Effect Of Sodium Fluoride On The Thyroid Follicular Cells And The Amelioration By Calcium Supplementation In Albino Rats: A Light And An Electron Microscopic Study.

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Abstract: Sodium fluoride was the first fluoride compound used in the fluoridation of drinking water and it is still commonly used for that purpose to prevent dental caries**.** It exerts toxic effects on many soft tissues and organs. The thyroid gland has a strong capacity for absorbing and accumulating fluoride. **Objective:** The present work aimed to study the histological and ultrastructural effect of sodium fluoride (NaF) on the thyroid follicular cells and the amelioration by calcium supplementation in albino rats. **Materials and methods:** Four groups of adult albino rats were used for this study. The 1st and 2nd groups were used as control. The 3rd group was treated with NaF in a dose of 10mg/kg bw/day orally by gavage once daily for 35 days. The 4th group was treated with NaF in the same dose for the same period followed by calcium chloride in a dose of 20mg/kg bw/day once daily for 35 days. The animals were anaesthetized and specimens from the thyroid gland were obtained and processed for light and electron microscopic examination. **Results:** The NaF had adverse effects on the follicular cells of the thyroid gland in NaF-treated group in comparison with that of the control groups where the follicular epithelium appeared with reduced cell height and the follicles had low colloid content. The basal membrane was ill defined. Colloid droplets appeared in the apical and basal parts of the cytoplasm. Overall cytoplasmic disorganization was observed with scattered stacks of rough endoplasmic reticulum and loss of mitochondria. The apical border showed pseudopods directed into the colloidal lumen. The nucleus appeared irregular, heterochromatic with deformed nuclear membrane. Co-administration of calcium to NaF-treated rats ameliorated the adverse effect of NaF. The follicular epithelium increased in height and most of the follicles contained colloid. The follicular cell regained some of its normal characteristic features with intact basal lamina. The cytoplasm showed rough endoplasmic reticulum, mitochondria and small apical vesicles. The nucleus appeared regular in shape and euchromatic with well-formed nuclear membrane and prominent nucleoli. **Conclusion:** Calcium supplementation can ameliorate the adverse effects of NaF on thyroid follicular cells in people at risk of high exposure.

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Key Words: Sodium fluoride, Calcium supplement, Thyroid follicular cells, Ultra structure.

1.Introduction

Fluoride is abundant in the environment and exists only in combination with other elements as fluoride compounds, which are constituents of minerals in rocks and soil [(1)]. Sources of fluoride include natural fluoride in food stuffs and water (fluoridated water usually at 1.0 mg/L) [(2)]. Fluoridated tooth pastes can provide another major source of fluoride intake, particularly to children. Tooth pastes contain 1.0 to 1.5 mg fluoride per gram. Fluoride containing mouth wash could contribute 0.2 to 0.4 mg fluoride per use. Fluoride tablets and topical gels represent additional sources of fluoride exposure [(3)] .The main source of fluoride for humans is the intake of ground water contaminated by geological sources (maximum concentration reaching 30-50mg/L).[(1)] Sodium fluoride was the first fluoride compound used in the fluoridation of drinking water and it is still commonly used for that purpose to prevent dental caries.[(4)] In addition to the well known effects of fluoride on the skeleton and on teeth, it exerts toxic effects on many other soft tissues and organs.[(5)] Although fluorosis has been investigated for many years, there are relatively few studies of its effect on the endocrine glands such as the thyroid gland. Liu *et al*.[(6)] found that thyroid gland has a strong capacity for absorbing and accumulating fluoride.

Fluoride disturbs the synthesis and secretion of thyroid hormone, interferes with the activity of enzymes that catalyze the conversion of thyroxin into the active thyroid hormone leading to perturbation of circulating thyroid hormone.[(7)] Relative to the amount of fluoride ingested, high concentration of cations that form insoluble complexes with fluoride (e.g., calcium, magnesium) can markedly decrease gastrointestinal fluoride absorption.[(8)] Fluoride can alter calcium homeostasis in human population and calcium also plays an important role in a wide range

of cellular alternations induced by fluoride.[9] The treatment with vitamin C and D and calcium showed significant improvement in skeletal, clinical and biochemical parameters in children consuming water containing 4.5 ppm of fluoride.[10] The present work aimed to study the histological and ultrastructural effect of sodium fluoride (NaF) on the thyroid follicular cells and the amelioration by calcium supplementation in rats.

