Research Article

Histological and Ultrastructural Study of the Protective Effect of Antioxidants on the Testis of Adult Albino Rat after Treatment with Aluminium Chloride

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Abstract
Different forms of aluminum are environmental xenobiotic that induce free radical-mediated cytotoxicity and reproductive toxicity. Selenium and vitamins A, C and E have been reported to be important antioxidants. Therefore, this study aimed at elucidating the protective effects of selenium and vitamins A, C and E against reproductive toxicity of aluminum chloride in male rats. The first group served as control group, group II received aluminum chloride (١٠٠ mg\kg\body weight) intra peritoneal injection every other day for five weeks, group III received ١ ml selenium ACE (antioxidants) given daily by gastric intubation for five weeks, and group IV co-administered with aluminum chloride and selenium ACE in the same doses in ٢nd and ٣rd groups. Aluminum chloride caused a decrease in body and testis weights, sperm concentration, motility and viability. Histopathological examination of aluminum-treated group revealed apparent alterations in the testis seminiferous tubules. Selenium ACE antagonized the harmful effects of aluminum chloride.
Key words: Aluminum chloride, Antioxidants, Selenium, Vitamins A, C, E, Testis.

Introduction
The impact of aluminum (Al) intoxication on human and animal health has been increasingly alarmed in recent years. Aluminum is known to cause toxic effects to the male reproductive organs (Domingo et al., ١٩٩٣).

Al is a ubiquitous element found in every food product. The sources of Al are especially corn, yellow cheese, salt, herbs, spices, tea, cosmetics, aluminum ware and containers. In addition, it is added to drinking water for purification purposes and is also present in medicines (Ochmanski and Barabasz, ٢٠٠٠).

Aluminum compounds have many medical implications as, antacids, phosphate binders, buffered aspirins, vaccines, antiperspirants and allergen injection (Exley, ٢٠٠٣).Aluminum accumulation in target organs has been associated with damage of testicular tissues of both humans and animals. High concentrations of aluminum in human spermatozoa and seminal plasma are correlated with decreased sperm motility and viability (Dawson et al., ١٩٩٨).

Selenium and vitamins A, C and E are a naturally occurring antioxidant nutrient that plays an important role in animal health by inactivating harmful free radicals that are produced through the normal cellular activity and from various stresses. The antioxidant function of these micronutrients could enhance immunity by maintaining the functional and structural integrity of important immune cells (El-Demerdash et al., ٢٠٠٤).

The present study aimed to determine the reproductive toxicity of aluminum chloride in adult male rats and evaluate the protective effect of Selenium ACE against the possible testicular dysfunction caused by aluminium chloride.

Materials and methods

Forty adult male albino rats, weighing (١٨٠-٢٠٠ gm) were used in the present study. Rats were kept for two weeks as acclimatization period before the start of experiment in animal house of anatomy department, Faculty of Medicine, El-Minia University. All rats were handled in accordance with the standard guide for the care and use of laboratory animals. The rats were subdivided into four groups as following:

Group I (negative control): ١٠ rats administered ٣ ml distilled water orally once daily.

Group II: ١٠ rats, each received aluminum chloride (١٠٠ mg/kg/body weight) intraperitoneal injection every other day for five weeks (Krasoudkii, et al., ١٩٧٩).

Group III: ١٠ rats, each received ١ ml selenium ACE (antioxidants) given daily by gastric intubation for five weeks.

Group IV: ١٠ rats, each was co-administered with aluminum chloride and selenium ACE in the same doses in ٢nd and ٣rd groups.

Selenium ACE is a group of antioxidants, available as tablets produced by Wassen Company. Each tablet of selenium ACE contains ١٠٠ ug of selenium, ٤٥٠ ug of vitamin A, ٩٠ mg of ascorbic acid and ٣٠ mg of vitamin E. Each tablet was crushed and dissolved in ١٠ ml of distilled water each rot received ٠.١ ml (Evangelou, et al., ١٩٩٧).

Body weight assessment: Body weight was measured and recorded for each rat in the beginning of the experiment and also before killing. The weight was done by using Sartorius balance capable of measuring with a precison of ٠.٠١ gm.

Testis weight: After dissecting the wall of the scrotum to get out the testes from each animal, the weight of the testes was measured by Sartorius balance. The data of weight are recorded.

Preparation for paraffin sections:

Pieces of testis were fixed in phosphate buffered saline (PBS, pH ٧.٣) containing ٣.٧٪ formaldehyde for ١ hour (h). Fixed samples were then washed with water, dehydrated in graded ethanol: ٧٠٪ ethanol for ١ h, ٨٠٪ ethanol for two times one hour each, ٩٥٪ ethanol for two times one hour each, ١٠٠٪ ethanol for three times one hour each, then cleared in xylene for three times one hour for the١st time then ٩٠ minutes twice and embedded in wax at ٦٠°C two times for two hours each. Finally, the samples were embedded in a paraffin wax in a labeled plastic cassette. Embedded samples were sectioned at ٥µm thickness, using a microtome. The sections were processed and stained with Haematoxylin and eosin and other sections were stained with Masson's trichrome stain (Bancroft et al., ١٩٩٨).

Preparation for the semi-thin and ultrathin sections:

Specimens for electron microscopic examination were immediately fixed in ٢.٥٪ glutaraldhyde buffered with ٠.١M phosphate buffered at pH ٧.٤ for ٢ hours and then postfixed in ١٪ osmium tetraoxide in the same buffer for another one hour. There were processed to prepare semi thin and ultrathin sections. One µm transverse semi-thin section was cut by RMC ultratome, stained with toluidine blue ١٪ and examined by light microscope. Ultrathin sections were mounted on copper grids stained with ٢٪ aqueous uranyl acetate for ٢٠ minutes, and lead citrate for another ٢٠ minutes, then examined and photographed using JEOL electron microscope equipped with a camera in the Electron Microscope Resarch Laboratory of Histology and Cell Biology Department, Faculty of Medicine, AssuitUniversity.

Assessment of sperm count, motility and viability:

Seminal content of epididymis was obtained by cutting of cudaepididymis using surgical blades and squeezed in a sterile clean watch glass. This content was diluted ten times with ٢.٩٪ sodium citrate dehydrate solution and thoroughly mixed to estimate the progressive motility and sperm concentration (Freund and Carol, ١٩٩٥).

Statistical analysis

Data were presented as mean ± standard deviation (SD). Statistical significance was

determined by unpaired student's t-test of unequal variance. Statistical differences between means were calculated by analysis of variance (ANOVA). Results were considered significant at p<٠.٠٥, according to Snedecor and Cochron (١٩٨٩). An IBM computer with a software system SPSS version ١٦ was used for these calculations.

Results

I. Gross measurements:

١-Body weights -:

In the present study, it has been found that the mean body weight of the aluminum-treated group is ٢٠٥gm. (٢١٪ reduction) while that of the control and Selenium ACE groups are ٢٦٠ gm. and ٢٦١ gm. respectively (Tables ١, ٢, ٣ and Figs. ١, ٢, ٣). This difference proved to be statistically highly significant (P < ٠.٠٠١).The weight difference in between aluminum-treated group and (aluminum + Selenium ACE) group appeared to be ١٩.٥٪ (P < ٠.٠٠١). (Tables ١, ٢ and Figs. ٢, ٣).The mean body weight of the (aluminum + Selenium ACE) treated group appeared to be ٢٤٥ gm. which is highly significant (٣٠٪) higher than that of the aluminum-treated group (P < ٠.٠٠١); (Tables ١, ٢, ٣ and Figs. ١, ٢, ٣).

Oral administration of Selenium ACE had no effect on body weight of rats, indicating its safe use under the experimental condition. No significant difference between Selenium ACE group and control group (Tables ١, ٢, ٣ and Figs. ١, ٢, ٣).

Table (١): Mean, Stander Deviation and p-value of Body weight.

		Mean	Stander Deviation	Minimum	Maximum	p-value
Initialbody weight/gm	Control	١٨٤.٥	٥.٧	١٧٥	١٩٥	٠.٣٨٨*
	Aluminum chloride	١٨١.٧	٤.٦	١٧٥	١٩٢	
	Selenium ACE	١٨١.١	٤.٤	١٧٥	١٩٠	
	Aluminum + Selenium	١٨٣.٧	٤.٤	١٧٩	١٩١	
Finalbody weight/gm	Control	٢٦٠	٨.٦	٢٥٠	٢٧٥	٠.٠٠١**
	Aluminum chloride	٢٠٥	٥.١	٢٠٠	٢١٣	
	Selenium ACE	٢٦١	٥.٤	٢٥١	٢٦٦.٥	
	Aluminum + Selenium	٢٤٥	٥.٤	٢٤٠	٢٥٥	
Body weight gain/gm	Control	٧٧.١	٥.٩	٦٧.٥	٨٥	٠.٠٠١**
	Aluminum chloride	٣٦.٦	٣.٣	٣١	٤٢	
	Selenium ACE	٧٩.٨	٥.٨	٦٨	٨٦.٥	
	Aluminum + Selenium	٦١	٤.٣	٥٤	٦٨	

*** P is not significant.**

****P is significant if < or = ٠.٠٥ and is highly significant if = or < ٠.٠٠١.**

Fig.١: Initial body weight.

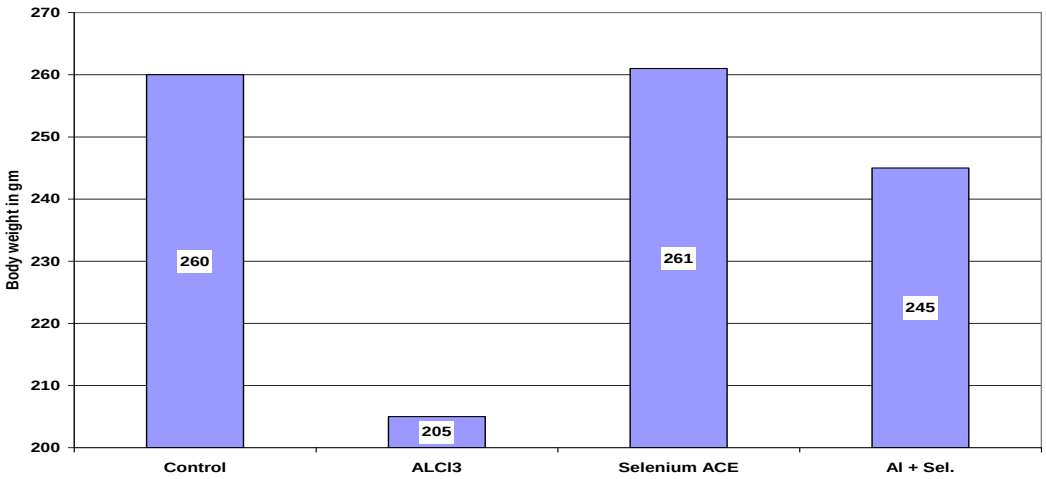

Fig.٢: Final body weight.

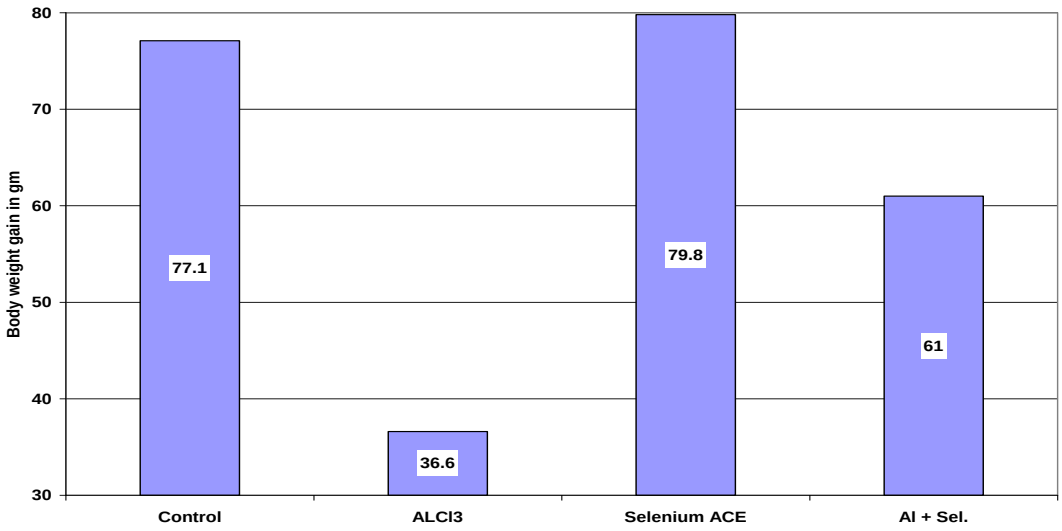

Fig. ٣: Body weight gain in gram.

