Original Article

Role of Montelukast in Alleviation of Toxicity Induced by Cadmium in Thyroid Gland of Adult Male Albino Rats. (Histological, Immunohistochemical and Biochemical Study)

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ABSTRACT

Background: Cadmium is a common toxic heavy metal of great concern for environmental pollution and human health. It is extensively used in many industries as radiation screens, rechargeable batteries and pigments. Montelukast is a powerful highly effective anti-inflammatory drug. Recently it shows significant antioxidant properties.

Aim of the Study: Evaluate the effect of Cadmium on thyroid glands and to assess the possible protective role of Montelukast

Materials and Methods: Forty adult male albino rats were divided into four equal groups received treatment for 6 weeks: (I) (Control group) received distilled water. (II) (Cadmium group)"3mg/kg". (III) (Recovery group) kept untreated for another 6 weeks after Cadmium administration with the same dose and period as pervious group. (IV) (Montelukast and Cadmium group) received Montelukast "10mg/kg" and Cadmium"3mg/kg". At the end, all animals were sacrificed. Thyroid gland samples were obtained for histological and immunohistochemical studies. Blood samples were obtained for hormonal assay. Data was statistically analyzed.

Results: Histological examination of thyroid glands of Cadmium treated rat's showed disorganization and irregularity in the follicles. The follicular cells appeared distorted with dark nuclei and some cell desquamated in follicular lumen. Colloid retraction within the follicles, cellular infiltration around congested blood vessels and marked collagen fibers deposition in connective tissue septae were detected. Significant increase in Caspase-3 immune expression in the follicular cell's cytoplasm was noticed. High significant decrease of thyroid hormone (T3, T4) level was noticed. These changes were still seen in the recovery group. Co-administration of Montelukast showed obvious protection against the hazardous effects of Cadmium.

Conclusion: Cadmium induces marked thyroid tissue damage via, fibrosis and apoptosis. These structural changes can be alleviated by combined treatment with Montelukast.

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INTRODUCTION

Cadmium is considered one of the most harmful heavy metals. It is a serious environmental and occupational contaminant that has very toxic effect even in small amounts to both humans and animals[1]. It has been classified as class I human carcinogenic agent[2]. It is extensively used in many industries as radiation screens, rechargeable batteries and pigments. As well as pharmaceutical products may be sources of Cadmium exposure. Unfortunately, we could be exposed to Cadmium environmentally, occupationally and by diet[3]. Significant concentrations of this metal in soil and water is due to its presence in the animal meat, fish, vegetables and fruit, so that contaminated food is the major source of population exposure to Cadmium[4]. Studies show that Cadmium accumulates in tissues and has a very long biological half-life in humans and other mammals. Cadmium intoxication leads to the development of lesions in many organs and tissues such as the liver, kidneys, lung, pancreas, testes and bones[5]. Evidences of oxidative stress in most reported studies of Cadmium toxicity are seen so, various types of antioxidants have been tried as possible protective agents for example selenium, zinc, vitamin C and E[6]. Montelukast is a selective leukotriene receptor antagonist that inhibits cysteine leukotriene. Montelukast is used medically for managing asthmatic patients. Recently researchers have tested it for its antioxidant effect in various conditions and hopeful results are obtained[7]. Montelukast show a neuroprotective effect through antioxidant, anti-inflammatory and anti-apoptotic mechanisms[8]. Also, Montelukast has protective effects against experimental induced nephrotoxicity[9]. So, our study was designed to evaluate the possible role of Montelukast as a novel protective against Cadmium toxicity on thyroid glands.

MATERIAL AND METHODS

Chemicals

Cadmium was obtained from Merck®, Egypt. Cadmium chloride anhydrous (99.995%-Cd pure white powder, 25gm package)

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Montelukast was obtained from Lelipel ®, Egypt (5mg chew tablets) produced by Globe Pharmaceutical New Launched.

Both chemicals were dissolved in distilled water. Continuous shaking was done for complete dissolving. The solution was prepared fresh every day.

Animals

Forty adult male albino rats were used in this study. They were weighing 185-200 gm. All animals were kept in special clean, properly ventilated cages in the animal house of the Faculty of Medicine, Menoufia University, Shebin el Kom, Menoufia, Egypt. Rats were treated in accordance with the guidelines approved by the Animal Care and Ethical Committee of Faculty of Medicine, Menoufia, University. They were divided randomly into four equal groups (10 animals each)

Group I (Control): received distilled water as a vehicle by oral gavage once daily for 6 weeks

Group II (Cadmium treated): received Cadmium at a dose of 3mg/kg/ day by oral gavage[6] once daily for 6 weeks.

Group III (recovery group): animals were kept untreated for another 6 weeks after Cadmium treatment.

Group IV (Montelukast + Cadmium): received simultaneously Montelukast 10mg/kg /day[9] and Cadmium 3 mg/kg/day by oral gavage once daily for 6 weeks.

Methods

Histological examination

On the time of scarification, rats were anesthetized via ether inhalation (2 ml) for approximately 2 min in a transparent acrylic jar[10]. Thyroid gland of each rat in all groups were excised and processed for light and electron microscope examinations.

Light microscopic examination: Tissues were fixed in Bouin's solution and processed for paraffin blocks. Sections of 5-6 µm were obtained and stained with hematoxylin and eosin (H & E), Periodic Acid Schiff reaction (PAS) and Mallory's trichrome (MT) stains[11]. Immunohistochemical staining of anti Caspase-3 was performed on sections of 4 µm thick. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. For antigen retrieval, citrate buffer was used after the sections were treated in a microwave. Sections were incubated overnight at room temperature with the primary antibodies of anti Caspase-3 mouse monoclonal antibody (Lab Vision, Fremont, CA USA). The procedure was completed using the streptavidin–biotin complex detection method. In each run controls slides were included. Negative controls were formed by excluding the primary antibody and replacing it with phosphate buffered saline (PBS)[12].

Electron microscopic examination; samples were excised rapidly and cut into one mm3 pieces, primary fixed in 3 % glutraldehyde and 0.1 M phosphate buffer at pH 7.4, then fixed in osmium tetraoxide, processed and embedded in epon. Semithin sections (1 µm thick) were stained with toluidine blue. Ultrathin sections (50-80 nm thick) were contrasted with uranyl acetate and lead citrate and then examined by using transmission electron microscope (Seo-Russia) in Tanta E.M Center in faculty of medicine, Tanta University[13].

Hormonal analysis

The blood samples were obtained from retro-orbital venous plexus and collected into clean glass tubes without anticoagulant. Samples were centrifuged to obtain sera and stored at-20°C until time of assay. Thyroid stimulating hormone (TSH), Total triiodothyronine (T3) and total tetra iodothyronine "thyroxine" (T4) hormone levels were measured by radioimmunoassay technique according to Kurian & Kapoor[14]. Values were reported as ng/mL for TSH and as ng/dL for T3 and T4. values were used for statistical analysis.

Morphometric and Statistical study

Ten images for each group were obtained at the same magnification (X400). Image analyzer software (Image J 1.47v national institute of health, USA) was used.

The diameter of thyroid follicles (follicular width) were measured by using standard methods of measurements. Follicular length was calculated at the two farthest points in the follicle then the follicular width was measured perpendicular to the follicular length. The mean of widths of the largest three follicles in each field were obtained.

We also used image J program to estimate PAS stain color intensity, percentage area of collagen and counting the number of the positive Caspase-3 immune stained follicular cells. The calculated numbers were used for comparison and statistical analysis.