2. Materials and Methods

1.Animals

Twenty four adult male albino rats weighing 120-130 g were obtained from the animal house unit in the Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room of controlled temperature (24.3°C) during the experimental period. The rats were provided *ad libitum* with tap water and fed with standard commercial rat chow. The animal procedures were performed in accordance with Guidelines for Ethical Conduct in the Care and Use of Animals.

2.Chemicals

Sodium fluoride (Sigma Chemical Company, ST Louis, MO, USA) and calcium chloride (E.P.I.CO, Egypt) were used in this experiment. NaF was administered in a dose of 10mg/kg bw/ day orally by gavages once daily. As many authors stated that, daily administration of NaF in a dose of 10 mg/kg bw/day for 30-35 days leads to a series of biochemical and pathological abnormalities as a result of oxidative stresses in ovary, pancreas and kidneys.[11, 12 & 13] Calcium chloride was administered in a dose of 20 mg/kg bw/day orally by gavages once daily for the same period(35 days) 4 hours after NaF treatment [14] .

3.Experimental design

After one week of acclimation, the animals were divided into 4 groups (6 rats in each group). The 1st group received distilled water orally by gavages once daily for 35 days and served as a negative control. The 2nd group received calcium chloride in a dose of 20 mg/kg bw/ day orally by gavages once daily for 35 days and served as a positive control. The 3rd group (NaF- treated rats) received NaF in a dose of 10mg/kg bw/ day orally by gavages once daily for 35 days. The 4th group (NaF-treated rats co-administered with calcium chloride) received NaF in a dose of 10 mg/kg bw/ day once daily for 35 days followed by calcium chloride in a dose of 20 mg/kg bw/ day once daily for 35 days. The rats administered the previous chemicals were anaesthetized by intra-abdominal injection of thiopental, then rapidly dissected and subjected to the following studies:

3.1.Histological study:

Specimens of thyroid gland were taken in a tissue block composed of thyroid gland, trachea and surrounding connective tissue and fixed in 10% natural buffered formaldehyde and processed for paraffin sections of 5 micron thickness. The sections were stained with Hematoxylin and Eosin.

3.2.Ultrastructural study :

Thyroid specimens were obtained and cut into blocks less than 1mm.The specimens were immediately fixed in 2.5% glutaraldehyde buffered with 0.1M phosphate buffer at pH 7.4 for 2 hours at 4°C and then washed with the phosphate buffer, post fixed in 1% osmium tetraoxide in the same buffer for one hour at 4°C . After washing in phosphate buffer, specimens were dehydrated with ascending grades of ethanol and then were put in propylene oxide for 30 minutes at room temperature, impregnated in a mixture of propylene oxide and resin(1:1) for 24 hours and in a pure resin for another 24 hours. Then, the specimens were embedded in embed-812 resin in Beem capsules at 60°C for 24 hours [16,17] . Ultrathin sections of about 50-70 nm thick were cut using diamond knives and mounted on a copper grids, stained with uranyle acetate and lead citrate [16,17]and examined using JEOL JEM 1010 transmission electron microscope in Electron Microscope Research Laboratory (EMRL) of Histology Department, Faculty of Medicine, Zagazig University.

3. Results

In control animals (in 1st and 2nd group) , the thyroid parenchyma examined by light microscopy showed the characteristic features of thyroid follicles which appeared several and variable in sizes. All the follicles were rich in colloid content. The follicles consisted of cuboidal epithelial cells lining the colloid containing lumina. Many blood capillaries appeared inbetween the follicles (Fig.1). By electron microscopic examination, the follicular cell showed the apical border with projecting microvilli towards the follicular lumen. The cytoplasm showed small apical vesicles, rough endoplasmic reticulum in both cisternal and vesicular form and mitochondria. Sub-epithelial basal lamina appeared regular and formed the base of the cell. The nucleus appeared euchromatic with regular and well formed nuclear membrane (Figs.2&3).

