Original Article

Histological and Ultrastructure Study of the Testes of Acrylamide Exposed Adult Male Albino Rat and Evaluation of the Possible Protective Effect of Vitamin E Intake

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Abstract

Acrylamide (AA) is a hazardous unavoidable gonadal toxin. Hence, the aim of this study is to clarify its harmful effects on the testis of adult albino rat by light and electron microscope and to evaluate the possible role of Vitamin E (Vit E) in the prevention of such effects. Thirty-five adult male albino rats were enrolled in this study. They were divided into three groups: Group I (control); Group II (AA exposed), and Group III (AA and concomitant Vit E treated group). Animals of Groups II and III were further subdivided into two equal subgroups (each subgroup included five rats): (a) rats were sacrificed after 4 weeks and (b) rats were sacrificed after 6 weeks. The testes of each rat were dissected out, processed, and examined by Hematoxylin and Eosin, Periodic acid–Schiff and Mallory's trichrome stains as well as electron microscopic study. The study revealed that AA induces testicular damage at the histological and ultrastructural level in the form of degeneration and arrested spermatogenesis. Moreover, decreased seminiferous tubules diameters and epithelial height were detected. These changes are maximally improved in Vit E treated group. Hence, we could conclude that AA causes degenerative changes of the testes of albino rats and arrest of spermatogenesis. The AA-induced histological and ultrastructural changes of the testes could be explained by oxidative stress. These effects changes are proportional to the duration of exposure. Moreover, it could be concluded that Vitamin E has a protective role against AA-induced testicular damage by its antioxidant and anti-apoptotic effects.

Keywords: Acrylamide, EM, testes, Vitamin E

Introduction

Acrylamide (AA) is a solid chemical compound (C3H5NO, and MW 71.08.AA). Its monomer form is highly toxic while its polymeric form is nontoxic.[1]

AA release to the environment may occur during poly AA production. Occupational exposure to AA is chiefly due to dermal contact during handling bags of the chemical, followed by inhalation of aerosols or dust.[2] Smoking is considered as another source of human inhalation exposure.[3]

The interest of the study of the health hazards of ACR is due to its multiple sources and unavoidable methods of exposure such as in drinking water, inhalation, skin absorption, and others.[4,5] In addition to the reported ACR effects as it is documented that it is neurotoxic,[6] gonadotoxic[7] and involved in carcinogenesis in experimental animals.[8]

The mostly acceptable mechanism of the harmful effects of AA is the oxidative stress and oxidative damage to critical macromolecules such as DNA and proteins besides cell damage or death.[9,10]

AA-induced testicular toxicity was studied in rodents.[11] Abnormalities of hormones of pituitary gland and testes as well as ultrastructure alterations were detected in these exposed animals. Moreover, impairment of testicular functions was reported in the form of diminished sperm concentration, increased sperm deformity index, reduced fertility potentials, and pregnancy rate.[12,13]

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The powerful antioxidant Vitamin E (Vit E), could have a protective effect on AA-induced damage of testicular tissue.[14] It has a protective effect at the cellular and systemic levels as it scavenges free radical and plays an important role in mutagenesis as well as repair of injury convinced by reactive oxygen species (ROS).[5,15]

Hence, the aim of the existing study is to elucidate the harmful effects of AA on the testis of adult albino rat by light and electron microscope and to assess the potential role of Vit E in prevention and/or reducing such effects.

Material and Methods

Chemicals

- AA monomer dry crystals (C3H5NO; 99.9% purity) was obtained from sigma chemical company
- Vit E pure in the form of gelatinous capsules (400 mg/capsule) was obtained from sigma chemical company.

Experimental animals

Thirty-five adult male albino rats (180–200 g in weight) were brought from the Nile center for researches. The animals were acclimatized for 2 weeks before the experiment. They were kept in plastic cages under adequate temperature and ventilation. All rats were provided with free access to water and food with a 12 h light/dark cycle.

Experimental design

The rats were classified into three groups:

- Group I (control group) comprised 15 rats. They are subdivided into three identical subgroups (each subgroup included five rats):
  - Subgroup Ia: Rats fed on basal diet and received saline intraperitoneally (IP) in a daily dose 10 ml/kg b. w for 6 weeks
  - Subgroup Ib: Rats fed on basal diet and received corn oil orally by gavage in a daily dose 4 ml/kg b. w for 6 weeks
  - Subgroup Ic: Rats fed on basal diet and received Vit E dissolved in 1 ml of corn oil orally for 6 weeks in a daily dose 400 mg/kg B. W. by nasogastric gavage.[15,16]

Animal sacrification was carried out by the end of the 6th week.

- Group II: (AA group): included 10 rats fed on basal diet and given AA dissolved in saline IP in a daily dose 10 mg/kg B. W.[17] Animals were further subdivided into two equal subgroups (each subgroup included five rats) according to the time of experiment termination:
  - Subgroup IIa: after 4 weeks from the onset of AA intake
  - Subgroup IIb: after 6 weeks from the onset of AA intake.
- Group III (AA and concomitant Vit E treated group): included 10 rats fed on basal diet and received AA and Vit E in concomitant with the same previously mentioned doses. Animals were further subdivided into 2 equal subgroups (each subgroup included five rats) according to the time of experiment termination:
  - Subgroup IIIa: at the end of the 4th weeks from the onset of AA and Vit E intake
  - Subgroup IIIb: at the end of the 6th weeks from the onset of AA and Vit E intake.

At the appropriate time, the testicles were dissected and prepared for light and electron microscopic studies.

Sample preparation and microscopic study

For light microscopic study: paraffin sections (5 μm thick) from testicular specimens were prepared and stained with Hematoxylin and Eosin (Hand E), Periodic acid–Schiff (PAS) and Mallory's trichrome stains.

For electron microscopic study: (1) Fixation of very small pieces (2 mm²) of the testicular sample in a mix of 2.5% glutaraldehyde and 2.5% paraformaldehyde (pH, 7.3) was carried out. (2) Semithin sections (1 μm) preparation and staining with toluidine blue (1%). (3) Finally, ultrathin sections (60–80 nm) were also, prepared, double-stained with uranyl acetate and lead citrate for examination and photographing with transmission electron microscope.

Morphometric study and statistical analysis

The diameter and epithelial height of seminiferous tubules were measured after 4 and 6 weeks of the experiment.

