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Harmful impact of Perfluorooctanoic acid on the Thyroid Gland of the Male Rat and the Preferential Protective Role of Curcumin

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Abstract

Perfluorinated compounds (PFC) are a large group of chemicals produced for several decades thatare widely used in many industrial and commercial processes. There are growing concerns about Perfluorinated chemicals, due to their global distribution, environmental persistence and bio-accumulative nature. The aim of the present study was to evaluate the harmful changes occurred in the structure of the thyroid gland of adult male albino rats after administration of perfluorooctanoicacid and to clarify the probable protective role of curcumin. **In this context.** Forty male albino rats, weighing 180–200 gm and pathogenically free were utilized**.** Animals subdivided randomly in to four groups, control, curcumin, PFOA group: rat was given PFOA andPFAO+Curcumingroup: rats received PFOA +curcumin. The initial and final body weight were recorded, and the serum samples were collected for estimation thyroid hormones(T3,T4,TSH).Thyroid sections were prepared for histopathological examination and immunohistochemical detection ofINOS and caspase 3. We also assessed oxidative enzymatic and lipid peroxidation biochemical profiles in tissue homogenate. Ultrastructure examination was performed by electron microscope.Morphometrical analysis were done to estimate the diameter and the highest of follicular cells and the % area of Mallory trichomestain, INOS and caspase-3 expression. We observed that Curcuminadministration before and along with the PFOArestored the final body weight, the normal thyroid hormonal levels, the thyroid histopathological and ultrastructuralchanges and significantly reduced INOS and caspase-3 immuno-expression. Additionally, Curcuminadministration rescued of the oxidative enzymatic activity, lipid peroxidation profiles. We concluded that curcumin exhibited perceptible antioxidant and antiapoptotic impacts on the thyroid cytomorphological, immunohistochemicaland biochemical effects induced by PFOA.

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1. INTRODUCTION

Perfluorooctanoic acid (PFOA) is an environmentally persistent Perfluorinated compound used in the manufacture of nonstick cookware and in coatings resistant to soil, water, stain, and grease. Human exposure to PFOA comes primarily from food, drinking water, dust, and air. PFOA is not metabolized in the human body and has a half-life of 2.3-3.4 years **(1).**

An accumulation of PFOA was documented in thyroid cells and a cytotoxic effect was observed after exposure to extremely high concentrations ofthese compounds**.** PFOA can mimic hormones so it is considered an endocrine-disruptingchemical that can target and disturb the hypothalamus–pituitary–thyroid axis.There is also an increasing evidence that PFOA interferes with endocrine homeostasis**(2,3).**

Thyroid hormones are of crucial importance for the normal function of nearly every organ, as they are involved in normal brain development, control of metabolism, and many other substantial aspects of normal adult physiology. Therefore, any changes in the function of thyroid gland or intervention with the ability of thyroid hormone to exert its action leads to adverse effects that may impact development, metabolism or adult physiology **(4).**

Curcumin is a yellow curry powder from turmeric (*Curcuma longa*) that is widely used in Indian and other Asian cuisines.Ithas been used as a therapeutic agent because of its attractive anti-inflammatory, antioxidant, and anticancer properties **(5).**It works through several mechanisms and it has been found to slow or stop enzymes that cause inflammation, like cyclooxygenase-2 and 5-lipooxygenase. Studies have shown that curcumin can modulate over 700 different genes **(6).**

Therefore, this study aimed to assess the possible hormonal, biochemical and structural changes in the thyroid gland of adult male albino rats after administration of perfluorooctanoic acid and to clarify the possible protective role of curcumin.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Perfluorooctanoic acid **(**PFOA) was purchased as a powder from Sigma-Aldrich Cairo, Egypt. It was dissolved in distilled water in a dose of 5 mg/kg BW/ day by gastric gavage for two weeks **(7).**

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Curcumin was purchased asa powder from Sigma-Aldrich Cairo,Egypt. It suspended in corn oil in a dose of 100 mg/kg BW/day by gastric gavage for 2 weeks **(8).**

2.2. Animals

Forty male albino rats, weighing 150–200 gm and pathogenically free were obtained fromZagazig scientific and medical research center (ZSMRC), Faculty of Medicine, Zagazig university. Rats were acclimatized for 15 days before starting the experimental procedures and housed in an animal care facility under room temperature (25 ± 1 °C) with 12-h light/dark cycles with free access to food and water. All the rats of the experiment were handled in accordance with the standard guide for the care and use of the Institutional Animal Care and Use Committee, Zagazig University ZU-IACUCwith a reference number(Zu-IACUC/3/F/99/2020).

2.3. Experimental design

Animals divided randomly in to four groups, each group consists of ten rats:

Control group:ratsreceived water and food for 2 weeks with no intervention.

Curcumingroup:eachrat was given curcuminsuspended in corn oil in a dose of 100 mg/kg BW/day for 2 weeks by gastric gavage.

Perfluorooctanoic acid (PFOA)group:each rat was given PFOAdissolved in distilled waterin dose of 5 mg/kg BW/ day by gastric gavage for two weeks.

PFOA +curcumingroup:rats received curcuminby gastric gavage in a dose of 100 mg/kg/day for one weeks before receiving PFOA and it was continued concomitantly with PFOA for another 2 weeks.

At the end of the experimental period for each group, On the following day morning, the rats were weighted and anesthetized by peritoneal injection of thiopental Na 50 mg\kg for the further processes:

2.4. Initial and final body weight assessment:

The weight of the ratsfromeach groupwere recorded at the beginning of the experiment and just before the time of sacrifice.

2.5. Serological study and hormonal analysis:

The blood was collected from the orbital sinus of rats under anesthesia. Then, clotted at room temperature then sera were separated by 20 minutes centrifugation at 3000 rpm and stored at -18 to -20°.The serum levels of TSH (ELISA kit catalog number: DEIA2211), free T3 (ELISA kit

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catalog number: EK7011), and T4 (ELISA kit catalog number: EK7013) were measured by ELISA in all animal groups at the time of sacrifice.

2.6. Tissue gathering

After blood collection, the neck was dissected and the thyroid gland from each rat was removed carefully for further processing.Fivethyroid specimens from5 rats of each group were immersed in formalin. The other five thyroid specimen from ethe remaining five rats from each group were divide into 2 portions. One portionpreserved at -80 °C for forthcoming homogenization. The second portion were quickly fixed in 2.5% glutaraldehyde buffer for preparation of semithinand ultrathin sections.

2.7. Oxidative stress and antioxidant biomarkers in lung tissues homogenates

The thyroid tissue specimens were minced and homogenized, distinctly, in ice-cold sodium and, potassium phosphate buffer (pH 8) at a concentration of 15% (w/v) in Scientific Research Centre, Faculty of Medicine, Zagazig University. The homogenate was centrifuged at 110000 × g for 20 min at 4 °C, and the resultantsupernatant was used.Estimation of lipid peroxidation by determination of malondialdehyde (MDA) content according to **Draper and Hadley (9).**Estimation of superoxide dismutase (SOD) and catalase (CAT) was determined according to**Sun.et al. (10)** and **Aebi., (11)** respectively.

2.8. Histological studies

2.8.1. Light microscopic study

2.8.1.1. Hematoxylin and Eosin (H&E) study

The thyroid gland was immersed immediately after excision in 10% formalin solution to be processed and embedded in paraffin blocks. According to **Bancroft and Gamble (12)** the thyroid tissue samples were fixed for 24h in 10% buffered formalin-saline (pH 7.2).Dehydrated by ascending grades of alcohols (50%, 70%, 90%, 95% and 100%) then cleaned in xylene. These tissues were embedded in paraffin block then 5µ sections of the tissues were prepared by using a microtome and stained by Hematoxylin-Eosin stain (H&E).

2.8.1.2. Mallory trichrome stain study

We utilized the paraffin blocks of formalin-fixed thyroid specimens for staining the collagen fibers by Mallory's Trichrome stainaccording to **Suvarna et al., (13).** The collagen fibers appear blue in color.

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2.8.1.3. Immunohistochemical study:

Immunohistochemistry was used for localizingtheinducible nitric oxide synthase (iNOS) protein (oxidative damage marker)and the Cysteine aspartic acid specific protease-3 (caspase- 3)protein (apoptosis marker) in paraffin sections according to **Blytheet al. (14).**

Paraffin sections were cut at 5μm thickness on positive-charged glass slides. They were incubated in 42°C oven for 24 hours. Sections were deparaffinized in xylene for one hour.They were hydrated in descending grades of alcohol, and then rinsed in distilled water. They were incubated in hydrogen peroxide block for five minutes. Sections were washed two times in PBS for five minutes each. Ultra V block was applied for ten minutes to block nonspecific background staining. Two drops of iNOS antibody attained from (rat monoclonal antibody, 1:500 dilution, Transduction Laboratories, San Diego, California, USA) and caspase-3 antibody attained from (a rabbit monoclonal antibody of IgG type antibody, dilution 1:100, delivered from Lab Vision Laboratories) were applied to each section, then they were incubated for hour and half at room temperature. Sections were washed twice in PBS, five minutes each. Two drops of secondary antibody were applied to each section and then incubated for twenty minutes at room temperature. Sections were washed three times in PBS, five minutes each. Two drops of DAB solution were applied to all sections, which were incubated for ten minutes. Sections were washed in distilled water and then counterstained with Mayer's hematoxylin for two minutes. Sections were washed in tap water, then dehydrated in absolute alcohol, cleared in xylene and mounted by DPX.