In treated animals with NaF, the thyroid parenchyma examined by light microscopy, appeared as several follicles which were lined by epithelial cells with reduced cell height and had low colloid contents with many peripheral vacuoles (Fig.4). By electron microscopic examination, the follicular cell reduced markedly in height and the basal membrane appeared ill defined. Colloid droplets appeared in the

apical and basal parts of the cytoplasm. Some of these droplets were fused and formed large colloid masses. Overall cytoplasmic disorganization appeared with few scattered stacks of rough endoplasmic reticulum and loss of mitochondria. The apical border showed pseudopods directed into the colloidal lumen. The nucleus appeared irregular, heterochromatic with deformed nuclear membrane (Fig.5,6,7 &8).

In NaF treated animals co-administered with calcium chloride, the follicles were lined by cuboidal cells and most of them contained colloid when examined by light microscopy (Fig.9). By electron microscopic examination, the follicular cells ameliorated and regained some of their normal characteristic features. The cell appeared with intact basal lamina and the cytoplasm showed rough endoplasmic reticulum, mitochondria, and small apical vesicles. The nucleus appeared regular in shape and euchromatic with well formed nuclear membrane and prominent one or more nucleoli (Figs.10&11).

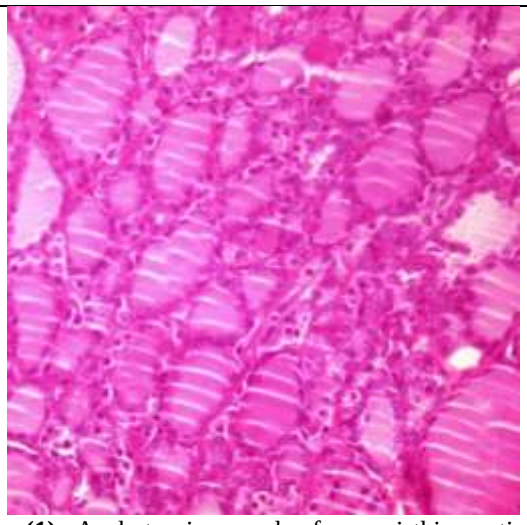


Fig. (1): A photomicrograph of a semi-thin section in the thyroid gland of control rat showing the thyroid parenchyma as several thyroid follicles. The follicles vary in size and all are rich in colloid content with cuboidal epithelial lining (H& E×400)

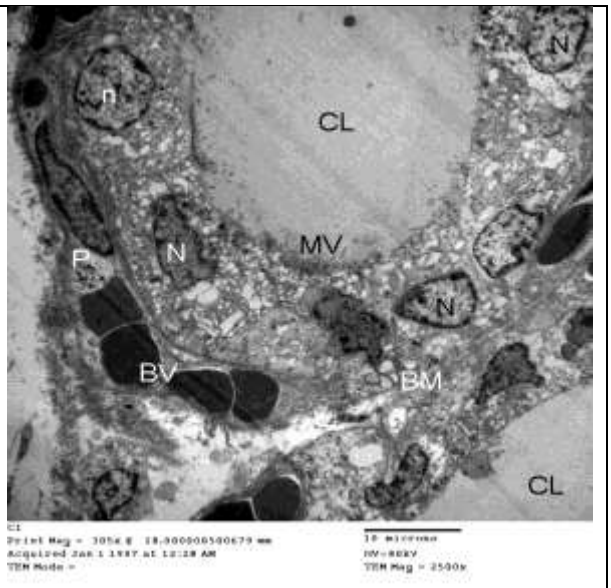


Fig.(2): An electron photomicrograph of ultrathin section in the thyroid gland of control rat showing parts of 2 adjacent thyroid follicles. The follicular cells show the apical border with microvilli (MV) projecting to colloidal lumen (Cl). Sub-epithelial basal membrane (BM) forms the base of the cells. The nuclei (N) appear euchromatic with regular and well formed nuclear membrane and prominent nucleolus (n). Blood vessels (BV) appear between the follicles and parafollicular cell (P) can be seen.