Testis weight: It is exhibited that there is a highly significant ٤٦٪ decrease (P <٠.٠٠١) in the mean testis weight (٠.٩٤ gm.) of the aluminum-treated group when compared with the control group (١.٧٥ gm.); (Tables ٤, ٥ and Fig. ٤). On comparing the aluminum-selenium ACE treated group as regard the mean testis weight (١.٥ gm.), with the aluminum treated group, a highly significant ٦٠٪ increase was observed in this value (P <٠.٠٠١); (Tables ٤, ٥ and Fig. ٤).Oral administration of selenium ACE alone didn't cause any significant effect on the weight of testis (١-٨ gm.) as compared with control group (Tables ٤, ٥ and Fig. ٤).

Table (٤): Mean, stander deviation and p-value of testis weight.

		Mean	Stander deviation	Minimum	Maximum	p-value
Testis weight/gm post experiment	Control	١.٧٥	٠.٠٨	١.٧٠	١.٩	٠.٠٠١**
	Aluminum chloride	٠.٩٤	٠.١٢	٠.٨١	١.١	
	Selenium ACE	١.٨٠	٠.٠٦	١.٨٠	١.٩	
	Aluminum + Selenium ACE	١.٥٠	٠.٠٧	١.٥٠	١.٧	

*** P is not significant.**
****P is significant if < or = ٠.٠٥ and is highly significant if = or < ٠.٠٠١.**

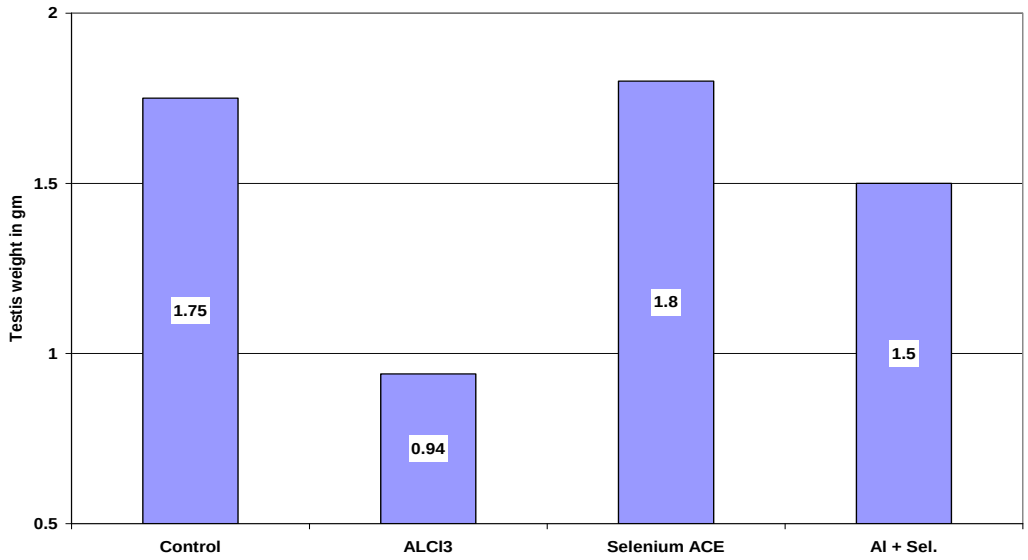

Fig.٤: Testis weight post experiment.

٣- Sperm count: In the present work, it has been found that the mean sperm count of control group ٥٩.٨ million, but had a highly significant elevation in sperm count ٦٦.٥ million (P <٠.٠٠١) in group Selenium ACE (Tables ٦, ٧ and Fig. ٥).In aluminum treated group, the mean sperm count ٢٥.٥ million showed highly significant decrease than the control group (P < ٠.٠٠١); (Tables ٦, ٧ and Fig. ٥).On the other hand, treatment with Selenium ACE in combination with aluminum chloride showed significant increase in sperm count compared to aluminum chloride treated group (P < ٠.٠٠١), and this means that Selenium ACE minimized the toxicity of aluminum chloride (Tables ٦, ٧ and Fig. ٥).

Table (٦): Mean, stander deviation and p-value of sperm count.

		Mean	Stander Deviation	Minimum	Maximum	p-value
Sperm count per million/ml	Control	٥٩.٨	٥.٢	٥٤.٠	٦٤.٢	٠.٠٠١**
	Aluminum chloride	٢٥.٥	١.٨	٢٢.٥	٢٧.٨	
	Selenium ACE	٦٦.٥	٤.٨	٥٥.٦	٧٠.٥	
	Aluminum + Selenium ACE	٤٥.٦	٣.٧	٤١.٠	٥١.٠	

*** P is not significant**
****P is significant if < or = ٠.٠٥ and is highly significant if = or < ٠.٠٠١.**

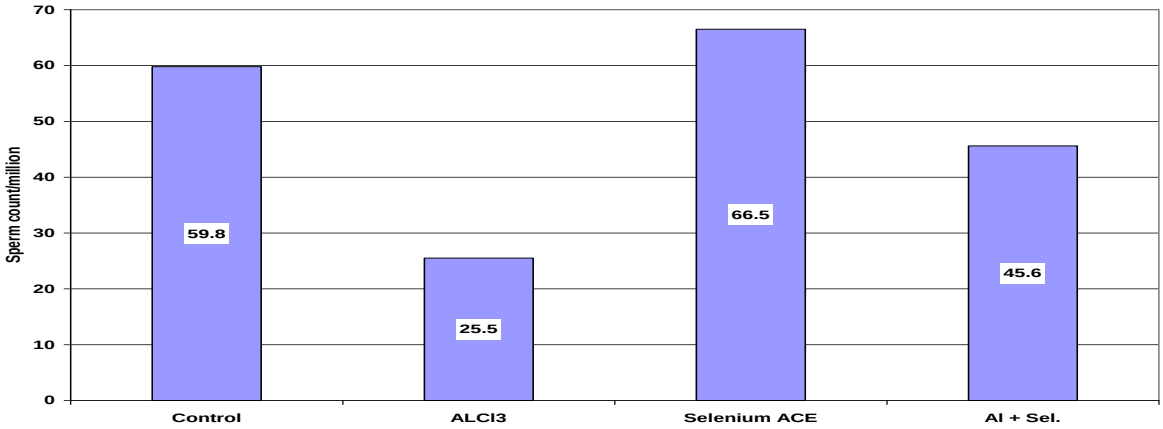

Fig. (٥): Sperm count %.

٤- Sperm motility and viability:
In the present work, the mean sperm motility and viability of Selenium ACE group ٨٧٪ and ٨٧.٣٪ respectively higher than that of control group; (Tables ٨, ١٠ and Figs. ٦, ٧). The mean sperm motility and viability of aluminum treated group ٤٣٪ and ٤٣.٥٪ respectively showed a highly significant decrease (P<٠.٠٠١) than control group (Tables ٨, ٩, ١٠, ١١ and Figs. ٦, ٧).Treatment with Selenium ACE in combination with aluminum chloride caused significantly improved the decline in sperm motility and viability compared to aluminum treated group (P < ٠.٠٠١) (Figs. ٦, ٧).

Table (٨): Mean stander deviation and p-value of sperm motility%.

		Mean	Stander deviation	Minimum	Maximum	p-value
Sperm motility%	Control	٨٦.٠	٣.٤	٨٠	٩١	٠.٠٠١**
	Aluminum chloride	٤٣.٠	٣.٧	٣٨	٤٨	
	Selenium ACE	٨٧.٠	٣.١	٨١	٩٠	
	Aluminum +Selenium ACE	٧٥.٧	٣.٧	٧٠	٨١	

*** P is not significant.**
****P is significant if < or = ٠.٠٥ and is highly significant if = or < ٠.٠٠١.**

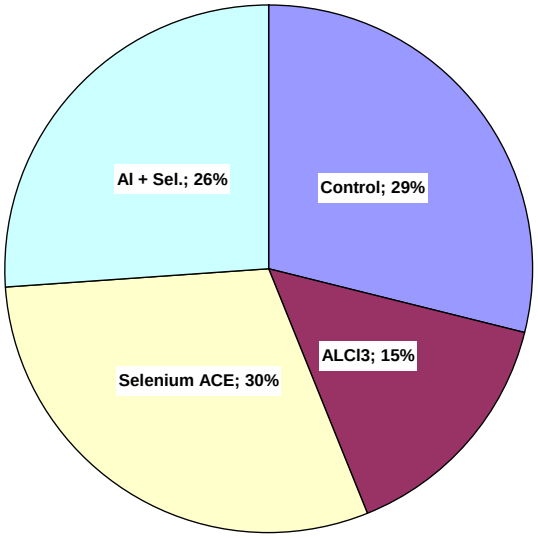

Fig. (٦): Sperm Motility%.

Table (١٠): Mean, stander deviation and p-value of sperm viability%.

		Mean	Stander Deviation	Minimum	Maximum	p-value
Sperm Viability %	Control	٨٦.٦	٣.٦	٨٠.٠	٩١.٠	٠.٠٠١**
	Aluminum chloride	٤٣.٥	٢.٦	٣٩.٥	٤٧.٠	
	Selenium ACE	٨٧.٣	٣.١	٨١.٠	٩١.٥	
	Aluminum +Selenium ACE	٧٢.٧	٢.٩	٧٠.٠	٧٧.٠	

*** P is not significant.**
****P is significant if < or = ٠.٠٥ and is highly significant if = or < ٠.٠٠١.**

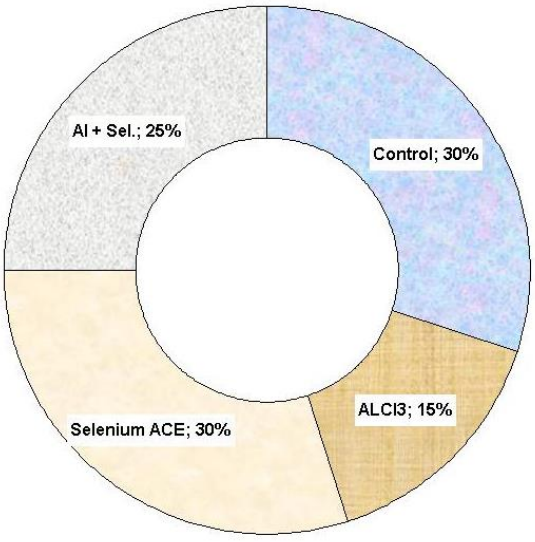

Fig. (٧): Sperm viability%.

II. Histological study:

١- The group I (control):

Light microscopic examination of control rat testis shows several rounded or oval seminiferous tubules with patent lumina separated by interstitial tissue (Figs. ٨ and ٩). The tubules are bounded by basement membrane (Fig. ٨). The wall of the seminiferous tubules is formed of ٤-٦ layers of germinal stratified epithelium. The lumen of seminiferous tubules contains spermatozoa (Fig. ٨ and ٩).