2.8.2. Electron microscopic study:

Preparation of thyroid tissue specimens for transmission electron microscope according to **Woods and Stirling (15).**The thyroid tissue was excised from each animal and cut into small pieces then fixed as soon as possible in cold bufferedformolgluteraldehyde (PH 7.2) for 24 hours.After2 rinses in phosphate buffer (PH 7.2), half an hour each, the tissues were post fixed in 1% osmic acid for one hour. The tissues were then washed twice in phosphate buffer, half an hour each.Dehydration was done in ascending grades of alcohol. The tissues were cleared in propylene oxide for twenty minutes.Finally, specimens were embedded in gelatin capsules filled with fresh Epon. The capsules were kept in oven 70°C for 48 hours to allow polymerization.Ultrathin sections were cut at 60 nm using a glass knife; they were then picked up on copper grids.The grids were then stained with uranyl acetate for twenty minutes, followed by lead citrate for ten minutes. They were then rapidly washed in distilled water to remove

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excess stain and preserved in labeled capsules.The ultrathin sections were examined with transmission electron microscope (TEM JEOL-JEM-1010 at 80 KV) and electron micrographs were taken from selected areas.

2.9.Histomorphometrical analysis

Perceptive five different non-overlapping H&E and immune-stained fields were utilized from five different sections of the thyroid tissue of each animal from each group.Weused ImageJ software analyzer computer system (Wayne Rasband, NIH, Bethesda, Maryland, USA) to measure the following.

1-Area percentage ofbothcaspase-3 and INOSimmuno-positive reaction along with the area percentage of Mallory Trichome-stained collagen fibers (magnificationx400).

2-The follicular cell height and follicular diameter in H&E-stained sections (magnificationx400).

2.10. Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 21 (SPSS Inc., Chicago, I11inois, USA). Quantitative data were expressed as mean± standard deviation (SD). Differences between mean values of experimental groups were tested with analysis of variance (ANOVA). Tukey's multiple comparison test was carried out as post hoc test of ANOVA. The results were considered statistically significant when the P value <0.05. Different stages of significance were considered P-value <0.05 was considered significant. P-value <0.001 was considered as highly significant. P-value > 0.05 was considered insignificant.

3. RESULTS

3.1. Body weight measurement

Table 1shown the Mean±SE of body weight (gm) among the different studied groups. Compared to the control group, PFOA rats revealed a significant decrease ($p<0.005$) in the values of the final body weights (170.87± 6.18 gm versus 230.15 ± 4.51 gm). In contrast, there was a significant increase in the values of the final bodyweightof PFOA+ Curcumin treated rats(205.47 ± 5.96) compared to the PFOA treated rats. Meanwhile. An insignificant difference in the final body weightbetweencontrol and curcumin groups was revealed.

3.2. Assessment of hormonal levels

The current study showed a significant decrease in the level of T3 and T4 in PFOA treated group compared to control group (21.57± 1.08 ng/ml, 1.27±0.12 µg/dl versus 72.85 ± 8.51 ng/ml,4.62 ±0.48 µg/dl)respectively. Incontrary, a significant increase in the level of T3 and T4 serum levels

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in PFOA treated with Curcumin group (57.22 ± 4.36 ng/ml) compared with PFOA group was revealed. In addition, there was no significant difference between control and Curcumin group **(Figs 1A, B)**.Concerning the level of TSH, a significant increase in PFOA group (12.27 ± 0.79 IU/ml) compared to control and Curcumin groups were revelated.However, in PFOA- Curcumin treated group, a significant decrease of TSH level (2.75 ± 0.41 IU/ml) compared with PFOA group was exhibited. There was no significant difference between control and Curcumin group **(Fig 1C)**.

3.3. Assessment of oxidative stress and antioxidant biomarkers in thyroid tissues homogenates

The obtained results showed a significant decrease in the level of tissue SOD in PFOA group compared to control group which were (7.13 ± 0.83 U/g versus 16.12 ± 2.43 U/g). There was a significant increase in the level of SOD in PFOA treated with Curcumin group (12.07 ± 1.04 U/g) compared with PFOA group. .Regarding the level of tissue CAT, there was a significant decrease in the level of CAT in PFOA group compared to control group which were (41.72± 3.39 U/g versus 80.72 ± 6.14 U/g). There was a significant increase in the level of tissue CAT in PFOA treated with Curcumin group (66.93 ± 4.17 U/g) compared with PFOA group. In addition, there was no significant difference between control and Curcumin group in the level of tissue CAT (Table 2 and Figure 6). Moreover, the level of MDA showed a significant increase in PFOA group compared to control group which were (75.81 ± 5.28 nmol/g versus 25.43 ± 2.58 nmol/g) **(Figs2A,B).** However, there was a significant decrease in the level of MDA in PFOA treated with Curcumin group (34.24 ± 3.44 nmol/g) compared with PFOA group. In addition, there was no significant difference between control and Curcumin group in the level of tissue SOD,CAT,MDA **(Fig2C).**

3.4. Histological results:

The control group and Curcumin treated group showed no significant differences between them in body weight and biochemical changes. So, they represented in our histological results as one group (control group).

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3.4.1. Light microscopic examination

3.4.1.1. Hematoxylin and Eosin stain results

The attainable histological resultsof H&E-stainedthyroidtissue of control rats showed a normal typical thyroid follicle of different sizes which filled with homogenous acidophilic colloid. The follicles are lined by flattened to cuboidal follicular cells with oval to rounded nuclei. Interfollicular cells and blood capillary are shown normally between the follicles**(Figure 3A)**. However, thyroid gland section in the group of rates treated with PFOA showed loss of architecture and irregular shape of thyroid follicles. The acini showed irregular shape and size with microcystic follicles and scanty amount of colloid. Follicular damage and absent of colloid can be noticed. Most of the follicular cells appear swollen and vacuolated. Interfollicular congested blood capillary is observed. In addition, there was extensive vacuolated colloid filling the follicular lumen in the thyroid sections of PFOA group **(Figure 3B, C, D)**. Thyroid gland treated with PFOA+ Curcumin showed preserved most of normal architecture of thyroid glands. Few follicles still revealed multiple layers of follicular cells with obliterated lumen. Other follicles revealed few colloids with few congested blood capillaries were shown between the follicles **(Figure 3E).** Concerning the morphometric parameter of H&E-stained section among the studied groups, there was a significant decrease in the thyroid follicle diameters and the height of thyroid follicular cells in PFOA group compared to control group e (**4.08±5.92**µm versus **8.41 ± 2.17and** (**3.92± 4.81 versus 7.13± 3.21**) respectively. However, there was a significant increase in the diameter of the follicles & the height of thyroid follicular cells in PFOA + Curcumin group (**6.73±2.52** µm) and (**6.02±4.34**) respectively compared with PFOA group. In addition, there was no significant difference between control and Curcumin group**(Figure 4A,B).**

3.4.1.2. Toluidine blue stain results

Examination of Toluidine blue stained sections of thyroid gland from the different studied groups were shown in **Figure (5).** Control group exhibited apparently normal thyroid follicles lined with one layer of cubical follicular cells with vesicular spherical. The follicles were filled with homogenous colloid of variable densities and surrounded by blood capillaries.Parafollicular cells were also seen that not reaching the lumen of the follicles with vesicular nuclei **(Fig.5A).** Thyroid gland of PFAO treated group demonstrated disrupted and fused thyroid follicles with desquamated epithelial cells in their lumen. Dark nuclei and vacuolated cytoplasm are seen in most of follicular cells in addition to, some flattened follicular cells with flat dark nuclei and parafollicular cell with vacuolated cytoplasm. Empty follicular cells from colloid, vacuolated colloidand congested blood capillaries were also seen between the follicles**(Figs.5B, C).** PFAO+ Curcumin group revealed nearly normal thyroid follicles filled with

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homogenous colloid and lined with a single layer of follicular cells with vesicular spherical nuclei and with parafollicular cells. The follicles were surrounded by blood capillaries. Few of the lining follicular cells exhibited dark irregular nuclei and vacuolated cytoplasm**(Fig.5D).**