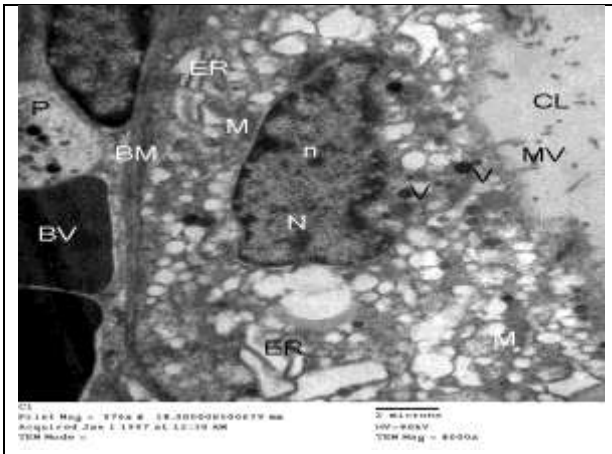
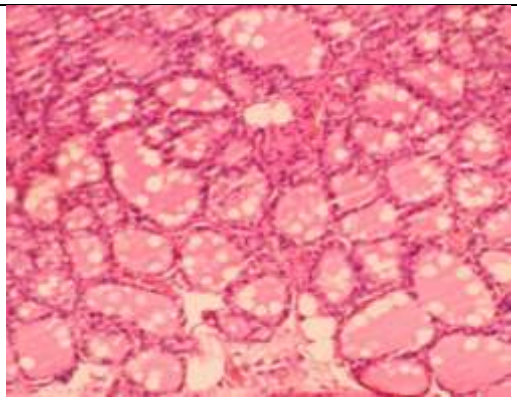


Fig.(3): An electron photomicrograph of a higher magnification of the previous figure showing the thyroid follicular cell.The cell show the apical border with microvilli (MV) projecting to the colloidal lumen (CL). Small apical vesicles (V), rough endoplasmic reticulum (ER) in both cisternal and vesicular forms and mitochondria (M) are seen. Subepithelial basal membrane (BM) forms the base of the cell. The nucleus (N) appears euchromatic with regular and well formed nuclear membrane. Blood vessels (BV) and parafollicular cell (P) are seen near to the base.



Fig(4): A photomicrograph of a semi-thin section in the thyroid gland of sodium fluoride treated rats showing the thyroid parenchyma as several thyroid follicles. The follicles are lined by epithelial cells with reduced cell height. Many follicles have low colloid with many peripheral vacuoles occupying parts of colloidal lumina . (H& E× 400**)**

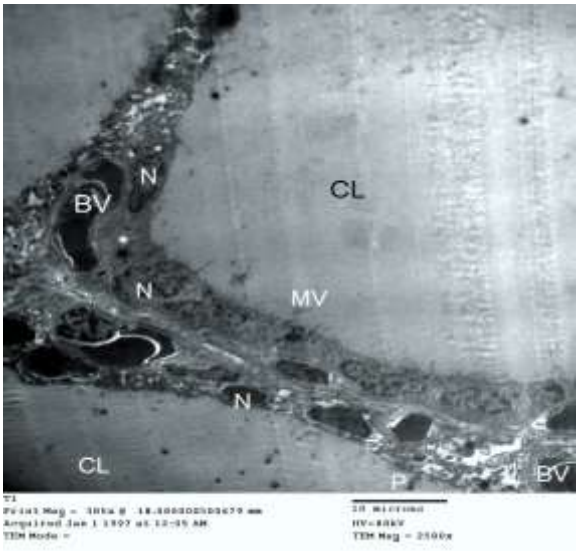


Fig. (5): An electron photomicrograph of ultrathin section in the thyroid gland of sodium fluoride treated rats showing parts of thyroid follicles . The follicular cells shows marked decrease in height with microvilli (MV) and pseudopod (P) projecting to the colloidal lumen (CL).The nuclei appear heterchromatic and irregular. Many blood vessels (BV) are seen