Data were statistically analyzed by SPSS (Statistical Package for the Social Sciences) program, version 20 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean and SD (standard deviation) and analyzed by using one-way analysis of variance (ANOVA) followed by post-hoc Tukey" test for comparison between the control and other groups. Differences were graded as significant if *P values* were < 0.05 and highly significant if *P value* was < 0.001[15].

RESULTS

Light microscop results

H&E stained sections: control group showed multiple thyroid follicles that were uniformly distributed and variable sized. Follicles were filled with acidophilic homogenous colloid with few peripheral vacuolation. The lining follicular cells were cuboidal with rounded vesicular nuclei and homogenous basophilic cytoplasm. Groups of interfollicular cells were also observed. (Figure 1a). Cadmium group showed disorganization of thyroid follicles

where, some follicles became distorted, irregular in shape, smaller in diameter and others appeared with empty lumen (Figure 1b). Ruptured follicular walls and some fused follicles were noticed and the colloid was interrupted in some follicles (Figure 1c) but other follicles exhibited markedly vacuolated colloid (Figure 1d). Follicular cells showed highly vacuolated cytoplasm (Figure 1b). Other follicular cells were seen desquamated into the lumen. (Figure 1d). thyroid follicles appear as concentric lamellae of flattened cells with dark pyknotic nuclei (Figure 1f). Cellular infiltration around markedly dilated congested blood vessels were seen in connective tissue septa (Figures 1d,e). Recovery group showed only slight improvement in regularity of follicular diameter and tissue arrangement but follicles still appeared empty with no colloid in their lumen and some follicles contained vacuolated colloid. Follicular cells still showed desquamation, multiple vacuolation and karyolysis (Figure 1g). Montelukast and Cadmium group exhibited obvious improvement in follicular diameter, follicles architectures and colloid homogeneity except that few follicles were still seen empty (Figure 1h).

PAS stained sections: Control group showed strong PAS reaction in the colloid and moderate reaction in the basement membrane (Figure 2a). Cadmium group revealed weak PAS reaction of both colloid and basement membrane. Most follicles showed colloid retraction (Figure 2b). Recovery group showed moderate PAS reaction in the colloid and the basement membrane. Some follicles showed decreased colloid (Figure 2c). Montelukast and Cadmium group revealed PAS reaction more or less similar to control (Figure 2d).

Mallory trichrome stained sections: Control group showed minimal collagen fibers deposition in the interfollicular septa (Figure 3a). Cadmium group revealed apparently thickened connective tissue septa with excessive deposition of collagen fibers around thyroid follicles and the blood vessels (Figures 3b,c). Recovery group still showed thickened interfollicular septa and deposition of large amount of collagen fibers. (Figure 3d). Montelukast and Cadmium group showed minimal amount of collagen fibers in the apparently thin septa (Figure 3e).

Caspase-3 stained sections

Control group showed negative Caspase-3 cytoplasmic immunostaining. (Figure 4a). Cadmium group showed intense positive Caspase-3 cytoplasmic immunostaining in the whole follicles (Figure 4b). Recovery group revealed moderately positive Caspase-3 immunostaining in some follicles (Figure 4c). Montelukast and Cadmium group showed weak positive Caspase-3 immunostaining (Figure 4d).

Transmission electron microscope results

Ultrastructural examination of thyroid follicular cells of control group showed a cuboidal cell with apical microvilli, and euchromatic oval or rounded nucleus. The cytoplasm contained numerous parallel rows of rough endoplasmic reticulum, free ribosomes, abundant mitochondria, secretory vesicles and lysosomes. The follicles were filled with homogeneous colloid with few colloid vacuoles and were bounded with thin regular basal lamina and surrounded by blood capillaries. Intact junctional complexes between follicular cells were also noticed. While parafollicular cell contained rounded euchromatic nucleus with granular cytoplasm and appeared between follicular cells and was seen surrounded by a basal lamina. Little interfollicular connective tissue was observed (Figure 5).

Cadmium group showed marked distortion of thyroid follicles, some follicles were filled with lamellated non-homogenous colloid and junctional complexes between follicular cells were disturbed (Figure 6a). Follicular cell appeared with sparse apical microvilli. Its nucleus appeared irregular and heterochromatic. The cytoplasm contained extensively dilated rough endoplasmic reticulum (Figures 6b,d). Some follicles were lined by more than one layer of follicular cells. One cell had rounded nucleus but others had irregular notched nucleus. Their cytoplasm contained lumps of rER (Figure 6c). Some cells showed foamy abundant cytoplasm and destructed mitochondria. (Figure 6d). Desquamation of follicular epithelial cell in the follicular lumen was noticed (Figure 6e). The interfollicular tissue contained congested dilated blood capillaries, large amount of collagen fibers, fibroblast, mast cell and other inflammatory cells (Figures 6f,g,h). The Para follicular cell was observed with vacuolated cytoplasm and few granules rested on thick irregular basal lamina (Figure 6h).

Recovery group showed that, follicular cell had sparse apical microvilli and rested on irregular thick basal lamina. Its nucleus appeared rounded, heterochromatic and notched. The cytoplasm contained dilated rough endoplasmic reticulum and multiple vacuolation (Figure 7a). Some cells had irregular shrunken nucleus and junctional complexes between adjacent cells appeared partially intact (Figure 7b). Damaged mitochondria and degenerated parafollicular cells were noticed (Figure 7c). Also; the interfollicular tissue contained large fibroblast, massive amount of collagen fibers (Figure 7b) and congested blood capillaries (Figure 7c).

Montelukast and Cadmium group revealed obvious improvement in the structure of follicles. Follicular cells appeared more or less similar to control (Figures 8a,b).

Morphometric and statistical results

Follicular diameter: The mean of follicular diameters of both Cadmium (GII) and recovery (GIII) groups showed high significant decrease ($P < 0.001$) when compared to control. But Montelukast and Cadmium (GIV) group showed a non-significant relation to control group ($P > 0.05$) (Table 1, Figure 1 i).

Color intensity of PAS stain: Mean of PAS color intensity of Cadmium (GII) and recovery (GIII) groups showed high significant decrease ($P < 0.001$). But

Montelukast and Cadmium group (GIV) showed non-significant difference(P> 0.05) in accordance to control (Table 2, Figure 2e).

Percentage area of collagen fibers: There was high significant increase (P< 0.001) of collagen fibers percentage in Cadmium (GII) and recovery (GIII) groups. Montelukast and Cadmium (GIV) group revealed non-significant difference (P> 0.05) in relation to control (Table3, Figure 3f).

Number of positive Caspase-3 immunostaining cells: Cadmium group (GII) showed high significant increase (P < 0.001) of positive Caspase-3 cells. Recovery group (GIII) showed significant increase (p< 0.05) of positive Caspase-3 cells. Non-significant relation (P> 0.05) could be detected in Montelukast and Cadmium group (GIV) (Table 4, Figure 4e).

Serum hormone levels: Cadmium (GII) and recovery (GIII) groups showed highly significant (P < 0.001) decrease in serum T3 and T4. However, there was a highly significant increase (P< 0.001) in serum TSH in the same groups as compared to control. Levels of TSH, T3 and T4 in Montelukast and Cadmium group (GIV) did not reveal any statistically significant value (P> 0.05) (Table 5, Figure 9).

