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Histological and Ultrastructural Studies on the Testis of Rat after Treatment with Aluminium Chloride

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Abstract: The present investigation was conducted to reveal the effect of aluminium chloride on the testis of rat. The aluminium chloride was administered through an intraperitoneal injection every other day at doses of 15 and 30 mg. / kg. body weight for five weeks**.** The testis of the experimental group, showed histological changes including severe damage within the seminiferous tubules and vascular degeneration on the spermatogenic and Sertoli cells cytoplasm. On the other hand, electron microscopy study revealed changes in the testis seminiferous tubules such as atrophy of the tubular membrane, mitochondria, endoplasmic reticulum, Golgi apparatus and nucleus. The multinucleated giant cells appeared in the cytoplasm of the treated rat testis with high doses.

Key words: Aluminim chloride, Rats, Testis and Ultrastructure

INTRODUCTION

Aluminium (Al) has for a long time been considered on an indifferent element from a toxicological point of view. However, it is unclear whether normal environmental levels of Al. especially Al. contained in food, exert many toxic effect without the aid of other substances, since the toxic effects of environmental factors are usually enhanced by their interactions with other factors (Slanina *et al.* , 1984). Aluminium toxicity has been well documented in the pathogenesis of many disorders in patients undergoing long term dialysis including dialysis encephalopathy (Alfrey *et al.* , 1976).

Most of experimental studies on aluminium toxicity in an animal model have been preformed with the use of this metal in a soluble form (Alfrey, 1984) or with certain metal (Ebina, *et al.* , 1984).

Aluminium compounds are widely used in medicine e. g. antacids, phosphate binders, buffered aspirins, vaccines and allergen injections (Cannata, *et al.* , 1983; Kaehny, *et al.* , 1997 & Lione, 1985).

It has recently been demonstrated that ingestion of aluminium compounds with either fruit juices or citric acid causes a marked increase in both gastrointestinal absorption and urinary excretion of Al in healthy subjects (Slanina, *et al.* , 1986 & Weberg, *et al.* , 1986). Aluminium has been tested for ant fertility in male rats and was found to show significant activity (Sharma, *et al.* , 2003).

ATSDR (1990) reported that aluminium is distributed mainly in bone, liver, testis, kidneys and brain.

Guo, *et al.* (2002) observed that reduced testis acetylcholinesterase (ACE) activity presumably plays an important role in oxidative damage of aluminium induced testicular toxicity. Mayyas, *et al.* , (2005) reported that histological changes in testicular sections of adult male mice after ingested aluminium chloride. And also, Libet *et al.* , (1995) studied the reproductive toxicology of aluminium in male mice and observed that some histological changes, including necrosis of spermatocytes and spermatids in the testis of male mice treated with aluminium nitrate. The toxic effects of aluminium chloride were reported earlier (Chinoy, *et al.* , 1996 & 1997). Chinoy, *et al.* (2005) studied the effect of sodium fluoride together with aluminium chloride to male mice for 30 days and found some structural alteration in the testis with formation of giant cells.

Krasovskii *et al.* (1979) studied the biological effects of lead and aluminium on rats and guinea pigs and observed that the lead and aluminium chloride caused gonad toxicity.

Memon *et al.* (1998)] reported the same toxicity on mice testis after the effect of sodium fluoride and /or aluminium chloride.

The present study was undertaken to determine the compartmentalization of aluminium in normal rat following intraperitoneally injection with aluminium chloride

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MATERIALS AND METHODS

Thirty male albino rats, weight about 100-150 gm. were divided to three groups of 10 rats each. One group of rats was a normal control group and the other two groups received 2 doses of intraperitoneal injections of aluminium chloride in saline 15 and 30 mg / Kg. body weight. Injections were given every other day for five weeks. The samples of normal and treated rats were collected for light and electron microscopic examination.

The testis was cut into Small pieces about 1 mm. thick. The tissues immersed for 3 to 5 mins. in cold (4^0C) phosphate buffered then fixed in 2% Para formaldehyde and 2% glutaraldehyde in 0. 02 M phosphate buffer and then after placed in fresh cold fixative for 24 hrs. in refrigerator. The tissue was rinsed in cold phosphate buffer (pH 7. 4) and post fixed in 2% aqueous osmium tetroxide for 2 hrs. at 4^oC. The samples were then washed in 0. 1 M phosphate buffer (2 times) then dehydrated in ethanol series and embedded in epoxy resin.