Electron microscopic examination of control rat testis shows spermatogonia which are characterized by large pale ovoid nuclei containing finely granular nucleoplasm. The nuclei are usually lying with their long axis parallel to the boundary tissue and near the tubular limiting membrane (Fig. ١٢). Primary spermatocytes are characterized by the presence of spherical nuclei with finely granular nucleoplasm and chromatin accumulation (Fig. ١٣).Spermatids are rounded cells with large spherical or oval nuclei which contain chromatin clumps in a lightly stained cytoplasm which contains mitochondria (Fig. ١٤).Sertoli cells had large euchromatic nuclei and prominent nucleoli, the cytoplasm contains abundant endoplasmic reticulum and spherical or cylinder shaped mitochondria (Fig.١٠). Leydig cells are large, rounded or polygonal cells which exhibit a abundant mitochondria(Fig.١٥).Cross sections in the middle pieces of sperms show a central axonemesurrounded by fibrous sheath mitochondrial sheath (Fig.١١).

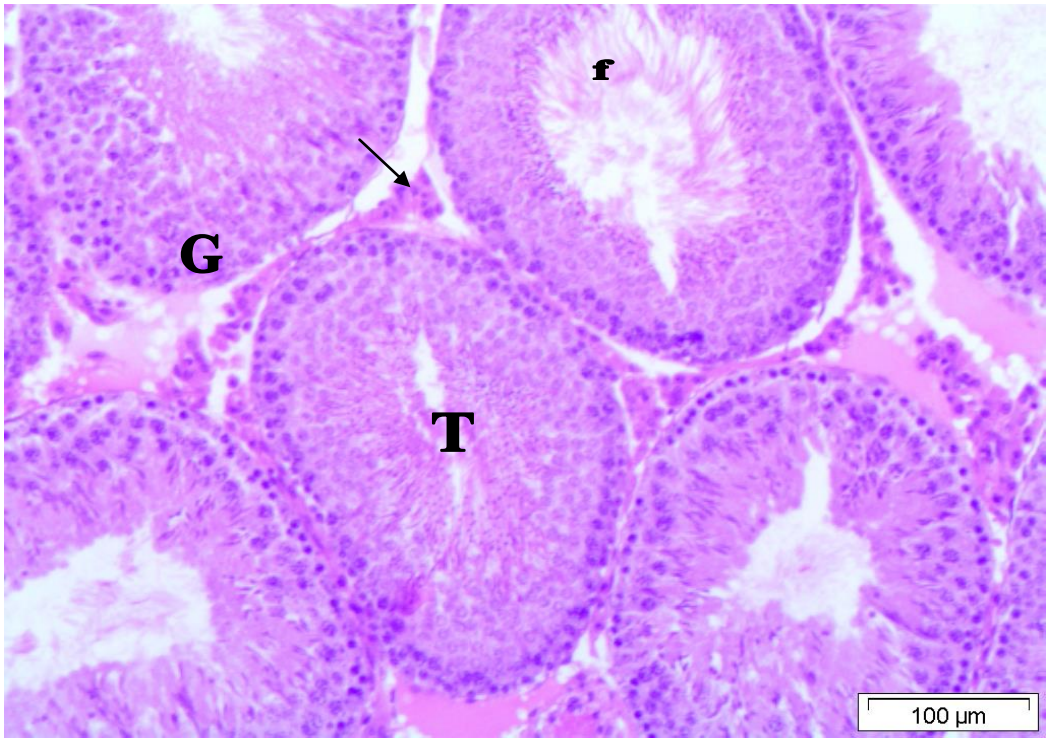

Fig. (٨): A photomicrograph of a section in the control rat testis, showing packed seminiferons tubules (T) lined by stratified germinal epithelium (G) and flagella (f). Thetubules are separated separted by a narrow interstitium containing clusters of interstitial cells of leydig (arrow). (Haematoxylin& Eosin X ٢٠٠).

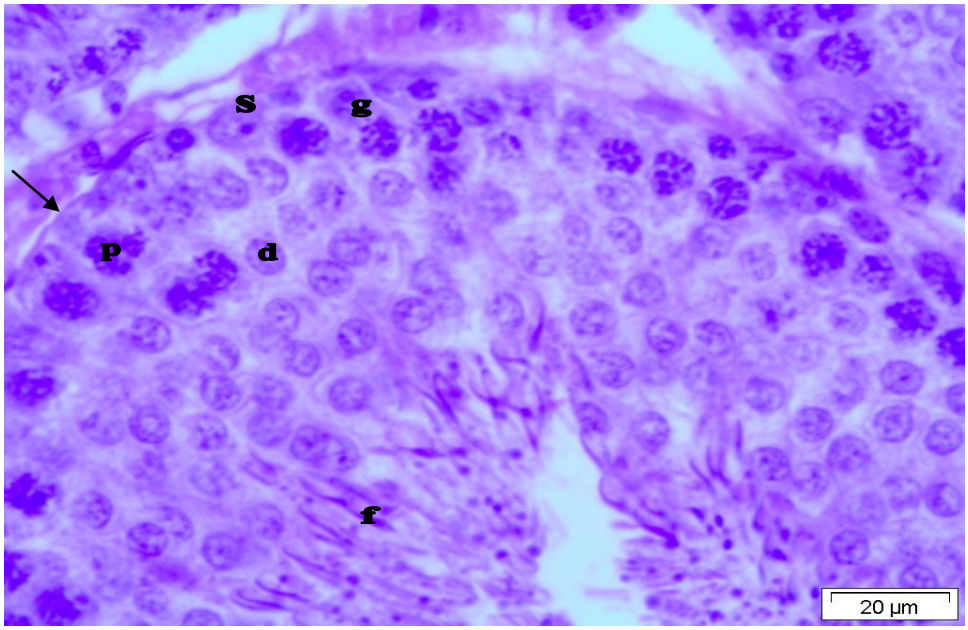

Fig. (٩): A photomicrograph of a section in the control rat testis, showing seminiferous tubules lined by spermatogenic cells: Sertoli cell (s) spermatogonia (g), primary spermatocyte (p) and spermatid (d), Flagella (f) rest on basement membrane (arrow) (Haematoxylin& Eosin X ٤٠٠).

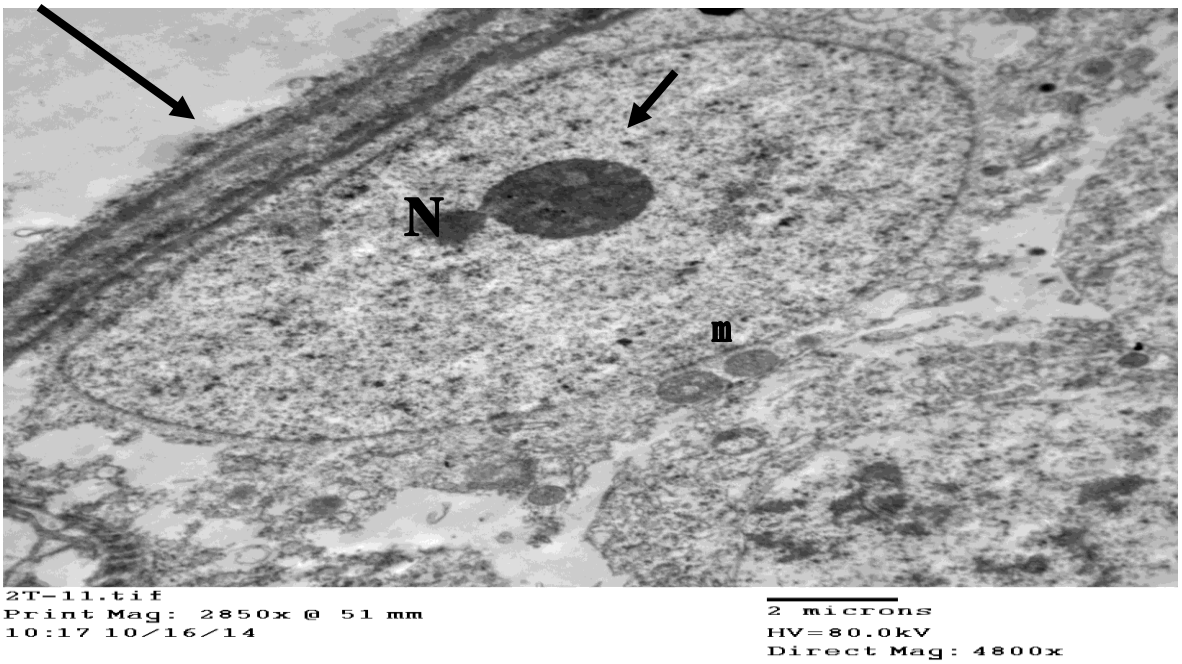

Fig. (١٠): An electron photomicrograph of control rat testis, showing sertoli cell with oval nucleus (N) well developed nucleolus (short arrow). It appears resting on the basement membrane (long arrow). Its cytoplasm contains mitochondria (M). X ٤٨٠٠.

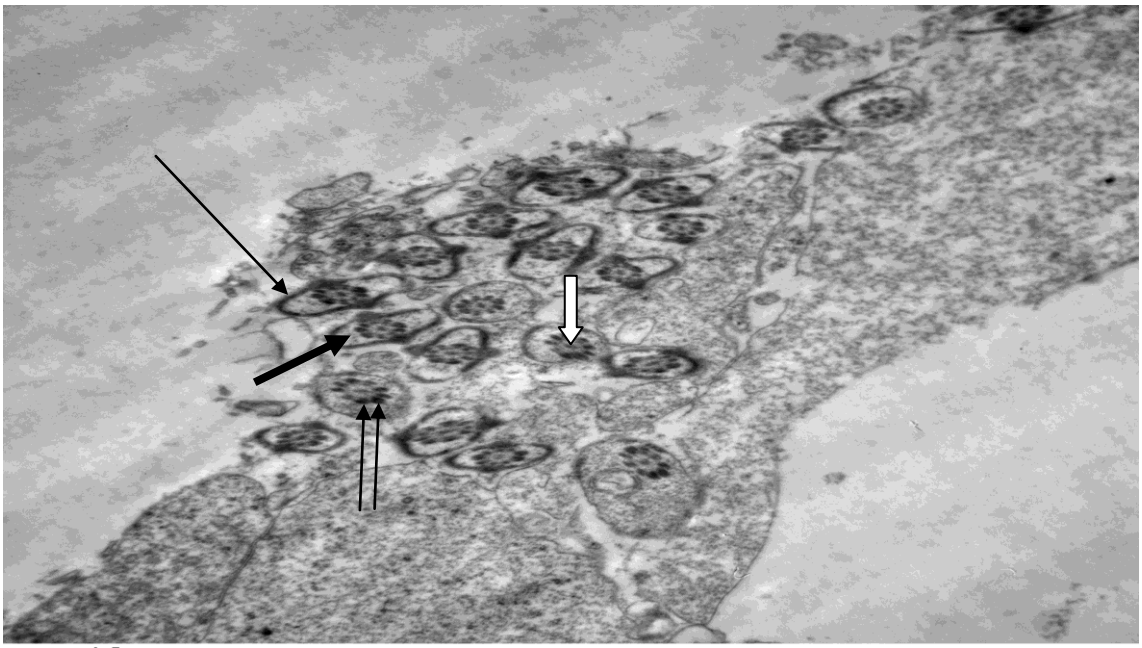

Fig. (١١): An electron photomicrograph of control rat testis, showing cross sections in the middle pieces (MP), of sperms. All pieces have a central axoneme (white arrow) surrounded by fiberons sheath (bold arrow) mitochondrial sheath (double arrows), peripheral cell membrane (long arrow), X ١٠٠٠٠..