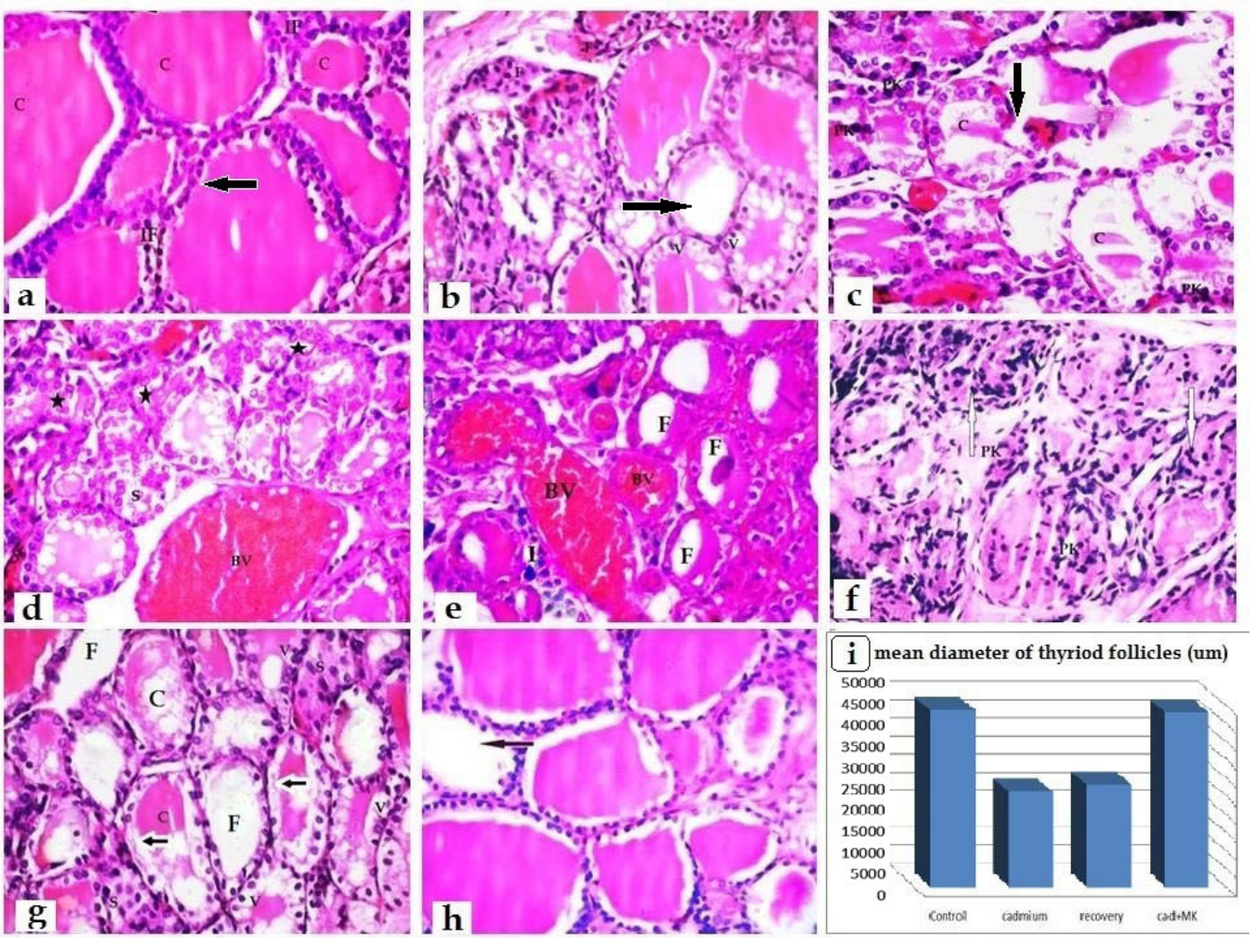

Fig. 1: H & E stained sections: a) Control group showing regular oval follicles filled with homogenous acidophilic colloid(C). Follicles are lined by simple cuboidal epithelium (arrow) notice: Groups of interfollicular cells (IF). (b-f) Cadmium treated group showing b): Follicles are irregular in shape and some are seen empty (arrow). The follicular cells have vacuolated cytoplasm (V) c): distorted follicles with ruptured follicular wall (arrow). Follicular cells showing deeply stained pyknotic nuclei (PK). The colloid(C) appears interrupted d): disorganized follicles (stars) with epithelial desquamation(S) and dilated congested blood vessels (BV) e): empty small sized thyroid follicles (F) and cellular infiltration (I) around congested blood vessels (BV) in C.T space. f): loss of normal architecture of thyroid follicles they appear as concentric lamellae of flattened cells (arrows) and many cells show pyknosis (PK). g) Recovery group showing multiple vacuolation in the follicular cells (V) and in the colloid (C) desquamated epithelial cells (S) appear within the follicular lumen. Notice: some cells with karyolytic nuclei (arrow) and some follicles appear empty with no colloid (F). h) Montelukast and Cadmium treated group showing regularly arranged thyroid follicles with normal architecture but few follicles appear empty (arrows). i) Mean values of thyroid follicles diameter in the different groups: Montelukast + Cadmium group (cad+Mk). (H&E.X400)

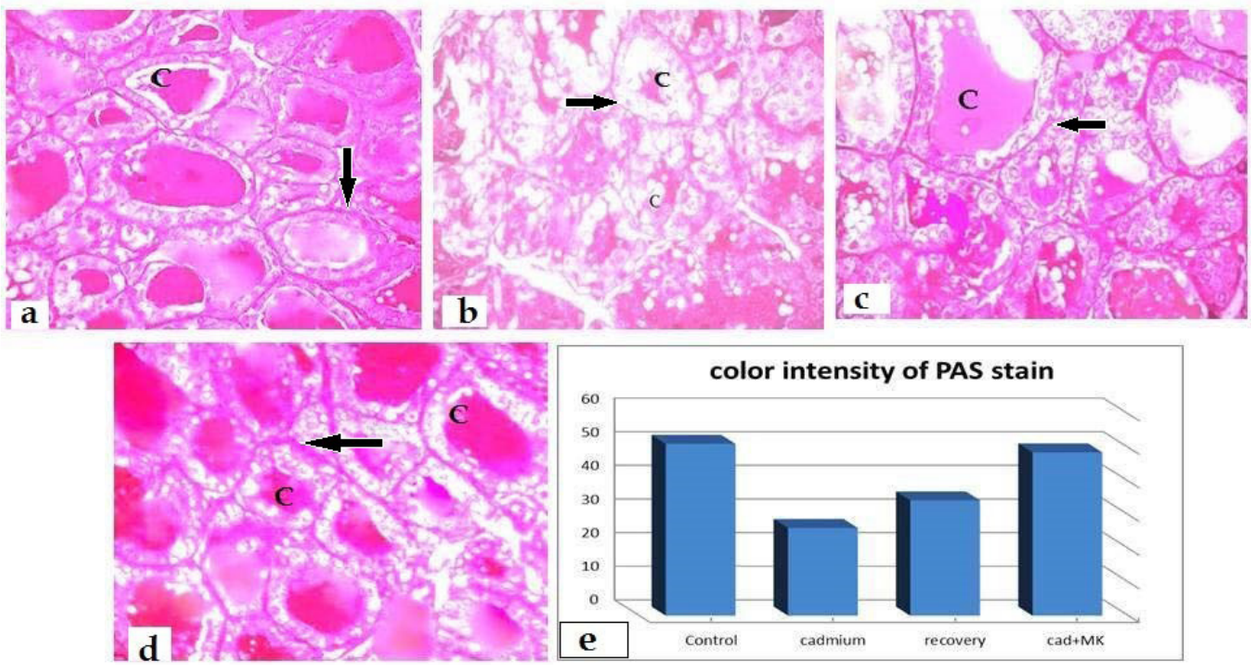

Fig. 2: Periodic acid Schiff (PAS) stained sections a:) Control group showing strong PAS reaction of abundant colloid "magenta in color" (C) and moderate reaction in the basement membrane (arrow). b:) Cadmium treated group showing weak PAS reaction in the colloid and most follicles show colloid retraction (C). The basement membrane shows weak PAS reaction (arrow). c:) Recovery group showing moderate PAS reaction of colloid and decreased colloid in some follicles (C). The basement membrane shows moderate reaction (arrow). d:) Montelukast and Cadmium treated group showing strong PAS reaction in the colloid (C) and the basement membrane (arrow). e:) Mean values of PAS color intensity in the different group: Montelukast + Cadmium group (cad+Mk). (PAS X400)

