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TRANSMISSION ELECTRON MICROSCOPY STUDY OF OVARIAN FOLLICLES IN RATS EXPOSED TO FLUORIDE

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ABSTRACT

Background: Fluorine, very active chemically, reacts with most metal elements to generate different fluorides widely found in nature.[1] Fluoride plays a key role in prevention and control of dental caries. However, fluoride has a tendency to accumulate in organisms if the exposure persists over time, even at low concentration.[2] Curcumin is an active component in turmeric rhizomes (Curcuma longa Linn.) and is a poly phenolic curcuminoid that accounts for 3-5% of turmeric[11] and is reported to have broad medicinal value and used in the treatment of various ailments. **Objectives:** The objective of this study was to elucidate the potential effects of curcumin on fluoride induced ultra pathological changes in ovary of female rats. **Materials and Methods:** Wistar albino female rats weighing150-200g were randomly divided into six rats in each group. The rats in experimental groups treated with 300 and 600mg NaF / kg b.w. / day by oral gavage for 40 days. The control animals were administered 1 ml deionized water/kg bw/day for the same duration. Rats treated with NaF for 40 days were post-treated with 200 mg /kg bw /day of Curcumin alone respectively. All the animals were sacrificed under ether anaesthesia. The ovary tissues were dissected out, washed in normal saline, and processed for transmission electron microscopic examination. **Results:** The transmission electron microscopic features of fluoride ovarian toxicity with 300 mg NaF/kg bw /day, the ovariansurface epithelium showed destructed microvilli on the surface of oocytes. The nucleus had distorted, irregular structure and cytoplasmic lamellae in oocyte became much more abuntant. In atretic follicle, mitochondria showed vacuolation, dilated endoplamic reticulum, and shredded ribosomes. The animals treated with 600 mg fluoride /kg bw/day showed degeneration of zonapellucida and enlargement of perivitelline spaces. Apoptotic granulosa cells spilling in the antrum alongwith accumulation of variable sized electron dense deposits were present. The cytoplasmic lamellae exhibited vauolation. The irregular shaped nuclei showed convolution of nuclear membrane. The animals post-treated with 200 mg/kg bw/day of Curcumin showed restoration of irregular nuclear membrane of oocyte. The vasicles were uniformly distributed in the cytoplasm. The swollen mitochondria become normal. The tunicaalbuginiaexhihited regular appearance alongwith restoration of nucleus and mitochondria.

KEYWORDS: Curcumin, Ovary, Transmission electron microscopy, Sodium fluoride, Wistar female albino rats.

INTRODUCTION

Fluorine, very active chemically, reacts with most metal elements to generate different fluorides widely found in nature.[1] Fluoride plays a key role in prevention and control of dental caries. However, fluoride has a tendency to accumulate in organisms if the exposure persists over time, even at low concentration. Dental and skeletal lesions are the major recognized effects of endemic fluorosis, caused by chronic persistent fluoride exposure through ingestion or inhalation and, most commonly as a result of high fluoride levels in drinking water or industrial exposure from dust.[2] Mild forms of dental fluorosis are evidenced by the appearance of white horizontal striations on the tooth surface or opaque patches of chalky white discolorations.[3]

Excessive fluoride intake can also affect hormone secretion and soft tissues such as thepancreas[4], liver[5], brain[6] The relationship between fluoride ingestion and reproductive structure and function has also received attention.[7] Fluoride exposure can increase the number of abnormal spermatozoa and reduce sperm quality and quantity in rats, mice and humans.[8,9,10]

Curcumin is an active component in turmeric rhizomes (*Curcuma longa* Linn.) and is a polypenolic curcuminoid that accounts for 3-5% of turmeric[11] and is reported to have broad medicinal value and used in the treatment of various ailments. Some studies have also demonstrated tharcurcumin and its analogues exert a stimulatory effect on ovarian fuctions. However, the effects of curcumin on experimentally fluoride induced ovarian ultrastructural

have not been reported. In this study we investigate the protective effect of curcumin on fluoride induced ultrastructural alterations in ovary of female rats.

MATERIALS AND METHODS

Young female Wistar albino rats, weighing 150-200 g were housed in polypropylene cages with stainless grill tops and fed standard rat pellet diet (Hindustan lever limited, India) and water was given *ad libtium*. The group I and II rats were administered 300 and 600 mg of NaF/kg bw/day orally by gastric tube for 40 days. The controlanimals received 1 mL. deionised water/kg bw/day for the same period. The fluoride treated animals were post-treated with 200 mg/kg bw/day of Curcumin for 20 days. The positive control group received Curcumin alone for 20 days. All the animals were sacrificed under ether anaesthesia. The ovarian tissues were dissected out, washed in normal saline, and processed for transmission electron microscopic examination.

Transmission electron microscopy

The ovarian tissues were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.3) for 4-6 hours at 4°C by the method of Karnovsky.[12] The tissues were postfixed in 1% osmium tetraoxide for 1 hour, dehydrated in acetone, infiltrated and embedded in araldite CY 212 (TAAB, UK), cut with an ultramicrotome (Leica Ultracut UC7, Austria), and stained with aqueous toluidine blue. The ultra-thinsections were stained with 8% uranyl acetate and lead citrate. All the studies were performed under a Tecnai G2 20 high resolution transmission electron microscope (Fei Company, The Netherlands) at an operating voltage 200 kV. A CCD camera (Megaview III, Fei Company) using TIA software was attached to the microscope for digitally acquired images. The primary magnification of the electron microscopic examination was X1100, X1550, X2550 for ovary.

Ethical Aspects

Experimental protocols and procedures used in this study were approved by the Institutional Animal Ethical Committee of Punjabi University, Patiala(Animal maintenanceandRegistrationNo.107/GO/ReBi/S/99/CPCSEA/2017-31).

