Original Article

Light and Electron Microscopic Study on the Possible Protective Effect of Pomegranate Peel Extract on the Pituitary- Thyroid Axis Exposed to Monosodium Slutamate in Adult Male Albino Rats

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ABSTRACT

Introduction: Monosodium glutamate is one of our new high-tech food additives. Nowadays, use of natural antioxidant as pomegranate peel extract has become an attractive therapeutic strategy for reducing the risk of disease.

Aim of Work: This study was designed to assess the effects of MSG on the pituitary thyroid axis and the possible protective role of pomegranate peel extract.

Material and Methods: Forty adult male albino rats were divided into three groups. Group I: (control) that was subdivided into two subgroups: I A, and I B. Group II (MSG treated) received MSG at a dose of (6mg/g./day). Group III (MSG & Pomegranate peel treated) received PPE &MSG as same dose as subgroup I B and group II. At the end of the experiment, pituitary and thyroid glands were dissected out and processed for biochemical, histological, immune-histochemical, and electron microscopic studies.

Results: MSG caused thyroid follicles distortion and degeneration. Follicular cells possessed vacuolated cytoplasm, and pyknotic nuclei. Decreased colloids & inflammatory infiltrates were detected. By E/M, follicular cells showed dilated RER cisternae, degenerated mitochondria, & depleted microvilli. While, basophils of pituitary gland appeared degenerated with vacuolated cytoplasm. By E/M, thyrotrophs showed dilated RER cisternae, dilated Golgi apparatus, and degenerated mitochondria. There was a highly significant decrease (*P value*<0.001) in the mean serum level of T3, T4 and TSH in Group II as compared with group I. While group III showed a highly significant increase (*P value*<0.001) in the same parameter as compared with group II. The administration of PPE induced improvements of these changes.

Conclusion: This work demonstrated that pomegranate peel extract improves the histological & biochemical changes induced by MSG on the pituitary thyroid axis.

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Key Words: Monosodium glutamate, pituitary thyroid axis, pomegranate peel extract.

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INTRODUCTION

Fast food consumption has become a common phenomenon all over the world. The modern lifestyle had switched from natural foods to fast foods intake that exhibits low dietary fibers and high energy[1,2].

Nowadays, we are depending more and more on the processed foods which contain chemicals known as food additives. These are substances added to food to introduce a special color or taste attracting consumers, especially children. Monosodium glutamate (MSG) is the most common used food additive giving a special aroma to processed food[3]. Monosodium glutamate (MSG) is a sodium salt of non-essential amino acid glutamic acid[4].

MSG is widely used as flavor enhancer not only in food industry, restaurants but also at homes. Therefore, the majority of canned and fast food as flavored tuna, flavored chips, canned soups or sauces, prepared meals, vegetarian burgers, fresh sausages, and manufactured meats containing variable concentrations of MSG[5].

Thyroid gland is responsible for production, release, and storage of thyroid hormones triiodothyronine (T3) and thyroxine (T4)[6]. Thyroid hormones play a main role in various physiological processes as normal growth, metabolism, and mental development so, any disproportion in their levels might lead to various clinical conditions[7].

Thyroid hormones biosynthesis and secretion are regulated by negative feedback mechanism of hypothalamus- pituitary- thyroid axis[8]. Pituitary thyrotrophs is responsible for synthesis and secretion of thyroid stimulating hormone (TSH) which regulates thyroid gland activity[9].

Unfortunately, studies reporting the effect of MSG on the morphology and function of pituitary-thyroid axis are very controversial. Some scientists studied the long-term effect of MSG on neonatal rats and observed non-remarkable histo-pathologic changes in thyroid gland morphology. Others showed a picture of a typical hypothyroidism in mice after receiving variable doses of

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MSG[5]. On the other hand, a picture of enhanced thyroid function was detected in MSG treated adult rats[10].

Nowadays, natural products play an impressive protective role with the clinical therapeutic regimens. A lifestyle-related health conditions can be reduced by ingesting healthy diets containing plenty of vegetables and fruits[11].

Pomegranate (Punica granatum L.) is a member of the family of Punicaceae, one of the most ancient fruits that are widely grown in Mediterranean regions[12]. Pomegranate fruit is considered as a food medicine because of its high nutritive value and health benefits[13]. Pomegranate peel extract is a rich source of natural antioxidants, and bioactive compounds, as flavonoids, proanthocyanidin, ellagitannins and phenolics compounds as well as minerals mainly sodium potassium, calcium, nitrogen, magnesium and phosphorus[14].

So, this study was designed to evaluate the histological, histochemical and immunohistochemical changes within the pituitary thyroid axis by using MSG and the possible protective role of Pomegranate peel extract powder in male albino rats.

MATERIALS AND METHODS

Animals

Forty adult healthy male albino rats of average weight 180-200 grams were used in the experiment. The animals were obtained from the animal house at Faculty of Medicine, Menoufia University one week before the start of the experiment to be acclimatized with the laboratory conditions. All animals were kept in clean, properly ventilated cages under similar environmental conditions at room temperature having free access to laboratory rat chow diet and water ad-libitum.

Chemicals

Monosodium glutamate (MSG) was obtained from sigma chemical company in the form of white crystalline powder. Pomegranate peel extract (PPE) was prepared from fresh pomegranate fruits that were obtained from the market in Sheibin El-kom, Menoufia by the following procedure.

Preparation of Pomegranate peel powder: Fresh pomegranate fruits were collected from the market and cleaned. The pomegranate peel extracts were isolated, divided into small pieces and air dried. The dried peels were then grinded into fine powder that was stored in a clean airtight container[14].