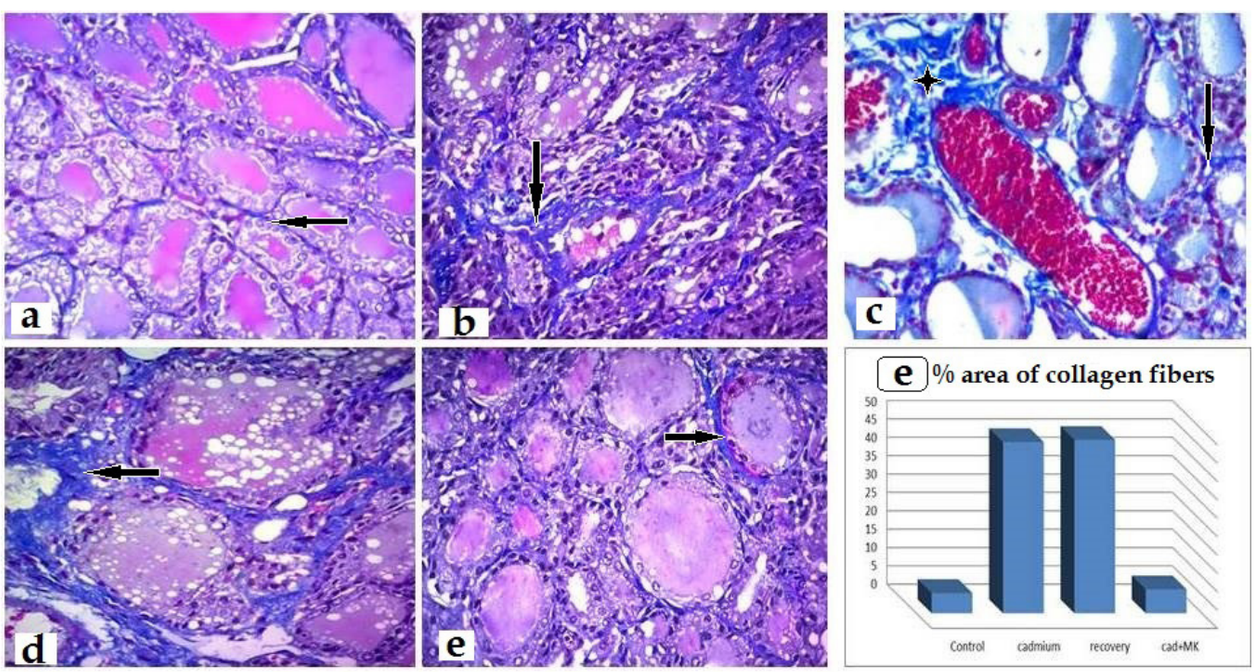

Fig. 3: Mallory trichrome stained sections a) Control group showing thin interfollicular connective tissue septa with minimal collagen fibers deposition (arrow). b&c) Cadmium treated group showing thickened connective tissue septa with excessive deposition of collagen fibers around thyroid follicles (arrow) and the blood vessels (star). d) Recovery group showing thickened interfollicular septa and deposition of large amount of collagen fibers (arrow). e) Montelukast and Cadmium treated group showing thin interfollicular septa with minimal collagen deposition (arrow). f) Mean values of percentages area of collagen fibers in the different groups: Montelukast + Cadmium group (cad+Mk). (MT x 400)

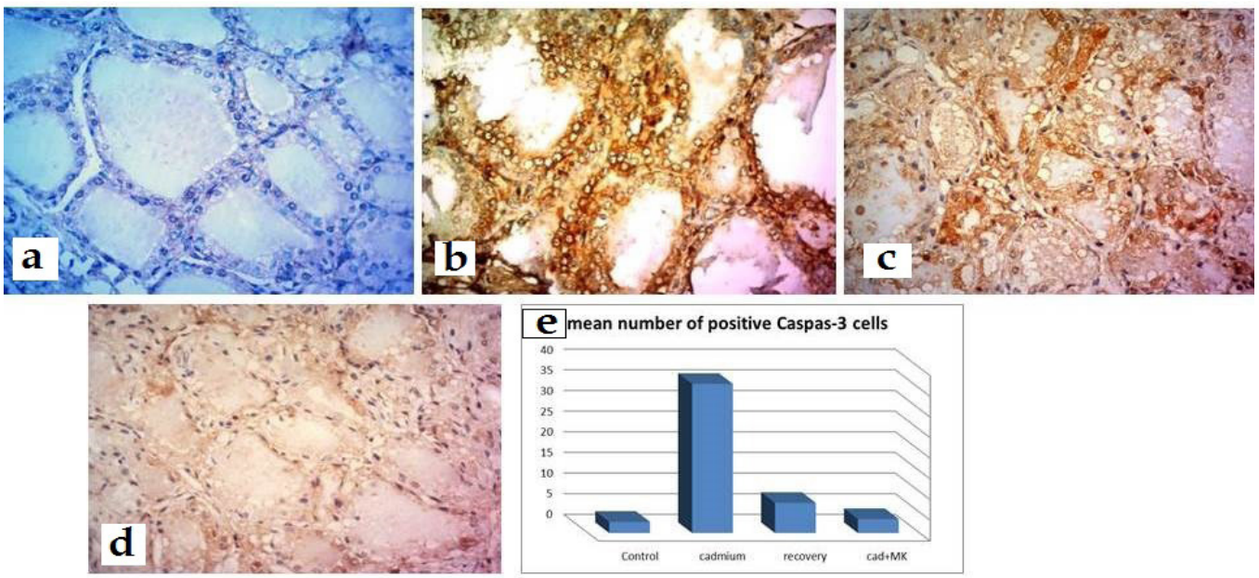

Fig. 4: Caspase-3 – immunostaining sections a) Control group showing negative aspase-3 immunostaining. b) Cadmium treated group showing intense positive caspase-3 immunostaining. c) Recovery group showing moderate positive caspase-3 immunostaining. d): Montelukast and Cadmium treated group showing weak positive caspase-3 immunostaining. e) Mean values for the number of positive caspase -3immunstained cells in the different groups: Montelukast + Cadmium group (cad+Mk). (Caspase-3 x 400)