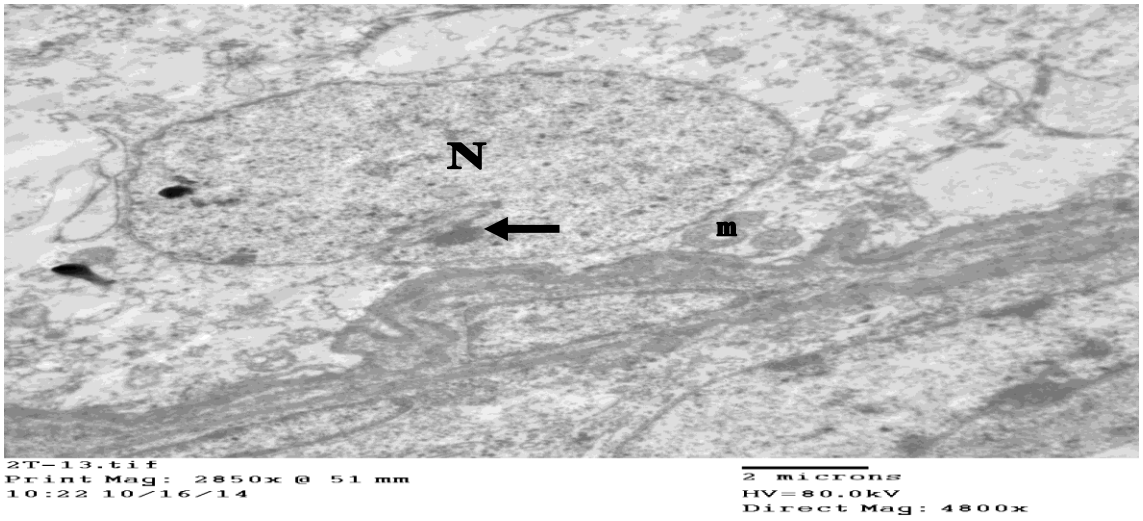

Fig. (١٢): An electron photomicrograph of control rat testis, showing spermatogonium with large rounded nucleus (N) of dispersed granular chromatin, nucleolus (arrow) and its cytoplasm contains mitochondria (M). X ٤٨٠٠.

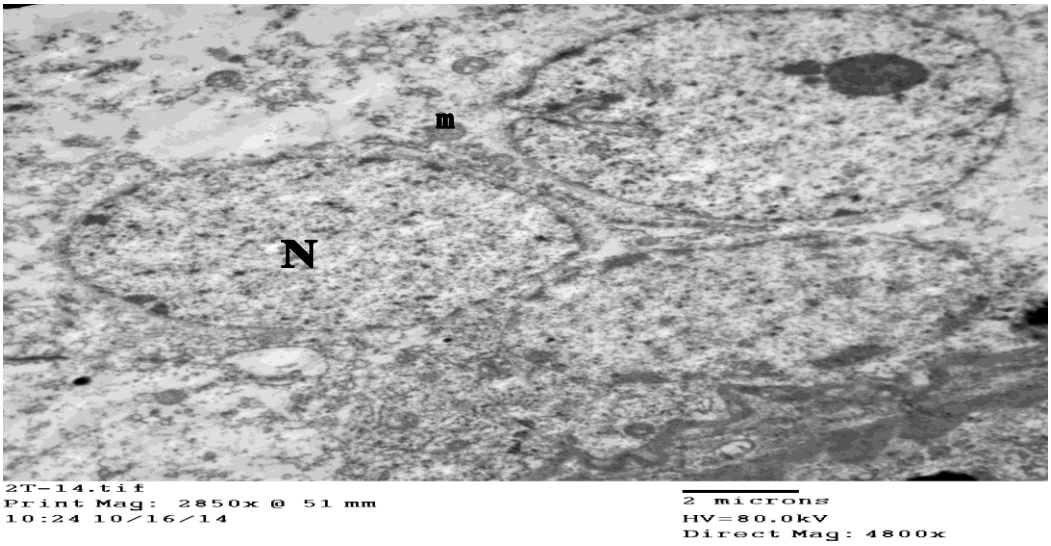

Fig. (١٣): An electron photomicrograph of control rat testis, showing spermatocyte rounded nucleus (N) with fine granular chromatin, the cytoplasm contains multiple mitochondria (M). X ٤٨٠٠.

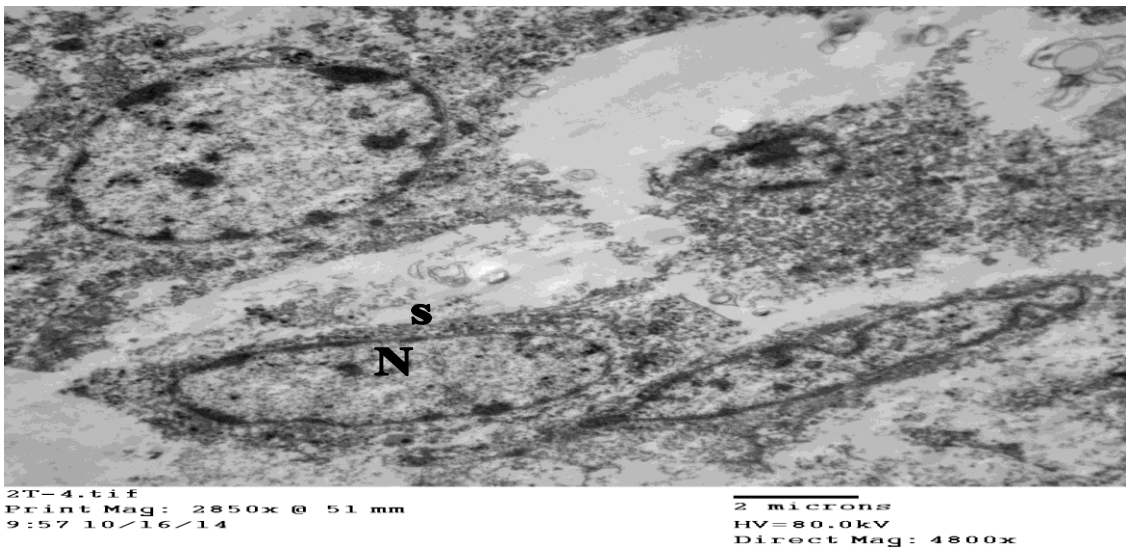

Fig. (١٤): An electron photomicrograph of control rat testis, showing elongated spermatid with piriform shaped nucleus (N) with condensed chromatin. X ٤٨٠٠.

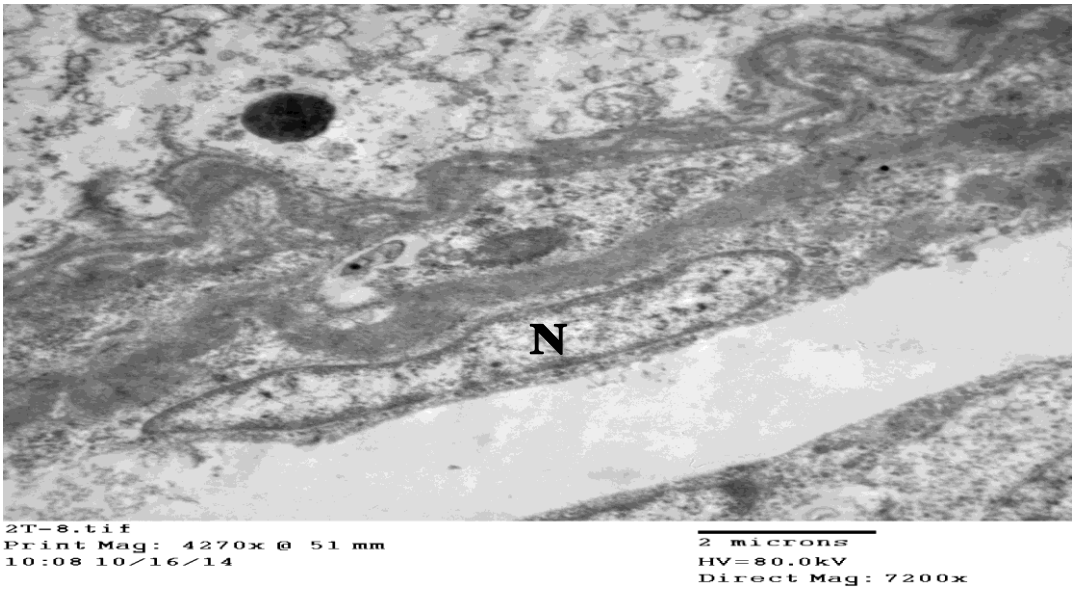

Fig. (١٥): An electron photomicrograph of control rat testis, showing oval elongated nucleus (N) of interstitial cell of Leydig with thin peripheral rim of chromatin. X ٧٢٠٠.

Group II (aluminum- treated):

Light microscopic examination of aluminum-treated rat testis shows thickened tunica albugenia (fig. ١٦) and the seminiferous tubules were distorted, shrunken and separated by a wide interstitium (figs. ١٨ and ١٩). Marked reduction in the thickness of the germinal epithelium with severe degenerative changes was observed (figs. ١٧, ١٨ and ١٩). The germ cells were vacuolated, separated from each other and rested on irregular base mentmembrane (figs. ١٧, ١٨, ١٩).

Electron microscopic examination of aluminum treated rat testis shows many intercellular spaces and vacuolations between the germinal epithelial lining of the seminiferous tubule (fig. ٢٠). Sertoli cellshowed irregular indented euchromatic nucleus, cytoplasmic vacuolations and degenerative mitochondria and lipid droplets (fig. ٢١).The spermatocyte showed an irregular nuclear membrane and clamped chromatin, their cytoplasm contained damaged mitochondria and vacuolated cytoplasmic organelles (figs. ٢٠). The spermatids showed highly distorted nuclear membrane and accumulated chromatin in the form of coarse granules. The mitochondria are distributed throughout the cytoplasm (fig. ٢٢). Cross sections in the middle pieces of sperms showed marked distortion of the central axoneme, fibrous sheath and mitochondrial sheath (fig. ٢٣).

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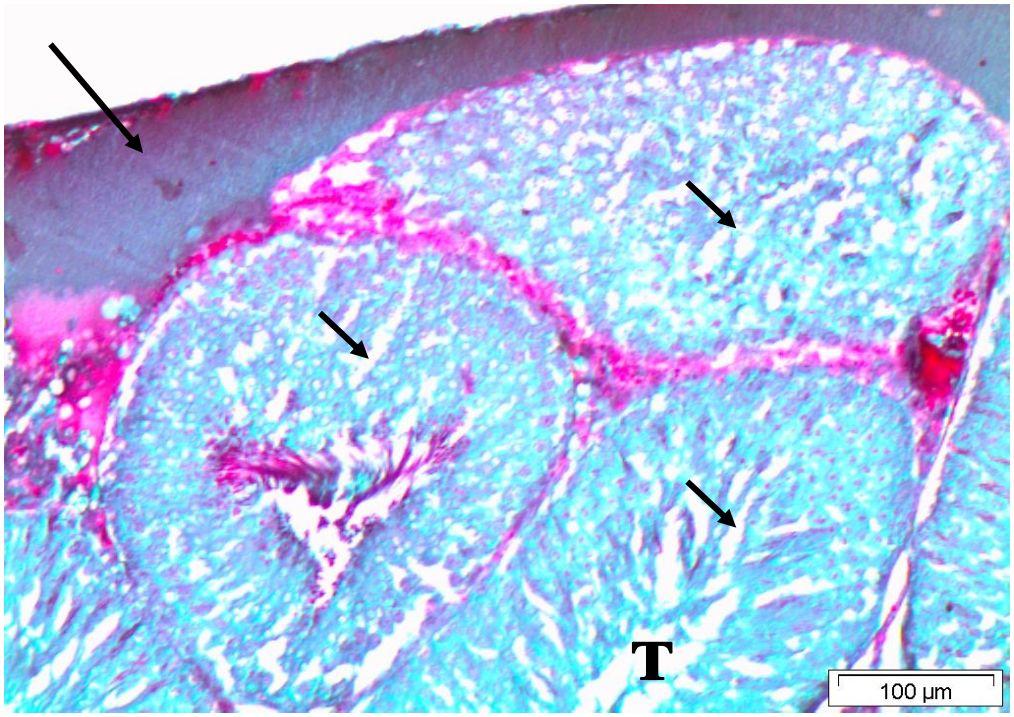

Fig. (١٦): A photomicrograph of a section in the testis of an albino rat of group II, showing some seminiferous tubules (T) with marked Vacculations, whereas others have exfoliated germ cells (short arrows) increase in thickness of tunica albugenia (long arrow) (Masson's trichrome X ٢٠٠).

