). The basement membranes of seminiferous tubules were intact and regular. Vacuolated germ cells and sertoli cells were observed and free spermatozoa appeared near tubular lumina of some seminiferous tubules (Figs.1h).

Figure 1: Photomicrograph of a transverse section of a) control rat testis showing parts of seminiferous tubules (T) lined with stratified germinal epithelium (Ge) and surrounded by interstitium (arrow). b) Higher magnification of (a) showing stratified germinal epithelium formed of spermatogonia (Sp), Sertoli cells (S) primary spermatocytes (P), rounded spermatids (Rs) mature spermatids (Ms) and free spermatozoa (Z). Note the seminiferous tubules covered with thin basal lamina (arrow) and interstitial cells of Leydig (L). c) testis of a rat treated with aluminum hydroxide showing some seminiferous tubules have disturbed basement membrane (arrow) and disorganized germinal epithelium. d) testis of a rat treated with aluminum hydroxide showing undifferentiated germ cells with small size and deeply stained nuclei ( arrow), multiple intercellular spaces (*) and interstitial vacuolation (arrow). Note the presence of free spermatozoa (Z) (H& E; a,c; X 100 and b,d; X 400).
Figure 2: Photomicrograph of a transverse section of e) testis of a rat treated with aluminum hydroxide showing loss of the normal arrangement of seminiferous germinal epithelium with multiple intercellular spaces, maturation arrest (Ma), desquamated epithelial cells in the lumen (arrow) and some of them filled with hyaline material (H) f) Higher magnification of (e) showing decreased germinal epithelium thickness (Ge), germ cells are undifferentiated from each other with small size deeply stained nuclei surrounded with thin rim of cytoplasm (arrow). Note the central area of seminiferous tubule filled with hyaline material (H) and thickened wall of blood vessels (Bv). g) testis of a rat treated with aluminum hydroxide and vitamin E showing the seminiferous tubules covered with intact capsule (Ca) and separated from each other by large amount of hyaline material (H). h) Higher magnification of (g) showing thickness of germinal epithelium (Ge) with vacuolated germ cells (arrow) and sertoli cell (S). Note the presence of mature spermatids (M) and free spermatozoa (Z) (H&E; e,g X 100 and f,h; X 400).

**Periodic Acid-Schiff’s (PAS) method**

In the testes of control untreated rats, strong PAS positive reaction was mainly localized in the capsule, basement membrane of seminiferous tubules and in interstitial cells of Leydig (Figs. 2 a& b). The testes of aluminum hydroxide treated rats showed marked depletion of PAS-positive materials in the basement membrane of seminiferous tubules, spermatogenic cells and in the interstitial cells of Leydig (Figs. 2c&d.). On the other hand, treatment with vitamin E and aluminum hydroxide showed a marked increase of PAS-positive materials in the capsule, basal lamina and interstitium (Figs. e& f).

**Immunohistochemistry: (Proliferating cell nuclear antigen immunostaining)**

Testicular tissues of control rats showing positive immunostaining (brown nuclear reaction) in most of the basal germ cells and leydig cells (Figs. 3 a, b). Sections of testicular tissues obtained from rats treated with aluminum hydroxide showed that, most of germ cells and interstitial cells of Leydig were negatively stained (Figs. 3c& d). In vit. E-treated group, germ cells and interstitial cells showed marked increase in immunopositive material (Figs. 3e& f).
Figure 3: Photomicrograph of a transverse section of a& b) control rat testis showing PAS positive material appeared in capsule (Ca), basement membrane of seminiferous tubules (arrow) and in the interstitial cells of Leydig (L) (PAS; a: X 100; b: X 400). c& d) testis of a rat treated with aluminum hydroxide showing decreased PAS positive materials in the capsule (Ca), basement membrane of seminiferous tubules (arrow) and in the interstitial cells of Leydig (L) (PAS; c: X 100; d: X 400). e& f) Photomicrograph of a transverse section in the testis of a rat treated with aluminum hydroxide and vitamin E showing marked increase in PAS positive material in capsule (Ca) and interstitial spaces. (PAS; a: X 100; b: X 400).