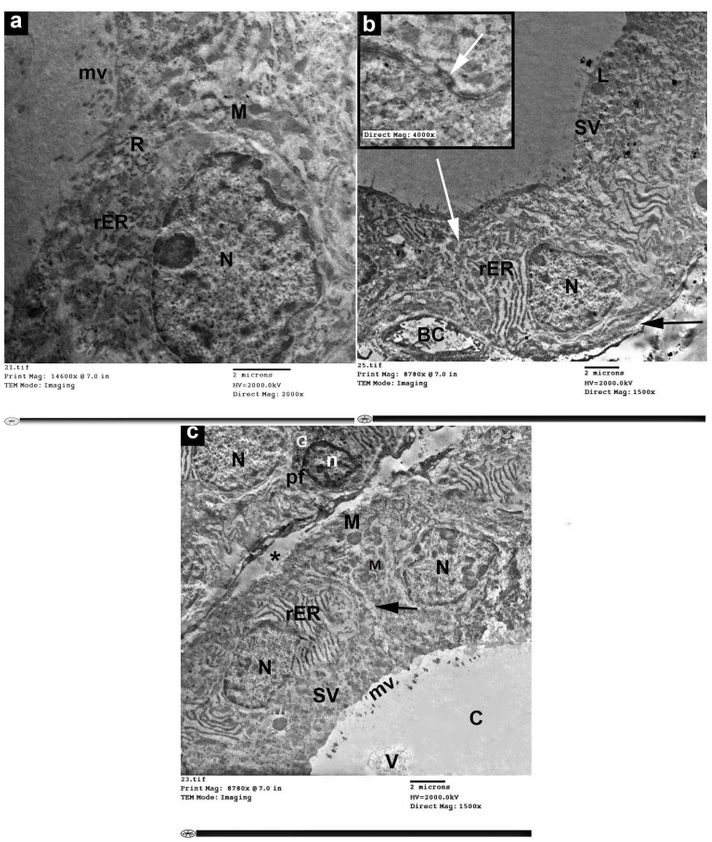

Fig. 5: Electron micrographs of control rat showing: Thyroid follicular cell with apical microvilli (mv) and have oval euchromatic nucleus (N), rough endoplasmic reticulum (rER), numerus free ribosomes (R) and mitochondria (M). x 2,000. Follicular cell has oval euchromatic nucleus (N), numerous parallel arrays of rER, secretory vesicles (SV) and lysosomes (L). Follicle is surrounded by thin regular basal lamina (black arrow) and blood capillary (BC) x 1,500. Inset: junctional complexes between adjacent cells appear intact (white arrow) x 4,000. Two thyroid follicle. The lower follicle filled with moderately dense colloid (C) with peripheral vacuole (V). Apical microvilli (mv) and intact junctional complexes (arrow) appear. The cytoplasm contains numerous mitochondria (M), secretory vesicles (SV), (rER) and euchromatic nucleus (N). The upper follicle showing euchromatic nucleus (N). Parafollicular cell (pf) appears with rounded euchromatic nucleus (n) and granular cytoplasm (G). Notice: narrow interfollicular space (star). x 1,500

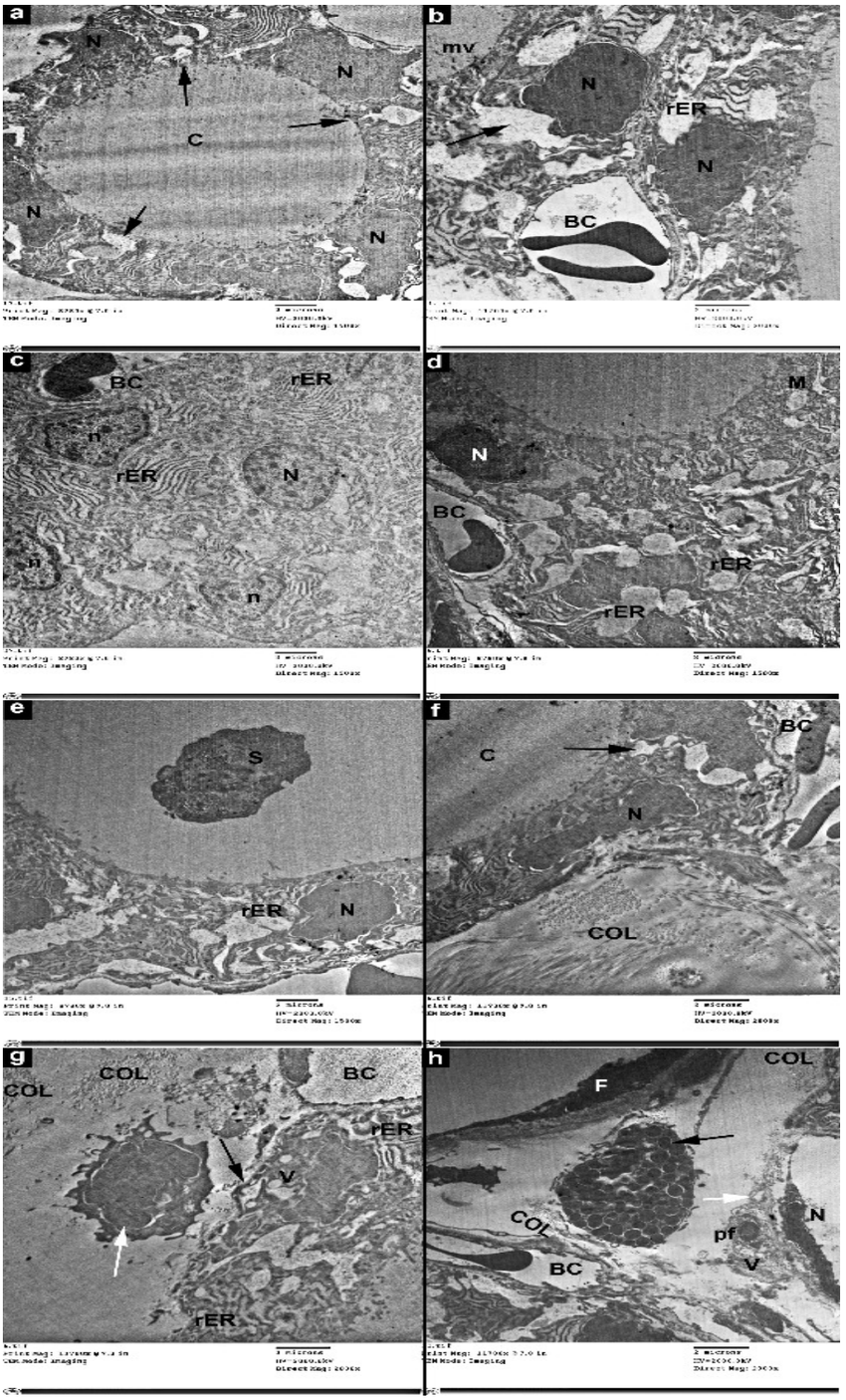

Fig. 6: Electron micrographs of Cadmium treated group showing a) Complete thyroid follicle with disturbed junctional complex between cells (arrow). Follicular cells have electron dense flattened nuclei (N) Colloid (C) appears lamellated non- homogenous. x 1,500 Follicular cells with sparse apical microvilli (mv), nuclei appear notched and heterochromatic (N). The cytoplasm contains extensively dilated rough endoplasmic reticulum (rER) with fine granules inside it (arrow). Notice: congested capillaries (BC) (arrow). x 2,000 Thyroid follicle lined by more than one layer of follicular cells one cell has rounded nucleus (N) and others exhibit irregular nuclei (n). Cytoplasm contains lumps of numerous (rER). Notice: dilated congested blood capillary (BC). x 1,500 Dilated (rER), destructed mitochondria(M) and disorganized follicular cell with electron dense nucleus (N) x 1,500 e) Desquamated epithelial cell (S) in the follicular lumen, dilated rER containing fine granules in their lumen and electron dense irregular nucleus(N). x 1,500 Follicular cell with flattened condensed nucleus (N) and distorted Junctional Complex (arrow). Massive collagen fibers deposition (COL) and congested blood capillaries (BC) around the follicles. x 2,000 Follicular cell having cytoplasmic vacuolation (v) dilated (rER) and resting on highly disturbed basal lamina (black arrow). Cellular infiltration as neutrophil (white arrow), collagen (COL) and congested capillaries surround the follicles (BC). x2,000 Wide inter follicular space congaing mast cell (black arrow), degenerated fibroblast (F), collagen (COL) and blood capillary (BC) Notice: Para follicular cell (pf) with few granules (G) and vacuolated cytoplasm (v) present between degenerated follicular cell (N) and irregular basal lamina (white arrow). x 2,000