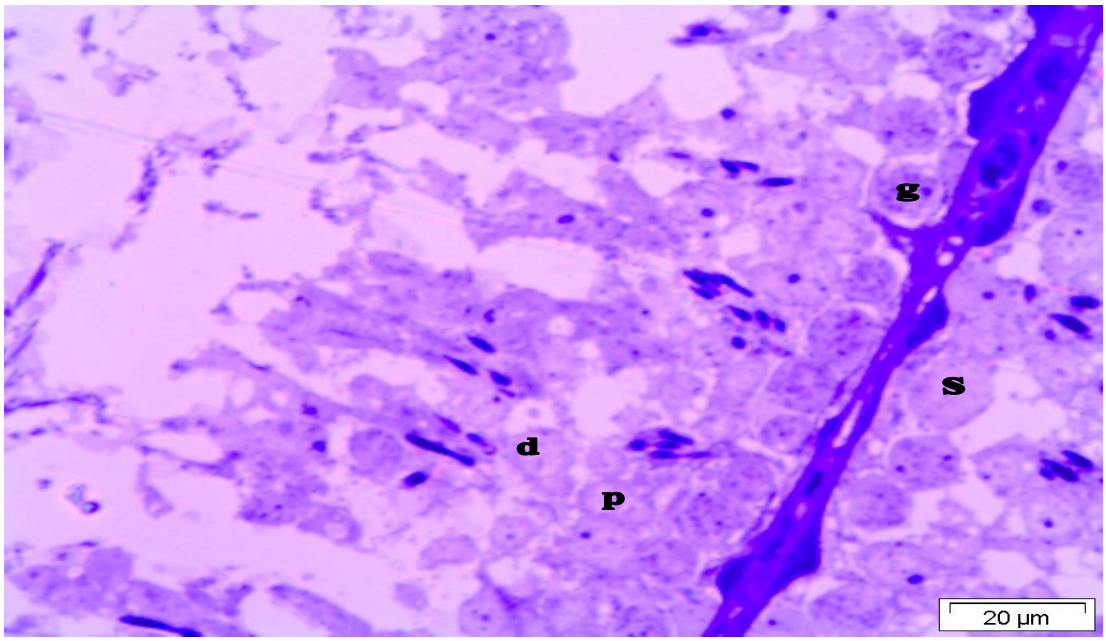

Fig. (١٧): A photomicrograph of a semi-thin section in the testis of an albino rat of group II, showing sertoli cells (S) with vacculated cytoplasm, spermatogmia (g) spermatocyte (p) and spermatid (d) with less chromatin (arrow) (Toluidine blue, X ٤٠٠).

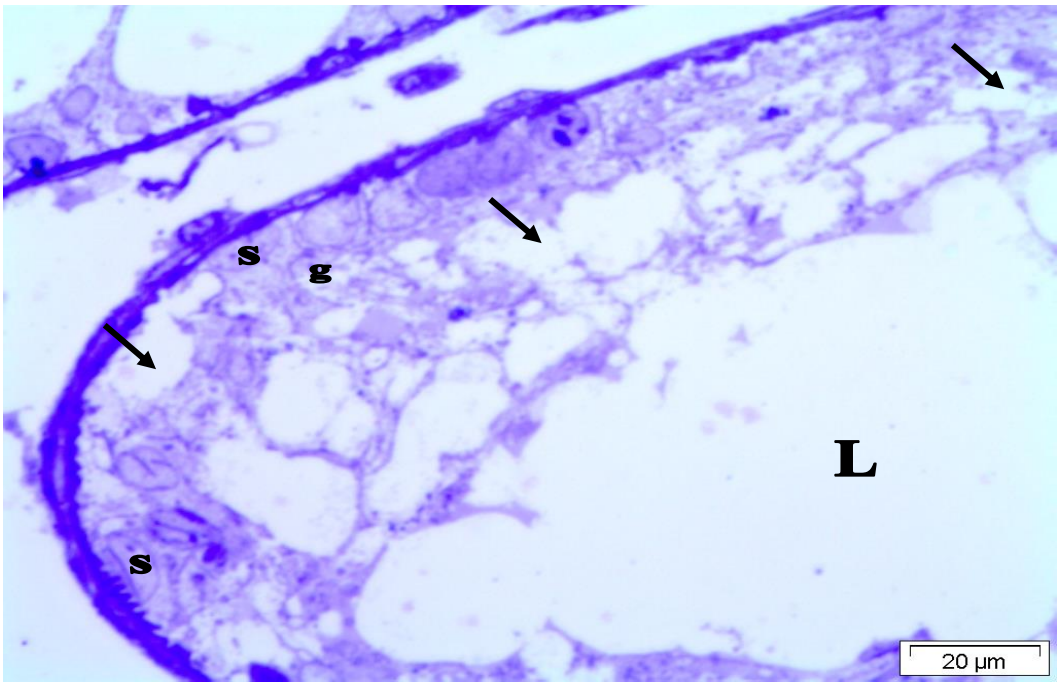

Fig. (١٨): Photomicrograph of semi-thin section of seminiferons tubules with thin epithelium, Sertoli cells with vacculated cytoplasm (s), spermatogonium with less chromatin (g) wide lumen (L), tubular atrophy, necrosis and degeneration of spermatogenic cells (arrows) (Toluidine blue, X ٤٠٠).

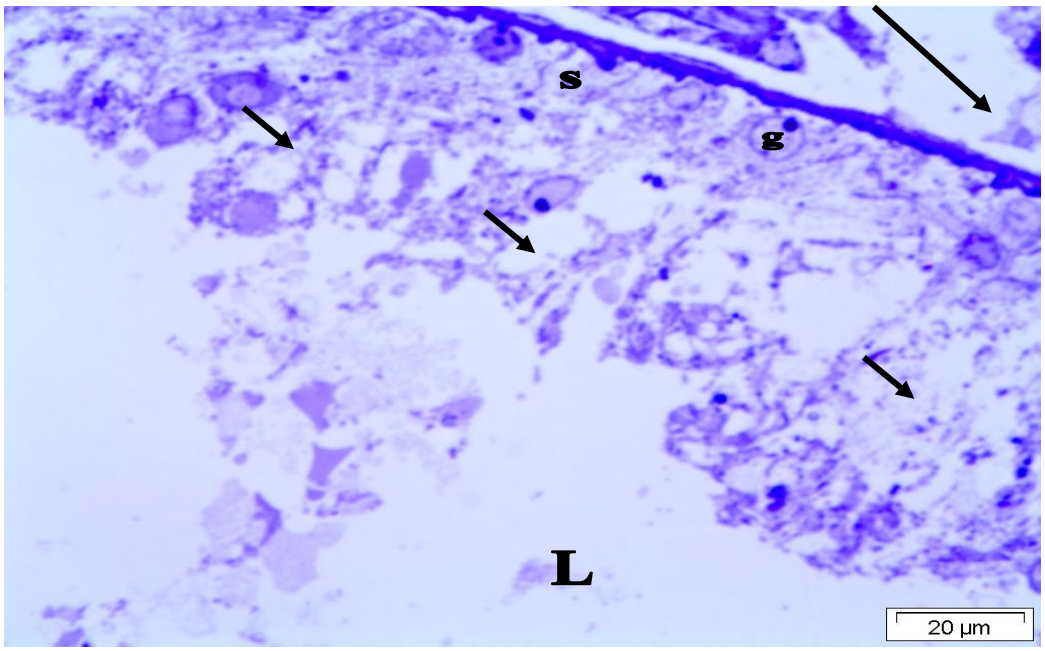

Fig. (١٩): Photomicrograph of semi-thin section of seminiferons tubules with thin epithelium, wide lumen (L) tubular atrophy and necrosis and degeneration of spermatogenic cells (short arrows) interstitial oedema (long arrow) (Toluidine blue, X ٤٠٠).

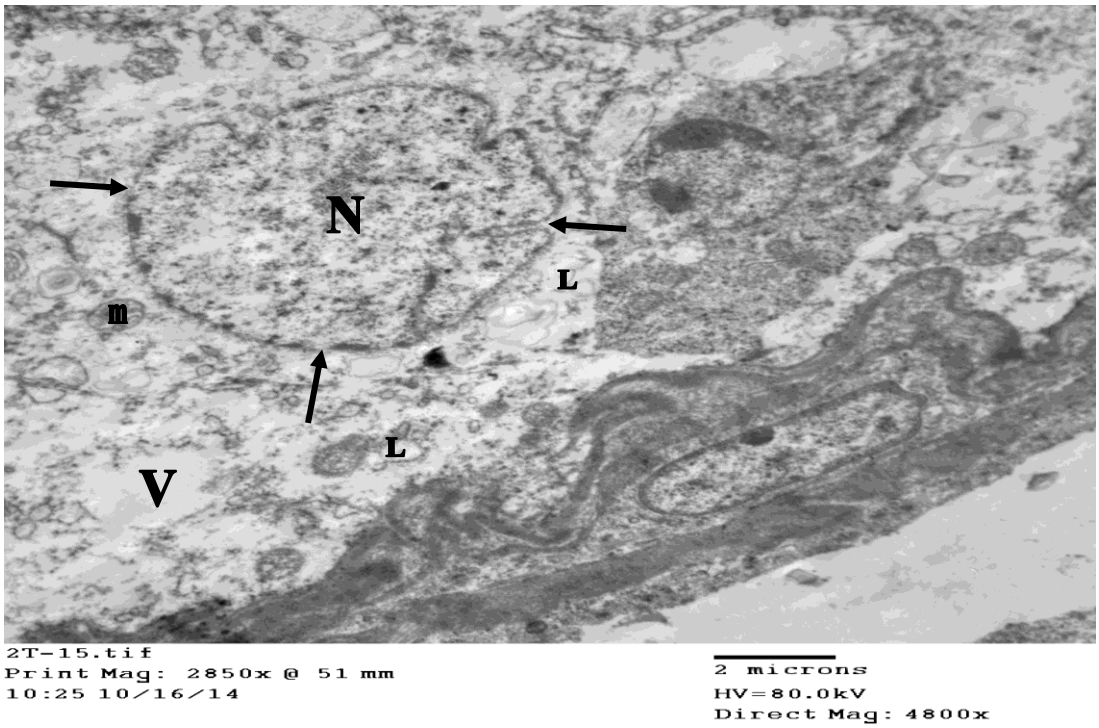

Fig. (٢٠): An electron photomicrograph of the testis of an albino rat of group II, showing primary spermatocyte with shrunken indented nucleus (N) with large pores in nuclear membrane (arrows) damaged mitochondria intracellular vacoules (V) and lipid droplets (L) X ٤٨٠٠.