3.4.1.3. Mallory stain results

Examination of Mallory trichrome stained thyroid gland sections from the different studied groups were shown in **Figure (6).**The control group revealed minimal collagen fibers in the interfollicular spaces **(Fig. 6A)**. In the PFOA treated group abundant collagen fibers are observed between the follicles **(Fig. 6B)**. While in the PFOA + Curcumin treated group a restoration of the normal collagen fibers distribution was observed **(Fig. 6C).**Statistical analysis by ANOVA & Turkey-Kramar post hoc test of the area % of collagen fibers in Mallory trichrome stained sections showed a significant increase ($P<0.05$) in the mean values of area % of collagen fibers in the PFOA treated group as compared to the control group. In the PFOA + Curcumin treated group the mean values showed a significant decrease ($P<0.05$) in relation to the PFOA treated group. These mean values were still significantly ($P<0.05$) differentfrom the control group **(Fig. 6D).**

3.4.1.4. Immunohistochemical results of INOs and Caspase-3

Examination of caspase immuno-stained thyroid gland sections from the different studied group were shown in **Figures (7, 8).**The control group revealed weak immuno-expression of caspase-3 in follicular cells detected by weak & localized brown cytoplasmic staining of (**Figs. 7A,8A)**respectively. In the PFOA treated group strong immuno-expression of caspase was observed in follicular cells as detected by strong & extensive brown cytoplasmic staining (**Figs. 7B, 8B)**. While in the PFOA + Curcumin treated group a moderate immune-expression of caspase in follicular cells was seen **(Figs. 7C, 8C).**Statistical analysis by ANOVA & Turkey-Kramar post hoc test of the % area of INOS and caspase -3 immunostaining showed a significant increase ($P<0.05$) in the mean values ofthe % area of INOS and caspase-3 immunostaining in the PFOA treated group as compared to the control group. In the PFOA + Curcumin treated group the mean values of the % areaof both INOs and caspase-3 showed a significant decrease ($P<0.05$) in relation to the PFOA treated group. These mean values were still significantly ($P<0.05$) different from the control group **(Figs. 7D, 8D).**

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3.4.2. Electron microscopic examination

Examination of ultrastructure of control thyroid gland revealed that some follicular cells were inactive and low in height or flatwith prominent nucleolus and clumps of heterochromatin. Oval and irregular euchromatic nuclei with can be noticed. Their cytoplasm shows cisternae of rough endoplasmic reticulum dense lysosomal granules and scattered mitochondria. Their apical border shows a moderate or a small number of microvilli projecting into the lumen. Blood capillary can be seen beneath the thin basal lamina. Para follicular cell with euchromatic nucleus and scattered mitochondriawas seen. Follicular cell with euchromatic nucleus, apical lysosome, rough endoplasmic reticulum, lysosomes and scattered mitochondria. The apical border shows a moderate or a small number of microvilli projecting into the lumen**(Figs. 9A, B).**

In contrast, thyroid gland from PFOA group exhibited follicular cells with euchromatic, heterochromatic, and apoptotic nuclei. Numerous dilated irregular cisternae of rough endoplasmic reticulum could be seen. Extensive electron lucent areas and vacuoles could be noticed.Their apical borders showed an extensive number of aggregated microvilli bulging into the lumenpolymorphic dense lysosomal granules were seen in the cytoplasm of follicular cells. Extensive apical lysosome was noticed in an apoptotic follicular cell. Scattered mitochondria were observed in the cytoplasm of the follicular cells. A blood capillary was seen indenting the epithelial lining of the follicle**. (Figs. 9 C, D, E).**

However, thyroid gland from PFOA +curcumin group exhibited thyroid follicular cell with euchromaticnucleus,normal, and slightly dilated rough endoplasmic reticulum, few lysosomes & scattered mitochondria. The apical border shows a moderate or a small number of microvilli projecting into the lumen**(Fig. 9F).**

DISCUSSION:

Thyroid gland hormones have a major role in metabolism, growth and development throughout life. Hypothyroidism is one of the most common endocrine diseases mainly caused by abnormalities in the thyroid gland which led to decrease in production and secretion of the thyroid hormones. Oxidative cellular damage and oxidative stress with free radicals' generation are hallmarks of thyroid dysfunction **(16).**

Perfluorooctanoic corrosive (PFOA) are industrious in the climate because of their solid carbon-fluorine (C-F) bonds **(17).** PFOA have been related with a few unfavorable impacts like

formative poisonousness, liver harmfulness, endocrine interruption, and immunotoxicity in different test models.

The support of thyroid chemical equilibrium is basic to ordinary development and advancement of organic entities.PFOA are known as thyroid chemical disruptors **(3).**

The medical uses of natural herbs are gradually increasing due to their antioxidant, anti-inflammatory, anti-bacterial and anti-cancer properties **(18).** The yellow pigment curcumin is one of natural polyphenols which is extracted from turmeric **(19).** In several studies' curcumin was used as hypolipidemic, anti-infectious, anticancer, anti-inflammatory due to antioxidant properties **(20).**

Higher incidences of hypothyroidism were reported in female **(21),** however, male rats were recruited in this study as males are more vulnerable to occupational exposure and to avoid the possible interfering effects of female hormones.

Therefore, the present work was designed to explore the role of Curcumin in prevention of the possible biochemical, histopathological and immuno-histochemical, changes in the thyroid gland after administration of perfluorooctanoic acid (PFOA) in adult male albino rats.

In the current study,a significant decrease in the mean values of the final body weights in PFOA group was recorded as compared to control group. Additionally

The biochemical profile resultsrevealeda significant decrease in the level of T3 & T4 and a significantcompensatoryincrease of TSH in PFOA group compared to their corresponding values in the control group. These results agree with **Mohamed and Mogeda, (22)** who stated a significant decrease in serum T3 and T4 levels and a significant increase in TSH level. This is explained by **Mescher (23)** who stated that elevated TSH stimulates the follicles to synthesize and secrete more hormones into the circulation in an attempt to compensate for decreased thyroid hormones similar to what occurred with chronic iodine deficiency.

in PFOA treated with Curcumin group, a significant increase in the level of T3 & T4 and a significant decrease in the level ofTSH compared with PFOA group was recorded. These results agree with **Mohamed and Mogeda, (22)** who reportedtheimprovement of the thyroid hormonal profile where the mean values of T3 and T4 were significantly increased and the mean value of TSH was significantly decreased in hypothyroid group treated with curcumin compared to hypothyroid group. In addition, we recorded non-significant difference in the mean values of T3,

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T4& TSH between the control & PFOA+ curcumin group. This is in accordance with **Aboul-Fotouh et al. (8).**

Similarly, **Aboul-Fotouh et al., (8)** reported that the mean values of T3 and T4 were significantly increased and the mean value of TSH was significantly decreased in curcumin treated group compared to hypothyroidism groups and recovery group but still with significant differences versus control group. These results suggested better improvement in the thyroid function with Curcumin.

Moreover, a significant decrease in the mean values of SOD and CAT levels in the homogenate of the thyroid tissues in PFOA group compared to control group was observed. These results agree with **Nasra (24) & Mohamed and Mogeda (22)** who recorded a similar observation in the hypothyroid rat's group that exhibited a significant decrease in SOD, GSH, CAT, GST and GPx activities.

In contrast, the mean values of SODandCAT thyroid tissue levels showed a significant increase in PFOA + Curcumin treated group as compared with PFOA group. This is in accordance with **Nisar et al. (21)** who recorded that curcumin could reduce or prevent oxidative damage caused by toxicants and oxidative materials.**Additionally, Sajadian et al., (25)** reported that the normal level of these parameters except GSH level which was significantly decreased in the hypothyroid group compared with control groups.

Moreover, the mean values of MDA thyroid tissue levels showed a significant increase in the in PFOA group compared to control group. these results in agreement with **Mohamed and Mogeda (22) &**who stated that hypothyroidism induced a significant increase in lipid peroxidation.

However, upon administration of Curcumin, a significant decrease in the level of MDA as compared to PFOA group was reported. In a line with our result **Jagetia et al. (26)**reportedthat Curcuminhas the ability to reduce the lipid peroxidation & increase the activity of the antioxidant enzymes thus improving the antioxidant status ..

Alterations in the thyroid gland function in the PFAO group were established by the histological examination of the thyroid tissue. The thyroid tissues exhibited a various of different morphological features: loss of normal architecture and irregular shape of thyroid follicles. The acini showed irregular shape and size with microcystic follicles with scanty amount of colloid were seen. Follicular damage and absent of colloid were also noticed. Fibrous tissue appears to

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be increased in the stroma between thyroid follicles. The majority of the follicular cells likes as swollen and vacuolated follicles. While other follicles are lined by multiple layers of follicular cells with obliterated lumen and show loss of their nuclei. Interfollicular congested blood capillary is observed. In addition, there was marked and vacuolated colloid filling the follicular lumen in the thyroid sections of PFOA group.