RESULTS

The transmission electron microscopy of antral follicle in ovary of control rat showed spaces between adjacent granulosa cells filled with ooplasm , nucleus and mitochondria (M) (Figures1 and 2). Granulosa cells displayed spherical euchromatic nuclei containing prominent nucleolus and condensed chromatin. The mitochondria with intact cristae and uniform membrane structure were visible (Figure 3). Theca cells exhibited typical steroid - expressing cell characteristics with lipid droplet content in the follicles. The small number of lipid vacuoles were visiblein these cells. A few homogenous and small lysosomes were present (Figure 4).

In rats treated with 300mgNaF/kg bw/day for 40 days, the ovarian surface epithelium showed destructed microvilli on the surface of oocytes.The nucleus had distorted, irregular structure (Figure 5). The cytoplasmic lamellae in oocyte became much more abundant. In atretic follicle, the mitochondria showed vacuolation. The endoplamic reticulum (ER) was dilated. The shredded ribosomes (R) were present in groups (Figure 6). The changes in the architecture of the granulosa cells were demonstrated by mitochondrial vacuolation, significant expansions of the endoplasmic reticulum, and presence of necrotic cells (Figure 7).

In animals treated with 600 mg fluoride /kg bw/day for 40 days, the oocyte showed degeneration of zonapellucida and enlargement of perivitelline spaces. Apoptotic granulosa cells spilling in the antrum were seen. There was accumulation of variable sized electron dense deposits (Figure 8). In the theca cell layer, there was an increase in intercellular space accompanied by irregular nucleus (N) with convolution of nuclear membrane. The large number of lysosomes (L) having electron dense material were present. The collapsed cytoplasmic lamellae, dilated endoplasmic reticulum, and aggregation of ribosomes were visible. The cytoplasmic lamellae exhibited vacuolation. The irregular shaped nucleus showed convolution of nuclear membrane (Figure 9). The granulosa cells exhibited euchromatic nucleus (N), The darkly stained degenerated mitochondria, begin to lose their cristae. There was expansion of endoplasmic reticulcum (ER). There were expanded intercellular spaces between granulosa cells (Figure10).

In animals treated with 300 mg fluoride for 40 days followed by 200 mg/kg bw/day Curcumin for 20 days, there was restoration of irregular nuclear membrane of oocyte. The swollen mitochondria become normal. The vesicles were uniformly distributed in the cytoplasm (Figure11). Ultrastructural evaluation of granulosa cells showed normal nucleus (N), lysosomes (L), mitochondria (M), smooth and granular endoplasmic reticulum, and decreased occurrence of vacuoles and intermediate filaments (IF) (Figure 12). The ovary also contained several corpora lutea composed of large and round cells with a prominent nucleus (N). The luteal cells contained mitochondria, an extensive rough endoplasmic reticulum (RER), numerous free ribosomes, lipid droplets and a few number of lysosomes (Figure 13). Granulosa cells had normal nucleus (N), mitochondria and free ribosomes. The large number of electron lucid vesicles was present in the cytoplasm (Figure 14).The theca interna cells were well organized and showed normal nuclear and cytoplasmic outlines with preservation of nucleus (N), nucleolus and mitochondria (Figure 15). The germinal epithelium had condensed nucleus with condensed chromatin, nucleolus, mitochondria and several numbers of lipid vacuoles (Figure 16).

In rats treated with 600 mg fluoride for 40 days, followed by 200 mg/kg bw/day Curcumin for 20 days, the tunica albuginea exhibited regular appearance alongwith restoration of nucleus and mitochondria. In cytoplasm, large number of lipid vacuoles were present (Figure 17). The uniform zonapellucida layer integral with the adjacent follicular cells was visible. In oocyte, the mitochondria showed preservation of cristae (Figure 18). The granulosa cells were normal had round nucleus and high proportion of cytoplasm. The nuclear membrane was markedly wavy with enlargement of perinuclear space (Figure 19). The granulosa cells displayed normal nucleus, mitochondria and extensive formation of lipid vacuoles (Figure 20).The theca cells have normal nucleus and mitochondria. There was formation of many cavities in theca interna (Figure 21).

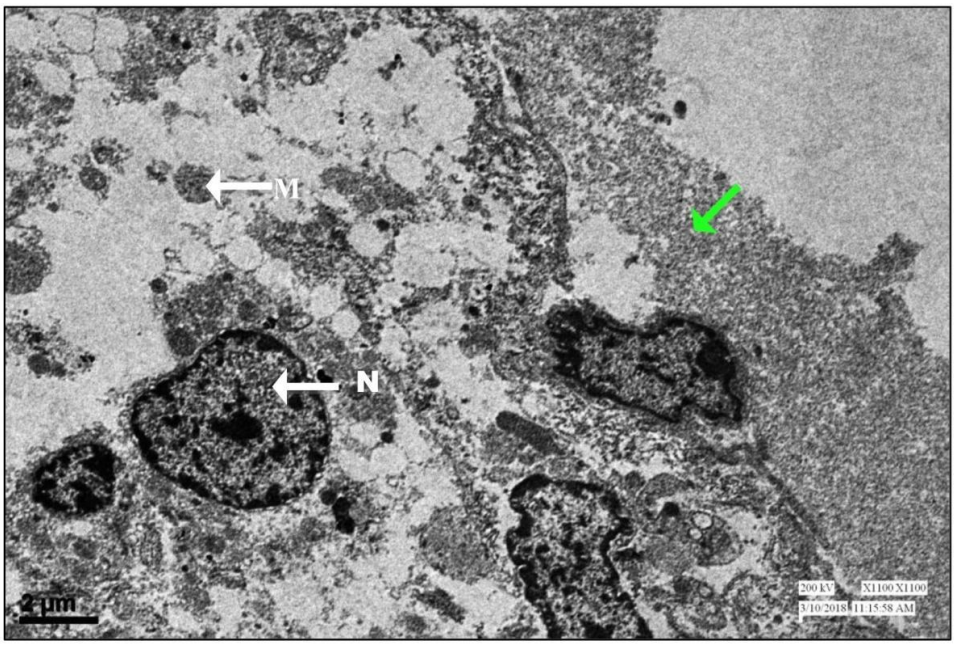

Figure 1: Electron micrograph of antral follicle in ovary of control rat showing spaces between adjacent granulosa cells filled with follicular fluid with clear ooplasm ( ), nucleus (N) and mitochondria (M). X 1100.