Figure 4: Photomicrograph of a transverse section of a) and b): testis of a control rat showing positive immunostaining (brown nuclear reaction) in most of the basal germ cells in the seminiferous tubules (arrows) and interstitial cells (L). c and d). testis of a rat treated with aluminum hydroxide showing few immunopositive germ cells e): testis of a rat treated with aluminum hydroxide and vitamin E showing marked increase in immunopositive material in the germ cells and interstitial cells f) Higher magnification of (e) showing numerous immunopositive cells in germ cells (arrows) and interstitial cells (L) (PCNA immunohistochemical staining; a,c,e X 100 and b,d,f).
**Morphometric analysis**

The main diagonal diameter of seminiferous tubules

Statistical analysis of the results showed a significant decrease in the main diagonal diameter of seminiferous tubules in aluminum hydroxide-treated group (G2), when compared with the control group (G1) (P ≤ 0.05). Moreover, there was highly significant increase in the main diagonal diameter of seminiferous tubules of rats administrated aluminum hydroxide and vitamin E (G3) when compared with that of aluminum hydroxide-treated rats (G2) (P≤0.05) (Table 1 and Graph 1).

### Table 1. Mean values of the diagonal diameter of seminiferous tubules:

<table>
<thead>
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<th>Mean ± SD</th>
<th>Range</th>
<th>ANOVA</th>
<th>P value</th>
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<td>262.3±5.86</td>
<td>258-269</td>
<td>20.6</td>
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<tr>
<td>Treated group</td>
<td>216.6±14.43</td>
<td>200-225</td>
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<tr>
<td>Protected group</td>
<td>297±17.57</td>
<td>284-317</td>
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Histogram 1. Diagonal diameter of seminiferous tubules

**Counting number of the interstitial cells**

Statistical analysis of the results showed a significant decrease in the number of interstitial cells in aluminum hydroxide-treated rats (G2) compared with control group (G1) (P≤0.05). Moreover, the number of interstitial cells showed, a highly significant increase in rats treated with aluminum hydroxide and vitamin E (G3) as compared with the control group (G1) (p≤ 0.05) (Table 2 and Graph 2).

### Table 2. Mean values of the interstitial cell number:

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<th>Range</th>
<th>ANOVA</th>
<th>P value</th>
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<td>Control group</td>
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<td>15-19</td>
<td>33.3</td>
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<tr>
<td>Treated group</td>
<td>11±1</td>
<td>11-13</td>
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<td>Protected group</td>
<td>25±3</td>
<td>22-28</td>
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Histogram 2. Number of interstitial cells
Measuring Perimeter of the nuclei in the cells of seminiferous tubules:

Statistical analysis of the results showed non-significant decrease of the perimeter of the nuclei in the cells of seminiferous tubules of aluminum hydroxide (G2) and aluminum hydroxide plus vitamin E treated rats (G3) when compared with control group (G1) \((p \geq 0.05)\). On the other hand, there was a significant increase in the perimeter of the nuclei in rats administrated aluminum hydroxide and vitamin E (G3) when compared with that of aluminum hydroxide treated group (2) \((p < 0.05)\) (Table 3 and Graph 3).

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
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<th>(P) value</th>
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<td>Control group</td>
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<td>43-48</td>
<td>115.17</td>
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<tr>
<td>Treated group</td>
<td>17±1</td>
<td>16-19</td>
<td></td>
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<td>Protected group</td>
<td>28±1</td>
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Table 3. Mean values of Perimeter of the nuclei:

The main thickness of testicular epithelium (tubular wall thickness)

Statistical analysis of the results showed significant decrease in the tubular wall thickness of aluminum hydroxide-treated rats (G2), when compared with the control group (G1) \((P \leq 0.05)\). On the other hand, there was a significant increase in the tubular wall thickness in rats administrated aluminum hydroxide and vitamin E (G3) when compared with aluminum hydroxide-treated rats (G2) \((P \leq 0.05)\) (Table 4 and Graph 4).