1. Slides were photographed by Olympus®digital camera (Olympus®microscope with 1/2X photoadaptor and ×40 objective). Then, the images were analyzed (Intel®CoreI3®based computer using Video Test Morphology®software [Russia]) with a specific built-in routine for distance measurement
2. Five slides from each rat were prepared, five random fields from each slide were analyzed
3. All measurements were calibrated against a micrometers slide which had been photographed with the same system under the same magnification. This enables us to obtain the measurements in μm instead of pixels
4. In each image, five measurements for the thickness of epithelium and seminiferous tubule diameter for the same tubule were taken in different places using the manual line tool
5. Data were exported as Excel sheet.

Data were analyzed using SPSS program (Statistical package for social science-version 17.0). Data are presented as a mean ± standard deviation. One-way analysis of variance is the test used to compare between more than two groups followed by *post hoc* Tukey test. $P < 0.05$ is the statistically significant value.[18]

Results

Histological results

Hematoxylin and eosin-stained sections

Group I

H and E-stained testicular samples of the negative control group (subgroup Ia), corn oil treated group (subgroup Ib), and

Vit E treated group (subgroup Ic) revealed the same histological features of the testis. The testicle appeared covered by a capsule of connective tissue (the tunica albuginea). Testicular parenchyma consisted of seminiferous tubules which appeared rounded or oval with regular contour. The interstitial spaces in-between the tubules contain a delicate loose C. T and Leydig cells [Figure 1].

The seminiferous tubules were enclosed by a basement membrane encircling myoid cells and lined by stratified spermatogenic cells and supporting Sertoli cells. In control rats, complete spermatogenesis was established, spermatogonia were seen close to the basement membrane with their dark nuclei, primary spermatocytes were the largest cells, the spermatids appeared smaller than primary spermatocytes and lying near the lumen. The lumen enclosed large sperms, and some of these sperms were attached to the apex of Sertoli cells. Sertoli cells appeared tall cells with pale nucleus with prominent nucleolus [Figure 2].

Group II (acrylamide exposed group)

Subgroup IIa (after 4 weeks) H and E-stained sections of 4 weeks AA exposed rats' testicles revealed the irregular outline of the seminiferous tubules. Many degenerating and apoptotic germ cells were observed. The basement membrane was thickened and irregular. Interstitial cells of Leydig had scanty cytoplasm with deeply stained or normal vesicular nuclei [Figure 3].

Subgroup IIb (after 6 weeks)

In this group, there was a complete loss of the normal histological structure of seminiferous tubules with complete degeneration of spermatogenic cells apart from some spermatogonia. Apoptotic cells were also detected. The basement membrane was thickened and irregular. Interstitial cells of Leydig had scanty cytoplasm with deeply stained nuclei [Figure 4].

Figure 1: Photomicrograph of control rat testis. It is covered by "tunica albuginea" (arrow) and contains seminiferous tubules (T) that appear circular with regular contour. The interstitium (I) contains a delicate loose C. T and Leydig cells (H and E, ×100)

Group III (acrylamide and concomitant Vitamin E treated group)

Subgroup IIIa (after 4 weeks)

Examination of the treated testis in this group revealed that the architecture of seminiferous tubules was nearly preserved. The basement membrane of tubules was slightly corrugated. In many tubules, there were normal spermatogonia, normal primary spermatocytes, and Sertoli cells with few attached mature sperms. Interstitial cells of Leydig had scanty cytoplasm and deeply stained or normal vesicular nuclei [Figure 5].

Subgroup IIIb (after 6 weeks)

H and E-stained sections of the testis of rats receiving AA and Vit E for 6 weeks revealed that the outline and architecture of most of the seminiferous tubules were highly preserved with enhanced spermatogenesis and normal appearance of spermatogonia, primary spermatocytes, and spermatids. In addition, Sertoli cell appeared normal with a lightly stained nucleus and prominent nucleolus. Interstitial cells of Leydig seemed to be normal with normal vesicular nuclei [Figure 6].

Periodic acid–Schiff's -stained sections

Group I

PAS-stained sections of the testicles of the control rats showed intense magenta red (PAS + ve) material in the basement membrane of the seminiferous tubules. The nuclei of early spermatids showed + ve PAS's particles at one pole. They were recognized as the acrosomic vesicles. Numerous elongated spermatids were observed attached to the apex of Sertoli cell with strong PAS +ve reaction [Figure 7].

Group II (acrylamide exposed group)

PAS-stained testicular sections of animals of

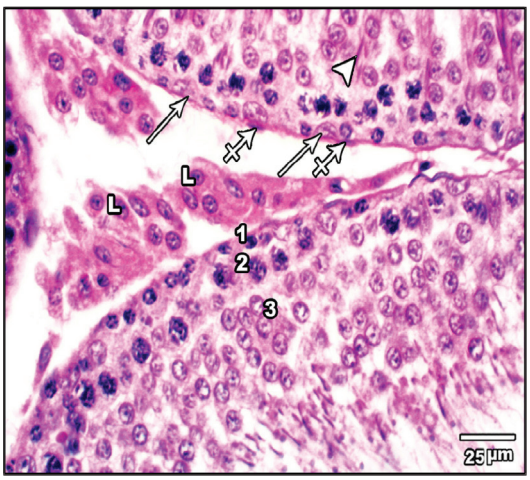

Figure 2: Photomicrograph of control rat testis showing seminiferous tubules lined with series of spermatogenic cells; spermatogonia,(1) primary spermatocytes (2) and round (early) spermatids.(3) Sertoli cells (arrow) are seen with attached sperms (arrow head). Tubules are surrounded by basement membrane enclosing myoid cells (crossed arrows). The interstitial spaces in-between the tubules contains interstitial cell of Leydig (L) having vesicular nucleus with prominent nucleolus (H and E, ×400)