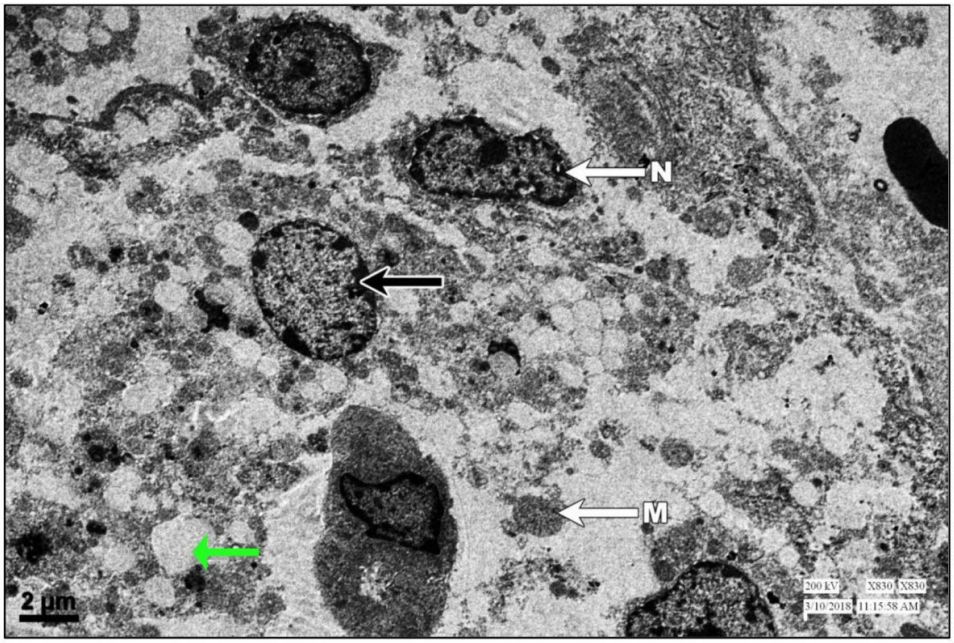

Figure 2: Electron micrograph of granulosa cell in ovary of control rat demonstrating the appearance of cytoplasm, mitochondria (M), nucleus (N) containing small amount of condensed chromatin ( ) and lipid droplets ( ). X 830.

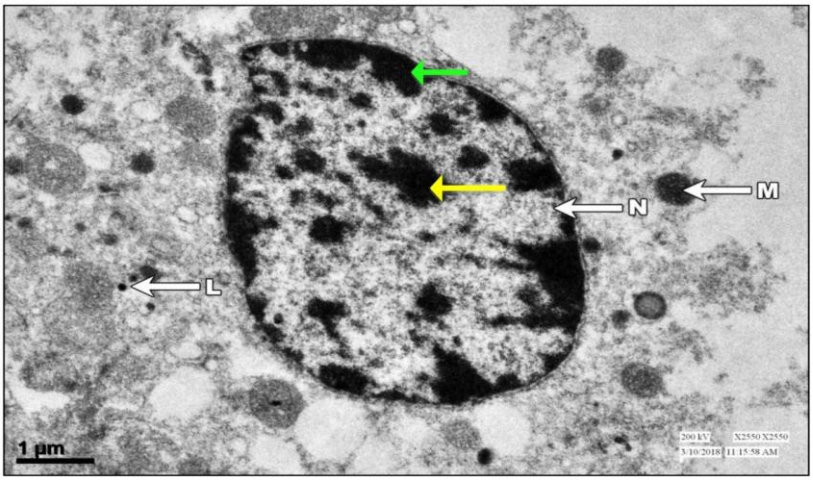

Figure 3: Electron micrograph of magnified view of granulosa cell in ovary of control rat with normal nucleus (N), nucleolus ( ) and condensed chromatin ( ). The cytoplasm contains mitochondria (M) and lysosomes (L). X 2550.

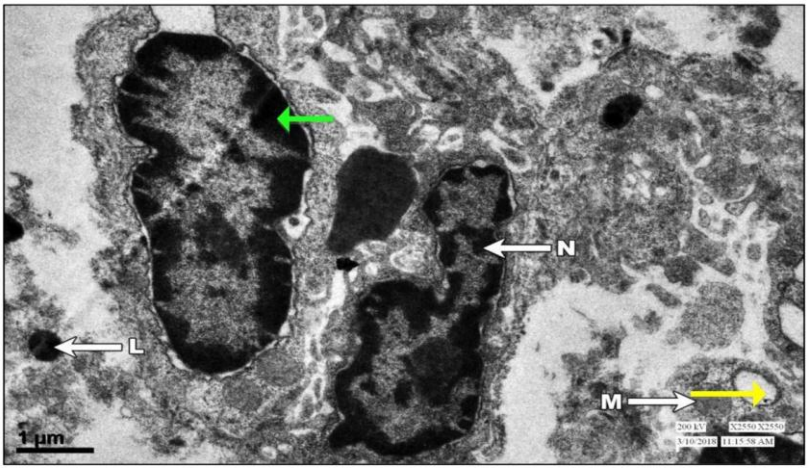

Figure 4: Electron micrograph of theca cells in ovary of control rat showing nucleus (N) with condensed chromatin ( ), Mitochondria (M), lysosomes (L) and lipid droplets ( ) are visible in the cytoplasm. X 2550.