Semithin sections (1um) were cut by RMC ultratome, stained with 0. 5% toludine blue in borax and examined under light microscope.

Ultrathin sections were mounted on copper grids stained with 2% aqueous uranyl acetate for 20 mins. , and lead citrate for another 20 mins. , then examined and photographed using electron microscopy (Joel 1200 Ex-II).

RESULTS AND DISCUSSIONS

Light Microscopic Examination:

Control Group:

The light microscopy examination of the testis of the control rats had normal structure and completely enveloped by a thick capsule, tunica albuginea, which is composed mainly of dense collagenous fibrous connective tissue. The structural components of the testis are the seminiferous tubules and interstitial tissues. the seminiferous tubules are two types of cells, the Sertoli cells, resting on the thin basal lamina (basement membrane) and the spermatogenic cells. These cells are many layers, namely, the spermatogonia, primary and secondary spermatocytes; spermatoids and finally mature spermatozoa. (Fig. 1).

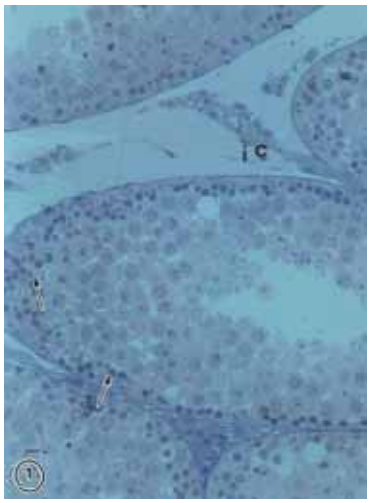


Fig. 1: Photomicrograph of a semithin section of the testis of a normal control rat, showing the different stages of spermatogonic cells in the seminiferous tubules (arrows) and the interstitial cells (IC). Toludine blue stain. X1200.

Treated Groups:

1. Low Doses:

After rats treated with the low dose (15 mg/kg body weight) of aluminium chloride for five weeks, revealed cellular changes. The seminiferous tubules had thickened in basement membrane together with focal areas of vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium and in the

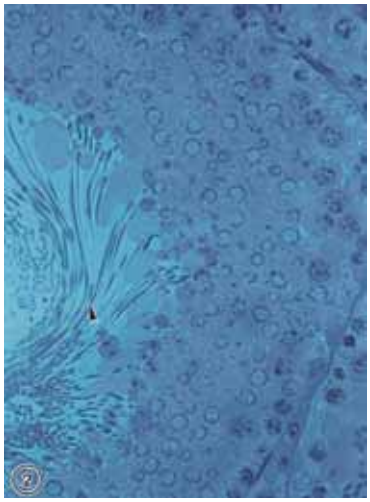
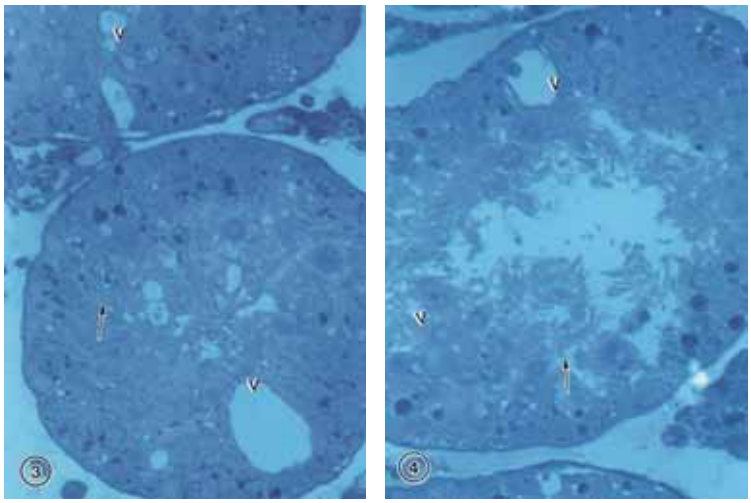


Fig. 2: Photomicrogh of a semithin section of rat testis after treated with low dose (15 mg/kg body weigh for 5 weeks) of aluminium chloride, showing a part of the damaged in seminiferous tubules cells and late spermatide heads appeared twisted (head arrow). Toludine blue stain. X3000.

Sertoli cells and abnormal distribution of spermatozoa showed in the Lumina of the seminiferous tubules (Fig. 2).