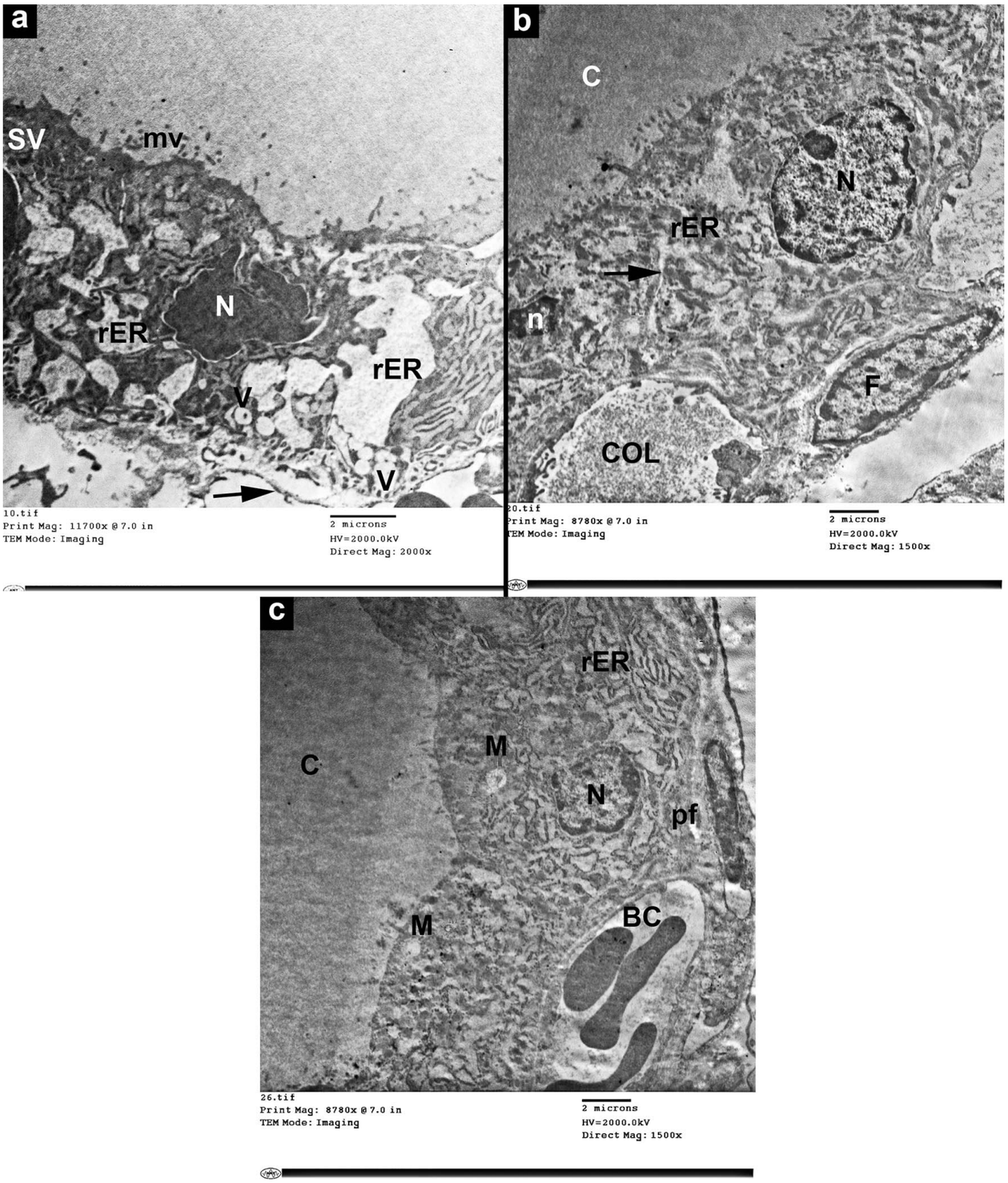

Fig. 7: Electron micrographs of recovery group showing. Follicular cell resting on irregular thick basal lamina with irregular and sparse apical microvilli (mv). Cell has dense notched heterochromatic nucleus (N). The cytoplasm contains dilated rough endoplasmic reticulum (rER) ,secretory vesicles (SV) and multiple vacuoles (V).x 2000 Two follicular cells one has irregular shrunken nucleus (n) but, other cell has regular rounded nucleus (N) Follicular cell has moderately dilated (rER). Junctional complexes between adjacent cells appear partially intact (arrow). The interfollicular space contains massive collagen fibers deposition (COL) and fibroblast (F). x 1,500 Thyroid follicular cell with dilated (rER), few damaged mitochondria (M) The nucleus appears shrunken condensed(N) notice: colloid(C), degenerated Para follicular cell (pf) and congested dilated capillaries (BC) x 1,500

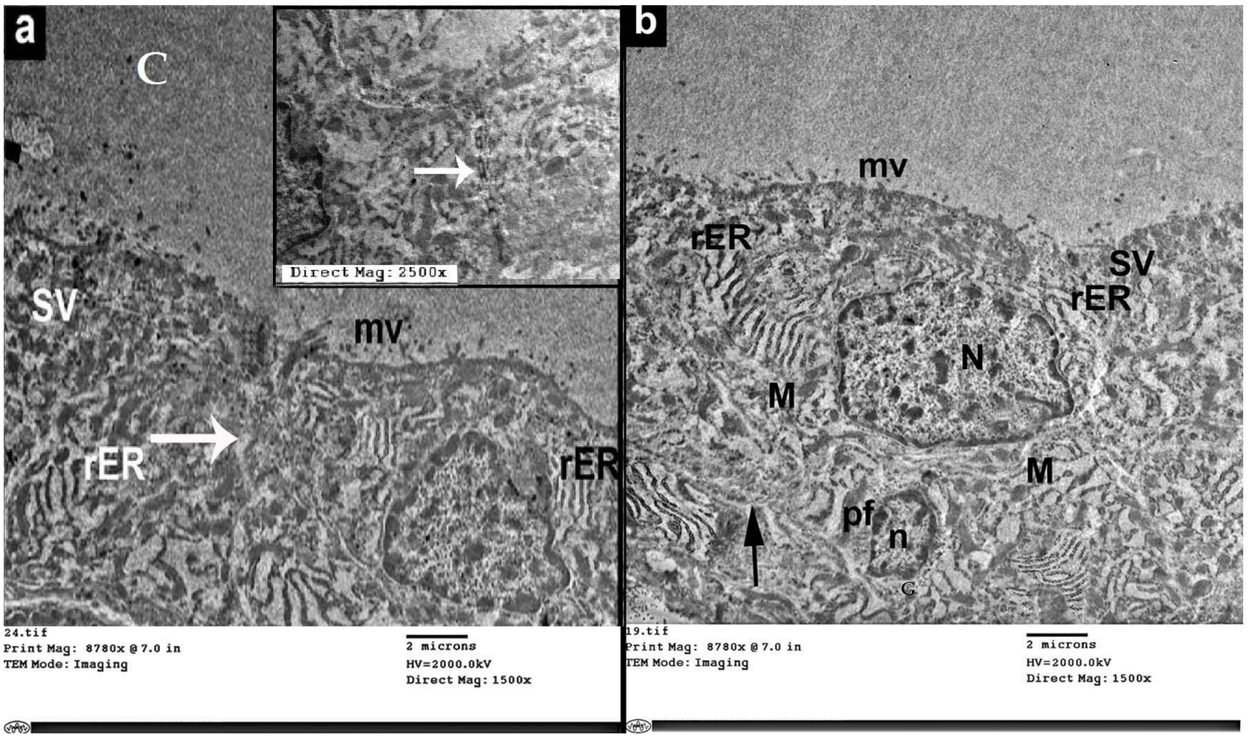

Fig. 8: Electron micrographs of Montelukast and Cadmium treated group showing. Follicular cells with numerous cisternae of rough endoplasmic reticulum (rER) and numerous secretory vesicles (SV). Its apical border shows microvilli (mv). Notice: homogenous colloid (C) in the follicular lumen x 1500 Inset: junctional complexes between adjacent cells appear intact (arrow) x 2,500. b) Follicular cells has apical microvilli border (mv), oval euchromatic nucleus (N) , rough endoplasmic reticulum (rER), numerous intact mitochondria (M) and secretory vesicles (SV) Notice: parafollicular cell (pf) resting on regular basal lamina(arrow) and has rounded nucleus (n) and granular cytoplasm(G) . x 1500