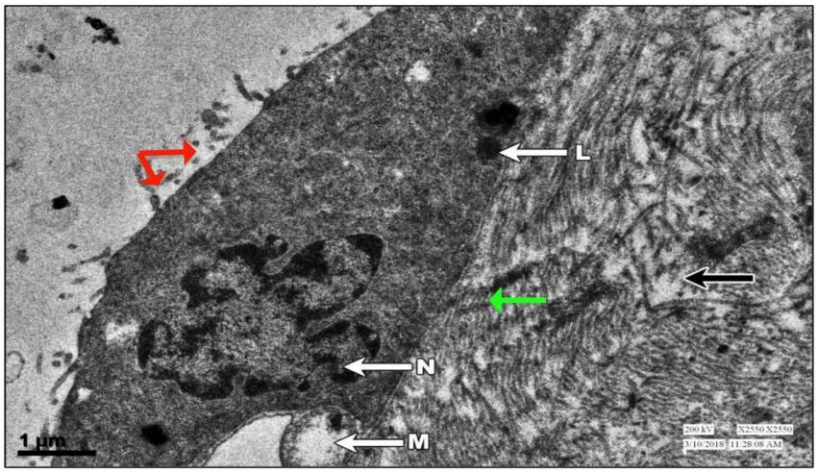

Figure 5: Electron micrograph of ovarian surface epithelium in ovary of rat treated with 300 mg fluoride for 40 days showing germinal epithelium which laid on the basement membrane ( ) that laid on ovarian stroma having increased prevalence of cytoplasmic lamellae ( ), depleted microvilli ( ), nucleus (N) and lysosomes with electron dense deposits. X 2550.

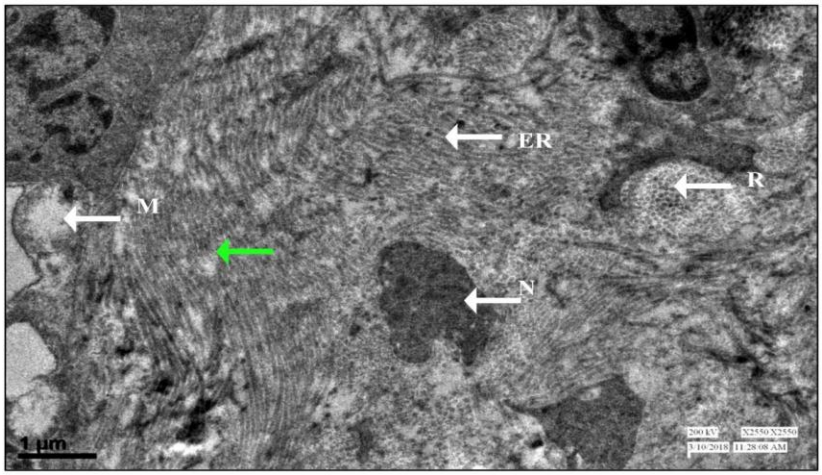

Figure 6: Electron micrograph of atretic follicle in ovary of rat treated with 300 mg fluoride for 40 days showing vacuolated mitochondria (M) with loose cristae, dilated endoplasmic reticulum (ER), ribosomes (R), and increased prevalence of lamellae ( ) in the cytoplasm of oocyte. X 2550.

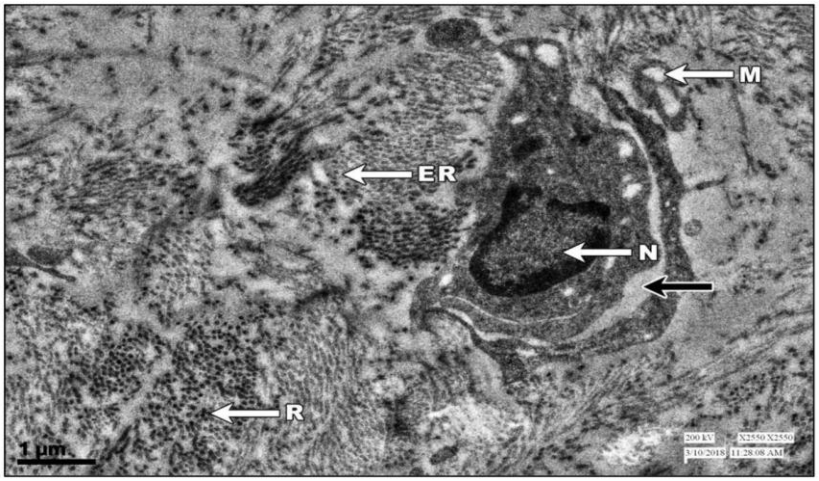

Figure 7: Electron micrograph of granulosa cell in ovary of rat treated with 300 mg fluoride for 40 days showing necrotic cells ( ), dilated endoplasmic reticulum (ER),vacuolated mitochondria (M) and ribosomes (R). X 2550.

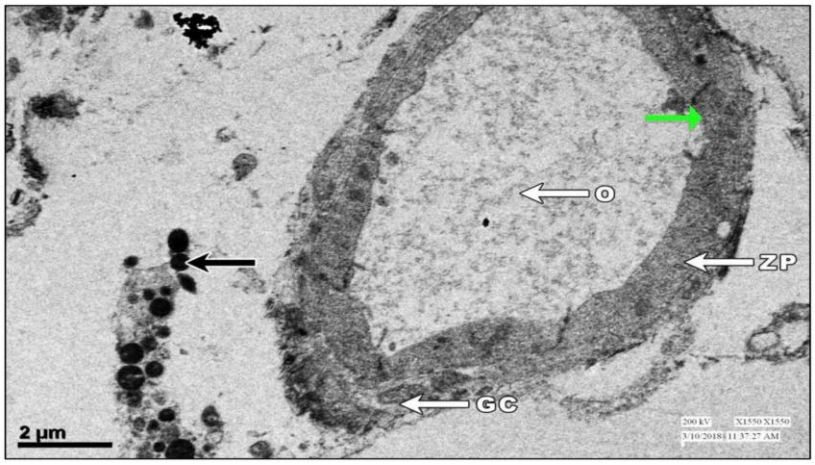

Figure 8: Electron micrograph of oocyte – zonapellucida (ZP) in ovary of rat treated with 600 mg fluoride for 40 days showing perivitelline membrane and intercellular spaces ( ), granulose cells (GC) and accumulation of electron dense deposits ( ) X 1550.