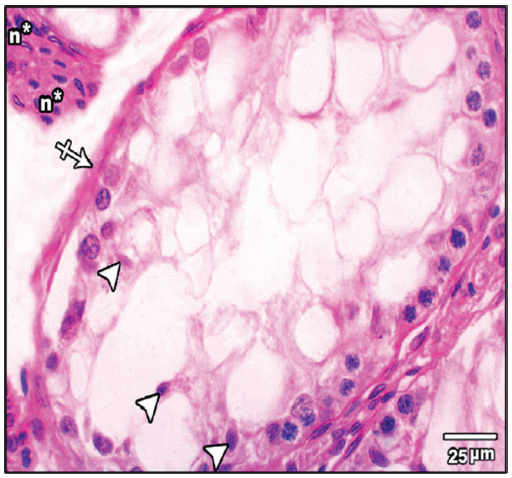

Figure 3: A photomicrograph of a section of testis of a rat from subgroup IIa (receiving acrylamide for 4 weeks) showing irregular contour of the seminiferous tubule, degeneration of spermatogenic epithelial series and apoptotic cells (arrow). Notice thick and irregular basement membrane (crossed arrow). Interstitial cells of Leydig have scanty cytoplasm with deeply stained nucleus (n*) or normal vesicular nucleus (n) (H and E, ×400)

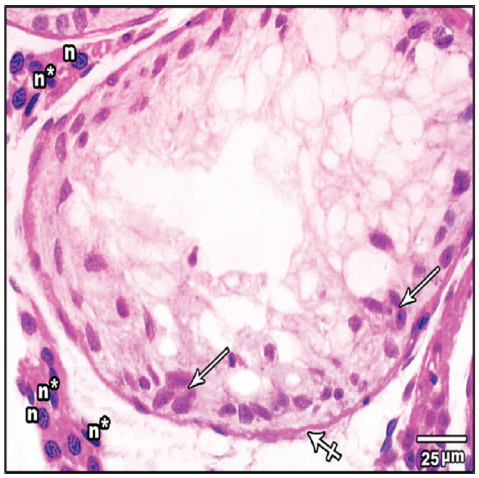

Figure 4: A photomicrograph of a section of testis of a rat from subgroup IIb (receiving acrylamide for 6 weeks) showing complete loss of normal architecture of the seminiferous tubule with complete degeneration of the spermatogenic epithelial series leaving remnants of apoptotic cells (arrow head). The basement membrane appear markedly disrupted and thickened (crossed arrow). Interstitial cells of Leydig have scanty cytoplasm with deeply stained nuclei (n*) (H and E, ×400)

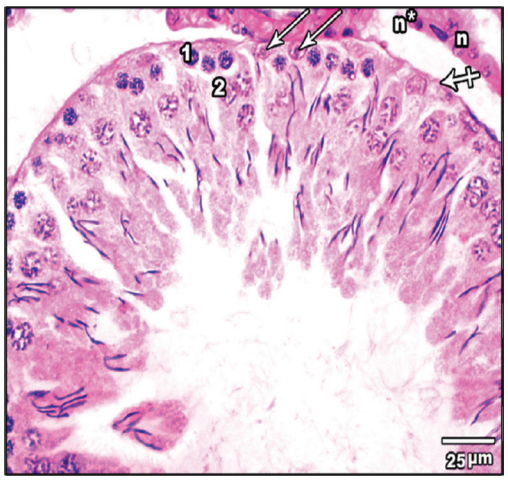

Figure 5: A photomicrograph of a section of testis of a rat from subgroup IIIa (receiving acrylamide and Vitamin E for 4 weeks) showing nearly preserved architecture of the seminiferous tubule. There is apparently normal spermatogonia,(1) primary spermatocytes (2), Sertoli cells (arrow). The basement membrane is slightly corrugated (crossed arrow). Interstitial cells of Leydig have scanty cytoplasm with deeply stained (n*) or normal vesicular (n) nuclei (H and E, ×400)

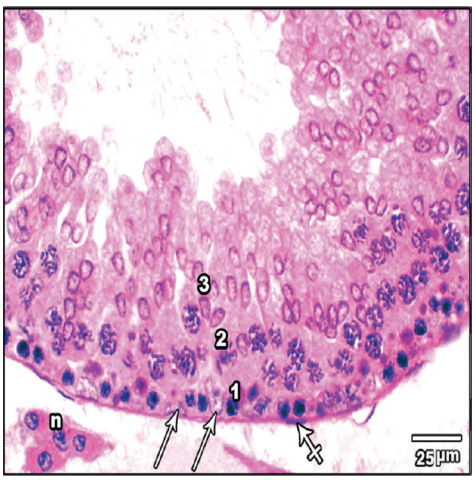

Figure 6: A photomicrograph of a section of testis of a rat from subgroup IIIb (receiving acrylamide and Vitamin E for 6 weeks). The architecture of seminiferous tubule is highly preserved with enhanced spermatogenesis and normal appearance of spermatogonia (1), primary spermatocytes (2), spermatids (3), Sertoli cell (arrow) with vesicular nucleus. Notice regular basement membrane enclosing myoid cells (crossed arrow). Interstitial cells of Leydig appear normal, having vesicular nucleus (n) with prominent nucleolus (H and E, ×400)

subgroup IIa (received AA for 4 weeks) showed disrupted and thickened PAS +ve basement membrane in addition to the absence of well-organized elongated spermatids [Figure 8]. In Group IIb (received AA for 6 weeks), degeneration of epithelial lineage of the seminiferous tubules, thick corrugated PAS +ve basement membrane and the absence of well-organized elongated spermatids or acrosomes were observed [Figure 9].

Group III (acrylamide and concomitant Vitamin E-treated group)

Examination of the treated testis in subgroup IIIa (animals received AA and Vit E for 4 weeks) revealed that the architecture of the seminiferous tubules was less preserved. The number of PAS +ve sperms in the lumen was decreased. Furthermore, there was slightly corrugated PAS +ve basement membrane [Figure 10]. In subgroup IIIb, PAS-stained sections showed enhanced spermatogenesis in most of the seminiferous tubules. The tubules were surrounded by regular PAS +ve basement membrane [Figure 11].

Mallory's trichrome-stained sections

Group I

Mallory's trichrome-stained sections of the testis of the control

rats showed blue stained collagen fibers nearly restricted to the tunica albuginea [Figure 12].

Group II (acrylamide group)

Testicular sections (stained by Mallory's trichrome) of animals of subgroup IIa (received AA for 4 weeks) revealed thickened irregular basement membrane in addition to degenerated spermatogenic epithelium [Figure 13]. In subgroup IIb (animals received AA for 6 weeks), the testis showed thickened irregular basement membrane and severe depletion of the spermatogenic epithelium [Figure 14].