2. High Doses:

After rats treated with high dose (30 mg/kg body weight) of aluminuim chloride for five weeks showed more exaggerated features of focal areas of spermatogenesis, arrest at the spermatid level, in the form of degenerative changes in the germinal cells together with few fragmented sperms in the lumen and acquired a thick, irregular basement membrane (Figs. 3 & 4).



Figs. 3, 4: Photomicrographs of semithin sections of the rat testis treated with high dose. (30 mg/kg body weigh for 5 weeks) of aluminium chloride showing severely damaged in seminiferous tubules cells (arrows) and vacuolation in the cells (V). Toludine blue stain. X1200. & X1200, respectively.

Electron Microscopic Examination:

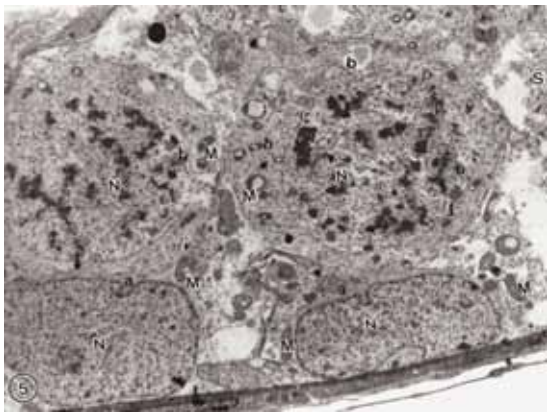
Control Group:

The electron micrographs of the control rats testis showed the following criteria:

Spermatogonia:

The spermatogonia are large diploid cells which lie against the boundary tissue of the seminiferous tubules and divide mitotically. They are two types of spermatogonia are A-type and B-type. The A-type spermatogonia 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, the chromatin is homogenous and the cytoplasm is scantly, granular, poor rough endoplasmic reticulum but abundance of ribonucleoprotein particles. The mitochondria are spherical or ovoid and the Golgi apparatus is simple (Figs. 5 & 6). The B-type cells are slightly smaller which contain rounded nuclei with more electron dense nucleoplasmic matrix than A-type and numerous chromatin clumps. The cytoplasmic organelles are similar to those described in the A-type (Fig. 5).

The intercellular bridges : The germinal cell population is characteristic by that cytoplasm does not separate completely, the cells remain linked in groups until the sperms are released. The cytoplasm in the bridge is similar to that of the germinal cells (Fig. 6).



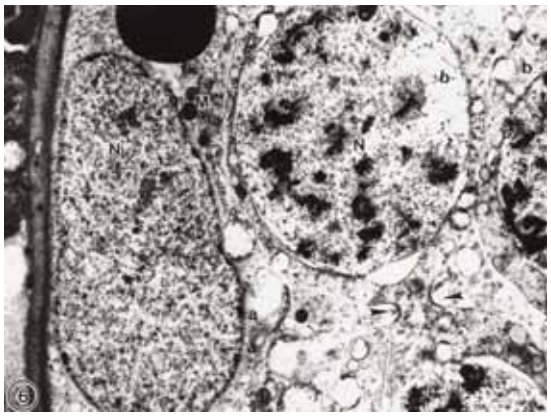


Fig. 5: Photo electron micrograph of the testis of a control rat, showing the basement tubular membrane (bt) of the seminiferous tubules, Sertoli cell cytoplasm (S), A and B types spermatogonia (A, B), spermatogonia with nucleus (N) and mitochondria (M). X9000.

Fig. 6: Photo electron micrograph of the testis of a control rat, showing A and B types spermatogonia (A, B), nuclei (N), mitochondria (M) and intercellular bridge (head arrow) between the two spermatogonium cells. X120000.

Spermatocytes:

The spermatocytes are two types, primary and secondary spermatocytes:

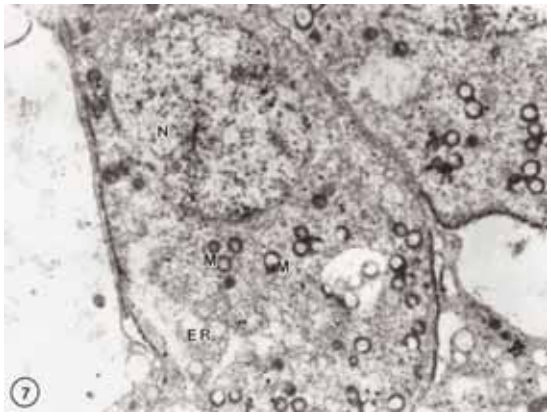
The primary spermatocytes are characterized by the presence of intercellular bridges between these cells, spherical nuclei with finely granular nucleoplasm and chromatin accumulation. The cytoplasm is scanty with little endoplasmic reticulum but small clumps of ribosome are distributed throughout the cytoplasm. The mitochondria are similar to those of spermatogonia in shape and they tend to aggregate in groups. The Golgi apparatus is found above the nucleus and is formed of fine vesicles and few cluster accumulated at one pole of the cell (Figs. 7 and 8).