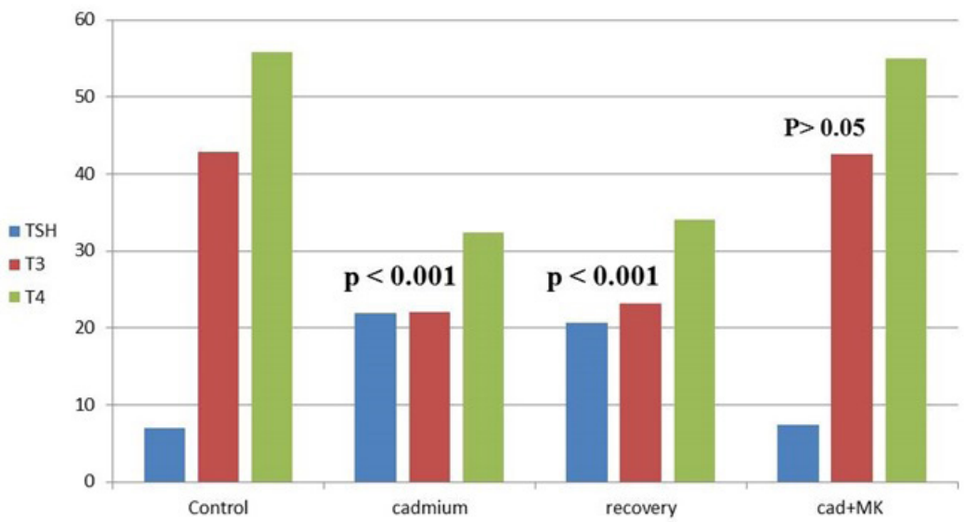

Fig. 9: Shows relatively significant diffrence in T3, T4 and TSH levels in different group

Table 1: Statistical means of thyroid follicles diameter (um) in various groups: Data was expressed as mean ±SD.

Diameter of thyroid follicles	Mean ± SD	T-test	*P- value*
Control	48556.4±609.2		
Cadmium	26027.8±550.2	19.280	P=0.000**
Recovery	28027.9±593.7	17.981	P=0.000**
Montelukast+ Cadmium	47928.4±522.0	1.658	P=0.117

P value >0.05 = Non-significant.
* Significantly as compared to control group ($p < 0.05$).
** High significant as compared to control group ($p < 0.001$).

Table 2: Statistical means of color intensity of PAS stain in various groups

Color intensity of PAS stain	Mean ± SD	T-test	*P- value*
Control	51.1±3.8		
Cadmium	26.1±0.2	20.004	P=0.000**
Recovery	34.3 ±2.7	14.034	P =0.00**
Montelukast+ Cadmium	48.6±1.4	1.912	P=0.075

Table 3: Statistical means of % area of collagen fibers in various groups

% area of collagen fibers	Mean ± SD	T-test	*P- value*
Control	5.5±1.1		
Cadmium	46.6±1.8	98.503.	P=0.000**
Recovery	47.2±0.8	196.983	P=0.000**
Montelukast+ Cadmium	6.5±1.2	2.004	P=0.063

Table 4: Mean number of positive Caspase-3 immunostaining cells of thyroid follicle

Number of positive Caspase- 3 cells	Mean ± SD	T-test	*P- value*
Control	2.7±1.3		
Cadmium	36.1±2.9	51.247	P =0.000**
Recovery	7.3±1.8	3.787	P =0.002*
Montelukast+ Cadmium	3.4 ±1.0	1.763	P =0.098

Table 5: Serum levels of TSH, T3 and T4 in different groups

Mean ± SD of	Control	Cadmium	Recovery	Montelukast + Cadmium
TSH ng/mL		21.9±1.2	20.7±0.9	7.4±0.1
T-test	7±1.3	9.956	10.942	1.691
P- value		P =0.000**	P =0.000**	P =0.111
T3 ng/dL		22.0±0.5	23.2±0.9	42.5±0.8
T-test	42.9±0.6	15.414	12.579	1.605
P- value		P =0.000**	P =0.000**	P =0.129
T4 ng/dL		32.4±1.1	34.0±2.3	55.0±1.0
T-test	55.8±0.4	15.768	10.298	1.534
P- value		P =0.000**	P =0.000**	P =0.146

DISCUSSIONS

Cadmium is a toxic metal that is present in the environment naturally and as a pollutant from industry, agriculture and many sources. Cadmium has potential hazardous effects on the endocrine glands. Cadmium concentrations in thyroid tissue are three times higher in people living in polluted areas[16]. Cadmium has a delayed clearance with high deposition rate in different body organs causing tissue damage through different mechanisms[17].

In our study, Cadmium induced high significant decrease in the diameter of thyroid follicles, distortion and degeneration of thyroid tissue, follicular epithelial desquamation, cytoplasmic vacuolation, nuclear pyknosis and colloid retraction. These results were previously reported by other researches[18,19] this was attributed to the oxidative stress caused by cadmium toxicity. In our study these changes were not improved after stopping Cadmium in recovery group and this might be an indicator that Cadmium had irreversible thyroid toxicity.

Cellular infiltration after Cadmium administration observed in our study was documented by Elblehi *et al*[17] and Ghonim *et al*[20]. The later reported an increase in the total leukocytic count in Cadmium treated rats and stated that this infiltration occurred as a defensive response to the degenerative changes caused by Cadmium toxicity.

Marked decrease of T3 and T4 levels occurred in Cadmium group in our study explained the result of PAS stained section of the same group where, PAS reaction was weak with colloid retraction. These findings were similar to the observation reported by previous studies[19,21]. Cadmium accumulated in mitochondria of follicular epithelial cells and distorted the oxidative phosphorylation and cellular respiration with subsequent loss the energy supply. Thus, the synthesis of colloid and thyroid hormone is inhibited[22].

High significant increase in collagen fibers deposition (fibrosis) at the interfollicular space was detected in Cadmium group and this was confirmed by the finding of Benvenga *et al*[18]. This fibrosis was not reversed by stopping Cadmium treatment in recovery group. Velickov *et al*[23] stated that Cadmium induced fibrosis by degranulation of mast cell with releasing of its cytokine tryptase. This cytokine stimulates fibroblast proliferation and collagen fibers formation. Also, Cadmium treatment

reported to decrease selenium. This deficiency may lead to activation of fibrosis through inflammatory reaction and excess stimulation of growth factor transforming[22].