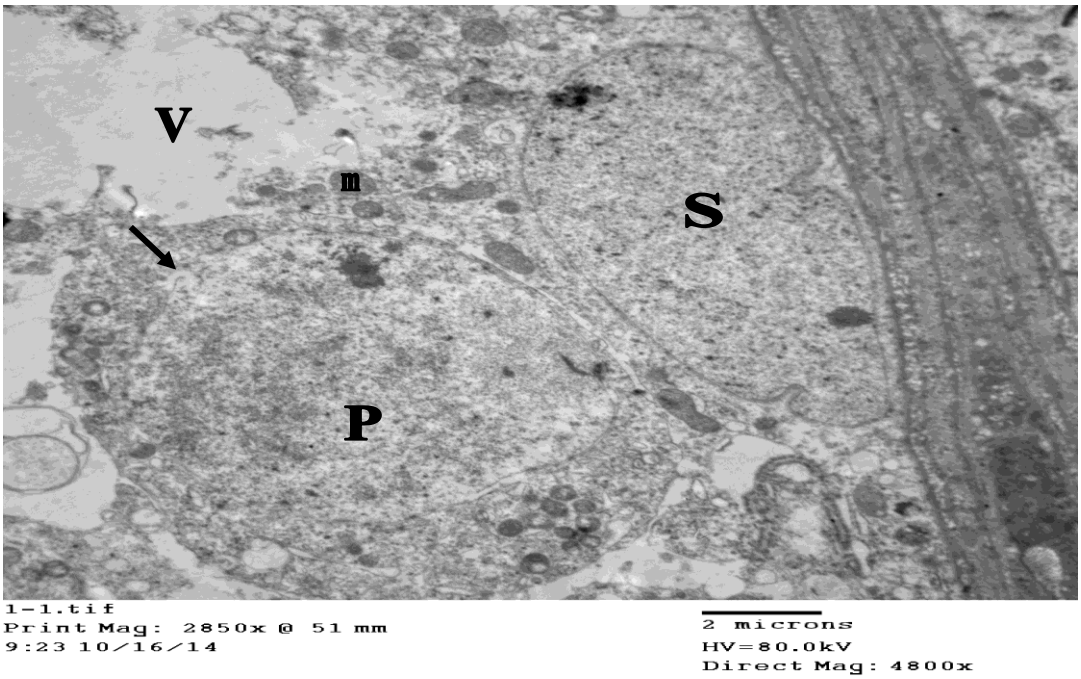

Fig. (٢١): An electron photomicrograph of the testis of an albino rat of group II, showing many intra cellular spaces or vacoules (V) between germinal epithelial lining of the seminiferous tubules Sertoli cells (S) with irregular indented euchromatic nucleus lies on the basement membrane and primary spermatocytes (P) with heterochromatin and large pores in nuclear membrane (arrow) the cytoplasm shows damged mitochondria(m) (x ٤٨٠٠).

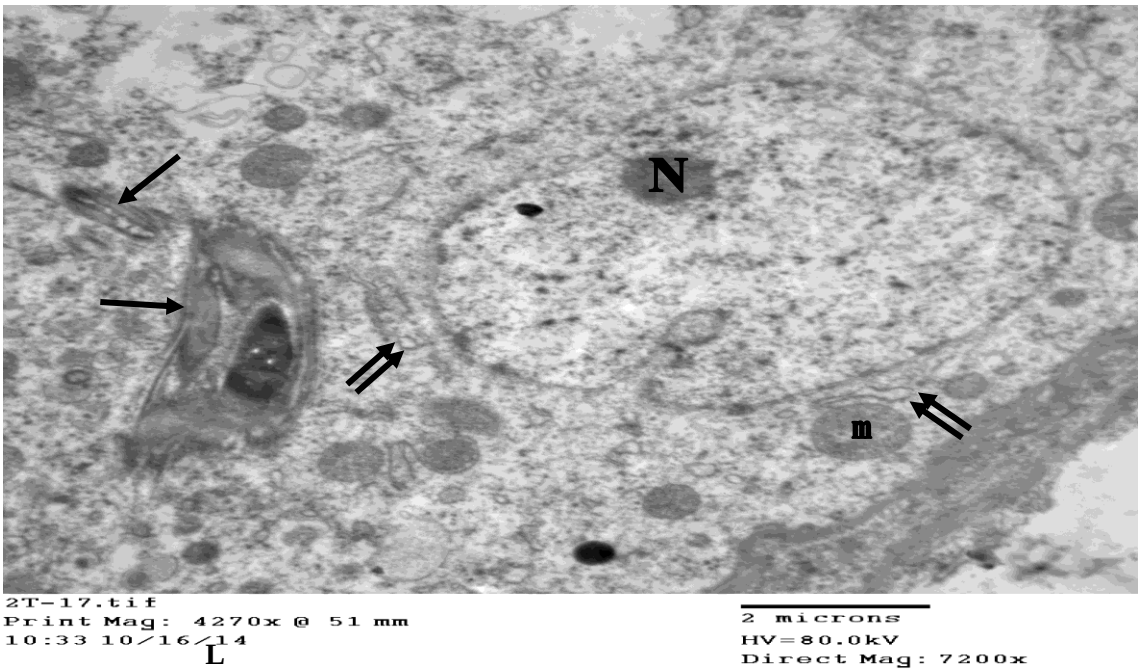

Fig. (٢٢): An electron photomicrograph of the testis of an albino rat of group II, showing spermatids with irregulareuchromatic indented nucleus (N). The cytoplasm contains heads of sperms (single arrow) mitochondria (m), droplets (L) and endoplasmic reticulum (double arrows) X ٧٢٠٠.

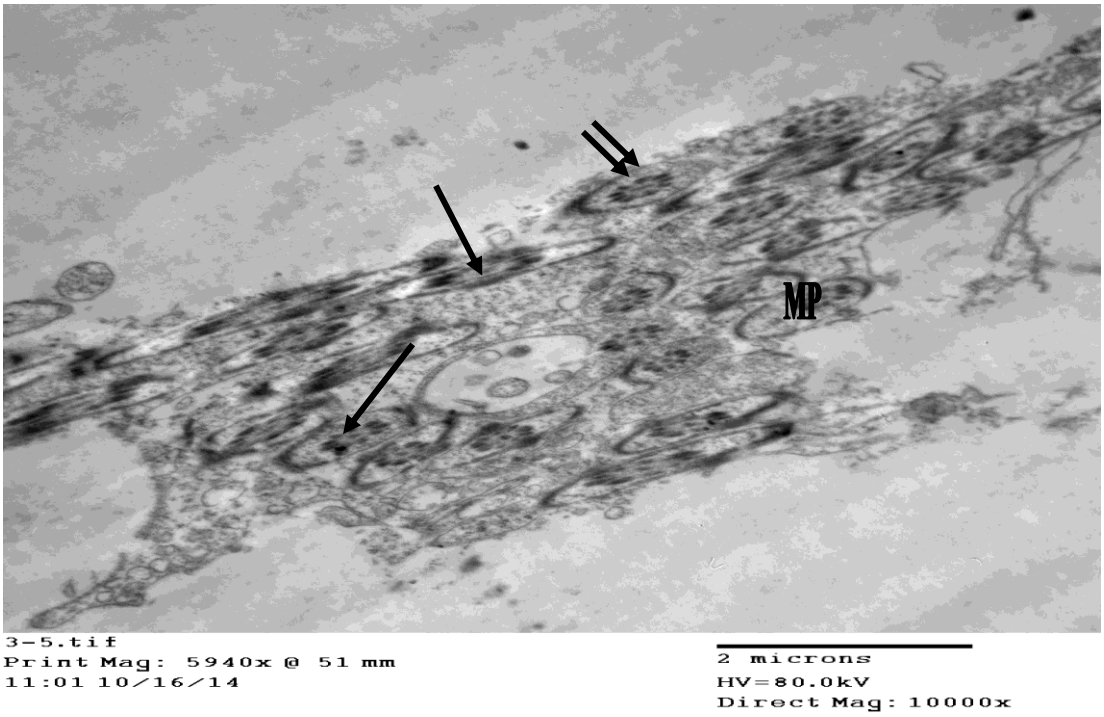

Fig. (٢٣): An electron photomicrograph of the testis of an albino rat of group II, showing cross sections in the middle pieces of sperms (MP), with marked distorsion of the central axoneme (long arrow), fibrous sheath (short arrow) and mitochondrial sheath (double arrows) X ١٠٠٠٠.

The group III (selenium ACE-treated):
Light microscopic examination of selenium ACE-treated rat testis shows several rounded or oval seminiferous tubules with patent lumina separated by interstitial tissue (Figs. ٢٤). The tubules are bounded by basement membrane (Fig. ٢٥). The wall of the seminiferous tubules is formed of ٤-٦ layers of germinal stratified epithelium. The lumen of seminiferous tubules contains spermatozoa (Fig.٢٤ and ٢٥).Electron microscopic examination of the same group showsSertoli cells resting on a basement membrane. Normal spermatogonia and primary spermatocytes with normal cytoplasmic organelles as mitochondria found in (Fig. ٢٧). Rounded spermatids with spherical nuclei and elongated spermatids with piriformshapd nuclei with dense chromatin (Fig. ٢٦) Leydig cells appear with oval indented nuclei with peripherally clumped chromatin and their cytoplasm contain normal mitochondria (Fig. ٢٦).

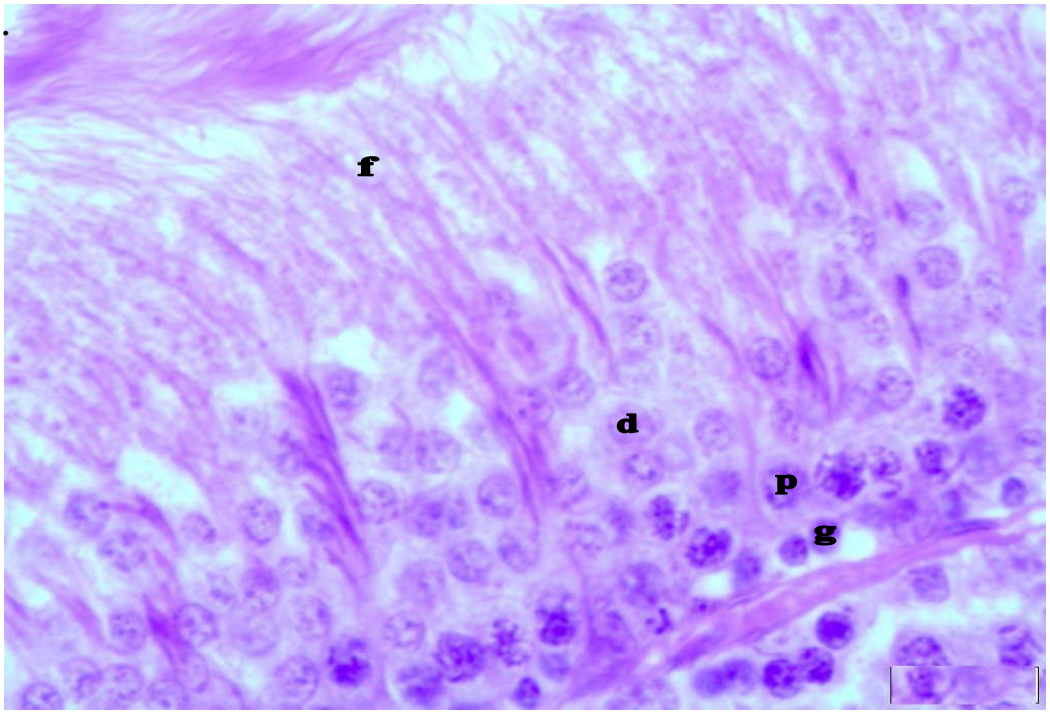

Fig. (٢٤): A photomicrograph of a section in the selenium ACE treated rat testis, showing spermatogonia (g) primary spermatocyte (p) spermatids (d) flagella (f) (Haematoxylin& Eosin X ٤٠٠).