Group III (acrylamide and concomitant Vitamin E treated group)

In subgroup IIIa (animals received AA and Vit E for 4 weeks), Mallory's trichrome-stained sections revealed the less preserved architecture of most of the tubules and decreased mature sperms in the lumen [Figure 15]. In subgroup IIIb, there were enhanced spermatogenesis, and the lumen was full of sperms [Figure 16].

Electron microscopic results

Group I

Electron micrographs of the testis of control animals revealed that the seminiferous tubule was surrounded by a basal lamina and lined by spermatogenic epithelium which followed the usual sequence of spermatogonia, primary spermatocytes, and spermatids. Spermatogonial cell appeared close to the basal lamina with a characteristic nucleus having peripheral clumped chromatin. The primary spermatocytes appeared as large rounded cell above the spermatogonia. The spermatid possessed a rounded nucleus with dark clumps of heterochromatin dispersed in the nuclear sap. Furthermore, early stage of spermiogenesis was noted in the seminiferous

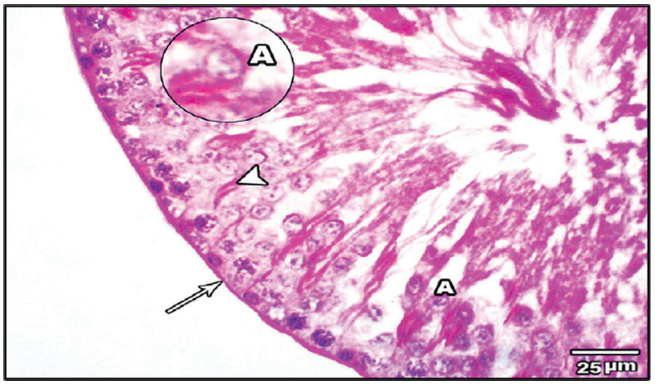

Figure 7: A photomicrograph of a section of control rat testis showing PAS +ve basement membrane (arrow) and Periodic acid–Schiff +ve elongated spermatids (arrow head). Periodic acid–Schiff +ve acrosomal caps (*A*) are also visible in some developing spermatids (PAS, ×400)

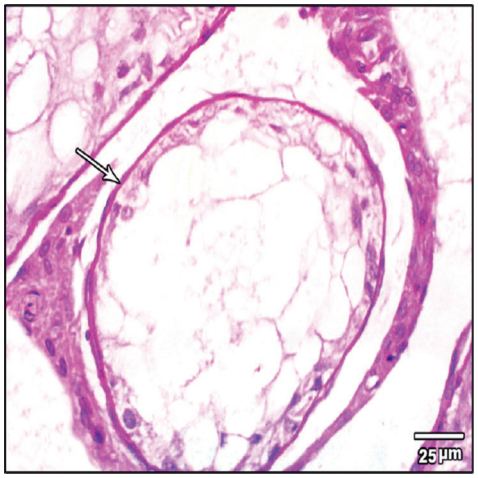

Figure 8: A photomicrograph of a section of testis of a rat from subgroup IIa (receiving acrylamide for 4 weeks) showing disrupted and thickened Periodic acid–Schiff +ve basement membrane (arrow) and absence of well-organized elongated spermatids (PAS, ×400)

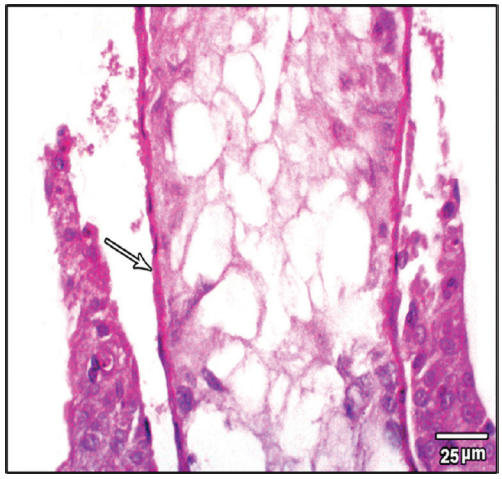

Figure 9: A photomicrograph of a section of testis of a rat from subgroup IIb (receiving acrylamide for 6 weeks) showing degeneration of epithelial lineage of seminiferous tubules, thick corrugated Periodic acid–Schiff +ve basement membrane (arrow). Notice absence of well-organized elongated spermatids or acrosomes (PAS, ×400)

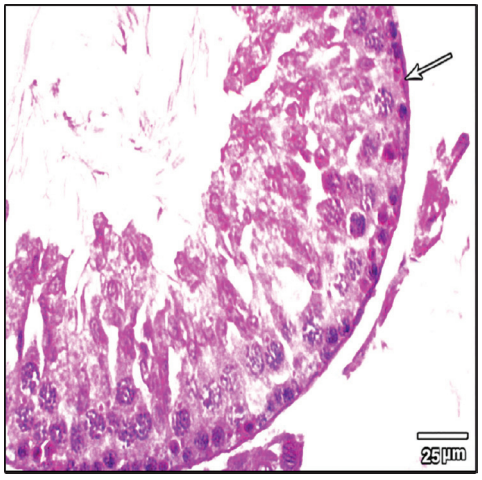

Figure 10: A photomicrograph of a section of testis of a rat from subgroup IIIa (receiving acrylamide and Vitamin E for 4 weeks) showing slightly corrugated Periodic acid–Schiff +ve basement membrane (arrow) (PAS, ×400)

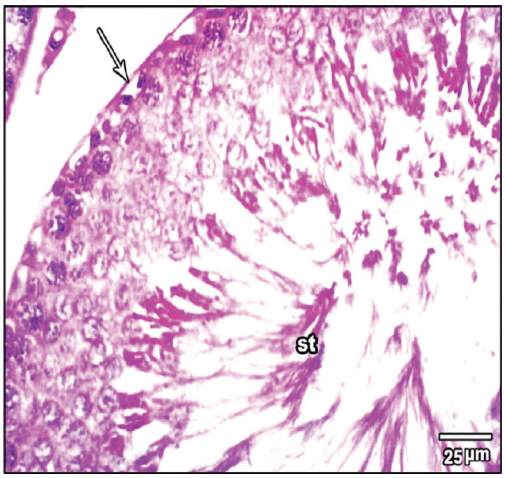

Figure 11: A photomicrograph of a section of testis of rat from subgroup IIIb (receiving acrylamide and Vitamin E for 2 weeks) showing enhanced spermatogenesis. The lumen is full of abundant Periodic acid–Schiff +ve elongated spermatids (st). Notice regular Periodic acid–Schiff +ve basement membrane (arrow) (PAS, ×400)