The secondary spermatocytes are rarely seen among the germinal cells of rat, their life span is short and enter into the second meiotic division producing the spermatids.

The present observation confirms the scarcity of these cells in the testis of rat. The 2ry spermaotcytes are smaller in size than the late primary spermatocytes. Their nuclei are spherical with centrally located clumps of chromatin substance. The mitochondria of the 2ry spermatocytes show great similarity to those of the spermatids

Spermatids:

The early spermatids are rounded cells with large spherical nuclei which contain chromatin clumps in a lightly stained cytoplasm and the endoplasmic reticulum is membrane bounded canaliculi and flattened vesicles; mitochondria at this stage tend to aggregate at the periphery of the plasma membrane (Figs. 9 & 10).



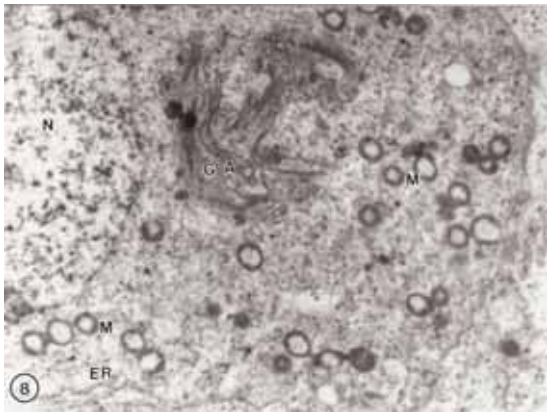
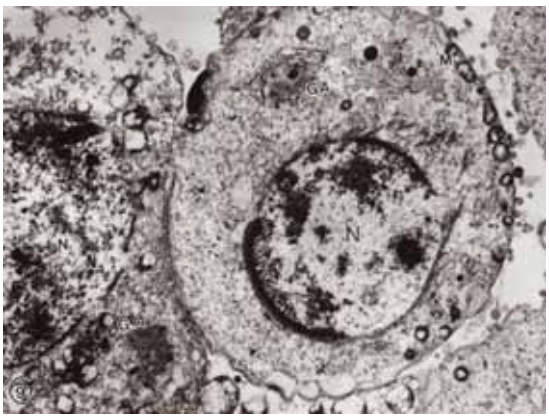


Fig. 7: Photo electron micrograph of control rat testis showing primary spermatocytes nucleus (N), mitochondria (M) and endoplasmic reticulum (ER). X15000.

Fig. 8: Photo electron micrograph of control rat testis showing primary spermatocytes nucleus (N), mitochondria (M), Golgi apparatus (GA) and endoplasmic reticulum (ER). X15000.



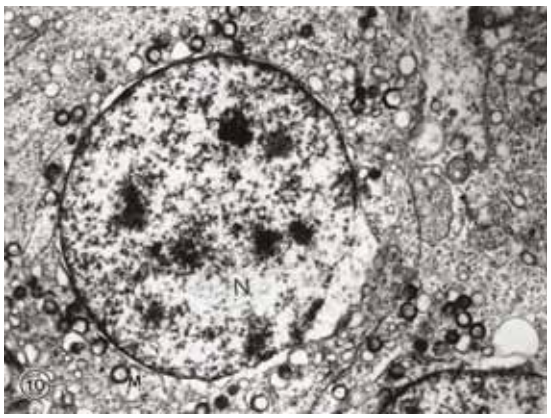


Fig. 9: Photo electron micrograph of control rat testis, showing spermatid nucleus (N), mitochondria (M) and Golgi apparatus (GA). X12000.

Fig. 10: Photo electron micrograph of control rat testis showing spermatid nucleus (N), mitochondria (M) and endoplasmic reticulum (ER). X12000.