Intense positive Caspase -3 cytoplasmic expressions in Cadmium group in the current study proved apoptosis. Karoui-Kharrat, *et al*[5] studied the effect of Cadmium on cell culture. They stated that Cadmium showed a significant effect on cell viability via necrotic and apoptotic pathway. Cadmium activated both the intrinsic and extrinsic apoptotic pathway[24]. In addition, it induced cellular damage through upregulation of the pro-apoptotic genes[16]

Light microscopic findings were confirmed by electron microscopic results. Cadmium exhibited considerable ultrastructural changes in thyroid follicular cells as marked dilatation of rough endoplasmic reticulum, absence of mitochondrial crests, heterochromatic nucleus, cytoplasmic vacuolation and distorted microvilli these changes were supported by other studies[19]. According to Sedky *et al*[25] Cadmium induced swelling of mitochondria and proliferation of endoplasmic reticulum leading to cytoplasmic vacuolation. Also, the nucleus condensation induced by Cadmium occurred as result of apoptosis[26].

The mitochondrial damage induced by Cadmium could be explained by disturbed ion homeostasis, where calcium influx rapidly into the cell. Additionally, it affects the enzymes of oxidative phosphorylation in the mitochondria thus alters the energy inside the cell[27].

Dilated rough endoplasmic reticulum was the most obvious in our results this could be explained by Gardarin *et al*[28] who proved that endoplasmic reticulum is the main target of Cadmium toxicity. Also, Cadmium induces endoplasmic reticulum stress in *vivo* and in *vitro* leading to induction of apoptosis[24].

Congested dilated blood capillaries were noticed. According to previous study[27] Cadmium might induce vascular injury due to oxidative stress.

In Cadmium treated group some follicles were lined by more than one layer of follicular cells contained numerous RER. This finding could be an indicator for cells proliferation. This result could be explained by the finding of Djordjevic *et al*[24] who stated that Cadmium induces tissue proliferation and cells trans-differentiations which may lead to carcinogenicity.

Cadmium enhances production of reactive oxygen species (ROS) superoxide ion, hydrogen peroxide and leads to oxidative stress. Increased ROS level cause damage to different cell component like proteins, lipids, membranes and cytoplasmic organelles as endoplasmic reticulum and mitochondria. Cadmium affect the metabolism of calcium, Zinc, iodine and Selenium thus it alters the structure of thyroid follicular and parafollicular cells[29].

Oxidative stress has been considered as the most important mechanism of Cadmium toxicity[5]. So, we tried Montelukast in our study as novel antioxidant against thyroid toxicity induced by Cadmium. Montelukast is one of the leukotriene receptor antagonists. It is the best prescribed cysteinyl leukotriene 1 antagonist as it is safe and high tolerable drug used in asthmatic patients (children and adults) for many years without documented side effects[30]. In the last few years, Montelukast attracted scientists to be assessed as a protective against many toxic agents as it has anti-inflammatory and antioxidant properties[6].

The anti-inflammatory effects of Montelukast in our study appeared in form of absence of both cellular infiltration and congested blood capillaries. As reference to Al-Amran *et al*[31] Montelukast can decrease pro-inflammatory mediator's formation. Also, it can inhibit infiltration and activation of neutrophils[32]. Montelukast can reduce the production of vascular reactive oxygen species "ROS". It gave obvious improvement in acute toxicities of different organs for examples; pituitary gland[6], liver[33], kidney[9], lung[34], and spinal cord[35].

Our results revealed that Montelukast decreased thyroid tissue fibrosis this was supported by the pervious study[34] that proved the protective role of Montelukast against pneumonitis and lungs fibrosis. This is supported by Mallory trichrome results in G (IV).

In our study significant decrease of Caspas-3 immunoexpressing after Montelukast administration indicated that Montelukast could have anti apoptotic property. This has been documented in previous studies. Where, Montelukast reduced apoptosis and Caspase-3 activation in the hippocampal injury induced by transient brain ischemia[8]. Also, it reduced the apoptosis of intestinal mucosa after intestinal ischemia[36]. As well as it exhibited anti-apoptotic effects against the testicular damage induced by doxorubicin[37].

Montelukast has the ability to reduce inflammatory cytokines, tumor necrosis factor α (TNF-α) and interleukin-6. It can ameliorate the changes in levels of the superoxide dismutase, myeloperoxidase and lipid peroxidation in the tissue[38]. Moreover, it has been reported to inhibit the activation of necrosis factor κB (NF-κB) in human monocyte/macrophage cell line[32].

CONCLUSION

Cadmium induced thyroid gland damage via, fibrosis and apoptosis. This damage was not reversed after stopping Cadmium for the same period thus we suggest further studies. Our results proved that Montelukast could protect thyroid gland from toxicity induced by Cadmium. So, we recommend further consideration of the role of Montelukast as a safe protective drug in peoples exposed to Cadmium in their environment.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دور المونتيلوكاست في تخفيف السمية المحدثة بواسطة الكادميوم في نسيج الغدة الدرقية في ذكور الجرذان البيضاء البالغة (دراسة هستولوجية وهستوكيمائية مناعية وكيميائية حيوية)

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المقدمة: يعد الكادميوم واحدا من المعادن الثقيلة السامة ذات التأثير القوي على التلوث البيئي وصحة الإنسان. يستخدم الكادميوم على نطاق واسع في العديد من الصناعات مثل شاشات الإشعاع، البطاريات القابلة لإعادة الشحن وكذلك الصباغات المختلفة. يعتبر المونتيلوكاست من أقوي المواد المضادة للالتهابات. بالاضافة الي أنه أثبت فاعليته كمضاد للأكسدة في الآونة الأخيرة.

الهدف من الدراسة: تقييم تأثير الكادميوم على الغدة الدرقية والدور الوقائي المحتمل للمونتيلوكاست

المواد المستخدمة وطريقة البحث: تم تقسيم أربعون من ذكور الجرذان البالغة إلى أربع مجموعات متساوية. جميعها تتلقى العلاج لمدة ٦ أسابيع. المجموعة الأولي (الضابطة): تلك التي تناولت الماء المقطر. المجموعة الثانية (مجموعة الكادميوم): تلقت العلاج بجرعة ٣مج/كجم. المجموعة الثالثة: (الاستشفاء) أبقت بدون علاج لمدة ٦ اسابيع اخري بعد تناول الكادميوم. المجموعة الرابعة (الكادميوم والمنتيلوكاست): تناولت المونتيلوكاست بجرعة ١٠مج/كجم والكادميوم بجرعة ٣مج/كجم. في نهاية التجربة تم ذبح الجرذان والحصول على عينات الغدة الدرقية للفحص النسيجي والهستوكيمائي المناعي كما تم سحب عينات الدم لقياس نسبة الهرمونات. كما تم تحليل جميع البيانات إحصائيا.

النتائج: أظهر الفحص النسيجي للغدة الدرقية في المجموعة المعالجة بالكادميوم عدم انتظام في شكل الحويصلات كما ظهر تغلظ في نواه تلك الخلايا المبطنة الحويصلة داكنه اللون كذلك ظهرت بعض الخلايا داخل الحويصلات .كما تم الكشف عن قلة المادة الغروانية بداخل الحويصلات. لوحظ تجمع لخلايا الالتهاب حول الأوعية الدموية المحتقنة مع ترسيب ملحوظ لألياف الكولاجين. لوحظت زيادة كبيرة في التفاعل المناعي للكسبيز caspase-٣ في سيتوبلازم الخلايا. كما ان نسبة هرمونات الغدة T٤,T٣ قلت بشكل كبير. ولا تزال كل هذه التغيرات النسيجية موجودة في مجموعة الاستشفاء. علي الجانب الاخر التناول المتزامن للمونتيلوكاست أدي الي حماية واضحة من تلك الآثار الخطرة.

الاستنتاج: يؤدي الكادميوم إلى تلف ملحوظ في أنسجة الغدة الدرقية وتليفها والموت المبرمج لخلاياها ومن الممكن التخفيف من حدة تلك التغييرات إلى حد كبير بواسطة التناول المتزامن للمونتيلوكاست.