Figure 13: A photomicrograph of a section of testis of a rat from subgroup IIa (receiving acrylamide for 4 weeks) showing distorted seminiferous tubule with degenerated spermatogenic epithelium. Notice thick and disrupted basement membrane (arrow) (Mallory's trichrome, ×400)

epithelium as the formation of acrosomes over the anterior hemisphere of the spermatid nucleus to form an acrosomal cap [Figure 17]. Sertoli cells were basely attached to the seminiferous tubules. Their cytoplasm appeared rich in mitochondria and lipid droplets. The nucleus had a prominent nucleolus [Figure 18]. Mature spermatozoa, interposed in recesses of Sertoli cells, could be seen with acrosome at the leading point of the head, condensed nucleus, neck, and the middle piece with the mitochondrial sheath [Figure 19].

Group II (acrylamide group)

Subgroup IIa (after 4 weeks)

In this group, some seminiferous tubules showed degenerative changes in the developing spermatogenic epithelium. The primary spermatocytes revealed irregular nucleus and vacuolated cytoplasm [Figure 20].

Figure 12: A photomicrograph of a section of control rat testis showing tunica albuginea (arrow) stained blue and interstitium (I) contains delicate loose connective tissue (Mallory's trichrome, ×100)

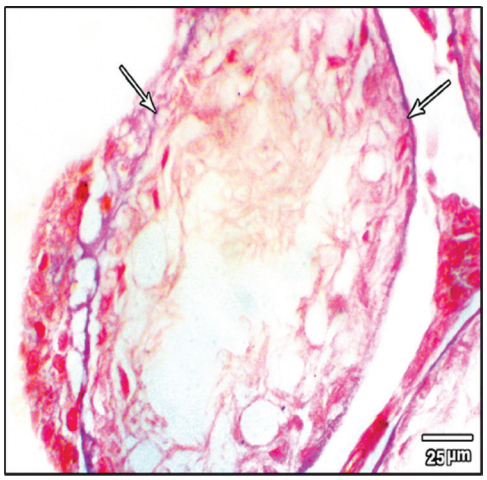

Figure 14: A photomicrograph of a section of testis of a rat from subgroup IIb (receiving acrylamide for 6 weeks). Notice degenerated epithelial lineage of seminiferous tubule and thickened irregular basement membrane (arrow) (Mallory's trichrome, ×400)

Subgroup IIb (after 6 weeks)

The majority of seminiferous tubules showed marked degenerative changes of the whole of the cell lineage of the seminiferous epithelium. Wide spaces between the cells were observed. Spermatids were distorted and degenerated with shrunken pyknotic nuclei, abnormal acrosomal cap, and acrosomal vesicle as well as vacuolation of the cytoplasm [Figure 21]. Few and incompletely developed sperms were observed in the tubular lumen with distorted or absent ectoplasmic specialization and abnormal vacuolation in the head [Figure 22].

Group III (acrylamide and concomitant Vitamin E treated group)

Subgroup IIIa (after 4 weeks)

Electron micrographs of the testis of animals received AA and Vit E for 4 weeks showed morphologically normal spermatogonia and primary spermatocytes. Regarding spermatids some were intact, and others were less developed [Figure 23].

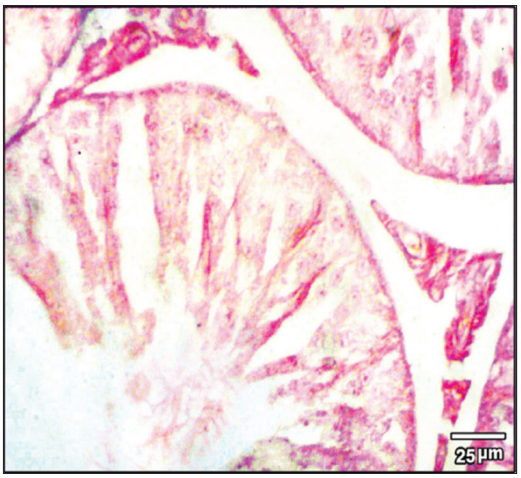

Figure 15: A photomicrograph of a section of testis of a rat from subgroup IIIa (receiving acrylamide and Vitamin E for 4 weeks) showing less preserved architecture of tubule and decreased mature sperms in the lumen (Mallory's trichrome, ×400)

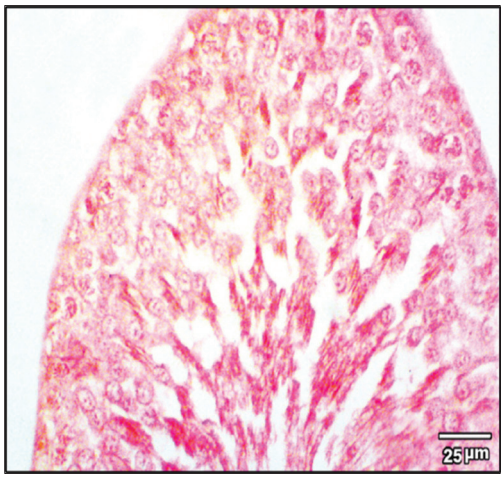

Figure 16: A photomicrograph of a section of testis of rat from subgroup IIIb (receiving acrylamide and Vitamin E for 2 weeks) showing enhanced spermatogenesis and abundant sperms in the lumen (Mallory's trichrome, ×400)

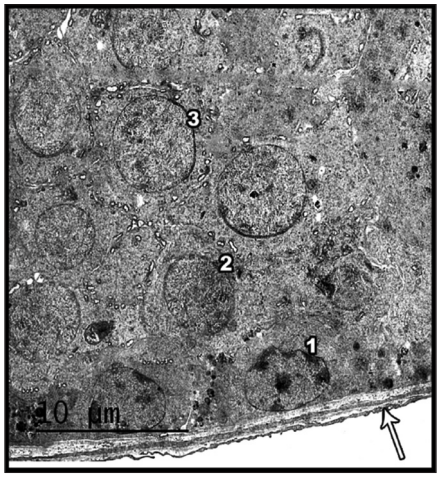

Figure 17: An electron micrograph of a portion of a seminiferous tubule of control rat testis showing spermatogonia (1), primary spermatocytes (2) and spermatids (3) at the phase of acrosomal formation. Notice surrounding basement membrane (arrow) (TEM, ×5500)