Sertoli Cells:

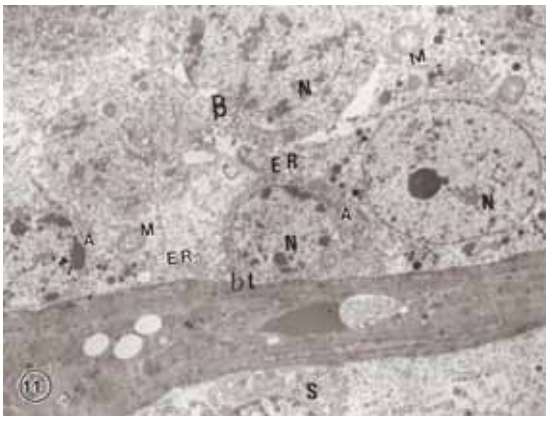
The cytoplasm of these cells extends from the basal lamina to the lumen of the seminiferous tubule and envelop the adjacent germinal elements. The nucleus is infolded and sometimes has a very irregular shape, the nucleoplasm is homogenous and the cytoplasm contains abundant endoplasmic reticulum, ovoid Golgi apparatus and spherical or cylinder shaped mitochondria. (Fig. 5).

Treated Groups:

1. Low Doses:

After rats treated with low dose of aluminium chloride, the seminiferous tubules, showed cellular alteration of cells than those in the control groups.

A and B types spermatogonia are less affected, the cytoplasm of both cells is more or less electron dense, the mitochondria are swollen and vacuolated, the chromatin particles clumps periphery at nuclear membrane, the endoplasmic reticulum became vascular rather than tubular, and the basement membrane of the seminiferous tubules were more thickened with fibrous connective tissue than in control groups. The Sertoli cells were less affected (Fig. 11).



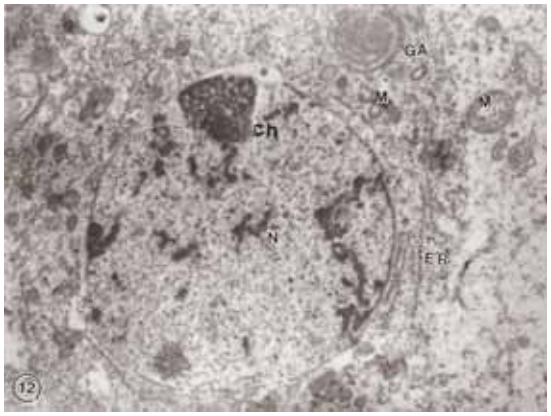
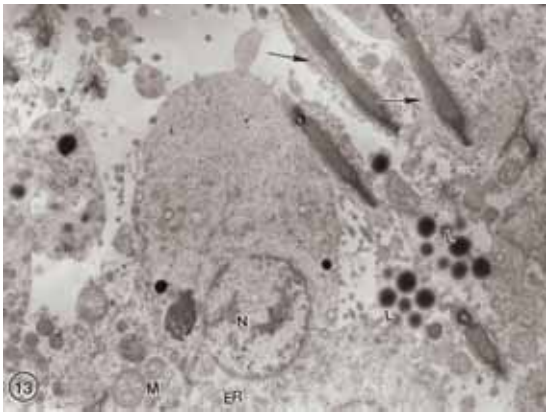


Fig. 11: Photo electron micrograph of rat testis after 5 weeks of treatment with low dose of aluminium chloride, Showing tubular basement membrane (bt), A and B types spermatogonia (A and B), nucleus(N), mitochondria(M), tubular endoplasmic reticulum (ER) and Sertoli cell (S). X6000.

Fig. 12: Photo electron micrograph of rat testis after 5 weeks of treatment with low dose, Showing primary spermatocytes, nucleus (N) with clumped chromatin (ch), dilated Golgi apparatus (GA), mitochondria (M) and tubular endoplasmic reticulum (ER). X15000.



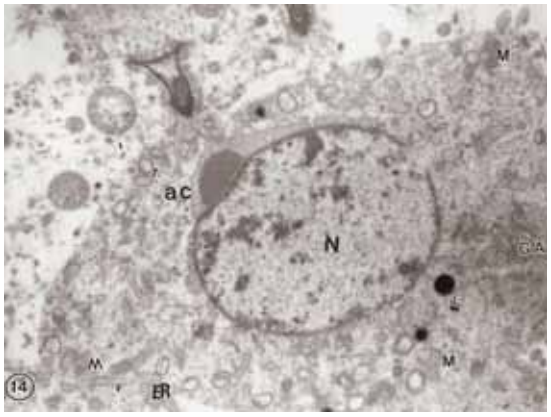


Fig. 13: Photo electron micrograph of spermatid cell after treatment with low dose, showing mitochondria (M), smooth tubular endoplasmic reticulum (ER), lipid droplet (L) and fragmented of spermatozoa (arrows). X18000.