In contrary to control and Curcumin groups that revealednormal histological architecture. Typical thyroid follicles of different sizes which loaded up with homogenous acidophilic colloid. The follicles were lined by smoothed to cuboidal follicular cells with oval to adjusted cores. Interfollicular cells and blood capillary are shown normally between the follicles. This pattern was previously described asnormalhistological features of rat thyroid and not in humans **(18)**

This is in agreement with **Abdul-Hamid and Moustafa (27)** who reported that cytoplasmic vacuolationsin most of the follicular cells and discontinuous basement membranes seen in stained sections and stated that thiscould be attributed to ROS induced cellular membranes insults, which ultimately generate self-sustaining lipid peroxidation .

Mahmood et al. (28) explained the follicles with scanty colloid & vacuolated colloid that observed in the PFOA group due to the response of the follicular cells totheelevation of the TSH with more rapid and continuous removal of thyroglobulin from the lumen of follicles in an attempt to compensate for decreased thyroid hormones in the blood leading to more vacuolations in colloid. But endocytosis of colloid usually proceeds at a rate greater than synthesis resulting in progressive depletion of colloid.

Stratification of the epithelium that observed in this study e epithelial stratifi cation of the follicular lining, observed in this study, should be a compensatory mechanism and might be contributed to elevation in the TSH level. TSH is accountable for follicular cells proliferative activity and release of growth factor that modifyfolliculogenisis**(Trabelsi et al. 2001)**.

In our study, thyroid gland treated with PFOA+ curcumin showed an improvement in the follicles of different size, most of them filled with homogenous acidophilic colloid. The follicles are lined by flattened to cuboidal follicular cells with oval to rounded nuclei. Low congested blood capillary is shown between the follicles. Few follicles still reveal multiple layers of follicular cells with obliterated lumen. Other follicles reveal few colloids. These suggest marked histological improvement induced by curcumin .

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Morphometric & statistical assessment of the thyroid follicle diameter among the studied groups revealed a significant decrease in the thyroid follicle diameters in PFOA group compared to control group. However, there was a significant increase in PFOA + Curcumin group compared to PFOA group.This finding might be explained by**El Bakry and Tawfik(30)** who reported even with the trial of synthesis by cell and endocytosis of the colloid the net result is decreased level of T3 and T4 versus control. In the same consequence, multiple thyroid follicles in the same group appeared irregular in shapes with disrupted basal laminae and wide inter follicular spaces and degenerative changes and collapse of some follicles following colloid depletion.
Moreover, morphometric & statistical assessment of the thyroid follicle height among the studied groups revealed decreased in the height of cells (from cuboid to squamous) that reflected the decreased activity of the follicular cells. . However, administration of Curcuminincreases the hight of follicular cells as reported by previous studies **(6).**

Also, **Patil and Dhurvey (31)** who revealed a continuation of stress onthyroidgland became exhausted as confirmed in the same group by the presence of some follicles lined with flat cells with flat nuclei indicating hypoactivity. Previous investigators correlated follicular epithelium height to the functional state of the thyroid gland.

Our morphometric results are agree witha study of **Aboul-Fotouh et al.(8)** who substantiated by morphometric analysis of follicular diameter, height of follicular cells and area percent of colloid which revealed no significant differences as compared to control group. These findings were consistent with the assumption that curcumin activity depends on the functional condition of the gland **(32) .**

In the present study Mallory's trichrome stained thyroid gland sections revealed minimal collagen fibers in the interfollicular spaces were observed in the stained sections from rats of control &curcumin groups. While in the PFOA group excess collagen fibers were observed in the interfollicular spaces. Fibrotic changes have been recorded in association with PFAO in lungs of exposed workers **(33)**

In addition, thyroid gland in PFOA + curcumin group exhibited minimal collagen fibers .Moreover,the mean values of area % of collagen fibers in PFOA + Curcumin treated group showed a significant decrease ($P<0.05$) in relation to the PFOA treated group. These mean values were still significantly ($P<0.05$) different from the control group. Curcumin has been reported to have anti fibrotic action by several studies **(34-36)**

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Regarding caspase-3 immunostaining of the thyroid gland, in control &curcumin groups, aweakimmuno-expression of caspase in follicular cells was observed. Thyroid gland of rats received curcumin were identify the normal immunoreactivity of the caspase-3 immunostaining of follicular cells of thyroid gland without any reactive changes.

However, in the PFOA group strong immuno-expression of caspase-3 was seen in follicular cells that revealed a significant increment of the % area of immuno-positive reaction..**According to Morshed & Davies(37)**, antioxidant & reactive oxygen species are both low in thyroid tissue. The unopposed increases in reactive oxygen species resultant oxidant antioxidant imbalance can induce sever inflammation and accordingly apoptosis may occur.
In contrast, up on administration of Curcumin with the PFAO, We observed a weak immuno-expression of caspase-3 in follicular cells. These mean values were still significantly ($P<0.05$) different from the control group. Curcumin was found to express antiapoptotic activity and to modulate the pro-apoptotic and anti-apoptotic signaling pathways **(38-40)**.

Concerning immunohistochemical expression of INOS, in control and curcumingroupsshowed normal immunohistochemical expression of INOS in the thyroid gland. Meanwhile, thyroid gland treated with PFOA showed upregulation of INOS immuno-expression that exhibited a significant increment in and the area percent of immuno-positive reaction.A positive reaction in nucleus of follicular cells of thyroid gland can be observed.PFOA toxicity was found to be associated with oxidative stress & increased levels of inos&reactive oxygen species **(41,42)**
We observed a moderate immuno-expression of INOS in follicular cells in the PFOA + Curcumin group. In addition, the mean values of the optical density for INOS in the PFOA + Curcumin group showed a significant decrease ($P<0.05$) as compared to PFOA group. These mean values were still significantly ($P<0.05$) different from the control group. **Ben et al. (43)** recorded that Curcumin could degrade INOS and suppresses its enzyme activity.

The Ultra structured results confirmed the results obtained by the light microscopic examination. The follicular cells nuclei were euchromatic, heterochromatic, and apoptotic. Numerous dilated irregular cisternae of rough endoplasmic reticulum and polymorphic dense lysosomal granules could be seen. Their apical borders show an extensive number of aggregated microvilli. Extensive electron lucent areas and vacuoles could be noticed. Some follicles were lined with low prismatic, cuboidal, or flattened epithelium with apparent decrease in epithelial height. That

was attributed to hypoactivity of the thyroid gland **(44)**. Comparable outcomes noticed already as disturbed basal laminae of the follicles, relapsed cores, and upset cell organelles**(28).**

The increased lysosomal content of the follicular cells in PFAO group could basically be reflect the enhanced cellular secretory activity induced by high levels of circulating TSH in response to decrease the formation of T3and **T4 (45)** .

Administration of curcumin in PFAO group improved the structural changes. Curcumin produced a significant enhancement in the thyroid histology, the follicular lumina confined colloid, and the follicular cells looked normally. Curcumin can moderate the synthesis of multiple inflammatory mediators, inflammatory cytokines as interleukin-6, and inflammatory enzymes as cyclooxygenase-2, thus it acts as a potent anti-inflammatory activity **(46).**

Pre-treatment with Curcuminmainly attenuated the PFAO associated thyroid histopathologicaldegenerationwith a little residual comparable change in the tissues as reported by **Abdelaleem, et al. (6).**

These findings could be attributed to the protective effect of curcumin due to its antioxidant and anti-inflammatory properties leading to regression of histological alterations caused by Perfluorooctanoicacid(PFOA) much more rapidly and more effectively.

CONCLUSION:

Collectively, in order of our results, Curcumin illuminated an effective and consistent protective agent against thyroid dysfunction, histopathological and biochemical changes in the thyroid tissues in the hypothyroidism induced by PFAO.More Studies are additionally expected to completely assess the efficacy and the security of reformulated curcumin, the underlying analogs of curcumin, as well as the mix of curcumin with existing treatments that may hold extraordinary guarantee later on.

No Conflict of interest.

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Table (1): The body weight of different studied groups:

Parameters	Control group	Curcumin group	PFOA group	PFOA + Curcumin group
B.W (gm)	230.15 ± 4.51	240.09 ± 5.02	170.87±6.18 *	205.47 ± 5.96 #

Values are expressed as mean ± SE, n=10. *: Significantly difference from control at p<0.05. #: Significantly difference from PFOA group at p<0.05. ANOVA followed by Tukey-Kramer post ANOVA.