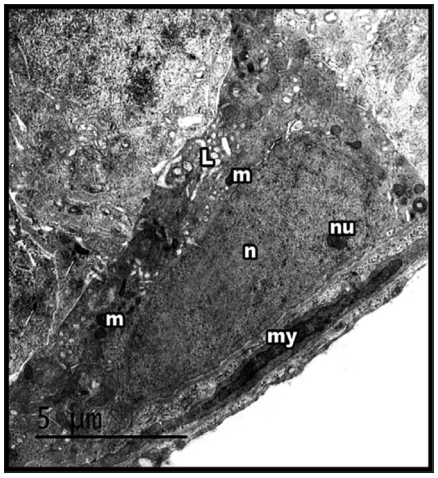

Figure 18: An electron micrograph of control rat testis. It shows a Sertoli cell. Its nucleus (*n*) shows a prominent nucleolus (nu) and its cytoplasm contains many mitochondria (*m*) and lipid droplets (*L*). Notice the basement membrane enclosing myoid cell (my) with central flat nucleus (TEM, ×11000)

Subgroup IIIb (after 6 weeks)

The majority of the seminiferous tubules showed the nearly normal histological appearance of spermatogenic epithelium, normal developing spermatids possessing acrosomal cap, and acrosomal vesicles [Figure 24]. Regarding the sperms, they were apparently normal with elongated condensed nucleus, acrosome at the leading point of the nucleus and ectoplasmic specialization [Figure 25].

Morphometric results

After 4 weeks from the onset of the experiment, there was significant ($P < 0.001$) decreased diameter of seminiferous tubules of testes in AA exposed group (111.93 ± 22.11 µm) and insignificant ($P = 0.61$) decrease in those of group treated with AA and Vit E (190.89 ± 20.80 µm) in contrast to controls. Furthermore, significant ($P < 0.001$) increase in seminiferous tubules' diameters was detected in the group treated with AA, and Vit E compared to AA exposed group [Table 1].

Moreover, after 6 weeks from the onset of the experiment, there was significant ($P < 0.001$) decrease seminiferous tubules' diameter in AA exposed rats (109.96 ± 10.78 µm) and significant ($P = 0.001$) decrease in those of the group exposed to AA and treated by Vit E (173.70 ± 17.18 µm) in comparison to control. And a significant increase in comparison to AA exposed group [Table 1].

Regarding the epithelial height of seminiferous tubules; After 4 weeks from the onset of the experiment, the epithelial height of the seminiferous tubules of the testis in control group was (49.33 ± 7.86 µm). There was significant ($P < 0.001$) decrease

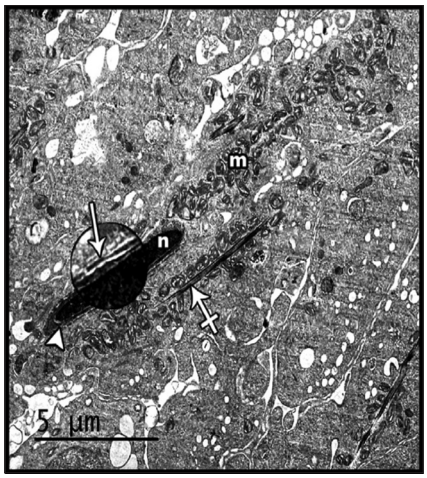

Figure 19: An electron micrograph of control rat testis showing two sperms, the upper one has elongated condensed nucleus (*n*) and acrosomal cap (arrow head) at the leading point of the head. The middle piece of the flagellum contains numerous mitochondria (*m*). Note ectoplasmic specialization (arrow). The lower one shows the axoneme (crossed arrow) surrounded with mitochondrial sheath (TEM, ×10,000)

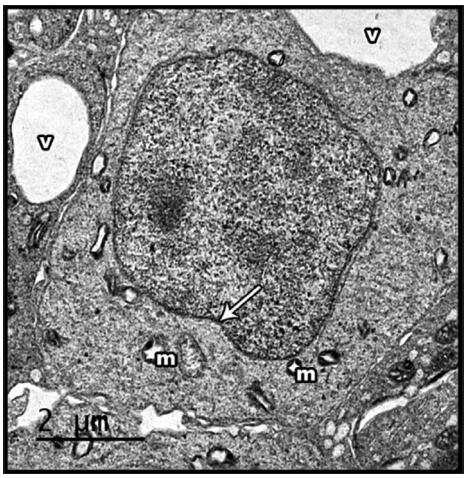

Figure 20: An electron micrograph of testis of a rat from subgroup IIa (receiving acrylamide for 4 weeks) showing primary spermatocyte with corrugated nuclear envelope (arrow). The cytoplasm contains numerous mitochondria (m). Notice vacuoles (v) in neighboring cells (TEM, ×17,500)

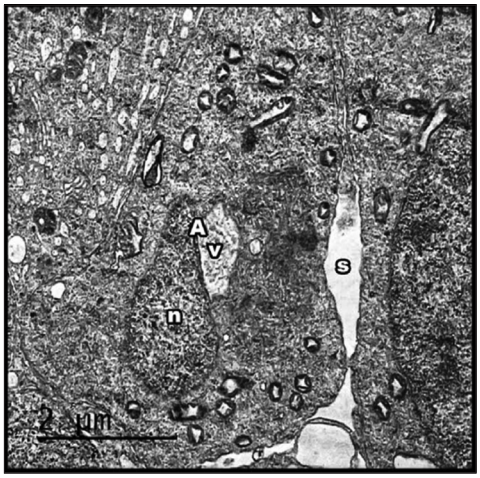

Figure 21: An electron micrograph of testis of a rat from subgroup IIb (receiving acrylamide for 6 weeks) showing severely distorted spermatid cell with shrunken pyknotic nucleus (n) and abnormal acrosomal cap (A) and acrosomal vesicle (v). Notice wide separation (s) between neighboring cells (TEM, ×22,500)