Fig. 14: Photo electron micrograph of spermatid cell after treatment with low dose, showing the acrosome (Ac) inverted towards the nuclear membrane, lipid droplet (L), mitochondria (M), Golgi apparatus (GA) and smooth endoplasmic reticulum (ER). X18000.

The primary spermatocytes were clearly affected at low dose of aluminium chloride, the cells are compact and are reduced in sizes if compare to control group, with flattened nucleus and cytoplasm. The smooth endoplasmic reticulum and Golgi apparatus are dilated, the mitochondria are condensed and appeared darkly outlined, the nucleus is less damaged and has chromatin condensation at periphery of the nucleur membrane which are irregular in some area (Fig. 12).

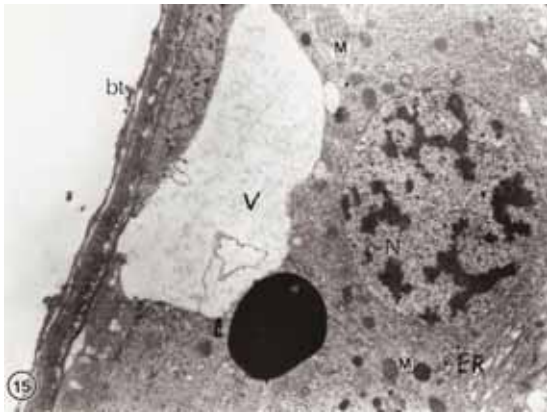
The spermatid showed little changes after low dose. The cytoplasm acquired a moderate electron density. Also, the smooth endoplasmic reticulum are dilated and occurred in a vesicular form , the nuclei appeared more or less reduced in size with coarse granular chromatin distributed within the karyoplasm. The acrosome inverted towards the nucleus and the outline was highly irregular, both the acrosomic granule and vesicle were detached from the nucleus (Fig. 14).

The Golgi apparatus cisternea were fused together in groups and appeared as elongated dense bodies. Lipid droplets are shown within the cytoplasm and the mitochondria still peripheral in location and the fragmented

spermatozoa appeared in the lumen (Figs. 13 & 14).

2. High Doses:

After the rats treated with high dose of aluminium chloride. The testis tubules showed more exaggerated features of focal areas of spermatogenesis arrest at the spermatid level, in the form of degenerative changes in the germinal cells.



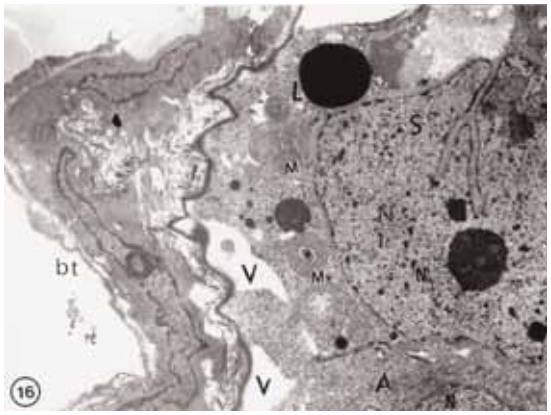
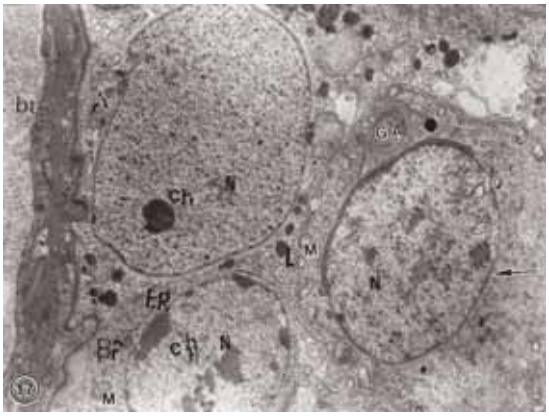


Fig. 15: Photo electron micrograph of treated rat testis with high dose, showing tubular membrane (bt), A and B types spermatogonia (A, B), nucleus (N), mitochondria (M), vacuolated cytoplasm (V), lipid droplet (L) and endoplasmic reticulum (ER). X12000.

Fig. 16: Photo electron micrograph of treated rat testis with high dose , showing Sertoli cell (S) with folded nuclear membrane and a part of A-type spermatogonia, cytoplasmic vacuoles (V), lipid droplet (L) and swollen mitochondria (M). X15000.