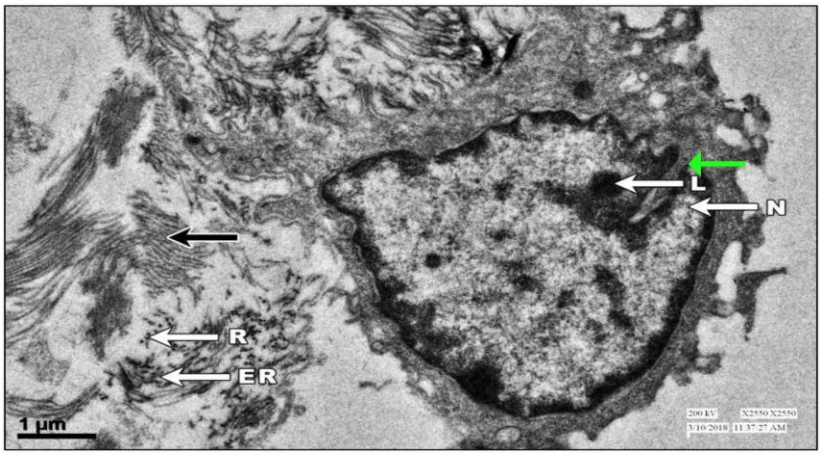

Figure 9: Electron micrograph of internal theca cells in ovary of rat treated with 600 mg fluoride for 40 days showing irregular nuclei (N) with torn of nuclear membrane ( ), dilated endoplasmic reticulum (ER), lysosomes (L) with electron dense deposits, aggregated ribosomes (R) and collapsed cytoplasmic lamellae ( ) resulting in vacuolation. X 2550.

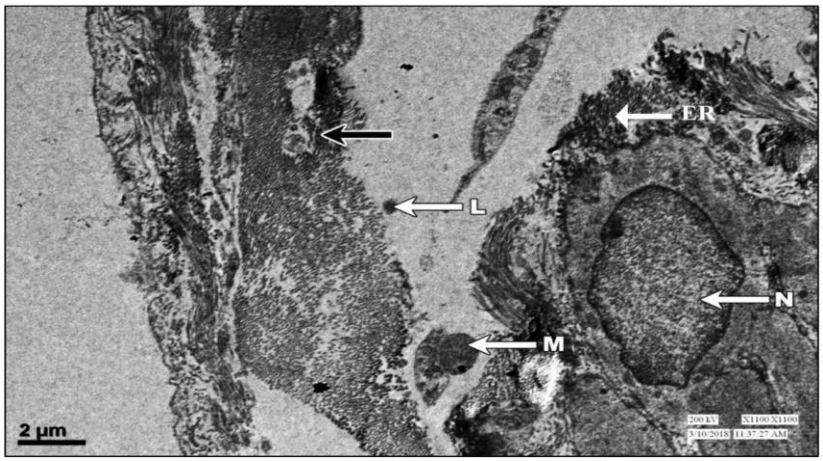

Figure 10: Electron micrograph of granulosa cells in ovary of rat treated with 600 mg fluoride for 40 days showing euchromatic nuclei (N). In their cytoplasm, mitochondria (M)with swollen and abnormal cristae, dilated endoplasmic reticulum (ER), lysosomes (L) and extensive intercellular vacuolation ( ). X 1550.

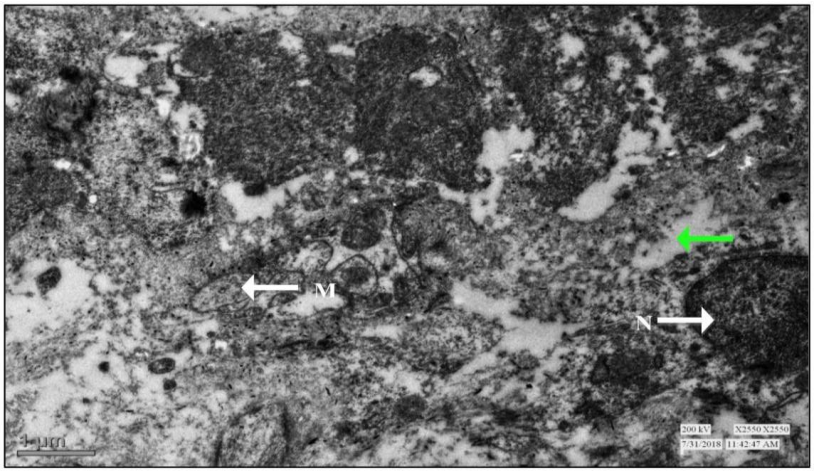

Figure 11: Electron micrograph of oocyte in ovary of rat treated with 300 mg fluoride for 40 days followed by 200 mg Curcuminfor 20 days showing preservation of nucleus (N), mitochondria (M) and presence of vesicles ( ) in the cytoplasm. X 2550.