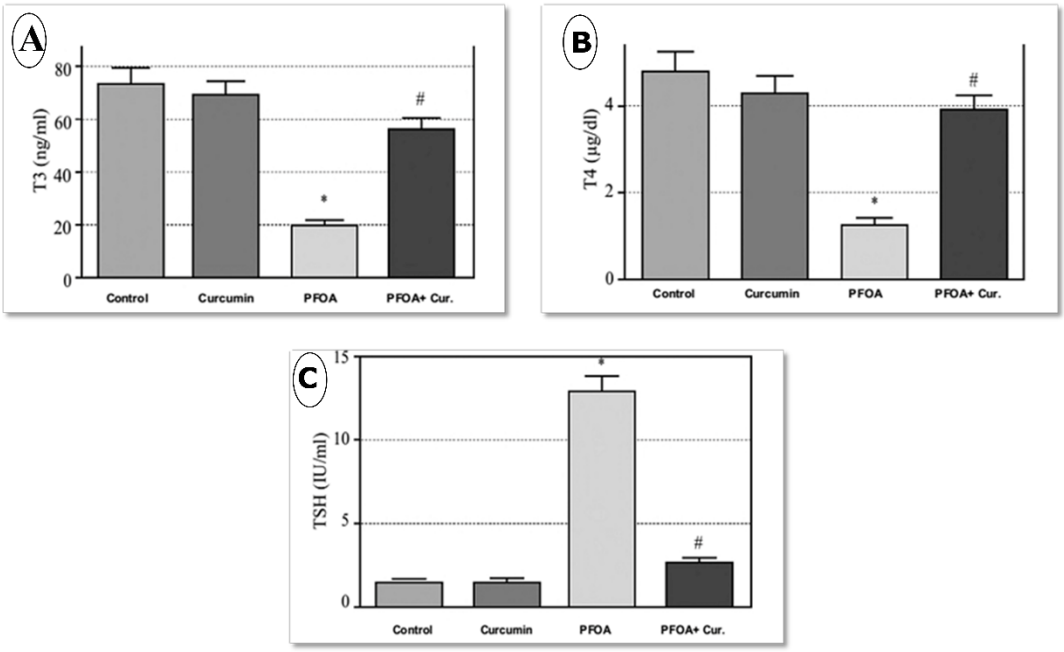

Figure 1. Bar charts showing the changes in the levels of(**A)**T3**, (B)**T4 and **(C)**TSH in different studied groups, control group, PFOA group and PFOA +Curcumin

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group.Statical analysis were performed using ANOVA &Turkey-Kramar post hoc testNumber of rats in each group (n=5)* Significant difference compared to the control group, P < 0.05.#Significant difference compared to the PFOA group, P < 0.05.

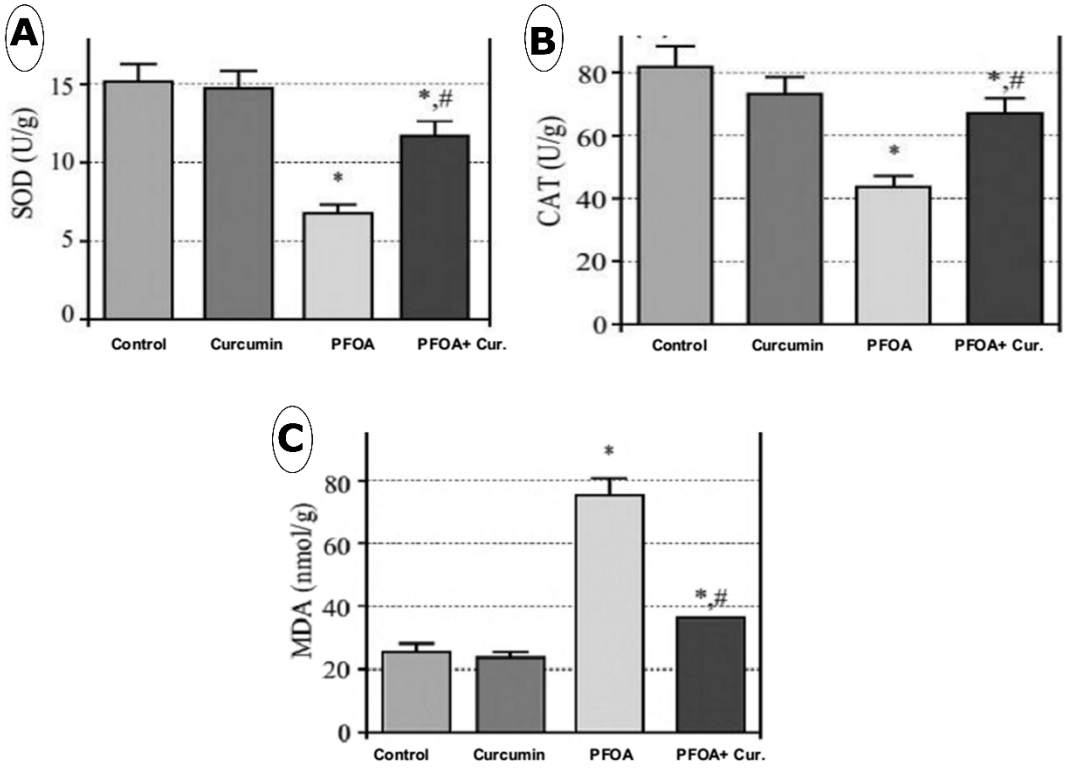

Figure 2. Bar charts showing the changes in the activities of **(A)** SOD, **(B)** CAT and **(C)** MDA in different studied groups, control, PFOAgroupandPFOA +Curcumingroups.Statical analysis were performed using ANOVA &Turkey-Kramar post hoc test.Number of rats in each group (n=5). * Significant difference compared to the control group, P < 0.05.#Significant difference compared to the PFOA group, P < 0.05.

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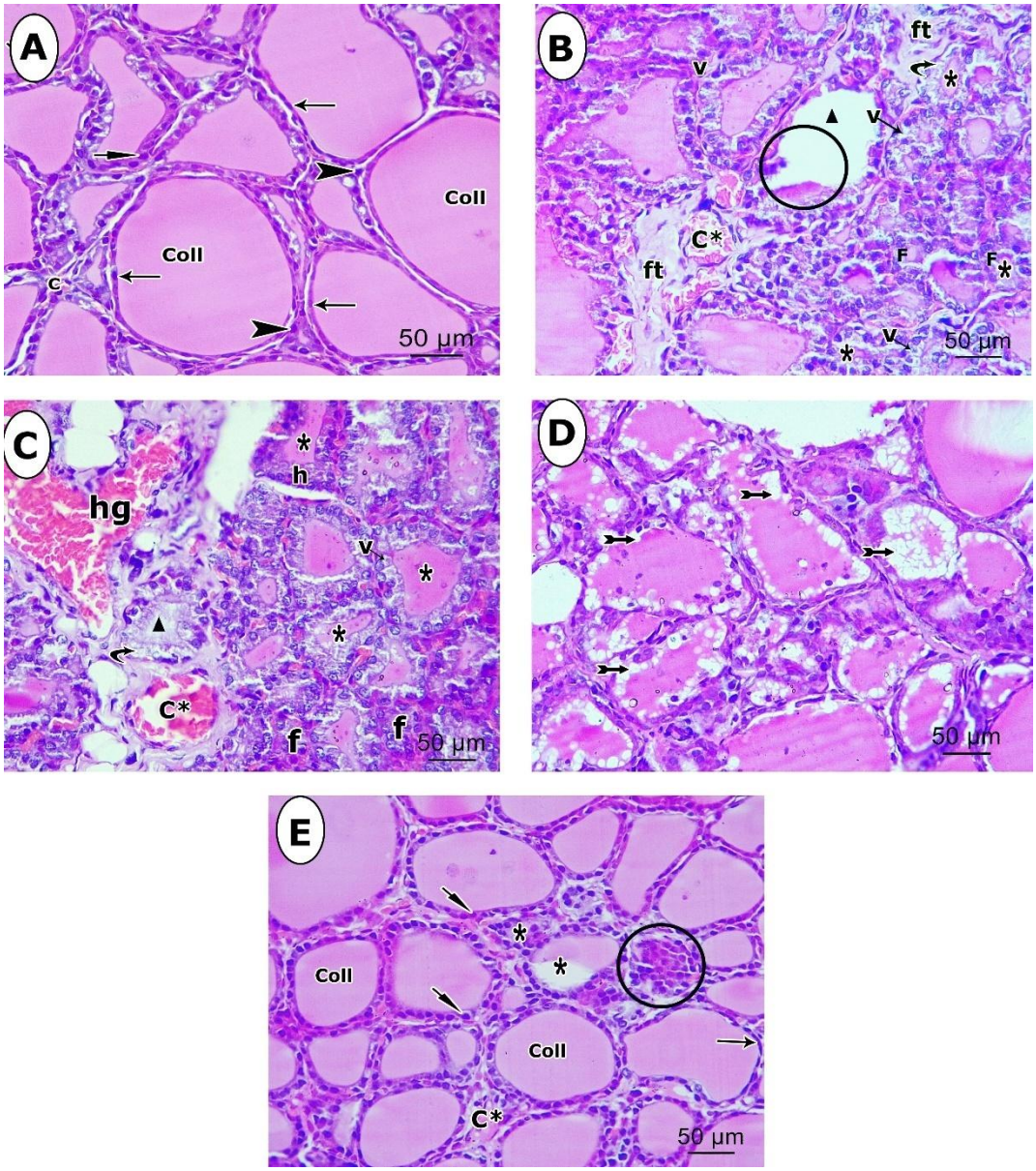