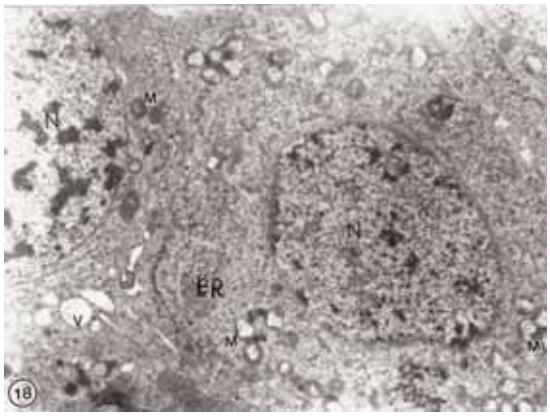
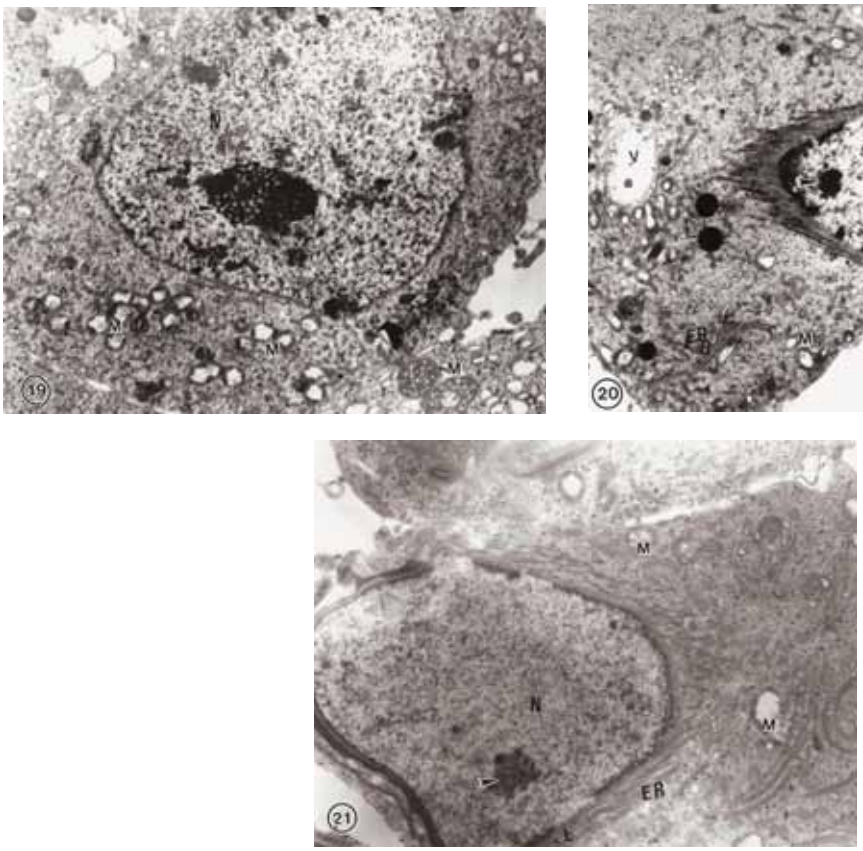


Fig. 17: Photo electron micrograph of treated rat testis with high dose, showing A and B types spermatogonia(A, B) and early spermatid (arrow), dilated tubular membrane (bt), nuclei (N), mitochondria (M), Golgi apparatus (GA), lipid (L) and endoplasmic reticulum (ER). X12000.

Fig. 18: Photo electron micrograph of treated rat testis with high dose, showing primary spermatocytes with irregular damage nuclear membrane (N), cytoplasmic vacuoles (V), damaged mitochondria (M) and smooth endoplasmic reticulum (ER). X18000.

Generally, the seminiferous tubules of rats which had high doses, showed strongly damaged tubules displaying advanced stage of injury in cells and a thick , irregular tubular basement membrane. (Figs. 15, 16, 17 & 18).

The spermatogonia of both types A and B are more affected than before after low doses, the cytoplasm containing large vacuoles and large lipid droplets (Figs. 15 & 16).



Figs. 19, 20 and 21: Photo electron micrographs of treated rat testis with high dose, showing degenerative and atrophy of spermatid cells, abnormal nuclear chromatin (arrows) and defects in acrosomal cap formation , degenerated mitochondria (M), dilated endoplasmic reticulum (ER) and cytoplasmic vacuoles (V). X18000, X. 15000 & X. 22500. respectively.