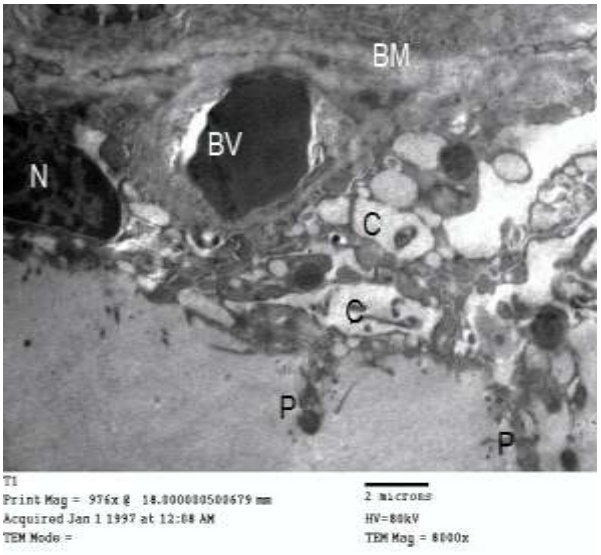


Fig.(6): An electron photomicrograph of a higher magnification of the previous figure showing ill defined basal membrane (BM) with nearby blood vessel (BV).The cytoplasm contains large colloid droplets (c). The apical border shows pseudopods(P). The nucleus (N) appears heterochromatic.

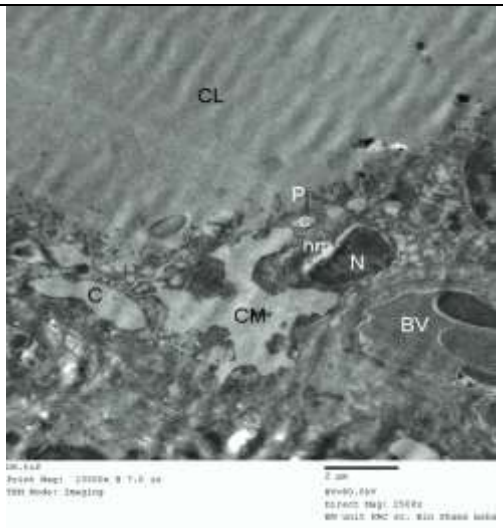


Fig.(7): An electron photomicrograph of ultrathin section in the thyroid gland of sodium fluoride treated rats showing the follicular cell with heterochromatic nucleus(N) . The cell shows marked decrease in height with ill defined basal lamina separating the cell from blood vessel (BV), colloid droplets (C) in the apical and basal parts of cytoplasm and large colloid masses (CM) appear with overall cytoplasm disorganization. A Pseudopod (P) appear arising and projecting to the colloidal lumen (CL). The nucleus appears heterochromatic with ill defined nuclear membrane (nm)

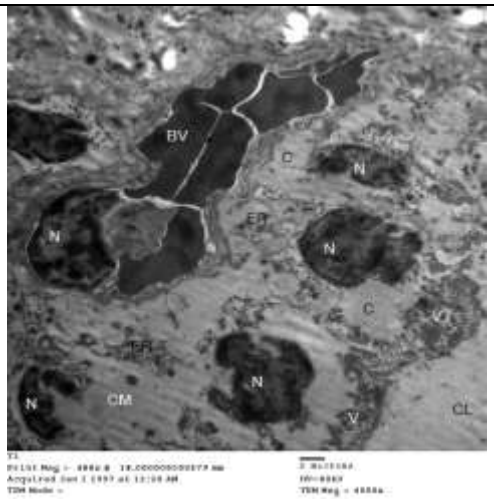


Fig.(8): An electron photomicrograph of ultrathin section in the thyroid gland of sodium fluoride treated rats showing the follicular cells . The cells shows ill defined basal lamina separating the cell from blood vessels (BV), colloid droplets (C) in the apical and basal parts of cytoplasm and large colloid masses (CM) appear separating scattered stacks of rough endoplasmic reticulum (ER) with loss of mitochondria and overall cytoplasm disorganization. Small vesicles (v) appear at the apical part of the cells. The nuclei (N) appear irregular in shape and heterochromatic