Figure 3. Photomicrographs of H&E-stained sections of thyroid gland from the different studied groups. **(A)**controlgroupshowingfollicles of different sizes filled with homogenous acidophilic colloid (Coll). The follicles are lined by flattened to cuboidal follicular cells with oval (arrow) to rounded (short arrow) nuclei. Interfollicular cells (arrowhead) and blood capillary (C) are shown between the follicles.**(B)**PFOA treated group showing irregular shape and size with microcystic follicles (F) and scanty amount of colloid (*). Follicular damage (circle) and absent of colloid (▲) can be noticed. Fibrous tissue (ft) increased in the stroma. Most of the follicular cells appear swollen and vacuolated (V), while others show loss of their nuclei (curved arrow). Interfollicular congested blood capillary (C*) is observed.**(B)**Another photomicrograph from PFAO treated ratsshowing interstitial tissues areas withhemorrhage (hg). Most of the follicular cells appear swollen and vacuolated (V) with few colloid (*) while others show loss of their nuclei (curved arrow) with absence of colloid (▲). The other follicle is lined by multiple layers of follicular cells with obliterated lumen (f). Sever dilated and congested blood capillary (C*) is observed.**(C)**Another photomicrograph from PFAO treated ratsreveals extensive vacuolated colloid (bifid arrow) filling the follicular lumen.**(E)**PFOA +Curcuminrats

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showing follicles of different size, most of them filled with homogenous acidophilic colloid (Coll). The follicles are lined by flattened to cuboidal follicular cells with oval (arrow) to rounded (short arrow) nuclei. Interfollicular cells (arrowhead) and congested blood capillary (C*) are shown between the follicles. Few follicles still reveal multiple layers of follicular cells with obliterated lumen (circle). Other follicles reveal few colloid (*).**H&E. Scale bar= 50 µm, x400.**

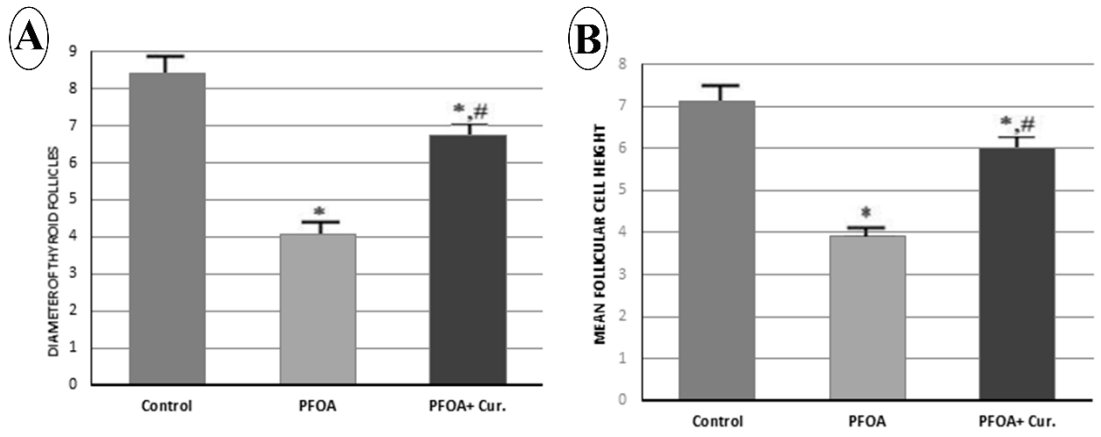

Figure 4. Bar charts illustrating the statistical analysis of the mean values ±SE. of the changes in (**A)** the diameter of follicular cells **(B)**the highest of the follicular cells of the thyroid gland in all different studiedgroupsusing ANOVA &Turkey-Kramar post hoc test. Number of rats in each group (n=5). * Significantly difference from control at p<0.05. #Significantly difference from PFOA group at p<0.

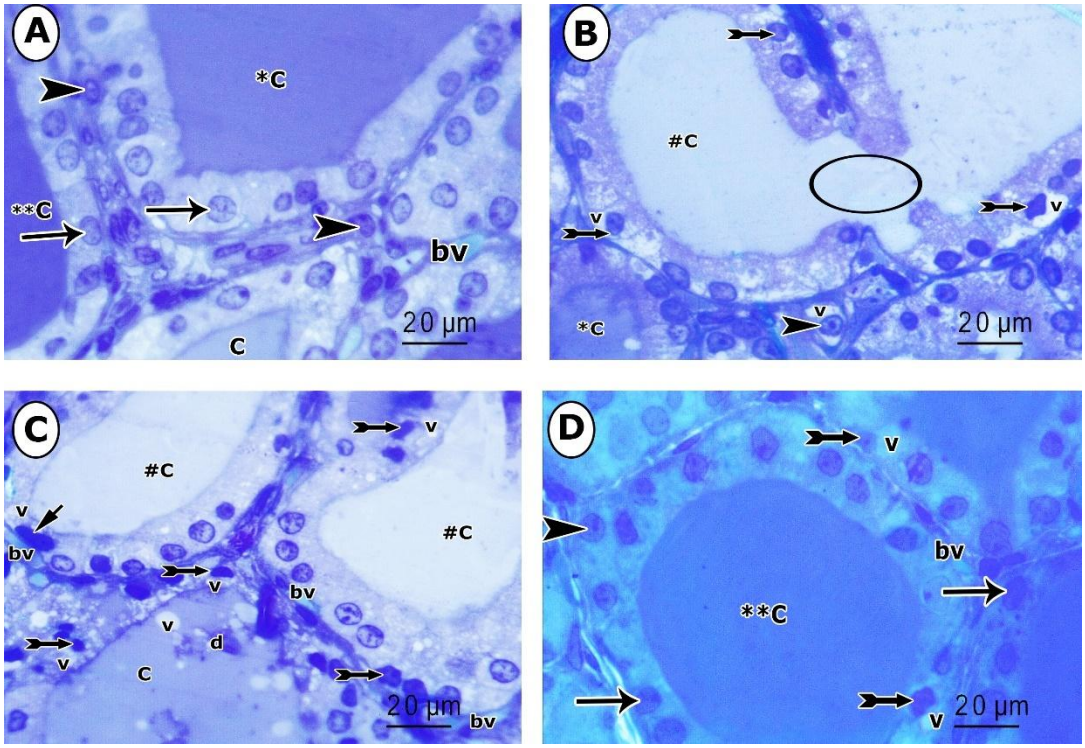

Figure 5. Photomicrographs of Toluidine blue stained sections of thyroid gland from the different studied groups.**(A)** Control group illustrating apparently normal thyroid

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follicles lined with one layer of cubical follicular cells with vesicular spherical (arrow). The follicles are filled with homogenous colloid of variable densities (C, C*, C**) and surrounded by blood capillaries (bv). Parafollicular cells (arrowhead) are also seen that not reaching the lumen of the follicles with vesicular nuclei (arrowhead). **(B, C)** Thyroid gland of PFAO treated group demonstrating disrupted and fused thyroid follicles (circle) with desquamated epithelial cells (d) in their lumen. Dark nuclei (bifid arrow) and vacuolated cytoplasm (v) are seen in most of follicular cells in addition to, some flattened follicular cells with flat dark nuclei (short arrow) and parafollicular cell with vacuolated cytoplasm.Emptyfollicular cells from colloid (#C), vacuolated (v) colloid(C) and congested blood capillaries (bv) are also seen between the follicles.**(D)** PFAO+ Curcumin group revealing nearly normal thyroid follicles filled with homogenous colloid (**C) and lined with a single layer of follicular cells with vesicular spherical nuclei (arrow) and with parafollicular cells (arrowhead). The follicles are surrounded by blood capillaries (bv). Few of the lining follicular cells exhibit dark irregular nuclei (bifid arrow) and vacuolated cytoplasm (v) **(Toluidine blue, X 1000).**