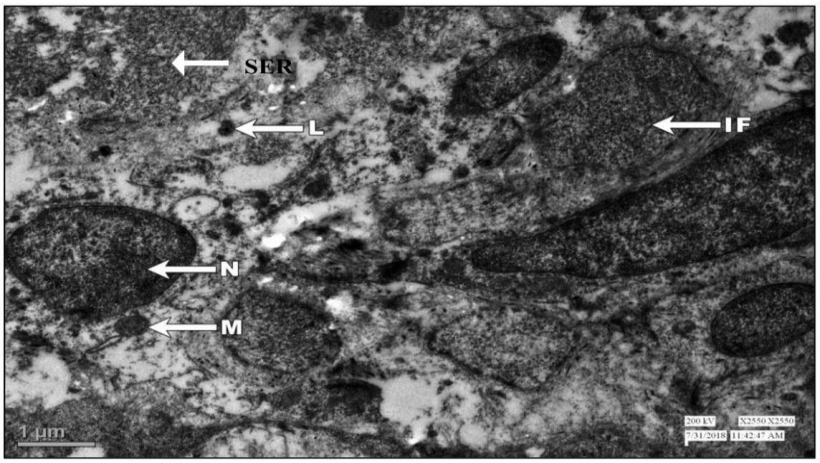

Figure 12: Electron micrograph of granulosa cell in ovary of rat treated with 300 mg fluoride for 40 days followed by 200 mg Curcumin for 20 days showing normal nucleus (N), mitochondria (M) and smooth endoplasmic reticulum (SER). In the cytoplasm, occurrence of vacuoles and intermediate filaments (IF). X 2550.

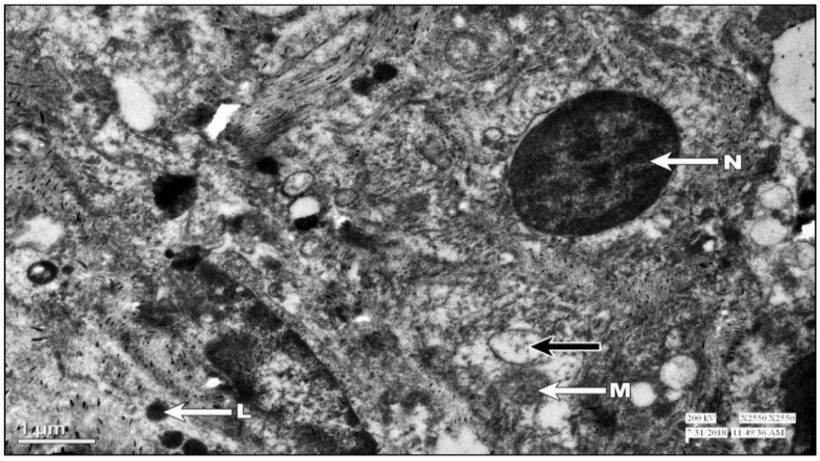

Figure 13: Electron micrograph of corpus luteum in ovary of rat treated with 300 mg fluoride for 40 days followed by 200 mg Curcumin for 20 days showing normal nucleus (N), mitochondria (M), free ribosomes and rough endoplasmic reticulum (RER). Some lysosomes (L) and lipid vesicles (⬆) were seen. X 2550.

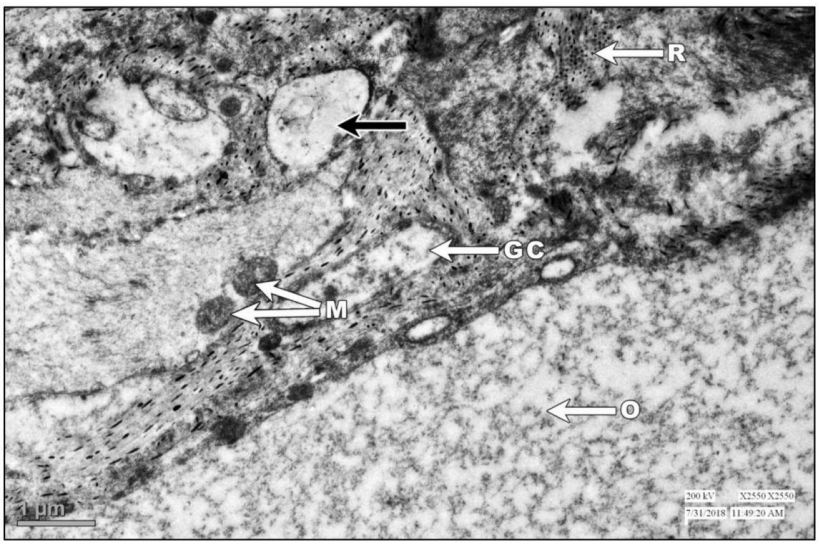

Figure 14: Electron micrograph of granulosa cells in ovary of rat treated with 300 mg fluoride for 40 days followed by 200 mg Curcumin for 20 days showing normal oocyte (O), granulosa cells (GC) have mitochondria (M) and ribosomes (R). In the cytoplasm number of electron lucid vesicles (⬆) were present. X 2550.

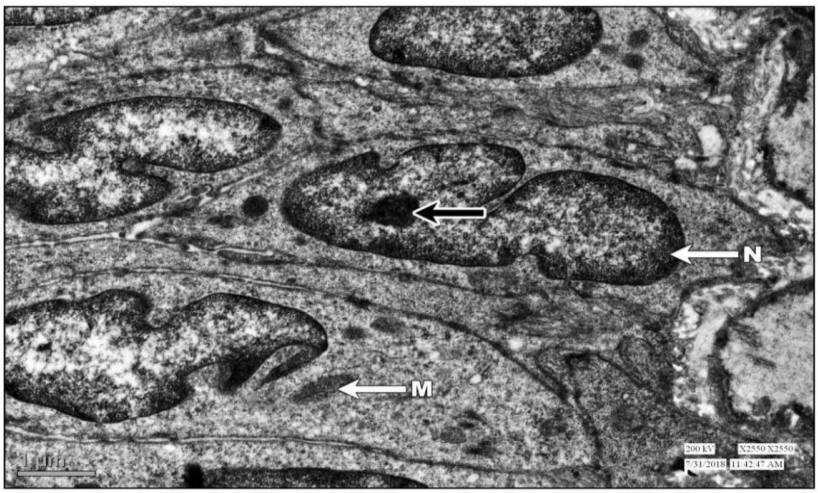

Figure 15: Electron micrograph of theca cells in ovary of rat treated with 300 mg fluoride for 40 days followed by 200 mg Curcuminfor 20 days showing normal arrangement of theca interna cells with normal nucleus (N), nucleolus and mitochondria (M). X 2550.