<table>
<thead>
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<th></th>
<th>Range</th>
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<th>ANOVA (Test of significance)</th>
<th>(P) value</th>
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<td>74±1.15</td>
<td>73-75</td>
<td>51.99</td>
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<td>Treated group</td>
<td>52.6±2.5</td>
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<td>Protected group</td>
<td>86.3±5</td>
<td>284-317</td>
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Table 4. Mean values of the thickness of testicular epithelium
**Area % of PCNA Results**

Statistical analysis of the results showed a very highly significant decrease in area % of PCNA of aluminum hydroxide-treated rats (G2) when compared to the control group (G1) ($P \leq 0.05$). There was highly significant increase of the area % of PCNA of aluminum hydroxide and vitamin E treated rats (G3) when compared to the corresponding testis of aluminum hydroxide group (G2) ($P \leq 0.05$). However, there were non significant difference in area % of PCNA of aluminum hydroxide and vitamin E treated rats (G3) when compared to the control group (G1) ($P \geq 0.05$) (Table 5 and Graph 5).

<table>
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<th>Group</th>
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<th>Range</th>
<th>ANOVA (Test of significance)</th>
<th>$P$ value</th>
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<td>Control group</td>
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<tr>
<td>Treated group</td>
<td>5.3±0.57</td>
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<td></td>
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<tr>
<td>Protected group</td>
<td>16.6±3</td>
<td>14-20</td>
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Histogram 5. Area % of proliferating cell nuclear antigen reaction.

**DISCUSSION**

The present histological study of rats orally administered aluminum hydroxide showed testicular damage, which lead to spermatogenic arrest. The seminiferous tubules were distorted with undifferentiated germinal epithelium. The spermatogenic cells were undifferentiated and separated from the basement membrane. The spermatogenic cells decreased in number to few layers, consequently the lumen of the tubules appeared wide and contained desquamated epithelial cells and no sperms were observed. Thickened blood vessels and hyaline materials appeared within most of the tubules. This results confirmed statistically by significant decrease in the tubular wall thickness of aluminum hydroxide -treated rat, when compared with the control group ($P \leq 0.05$). Most of the spermatogenic cells were undifferentiated and separated from the basement membrane with small size, deeply stained nuclei. Moreover, significant decrease in the number of interstitial cells in aluminum hydroxide-treated rats (G2) compared with control group (G1) ($P \geq 0.05$). In addition, significant decrease in the main diagonal diameter of seminiferous tubules in aluminum hydroxide-treated group, when compared with the control group ($P \leq 0.05$). The impairment caused by aluminum hydroxide was accompanied primarily by the prolonged accumulation of aluminum in the rat testes. Mahran et al. (24) reported a histological perturbation, in rats’ testes after aluminum chloride treatment, including severe damage within the seminiferous tubules and vacular degeneration on the spermatogenic and Sertoli cells cytoplasm.

Guo et al., (25) suggested that the nitric oxide produced by aluminum is responsible for allowing aluminum to enter the tight junctions that form the inter-Sertoli (so called blood-testis) barrier and accumulate in the testis. After entering into the testis it damage germ and Sertoli cells, damaging the seminiferous epithelium, with a decrease in its height, thus altering normal spermatogenesis and sperm production (3,26). Kutlubay et al. (27) observed that thinner germinal epithelium of the seminiferous tubules and almost absence of spermatids and sperm numbers in the lumen in Al-treated rats.

A great depletion of PAS-positive materials in the capsule, basement membrane of seminiferous tubules and in the interstitial cells of Leydig were observed. These results were in agreement with published data which found that after 2 weeks of aluminum treatment, deleterious effects and histopathological changes of testicular tissues were observed (28).
Depletion in testicular glycogen after aluminum hydroxide treatment was possibly attributed to the inhibition of phosphorylase activation or the depletion of certain other enzymes which could block androgen synthesis (29). A fall in glycogen level may be due to interference in glycogenolysis. Since glycogen is an energy source for general metabolism and constant supply of glucose is essential for proper functioning of testes (30).

In the current study, immunohistochemistry was used to map the distribution of PCNA its staining intensity was used to evaluate the proliferation of cells and the spermatogenic function of testes in case of male infertility (31). It has been reported previously that PCNA is differentially expressed during the cell cycle and reaches its maximum level during late G1/S phases and begins to decrease during late G2/M to G1 phases. Therefore, PCNA is considered a useful molecular marker for the assessment of germ cell kinetics (32).