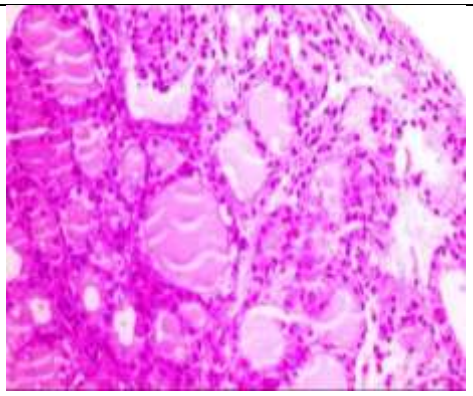


Fig.(9) :A photomicrograph of a semi-thin section in the thyroid gland of sodium fluoride treated rat co-administered with calcium chloride showing the thyroid parenchyma as several follicles. Most of the follicles contain colloids with cuboidal epithelial lining. (H& E× 400)

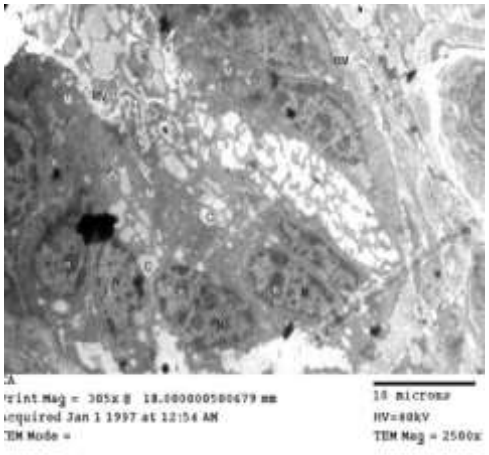


Fig.(10): An electron photomicrograph of ultrathin section in the thyroid gland of NaF treated rat co-administered with calcium chloride showing the follicular cell with intact basal membrane (BM). The cytoplasm shows mitochondria (M) and colloid droplets (c) .The nucleus (N) appears regular in shape and euchromatic with prominent one or more nucleoli (n) and well formed nuclear membrane

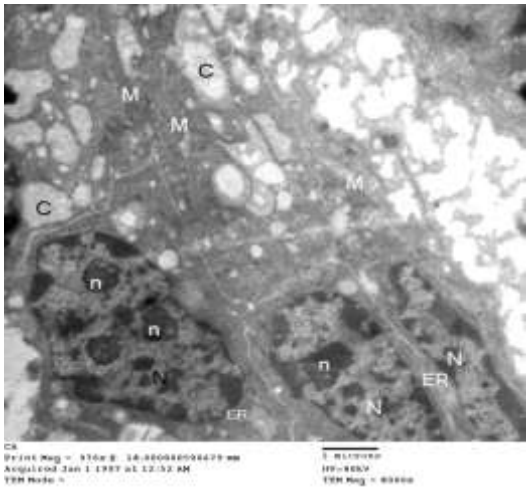


Fig.(11): An electron photomicrograph of a higher magnification of the previous figure showing rough endoplasmic reticulum (ER), mitochondria (M), small apical vesicles (V) and colloid droplets (C).The nuclei (N) appear regular in shape with prominent nucleoli (n)