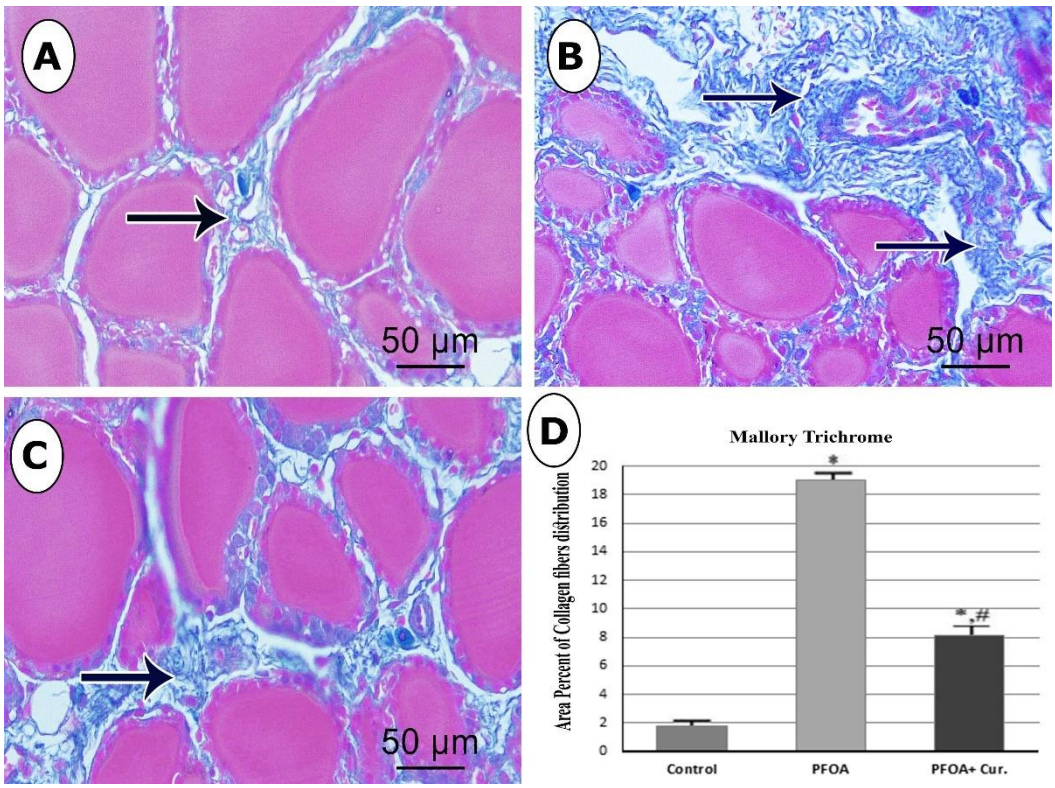

Figure 6. Photomicrographs of Mallory trichrome stained thyroid section from the different studied groups.**(A)** control group showing minimal collagen fibers in the interfollicular spaces.**(B)**PFOA treated group showing abundant collagen fibers are observed between the follicles. **(C)** PFOA + Curcumin treated group showing a restoration of the normal collagen fibers distribution. Arrow signifying the blue staining of collagen fibers. **(D)**Bar charts showing statistical analysis (using ANOVA

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&Turkey-Kramar post hoc test) of the area % mean values of collagen fibers in Mallory trichrome stained thyroid sections among different groups. * Significantly difference from control at p<0.05. #Significantly difference from PFOA group at p<0.

MT. Scale bar= 50 µm, x400.

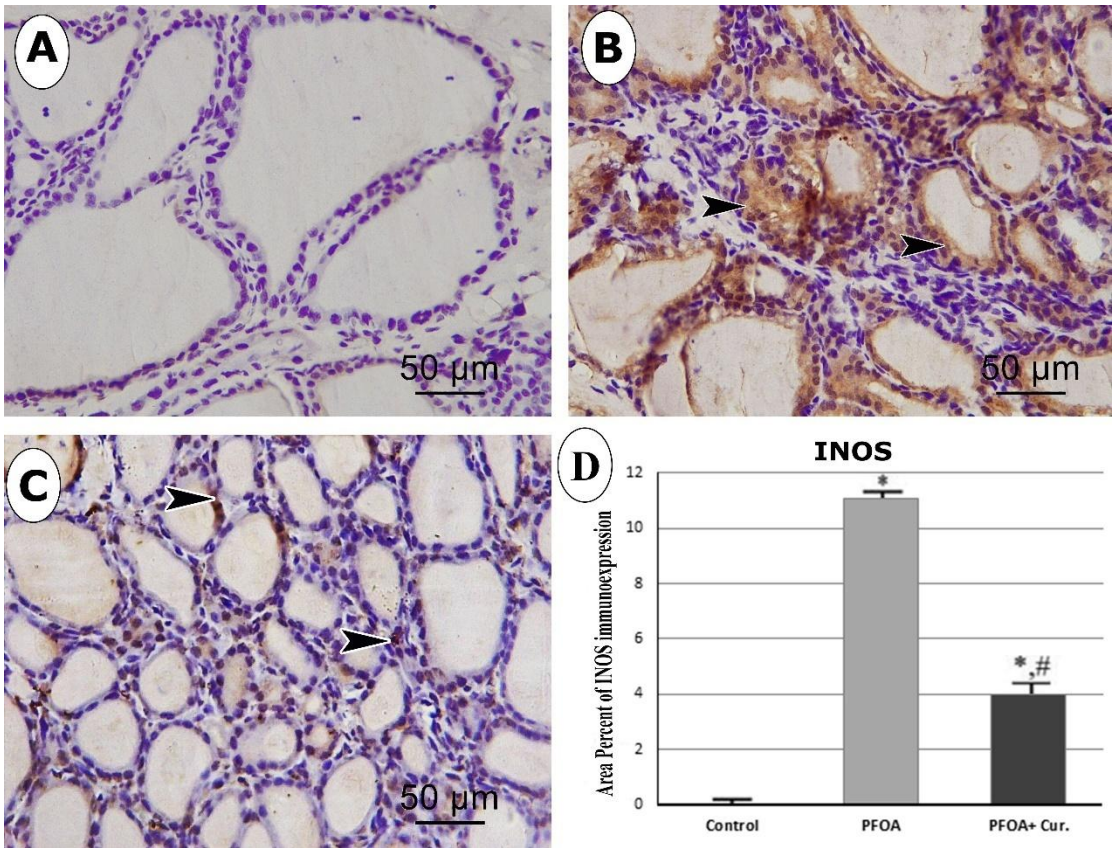

Figure 7. Photomicrographs of INOS immunohistochemical stained thyroidsection from the different studied groups. **(A):** control group showingweakimmuno-expression of caspase in follicular cells detected by weak & localized brown cytoplasmic staining. **(B):** the PFOA treated group showing strong immuno-expression of caspase in follicular cells detected by strong & extensive brown cytoplasmic staining (arrow).**(C):** the PFOA + Curcumin treated group showing a moderate immuno-expression of caspase in follicular cells. **(D):** Bar charts showing statistical analysis (using ANOVA &Turkey-Kramar post hoc test) of the optical density mean values of INOS immunostaining in thyroid sections of different groups**. *:** Significantly different from control at p<0.05. #: Significantly different from PFOA group at p<0.05. **Scale bar= 50 µm, x400.**

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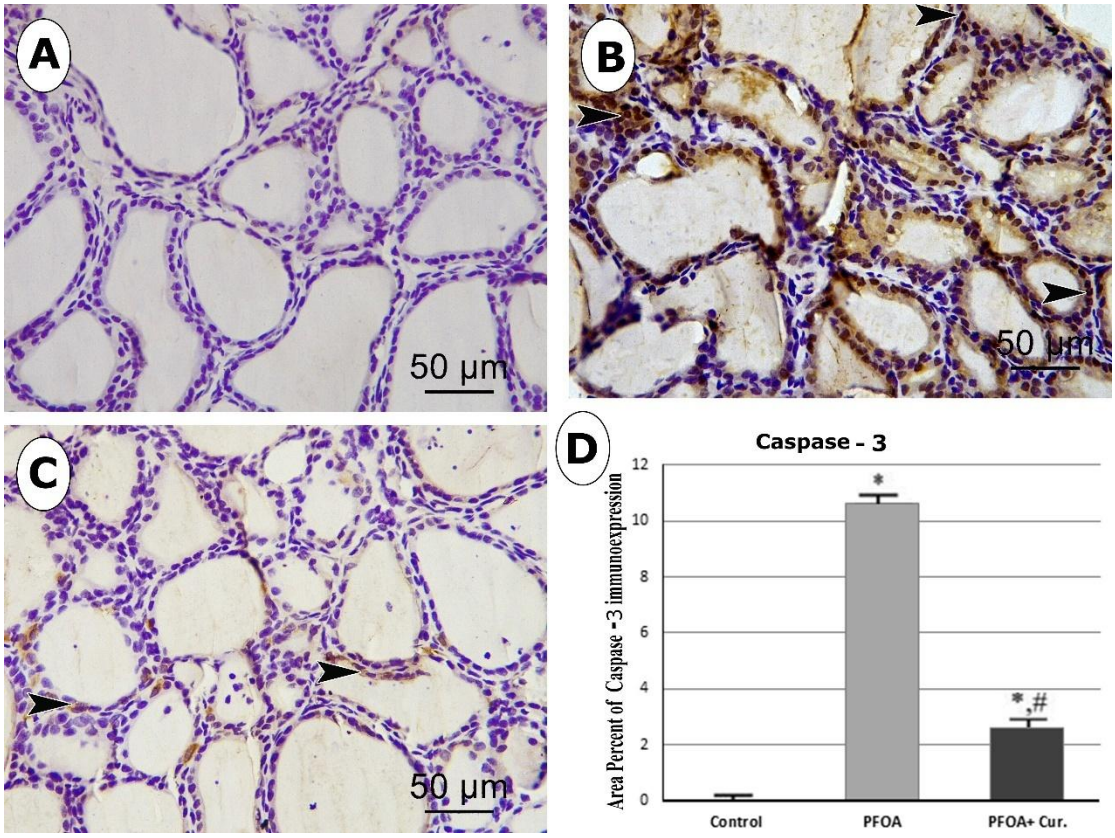