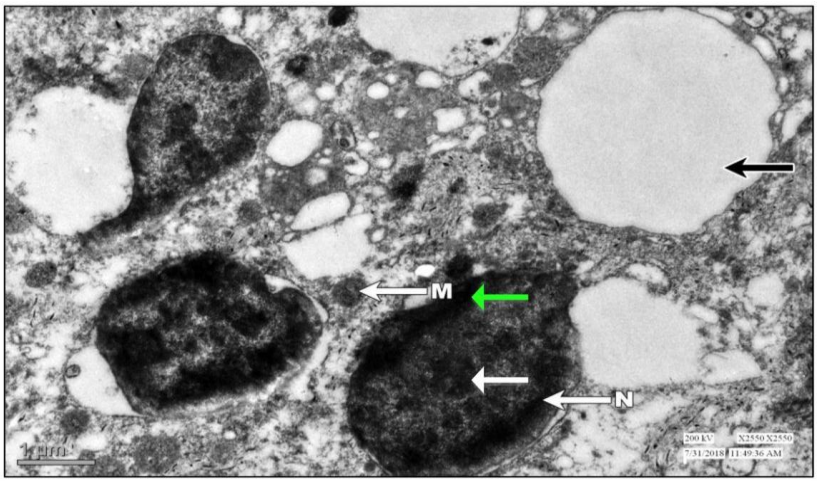

Figure 16: Electron micrograph of germinal epithelium in ovary of rat treated with 300 mg fluoride for 40 days followed by 200 mgCurcuminfor 20 days showing preservation of nucleus (N), with condensed chromatin ( ), nucleolus, mitochondria (M). Increased number of lipid vacuoles ( ) were seen in the cytoplasm. X 2550.

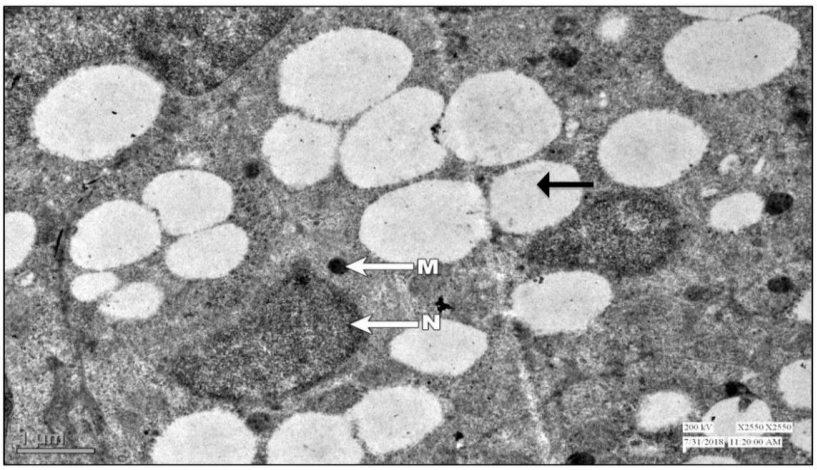

Figure 17: Electron micrograph of tunica albuginea in ovary of rat treated with 600 mg fluoride for 40 days followed by 200 mg Curcuminfor 20 days showing marked restoration in nucleus (N) mitochondria (M). Large number of lipid vacuoles ( ) were present. X 2550.

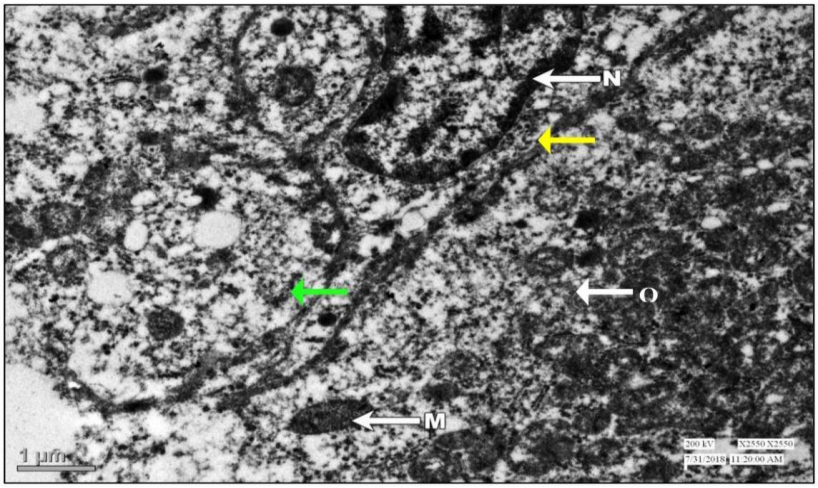

Figure 18: Electron micrograph of oocyte (O) and follicular cells ( ) in ovary of rat treated with 600 mg fluoride for 40 days followed by 200 mg Curcumin for 20 days showing regular zonapellucida ( ) numerous mitochondria (M) with preservation of cristae. X 2550.

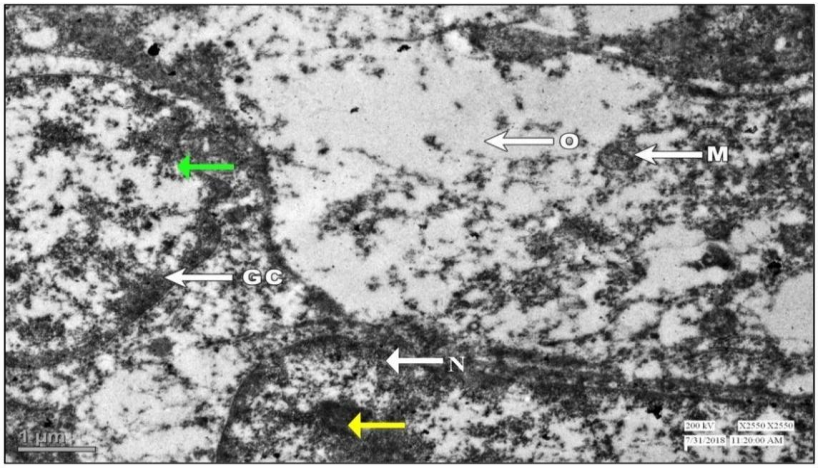

Figure 19: Electron micrograph of oocyte (O) and granulosa cell (GC) in ovary of rat treated with 600 mg fluoride for 40 days followed by 200 mg Curcuminfor 20 days showing enlargement of perinuclear space. normal nucleus (N), nucleolus ( ) mitochondria (M). Nuclear membrane was markdly wavy ( ) X 2550.