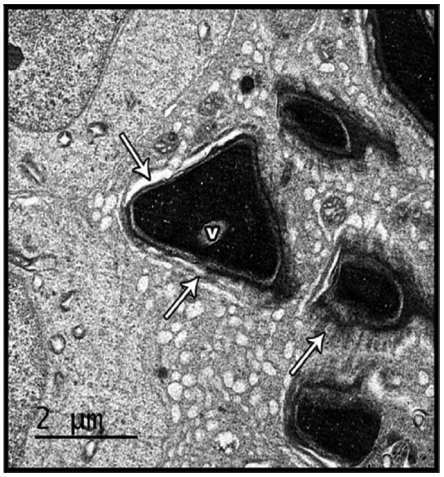

Figure 22: An electron micrograph of testis of a rat from subgroup IIb (receiving acrylamide for 6 weeks) showing morphologically abnormal sperms with distorted or absent ectoplasmic specialization (arrows) and abnormal vacuole (v) in the head (TEM, ×17,500)

in the epithelial height in AA exposed group (29.40 ± 2.98 µm) and significant ($P = 0.047$) decrease in those of the group treated with AA and Vit E (43.29 ± 3.99 µm) as compared to control. Furthermore, there was a significant increase ($P < 0.001$) in the epithelial height in the group treated with AA and Vit E compared to AA exposed group [Table 2]. After 6 weeks from the onset of the experiment, the epithelial height of the seminiferous tubules of the testis in control group was (50.31 ± 4.66 µm). There was significant ($P < 0.001$) decrease in the epithelial height in AA exposed group (24.30 ± 2.18 µm) and significant ($P < 0.001$) decrease in those of the group treated with AA and Vit E (40.67 ± 4.85 µm) as compared to control. Furthermore, there was a significant increase ($P < 0.001$) in the epithelial height in the group treated with AA and Vit E compared to AA exposed group.[2]

Discussion

AA has a reproductive toxic effect.[19-22] The testis-the most essential organs of the male reproductive system are highly sensitive to genetic, hormonal and environmental insults (e.g., X-ray exposure, infectious diseases, and toxicants).[23]

In the present study, the testicular damage in AA exposed rats was indicative for the harmful effect of AA. Seminiferous tubules have various degrees of degeneration of spermatogonia with the presence of numerous apoptotic cells. These findings are in agreement with that were reported by Al-Damegh.[24] Yang *et al*.[12] observed depletion of the

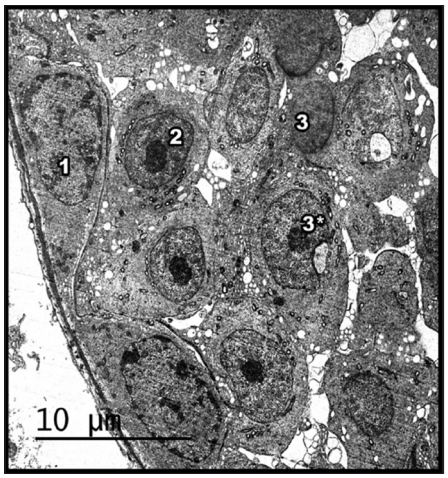

Figure 23: An electron micrograph of testis of a rat from subgroup IIb (receiv-ing acrylamide for 6 weeks) showing morphologically abnormal spermswith distorted or absent ectoplasmic specialization (arrows) and abnor-mal vacuole (v) in the head

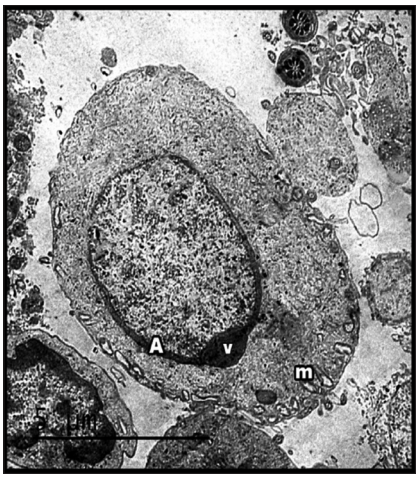

Figure 24: An electron micrograph of testis of a rat from subgroup IIIb (receiving acrylamide and Vitamin E for 6 weeks) showing normal developing spermatids with acrosomal cap (A) covering anterior two-thirds of the nucleus, acrosomal vesicle (v) and many mitochondria (m) in the cytoplasm (TEM, ×14,000)

spermatogenic cells and decrease in the epithelial height of seminiferous tubules in their work. Their results are coincide with our results. On the other hand, the increased epithelial height of rats treated with AA and Vit E compared to AA exposed group proved Vit E protective effect against AA possible oxidative effect.[25]

The findings of our study are commonly linked to testicular oxidative stress induced by AA. Jiang *et al*.;[26] Abd El-Halim and Mohamed[27] and Al-Serwia and Ghoneim[5] found that AA caused marked elevation of malondialdehyde level in testicular sample homogenate. ROS can affect various cell functions through inhibition of many cytosolic enzymes.[28] Moreover, apoptosis in the germ cells was found to be induced by AA.[29] It could be explained by its oxidative stress damage effect on germ cells and induction of apoptotic pathway.[30,31]

In the present study, morphologically abnormal sperms were revealed in rat testis treated with AA. Venkatesh *et al*.[32] documented ROS induced abnormal sperm morphology. AA-induced ROS production could lead sperm membrane lipioperoxidation, mitochondria damage and consequently, increased sperm deformity index.[33,34]

In addition to the oxidative stress mechanism of AA effect at the cellular level, inhibition of kinesin and dynein cytoskeletal motor protein families[35] induced by AA could explain the reproductive toxicity.[36,37] These findings were proved previously by Ali *et al*.[38] Lebda *et al*.,[13] and Al-Serwia and Ghoneim.[5]

Cytoskeletal inhibition by AA leads to decreased cholesterol uptake by Leydig cells and resultant lowered testosterone synthesis. In addition, AA-induced cytoskeletal dysfunction could down-regulate synthesis and/or transport of LH receptors to Leydig cells plasma membrane.[39]

The reduction in serum testosterone is documented to be accompanied by the histopathological changes that are represented by the production of high numbers of apoptotic cells, spermatogenic cells disintegration and decreased Leydig cell viability.[12] This could explain the findings of the current study.