Figure 8. Photomicrographs of caspase-3 immunohistochemical stained thyroid section from the different studied groups. **(A)**control group showing weak immuno-expression of caspase-3 in follicular cells detected by weak & localized brown cytoplasmic staining. **(B)**the PFOA treated group showing strong immuno-expression of caspase-3 in follicular cells detected by strong & extensive brown cytoplasmic staining (arrow). **(C)** PFOA + Curcumin treated group showing a moderate immuno-expression of caspase in follicular cells. **(D)** Bar chartshowing statistical analysis (using ANOVA &Turkey-Kramar post hoc test) of the optical density mean values of caspase immunostaining in thyroid sections of different groups. *: Significantly different from control at $p<0.05$. #: Significantly different from PFOA group at $p<0.05$. **Scale bar= 50 µm, x400.**

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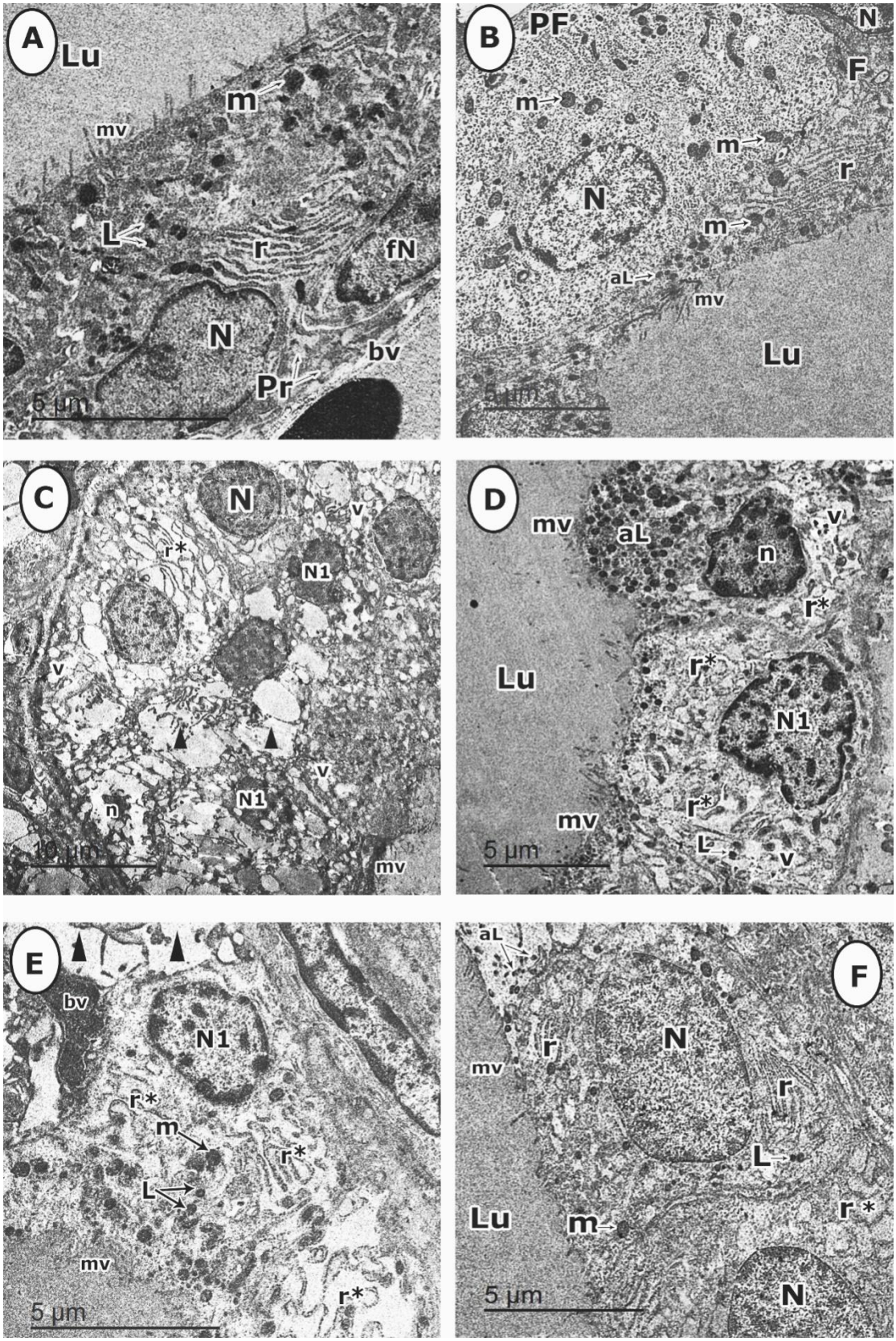

Figure9. Electron micrographs of thyroid gland from the different studied groups .**(A)**control group showing follicular cells has flat nuclei (fN) with prominent

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nucleolus and clumps of heterochromatin. Oval and irregular euchromatic nuclei (N) can be noticed. its cytoplasm shows cisternae of rough endoplasmic reticulum (r), dense lysosomal granules (L) and scattered mitochondria (m). Their apical border shows a moderate or a small number of microvilli (mv) projecting into the lumen (Lu). Blood capillary (bv) can be seen beneath the thin basal lamina. **(EMX 10 micron).(B)**Another electron micrograph from the control rats showing para follicular cell (PF) with euchromatic nucleus(N) and scattered mitochondria(m). Follicular cell (F) with euchromatic nucleus (N), apical lysosomes (aL), rough endoplasmic reticulum (r), and scattered mitochondria (m). The apical border shows a moderate or a small number of microvilli (mv) projecting into the lumen (Lu). **(EMX 5 micron).** Electron micrographs of thyroid gland from PFOA group showing**(C)**the follicular cells arranged in layers. Their nuclei are euchromatic (N), heterochromatic (N1), and apoptotic (n). Numerous dilated irregular cisternae of rough endoplasmic reticulum (r*) can be seen. Their apical borders show an extensive number of aggregated microvilli (mv). Extensive electron lucent areas (▲) and vacuoles (v) can be noticed **(EM× 10 micron)**. **(D)**showing the follicular cells,onehas heterochromatic nuclei (N1), and the other apoptotic (n). Numerous dilated irregular cisternae of rough endoplasmic reticulum (r*), polymorphic dense lysosomal granules (L) and vacuoles can be seen in the cytoplasm of both cells. Their apical borders show an extensive number of aggregated microvilli (mv) bulging in the lumen (Lu). Extensive apical lysosomes (aL) can be noticed in the apoptotic follicular cell **(EM× 10 micron)**. **(E)**showing columnar follicular cell that have heterochromatic nuclei (N1). Numerous dilated irregular cisternae of rough endoplasmic reticulum (r*), dense lysosomal granules (L), scattered mitochondria (m) and numerous vacuoles (v) can be seen. Their apical borders show a huge number of aggregated microvilli (mv). Blood capillary (bv) can be seen indenting the epithelial lining of the follicle. Electron lucent areas (▲) and vacuoles (v) can be noticed **(EM× 5 micron)**. **(F)**An electron micrograph of thyroid gland from PFOA +curcumin group showing thyroid follicular cell with euchromatic nucleus (N), normal (r) and slightly dilated rough endoplasmic reticulum (r*), Few lysosomes (L)& scattered mitochondria (m). The apical border shows a moderate or a small number of microvilli (mv) projecting into the lumen (Lu). **(EM× 5 micron)**