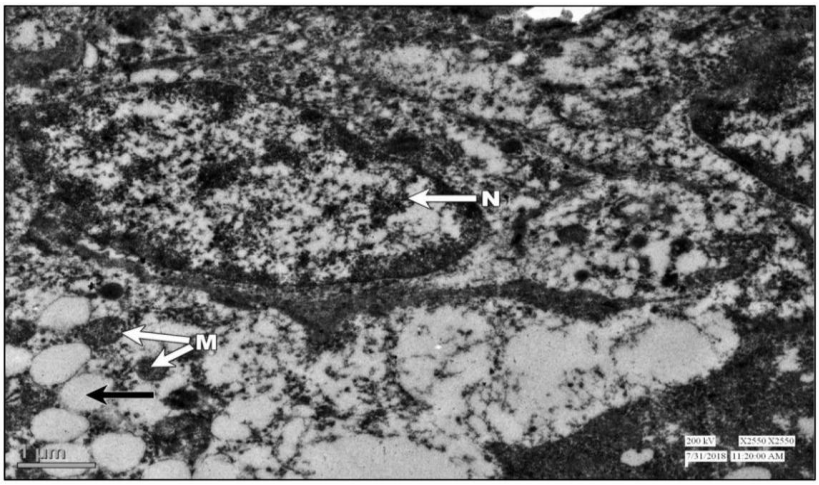

Figure 20: Electron micrograph of granulosa cells in ovary of rat treated with 600 mg fluoride for 40 days followed by 200 mg Curcuminfor 20 days showing normal nucleus (N) mitochondria (M). Numerous lipid vacuoles ( ) present in the cytoplasm. X 2550.

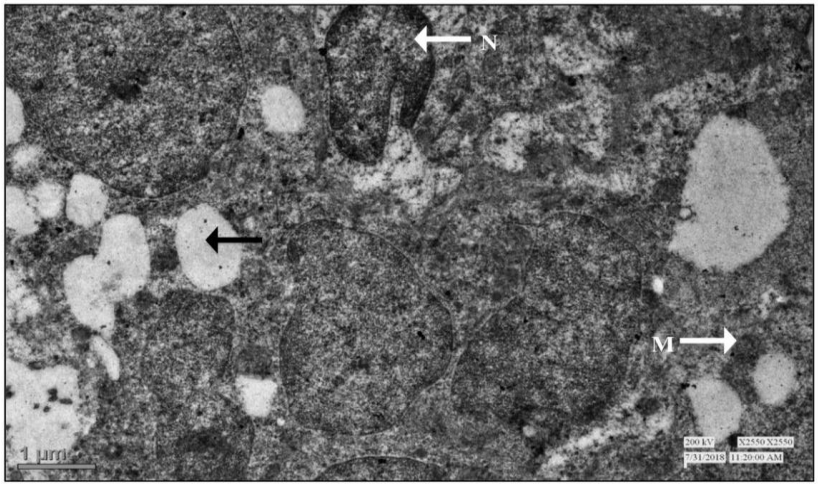

Figure 21: Electron micrograph of theca cells in ovary of rat treated with 600 mg fluoride for 40 days followed by 200 mg Curcuminfor 20 days showing normal nucleus (N) and mitochondria (M) alongwith formation of cavities (⬆) in the cytoplasm. X 2550.

DISCUSSION

The result of transmission electron microscopy in the present study showed that fluoride exposure increased degenerative changes in the ovarian follicles. There was cellular damage, mitochondrial and cytoplasmic vacuolation in granulosa cells in fluoride treated rats after 40 days. These findings are in agreement with the study reported earlier in fluoride treated mice.[13] The present study describes not only the ultrastructure of follicle in rat ovaries but also the increased degenerative changes within follicle committed to undergo apoptosis after fluoride exposure.[14,15] It was also noted the number of microvilli in oocytes were decreased in experimental group. It is known that the microvilli of oocytes and granulosa cells are in contact within the zonapellucida by gap junction and are involved in oocytes nutrition.

Presently many disruptions of cell architecture accompanied with several changes like vacuolations, significant expansion of endoplasmic reticulum, extensive mitochondrial alterations and necrotic cells, similar with those of previous study demonstrating the toxic effect of lanthanum and cerium in rats.[16]

The fluorotic female rats had reductions in follicle numbers and dysfunction of folliculogensis was well correlated with fewer primordial and atreticantral follicle.[17,18] The results obtained revealed numerous lysosomes present in the cytoplasm charged with an electron dense material in theca interna and granulosa cells. There was mitochondrial alterations, cytoplasmic vacuolization, and inflammatory reaction marked by the presence of macrophages.[16] The present study showed destructed microvilli on the surface of oocyte, irregular nucleus with convolution of nuclear membrane and abundant cytoplasmic lamellae.[19]

In the present study, when Curcumin was supplemented alongwith fluoride, a remarkable resurgence was observed in ovary. At the electron microscope level, this amelioration was in form of less noticable cytoplasmic vacuolation and disruption in the granulosa and theca cells, reduction in the inflammatory cell infilteration and preservation of microstructure. These ultrastructural findings were in the same line with the study that compared chromium intake with α-tocopherol.[20]

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