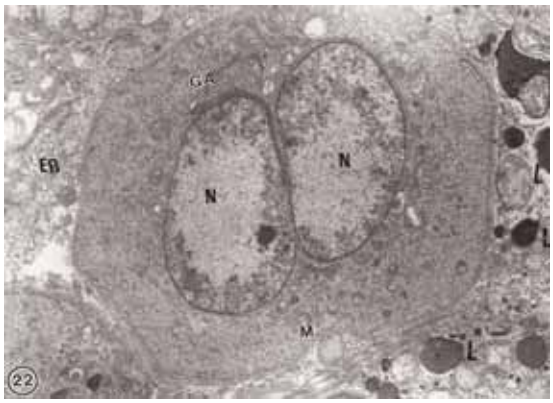


Fig. 22: Photo electron micrograph of treated rat testis with high dose, showing multinucleated giant cells with degenerative nuclei(N), Golgi apparatus (GA), smooth endoplasmic reticulum (ER) and lipids droplets (L). X12000.

The chromatins are clumped and the cytoplasm is vacuolated and condensed, the mitochondria are swelling, the endoplasmic reticulum was vesicular and dilated (Figs. 15, 16 & 17).

The Sertoli cells showed more damage as the spermatogonia (A and B types) in cytoplasmic vacuolation, degenerative mitochondria, dilation endoplasmic reticulum and lipid droplets (Fig. 16).

The spermatocytes showed clear degeneration changes for all cytoplasm, and the mitochondria are darkly outlined and condensed, the cytoplasmic organelles are vacuolated, the nucleus has irregular nuclear membrane and chromatin clamped. The Golgi apparatus and the endoplasmic reticulum are dilated and fragmented (Figs. 18 & 19).

The spermatids showed more severe damage than before after treatment with low dose the cells have a highly electron dense cytoplasm which is prominently defected and vacuolated (Figs. 17, 20 & 21).

The nuclear membrane is highly distorted and it appears ruptured in some areas, the chromatin is accumulated in the form of coarse granules. The mitochondria are distributed throughout the cytoplasm and damaged with fused cristea together and adhered peripherally to the outer mitochondrial membrane (Figs. 17 & 19).

The multinucleate giant cells appeared to be one of ultimate atrophy, Pyknosis and detachment. The cytoplasm are more pyknotic and the nuclei exhibited degenerative changes (Fig. 22).

The spermatozoa are absent in the testis seminiferous tubules after treatment with high doses of aluminum chloride.

Our results obtained in treated rat testis with low and high doses of aluminium chloride, agree with those obtained by other authors (Kamboji & Kar, 1964) who reported that, the seminiferous tubules are shrinkage and spermatogenic arrest at the primary spermatocytes or spermatogonial stage when treated mice with daily subcutaneous injection of 27. 4 mg/kg. of aluminium sulphate for 30 days.

Furthermore, (Alfrey, *et al.* , 1976; Krasovskii *et al.* , 1979 & Alfrey, 1984) have reported that, short term aluminium chloride exposures to rats and guinea pigs caused gonadal toxicity whereas in chronic exposure, the sperm density and motility were affected in agreement with the work of Chinoy, *et al.* (2005), who showed that the administration of sodium fluoride (NaF, 10 mg/kg. body weight) together with aluminium chloride ($AlCl_2$, 200 mg /kg body weight) to mice for 30 days, caused degenerative in structure of spermatogensis and formation of giant cells. These resulted agree with our results obtained after the treatment with aluminium chloride. The same results were recorded by , Libet *et al.* (1995), after the effect of alumiunium nitrite on mice and found that the spermatocytes and spermatids are necrosis.

Guo, *et al.* , (2002) have reported that aluminium caused suggested that ACE activity had a role in oxidative damage of Al-induced testicular toxicity in mice, after intraperitoneally exposed to 0. 13 or 35 mg. Al. /kg. body weight for a period of 14 days.

Mayyas, *et al.* (2005), reported the same resulted after the mice treatment with aluminium chloride, and found that destruction of the seminiferous tubules with large necrotic areas and degenerative cells.

Finally, in summary the results of this study demonstrate that the interperitoneal injection of aluminium chloride in rats , caused testis toxic as indicated by histological changes in the seminiferous tubules of those testis.

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