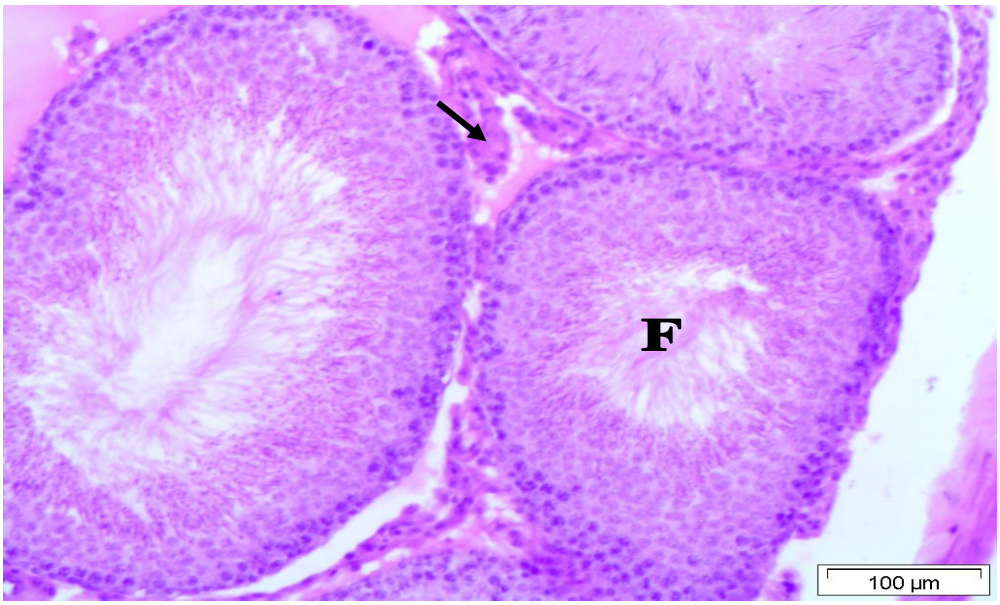

Fig. (٢٥): A photomicrograph of a section in the selenium ACE treated rat testis, showing seminiferons tubules with rounded, regular outlines notice sperm flagella (F) and narrow interstitium containing clusters of interstitial cells of Leydig (arrow) (Haematoxylin& Eosin X ٢٠٠).

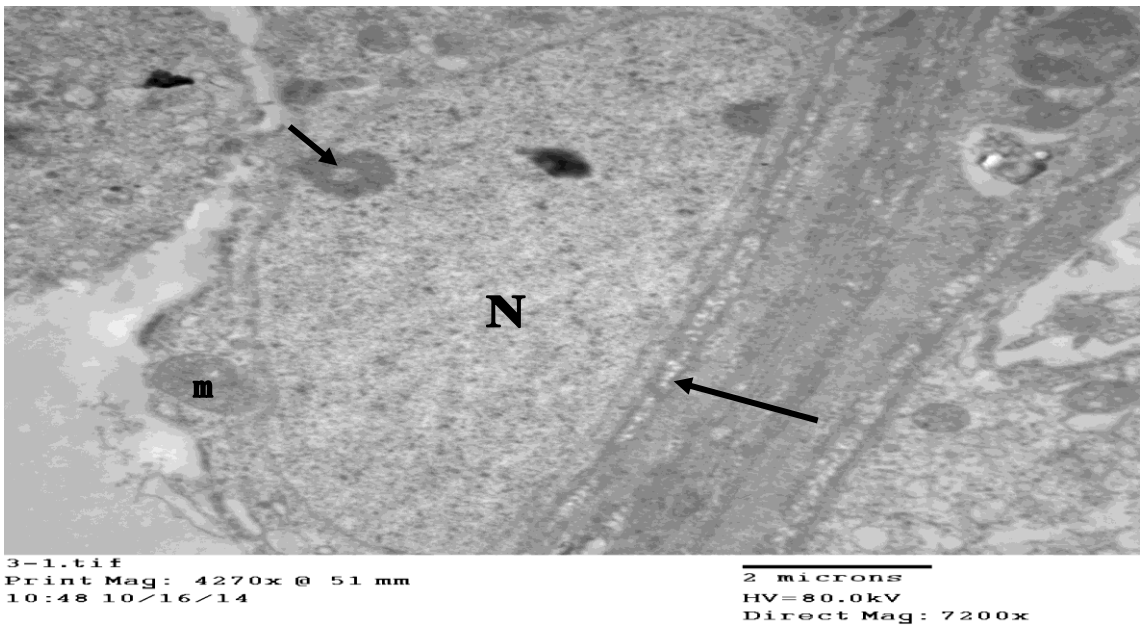

Fig. (٢٦): An electron photomicrograph of the selenium ACE treated rat testis, showing Sertoli cell with triangular nucleus (N) well developed nucleolus (short arrow). It appears resting on the basement membrane (long arrow). Its cytoplasm contains mitochondria (M) X ٧٢٠٠.

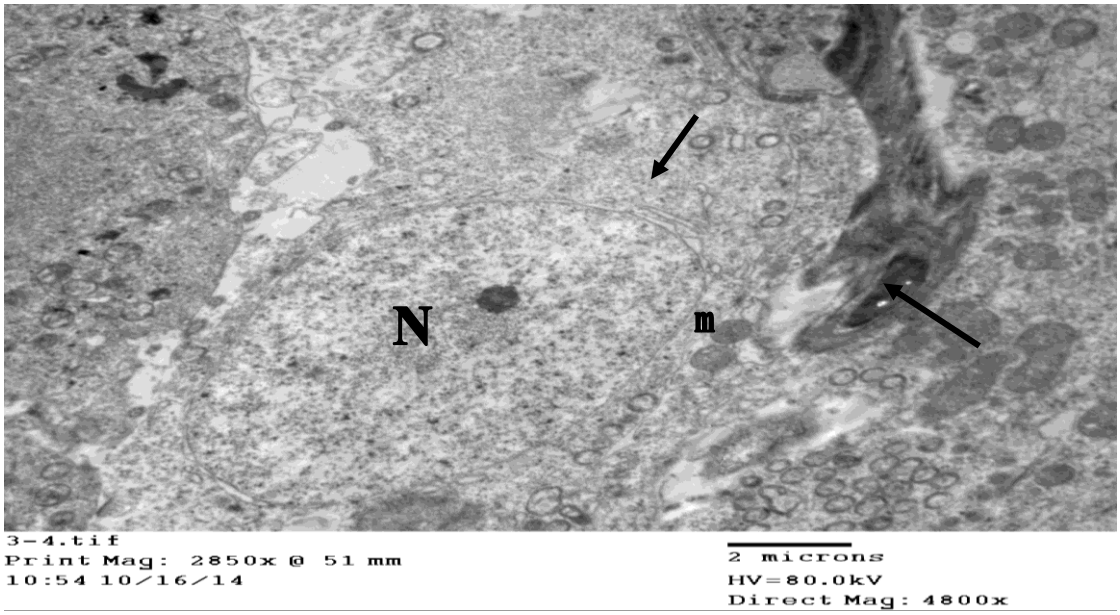

Fig. (٢٧): An electron photomicrograph of the selenium ACE treated rat testis, showing primary spermatocyte with large rounded nucleus (N) of dispersed granular chromatin and its cytoplasm contains mitochondria (m) and endoplasmic reticulum (double arrows) and head of sperm (long arrow) X ٤٨٠٠.

Group IV: (aluminum and Selenium ACE) group:

Light microscopic examination: showing improvement of the general histological picture of the testis, where multiple rounded seminiferous tubules with regular out lines are found (Fig. ٢٨). There are abundant whorly appearance of sperm flagella filling their Lumina (Fig. ٢٨) and some Leydig cells in the interstitial spaces (Fig. ٢٨). Electron microscopic examination shows spermatogonia, Sertoli cells, primary spermatocytes and spermatids apparently normal (Fig. ٢٩ and ٣٠).

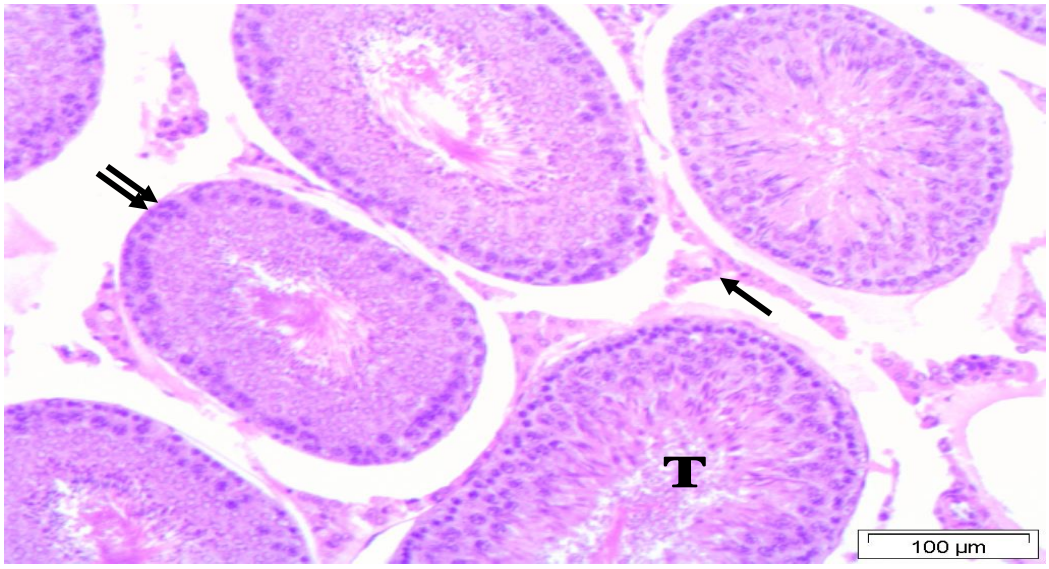

Fig. (٢٨): A photomicrograph of a section in the (aluminum + selenium ACE) treated rat testis, showing seminiferons tubules with rounded, regular outlines (T) and narrow interstitium containing clusters of interstitial cells of Leydig (arrow) and intact basement membrane (double arrows) (Haematoxylin& Eosin X ٢٠٠).

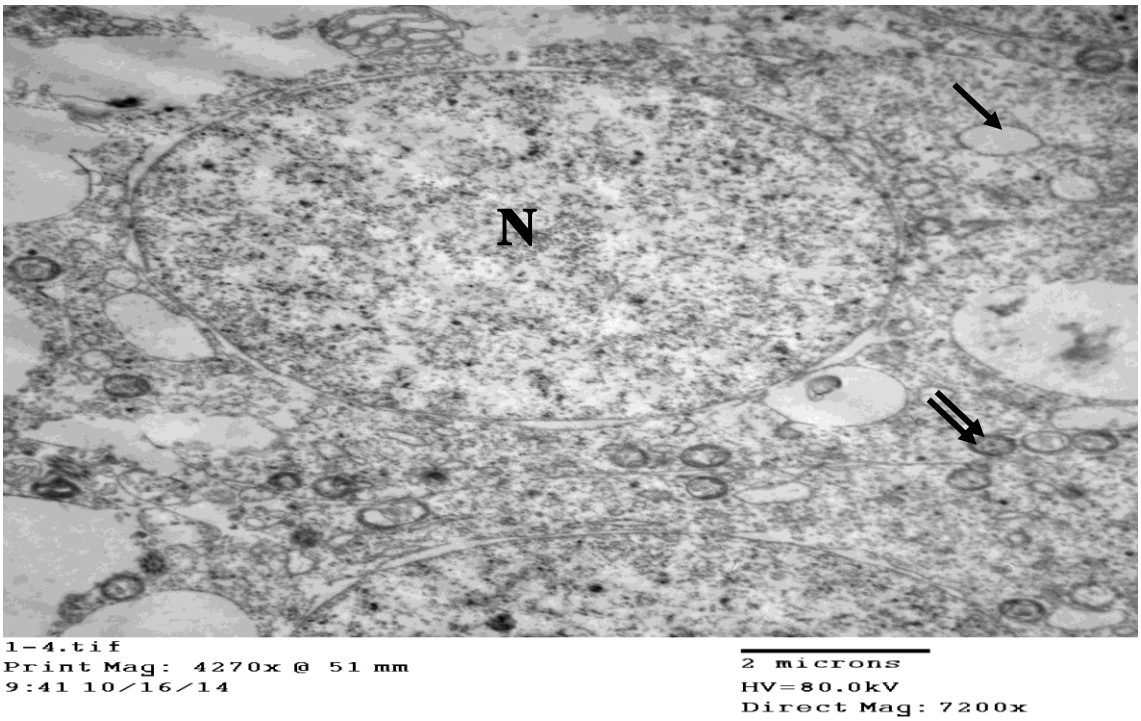

Fig. (٢٩): An electron photomicrograph of the (aluminum + selenium ACE) treated rat testis, showing primary spermatocyte with large rounded nucleus (N) of dispersed granular chromatin and its cytoplasm contains mitochondria (double arrows) and lipid droplets (arrow) X ٧٢٠٠.