4. Discussion

In the present study, the follicular cells in NaF treated rats examined showed marked decrease in cell height in addition to reduced colloid content. The decreased height reflects the decreased activity of the follicular cells[(18)]. This agrees with Liu *et al.*[(6)] who found that fluoride can induce structural changes and dysfunction in the thyroid gland. Also Yu[(19)] observed decreased serum T4 and increased TSH level in the residents of an endemic fluorosis area. The decreased colloid content observed in the present work is in agreement with Bouaziz *et al.*[(20)] who observed a decrease in the colloid content in mice treated with NaF in addition to increased follicular number and vascularity. The degenerative changes of the follicular epithelial cells examined can be explained by Barbier *et al.*[(21)] who suggested that, fluoride interacts with a wide range of cellular processes, such as gene expression, cell cycle, proliferation and migration, respiration, metabolism, ion transport, secretion, apoptosis and oxidative stress. At the ultrastructural level, the follicular cell in the present study showed adverse effects of NaF where the cytoplasm showed colloid droplets of variable size from large at the apical part to small at the base with appearance of pseudopods projecting to the colloidal lumen. These changes explain the decreased colloid content observed by light microscopy. The presence of pseudopods and colloid droplets in the cytoplasm suggested TSH stimulation of the follicular cells. This is supported by many investigators, who found that, the early response of TSH stimulated thyroid follicular cells is engulfment of colloid material from the follicular lumen into the apical cytoplasm of thyrocyte in the form of membrane bound colloid droplets. Administration of TSH to rats pretreated with thyroxin results in formation of pseudopods in the apical surface [(22,23,24)]. Also the cytoplasm of the follicular cells in the present study showed overall disorganization; the mitochondria were lost with scattered stacks of rough endoplasmic reticulum. This is in agreement with Zhan *et al.*[(25)] who mentioned that excessive fluorine injured structure of thyroid gland including mitochondria and endoplasmic reticulum. Also, Zhan etal.[(26)] investigated the effects of fluoride on the pancreatic acinar cells and found marked swollen mitochondria with loss of the cristae and the endoplasmic reticulum was markedly dilated. The loss of mitochondria in the present study could be explained by Shivarjashankara *et al.* [(27)] who found that, lipid peroxidation induced by NaF impairs a variety of intra and extra-mitochondrial membrane system that may contribute to apoptosis. Also, Barbier *et al.*[(21)] observed that, fluoride is thought to inhibit the activity of antioxidant enzymes such as superoxide dismutase(SOD), glutathione peroxidase and catalase. Moreover, fluoride can alter glutathione levels, often resulting in excessive production of reactive oxygen species (ROS) at the mitochondrial level, leading to damage of the cellular components. Excessive (ROS) production leads to macromolecule oxidation, resulting in free radical attack of membrane phospholipids with resulting membrane damage via induction of lipid peroxidation, mitochondrial membrane depolarization and

apoptosis. The nucleus of the follicular cell in the present study appeared irregular in shape and heterochromatic with deformed nuclear membrane. This is in accordance with Agha *et al.* [12] who found that, irregular shaped and heterochromatin nuclei are seen in the pancreatic cells of NaF administered group and this confirmed the induction of apoptosis. Also Yu [28] investigated the effects of fluoride on the ultrastructure in the tissue of liver, adrenal glands and thyroid glands of fetuses and found that the major pathological changes of cell nuclei were damaged and dilated vesicular dual-layer structure of nuclear membrane. Jacinto-Aleman[29] suggested that, excessive fluoride ingestion has been identified as a risk factor for fluorosis and oxidative stress and that can produce DNA fragmentation resulting in apoptosis.

Co-administration of calcium to the animals treated with NaF ameliorated the effects of NaF where the histology of the thyroid follicles regained to normal appearance. The follicular epithelium became cuboidal and most of the follicles contained colloid. Ultrastructurally, the follicular cell regained its normal height, the cytoplasm preserved some of its organelles; mitochondria, rough endoplasmic reticulum, and small dense apical vesicles. The basal lamina appeared intact and regular supporting the follicular cell. The nucleus appeared regular in shape and euchromatic with well-formed nuclear membrane and prominent nucleoli. This is in agreement with Sarkar *et al.*[14] who found that, co-administration of calcium and vitamin E with fluoride at the dose of 20 mg/Kg/day for 28 days of NaF resulted in a significant recovery from testicular disorders and oxidative stress in testis. Also, Teotia *et al.*[30] observed that, calcium nutrition provided 100% protection against the toxic effects of fluoride in deep bore drinking water supply with fluoride less than 0.5 ppm. The role of calcium against the effects of NaF to the thyroid follicular cells can be suggested by Ailani[31] who observed increase oxidative stress in cases of fluorosis with increasing drinking water fluoride concentration and that, the treatment with calcium, vitamin D and vitamin C resulted in a significant reduction in serum fluoride and superoxide dismutase (SOD) and increase in urinary fluoride. Dreisbach *et al.*[32] found that large quantities of supplemental calcium carbonate, which not only helps to de-acidify the body, but also binds fluorine assisting in safe excretion of fluorine and also helps to replenish body calcium stores depleted by fluorine. It could be concluded that, calcium supplementation can ameliorate the adverse effects of NaF on thyroid follicular cells in peoples at risk of high exposure.

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