Defective cytoskeleton proteins level could also be related to seminiferous tubules damage, including defective spermatogenesis and tight junction between the Sertoli cell and germ cell.[36] AA also disturbs genes of cell cycle control. This may result in histopathological abnormalities of the reproductive organs.[12]

In the existent study, there was apparent reduced sperms' amount in seminiferous tubules. Moreover, at the level of electron microscope, some sperms were malformed with distorted acrosome and abnormal vacuolation of the head. This finding agreed with Song *et al*.[40] They concluded that AA exposure could disrupt spermatogenesis process and hence favors production of spermatozoa abnormal morphology.

Moreover, Leydig cells in the interstitial tissue were decreased with scanty cytoplasm and dark nuclei. Pyknosis of nuclei were observed also in spermatogenic cells. It has been reported that subchronic exposure to AA directly damages Leydig cells.[40] Other authors, Friedman *et al*.[37] and Shipp *et al*.[4] documented necrosis of seminiferous tubules and nuclear vacuolation of germ cells in testicles of AA exposed mice.

In the current study, harmful effect of AA began to appear after 1 week of AA exposure (data not shown), became evident at the 4th week and increased progressively with long exposure where it caused severe diminution of the spermatogenic cells in seminiferous tubules by the end of the experiment, and this means that effect of AA was duration dependent. As mentioned by Mustafa,[41] AA degenerative effects in the testes is directly proportional to the period of AA exposure. It is proved also, that intraperitoneal route is more hazardous than the oral route.[41] These data were confirmed

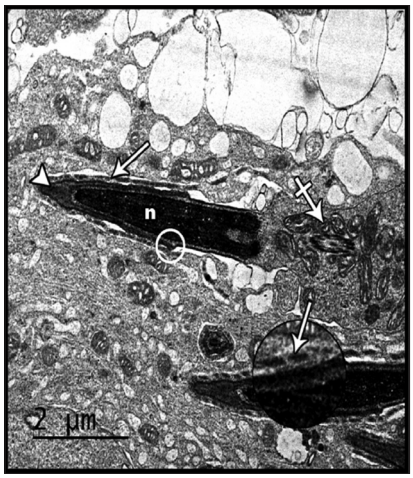

Figure 25: An electron micrograph of testis of a rat from subgroup IIIb (receiving acrylamide and Vitamin E for 6 weeks) showing 2 apparently normal sperms, both revealed elongated condensed nucleus (n), acrosome (arrow head) at the leading point of the nucleus and ectoplasmic specialization (arrows). Notice part of the flagellum (crossed arrow) and post acrosomal dense body (circle) (TEM, ×17,500)

by Shinomol *et al*.,[42] who stated that AA cytotoxicity is a dose and duration dependent.

Antioxidants are the main defense factors against oxidative stress induced by free radicals.[43] Vit E is the key antioxidant component of spermatozoa and membrane protectants against ROS[43,44] and could be considered as a good prophylactic agent against AA-induced toxicity.[45,46]

In the current work, testis of AA exposed rats and treated by Vit E showed that the architecture of most of the seminiferous tubules was highly preserved with enhanced spermatogenesis. The lumen of the tubule was full of sperms. Interstitial cells of Leydig appeared having normal vesicular nuclei and abundant cytoplasm. There was also proper differentiation of spermatids with acrosomal cap and acrosomal vesicle. Most of the sperms were morphologically normal. Our findings are supported by what is stated by Lee *et al*.[25] and Talebi *et al*.[47] Hasseeb *et al*.[48] also assured Vit E protective role against the hazardous effect of AA.

After 6 weeks of Vit E administration, the outline and architecture of most of the seminiferous tubules were highly preserved with enhanced spermatogenesis. Moreover, Leydig appeared normal with vesicular nuclei. Thus, the increased AA-induced free radicals generation in testes might have been neutralized by Vit E[15] as Vit E is chain-breaking antioxidant). This is enforced by other antioxidants chemicals, such as Vit C, that are important for regenerating antioxidant ability of α-tocopherol.[49]

In vitro experiments performed by Ross *et al*.[50] It is proved that Vit E protects spermatozoa from oxidative damage. It enhances sperm motility and performance. This is also, confirmed later by Ourique *et al*.,[51] and Liu *et al*.[52]

Finally, the current study could conclude that AA causes degenerative changes of the testes of albino rats and arrest of spermatogenesis. The AA-induced histological and ultrastructural changes of the testes. These changes are proportional to the duration of exposure. Moreover, it could be concluded, that Vit E has a protective role against AA-induced damage in testicles by its antioxidant and anti-apoptotic effects. Vit E protective effect is reflected on testicular histology and ultrastructure.

Table 1 Diameter of the seminiferous tubules of testes in control (C), acrylamide treated group (A) and group treated with acrylamide and vitamin E (A+V) after 4 & 6 weeks of starting experiment.

		Groups			*P*
		(C) group	(A) group	(A+V) group	
4wks	Mean±SD	201.02	111.93	190.89	<0.001**
		28.43	22.11	20.80	
	P1		<0.001**	0.61	
	P2			<0.001**	
6wks	Mean±SD	207.41	109.96	173.70	<0.001**
	P1	22.20	10.78	17.18	
			<0.001**	0.001*	
	P2			<0.001**	

SD: standard deviation P: Probability. Test used: One way ANOVA followed by *post-hoc* tukey *P*1: Significance relative to Control group. *P*2: Significance between (A) & (A + V) group. * Significance ≤0.05. ** High significance <0.001.

Table 2 Epithelial height of the seminiferous tubules of testes in control (C), acrylamide treated group (A) and group treated with acrylamide and vitamin E (A+V) after 4&6 weeks of starting experiment.

		Groups			*P*
		(C) group	(A) group	(A + V) group	
4wks	Mean ±SD	49.33	29.40	43.29	<0.001**
	P1	7.86	2.98	3.99	
			<0.001**	<0.047*	
	P2			<0.001**	
6wks	Mean ±SD	50.31	24.30	40.67	<0.001**
	P1	4.66	2.18	4.85	
			<0.001**	<0.001**	
	P2			<0.001**	

SD: standard deviation P: Probability. Test used: One way ANOVA followed by *post-hoc* tukey P1: Significance relative to Control group. P2: Significance between (A) & (A + V) group. * Significance ≤0.05. ** High sigbnificance <0.001.

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Conflicts of interest

There are no conflicts of interest.

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