In the present study, PCNA immunostaining was determined in basal germ cells of seminiferous tubules and Leydig cells in all groups except aluminum hydroxide group. Statistical analysis of the present results showed a decrease in area % of PCNA after aluminum hydroxide treatment. The decrease of PCNA immunostaining in aluminum hydroxide group may indicate that aluminum had an inhibiting effect on the proliferative activity in the seminiferous tubules. In the testes of aluminum hydroxide treated rats, most cells of seminiferous tubules and interstitial cells of Leydig were negatively stained. The decrease of PCNA in aluminum hydroxide group in comparison to the control group may indicate that the aluminum hydroxide had an inhibiting effect on the proliferative activity in the seminiferous tubules. This situation can be interpreted in the light of existing literature (33). Less PCNA expression in testicular epithelium was related to decreased DNA synthesis in the damaged testes (31).

Other authors have added that DNA synthesis assessed by PCNA is very useful in the pathological diagnosis of infertility, especially for the differentiation of hypospermatogenesis from partial germinal arrest (34).

The concomitant administration of vitamin E with aluminum hydroxide showed noticeable alleviation in histopathological changes induced by aluminum hydroxide in the structures of testis. The basement membranes of seminiferous tubules were intact and regular, vacuolated germ cells and sertoli cells were observed; free spermatozoa appeared near tubular lumen of some seminiferous tubules. Moreover, there was highly significant increase in the main diagonal diameter of seminiferous tubules, thickness of tubular epithelium, number of interstitial cells of rats, increase in the perimeter of the nuclei, compared to aluminum hydroxide-treated rats. The present results are in agreement with Kutlubay (27) who recorded that administration of vitamin E to aluminum hydroxide treated rats produced a marked increase in tubular parameters in testes. In the aluminum plus vitamin E treated rats, there were large numbers of spermatids and sperm in the seminiferous tubule lumen. Vitamin E has been proven to protect testicular tissues against experimental cryptorchidism in rats (35).

The primary mechanism of this effect of vitamin E may involve the scavenging of free radicals that cause lipid peroxidation. Vitamin E decreased the levels of free radicals and increased the antioxidant enzymes in plasma and different tissues of rats treated with aluminum (36). Moreover, The protective effect of vitamin E against the testicular toxicity of aluminum hydroxide showed moderate improvement after vitamin E administration and this may be due to the activity of vitamin E as antioxidant (37). The previous studies showed a decrease in the plasma and tissue (liver, kidney and brain) malondialdehyde (MDA) levels, and an increase in the antioxidant enzyme parameters (SOD, CAT, and GSH-PX) of animals that were administered vitamin E in association with a mixture of heavy metals (Pb, Hg, Cd and Cu), in comparison to the group that was administered a mixture of heavy metals alone (38).

The present results showed strong PAS reaction in capsule, basement membrane of seminiferous tubules and in interstitial cells of Leydig after treatment with vit.E. The present data were consistent with other workers (39), who reported that administration of vitamin E showed marked amelioration of nicotine testicular toxicity and increased PAS reaction in rat testes. In contrast, ethanol-vitamin E-treated rats decreased glycogen droplet in the seminiferous cells (40).

The administration of vitamin E protects against heavy metals-induced testicular oxidative stress and injuries (38). Vitamin E allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radical reactions, resulting in a marked decrease in the reactivity of free radicals (41).

The insufficient protective effect of vitamin E against aluminum hydroxide can be explained by Yalcinkaya et al. (42) who compared the antioxidant capacity of melatonin and vitamin E and determined that antioxidant capacity of both agents were dose-dependent. Vitamin E at 100 mg/kg b. wt was found to be ineffective to protect testicular tissues against radiation-induced damage. In addition, Partial protective role of vitamin E was recorded earlier during ethane dimethane sulfonate-induced testicular toxicity in rats (43).

CONCLUSIONS

The present study showed that exposure to aluminum hydroxide resulted in degenerative effects on the rats’ testes, and using vitamin E cannot completely prevent these effects of aluminum hydroxide, but it decreased to some extent the degenerative changes observed in testicular tissues.