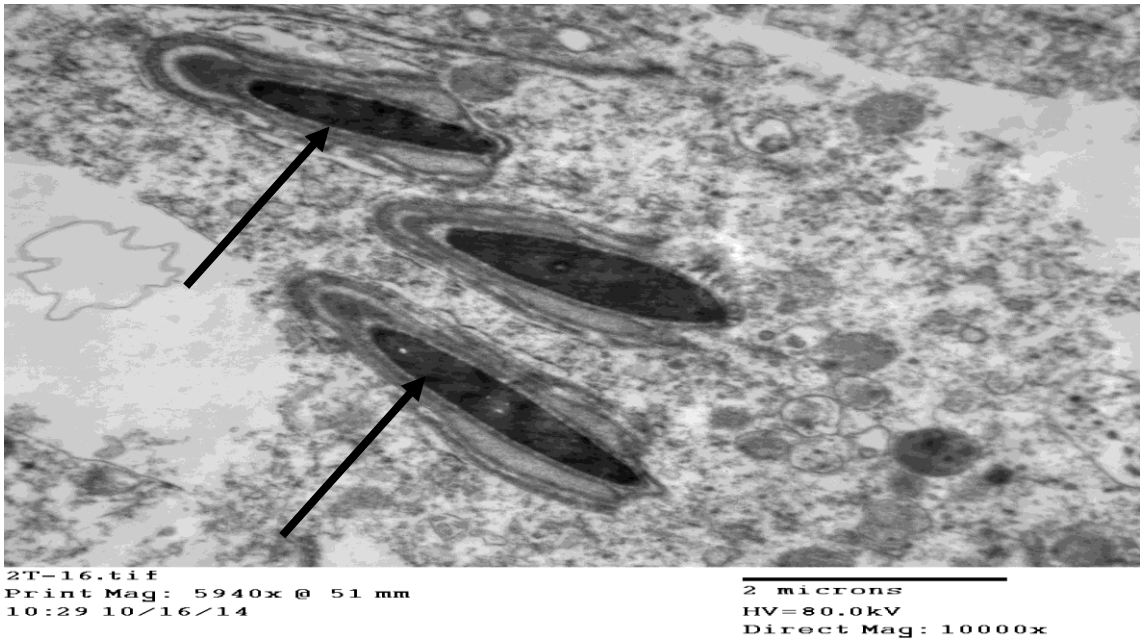

Fig. (٣٠): An electron photomicrograph of the (aluminum + selenium ACE) treated rat testis, showing elongated spermatid with piriform shaped nucleus (arrow) with condensed chromatin X ١٠٠٠٠.

Discussion

Body weight:

In the present work, there was an increase in the final body weight in the control group. This is in agreement with Odendaal and Reineeke, (٢٠٠٤) who reported that the mean weight of unexposed animals in the control group increased at the end of the experiment.

In the present work, results indicated a significant decrease in the body weight gain of aluminum-treated group ($p< ٠.٠٠١$) in rats group received aluminum chloride as compared with control, these results were in agreement with Kowalczyk et al., (٢٠٠٤) who found that during three months observation of rats receiving aluminum chloride, there was decrease in water and food intake and transient diarrhea occurred, which resulted in lowering of final body mass of animals in comparison to the controls (differences statistically significant).

The present results showed that oral administration of selenium ACE had no effect on body weight gain, this confirmed its safe use and agreed with Clifton, (٢٠٠٤). In aluminum-selenium ACE treated group, there was a significant increase in body weight compared with aluminum treated group. This is in agreement with Colombia et al., ١٩٩٤ who confirmed the protective role of ascorbic acid, selenium and vitamin E against aluminum toxicity.

Testis weight:

In the present study, the mean testis weight was ١.٨gm, ٠.٩٤ gm, ١.٨gm and ١.٦٥ gm in group I, group II, group III and group IV respectively, it was observed that there is a significant decrease in the testis weight of aluminum-treated group (group II) ($p< ٠.٠٠١$) relative to the other groups, these results are in agreement with Bataineh et al., (١٩٩٨) who found decrease in absolute and relative testes weights and seminal vesicles weights after aluminum chloride ingestion. The decrease in the reproductive organs weights could be due to the decrease in testosterone level.

Sperm count, motility and viability:

In the present study, aluminum-treated group induced highly significant decrease in sperm count, motility (%) and viability (%), with increase in dead and abnormal sperm count as compared to both control group, selenium ACE-treated and aluminum-selenium ACE treated group, this means that selenium ACE minimized the toxicity of aluminum. Previous studies by Bataineh el al., (١٩٩٨) and (Liobet et al., ١٩٩٥) showed that, necrosis of spermatocytes and spermatids which was observed in the testes of rat exposed to aluminum.

Liobet et al., (١٩٩٥) and Yousef et al., (٢٠٠٧) showed also that aluminum declined semen quality in vivo and vitro, and induced significant decrease in ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperm per ejaculate, packed sperm volume, normal and live sperm. Moreover, reactive oxygen species and oxidative damage may contribute to male infertility by reducing sperm function (Atessahin et al., ٢٠٠٥). The results of the present study were in agreement with that ofAdams et al., (١٩٩٤) & Bondy et al., (١٩٩٨) who proved that excessive aluminum oxide levels have been shown to suppress testosterone synthesis and to cause cytotoxicity of spermatozoa, with decreases vitality and mobility of sperm and an increase in morphological abnormalities of sperms. In the present work, alumnium-selenium ACE treated group significantly alleviated the decline in sperm count, motility and viability compared to aluminum-treated group ,these results were in harmony with the work of (Liobet et al., ١٩٩٥) who noticed that there were minimizing the hazardous effects of aluminum by selenium and vitamin E on spermatid counts and epididymis sperm counts. Hsu et al., (١٩٩٨) reported that vitamin E prevents the mobility and loss of spermatozoa by promoting a reduction in reactive oxygen species (ROS) production. Supplementation Vitamins C and E reduced the generation of ROS thereby significantly

escalating sperm count and declining the frequency of abnormal sperm (Acharva et al., ٢٠٠٢).

٢) Histological finding:

In the present study, examination of sections of the control rat testis showed several rounded or oval seminiferous tubules with patent lumina separated by interstitial tissue. The testis is surrounded with tunica albugenia which is formed of condensed collagen fibers. The tubules are bounded by limiting membrane. These findings are in agreement with Junqueira & Carneiro (٢٠٠٥). It was observed that the wall of the seminiferous tubules is formed of germinal stratified epithelium and supporting Sertoli cells. The spermatogenic cells were arranged in layers that occupy the space between the limiting membrane and the lumen of the tubule, they included spermatogonia, primary spermatocytes, spermatids and finally spermatozoa. These observations are in accordance with the finding reported by Bloom & Fawcett, (١٩٨٦) and Williams et al., (٢٠٠٥). Primary spermatocytes occupy the middle zone of the seminiferous epithelium. They were the largest cells in the tubules which appear rounded or oval in shape with large rounded or oval nuclei. These observations are in accordance with Cormack, (٢٠٠١). Two types of spermatids were seen near the lumina of seminiferous tubules. The early formed spermatids which appear small and rounded with peripheral nuclei, however the mature elongated spermatids appear with deeply stained nuclei. Variable numbers of spermatids are liberated in the lumina of seminiferous tubules. These findings are in agreement with Ronald et al., (١٩٩٦) who reported that the mature spermatids did not divide further but go through a cytological transformation in several steps to mature spermatozoa. Sertoli cells were observed on the limiting membrane, at intervals between the spermatogonia. They are large irregular cells approximately columnar with tapering apices towards the lumen. Their nuclei appear oval in shape with pale cytoplasm. This is in accordance with the finding reported by Williams et al., (٢٠٠٥).

The interstitial Leydig cells appeared triangular, ovoid or polygonal in shape with large eccentric nuclei. These findings are in agreement with the finding reported by Nistal et al., (١٩٨٤). The present study showed that ultrastructure of control group showed that Sertoli cells had large euchromatic nuclei and prominent nucleoli. Spermatogonia appeared with rounded nuclei resting on basement membrane. Primary spermatocytes had rounded nuclei and a thin rim of cytoplasm containing mitochondria. Spermatids had rounded or elongated nuclei with a condensed chromatin and numerous peripherally arranged mitochondria. Cross sections in the middle pieces of sperms showed a central axoneme surrounded by fibrous sheath and mitochondrial sheath, their cytoplasm contained mitochondria and lipid droplets. These results were in agreement with Junqueira & Carneiro (٢٠٠٥) .

In the present study light microscopic examination of aluminum-treated group showed thick tunica albugenia compared to the control group, many distorted and shrunken seminiferous tubules separated by a wide interstitial spaces. Marked reduction in the thickness of the germinal was observed in some tubules and exfoliated germ cells appeared in the Lumina of others. The germ cells were vacuolated, separated from each other and rested on irregular basement membrane. Spermatids were almost absent and sperm numbers are low or there were no sperm in the lumen. The same results were recorded by Liobet et al., (١٩٩٥) who stated that the effect of aluminum nitride on the testis of the mice and found that the spermatocytes and spermatids are necrosis.

Electron microscopic examination of aluminum-treated group showed irregularities in the nuclear membrane some damaged mitochondria, a decrease in the number of ribosomes, and an increase in the number of lysosomes in the Steroli cell cytoplasm. The primary spermatocytes had irregular euchromatic indented nuclei; their cytoplasm showed increase in the rough endoplasmic reticulum and

damaged mitochondria. Cross sections of mid pieces of sperms showed marked distortion of centralaxoneme, fibrous sheath and mitochondrial sheath. These observations are in agreement with the work of Chinoy et al., (٢٠٠٥), who showed that administration of aluminum chloride (٢٠٠ mg/ kg body weight) to mice for ٣٠ days, caused degenerative in structure of spermatogenesis and formation of giant cells.

Guo et al., (٢٠٠٢) has reported that aluminum caused testicular toxicity in mice, after intraperitoneally exposed to aluminum chloride (٣٥ mg /kg. body weight) for a period of ١٤ days. Mayyas, et al., (٢٠٠٥) also, reported the same results after the mice treatment with aluminum chloride, and found that destruction of the seminiferous tubules with large necrotic areas and degenerative cells.

In the present study, aluminum-selenium ACE treated group showed large numbers of spermatids, sperm in the seminiferous tubule lumen and the spermatogenetic cells and the Steroli cell cytoplasm showed an almost normal appearance. These observations are in agreement with Youssef et al., (٢٠٠٥) who stated that selenium ACE antagonize the toxic effects of aluminum at the histological level, thus potentially contributing to an amelioration of the testis pathology in the aluminum-treated rats. The ultrastructure of the testis in the selenium ACE group showed a normal appearance, these results in agreement with (Kutlubay et al., ٢٠٠٧) who reported that co-administration of aluminum plus vitamin E showed large number of spermatids and sperms in the seminiferous tubule lumen with normal germinal epithelium. These results strongly endorse the findings of Wenzelet al., (٢٠٠٤), who have reported that ascorbic acid dose-dependently inhibited the apoptotic response of cells.Dietary supplementation, particularly antioxidants, such as vitamin C, vitamin E and beta-carotene has the potential to improve the male reproductive outcomes by reducing the extent of oxidative damage (Wong et al., ٢٠٠٠).

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