Experimental protocol

The experimental period was 6 weeks. During this duration, the rats were randomly divided into three groups:

Group I (control group): animals were subdivided into two subgroups (each has 10 animals):

- Subgroup IA: animals did not receive any treatment.
- Subgroup IB (pomegranate peel treated group): The rats were treated with pomegranate peel powder at a dose of (500 mg/kg body weight) that was dissolved in distilled water and given by gastric tube once daily during experimental period[14]. Each animal received 2 ml of a solution formed by dissolving 1 gram of pomegranate peel powder in 20 ml distilled water orally by gastric tube once daily for 6 weeks.

Group II (MSG treated group) (n=10 animals): the rats of this group received MSG at a dose of (6 mg/g. body weight /day) dissolved in distilled water by gastric tube once daily during duration of experiment[15]. Each animal received 1 ml of a solution formed by dissolving 12 grams of MSG in 10 ml distilled water orally by gastric tube once daily for 6 weeks.

Group III (MSG and pomegranate peel treated group) (n=10 animals): the rats of this group received pomegranate peel powder at a dose of (500 mg/kg body weight) that was dissolved in distilled water and given by gastric tube once daily for experimental period[14] and MSG at a dose of (6 mg/g. body weight/day) for same duration[15].

Twenty-four hours following the final dose, all animals were weighted by digital scale and then anesthetized by an intraperitoneal injection of 40 mg/kg phenobarbital sodium[16]. The blood samples were collected from rat`s tail vein to measure the serum levels of T3, T4 and TSH hormones. Then, intra-cardiac perfusion was done by 2.5% glutaraldehyde with 0.1 mol/L phosphate buffer at pH 7.4 for partial fixation of the specimens. Thyroid and pituitary glands were carefully dissected, excised then washed with normal saline from the animals of all groups. The tissues and blood samples were subjected to the following studies:

I-Hormonal assay

A hormonal assay was performed to find out the serum levels of thyroid-stimulating hormone (TSH), T3 and T4 using commercially available Chemiluminescence Immunoassay (CLIA, catalogue no. ABIN504750; ABIN, Canoga Park, C.A. USA) following the manufacturer's instructions. This was done in central lab, Faculty of Medicine, Menoufia University

II-Light microscopic studies

Histological study: Specimens of thyroid and pituitary glands of each animal were fixed in 10% formol saline, dehydrated, cleared, and embedded in paraffin wax. Five µm thickened sections were cut and stained with hematoxylin and eosin (Hx &E) for routine histological examination of general architecture of thyroid and pituitary glands[17], Mallory's Trichrome stain for detection of collagen fibers within the thyroid gland[18].

Histochemical study: Periodic Acid Schiff (PAS) stain of thyroid sections for detection of muco-polysaccrides[19].

Immunohistochemical study:

1. Anti-BAX immunomarker expression for thyroid sections: The primary anti-Bax antibody used was rabbit polyclonal antibody (1/50 dilution, Abcam). It is a marker of apoptosis. Brown cytoplasmic staining was recorded as positive reaction. Hodgkin's lymphoma was used as a positive control. Negative control sections were prepared without using the primary antibodies[20].
2. Immuno-staining for the β-chain of thyroid stimulating hormone (TSH) for thyrotrophs of anterior pituitary gland:

A monoclonal anti-TSH antibody at a dilution of 1:200 (Thermo Fisher, Fremont, California, USA) was used for TSH immunostaining. This antibody recognizes thyrotrophs by cytoplasmic reactions. Brown cytoplasmic reaction is considered positive, whereas the nuclei appeared blue. Anterior pituitary tissue was used as positive control. Negative control sections were prepared without using the primary antibodies[21]. The slides were counterstained with Mayer's hematoxylin, dehydrated, and mounted with DPX (BDHLtd, Poole, UK)

III- Electron Microscopic study

Specimens of the thyroid and pituitary glands were rapidly cut into small pieces (1mm), and then were immediately fixed in 3% glutaraldehyde buffered with 0.1 mol/L PBS at pH 7.4 for 3 hours at 4°C and post fixed in 1% osmium tetroxide in the same buffer for 2 hours at 4°C. The tissues were then dehydrated in ascending grades of alcohol and embedded in epoxy resin to cut semithin sections (1μm thick) which were stained with 1% toluidine blue then examined by light microscope[22].

Ultrathin sections were stained with uranyl acetate and lead citrate to be examined and photographed using transmission electron microscope TEM (JEOL, JEM-2100, Tokyo, Japan)[23]. In the Faculty of science, Alexandria University, Alexandria, Egypt.

IV: Morphometrical study

For quantitative evaluation, 10 non-overlapping fields were chosen for each animal. Image analysis was done using ″ Leica Quin 500 ″ software image analyzer computer system (Leica image system Ltd; Cambridge, England) present in the human anatomy & embryology department, Faculty of Medicine, Menoufia University. The measured data were undertaken using PAS, Mallory`s Trichrome and immune-histochemical sections at a magnification of 400 for the first four parameters. For quantitative evaluation, the following illustrated parameters were calculated:

- Area % of collagen fibers in Mallory`s Trichrome -stained sections.
- Area % of colloid in PAS-stained sections.
- Color intensity of Bax-positive immunoreactions in Bax- immunostained sections.
- Number of positive-stained thyrotrophs in TSH-immunostained sections.
- Number of electron dense granules within the cytoplasm of EM pictures of thyrotrophs.

Statistical analysis: the body weight (g.), biochemical and morphometrical data were expressed as mean ±SD. The student t-test was used to evaluate the significant change in each parameter in the experimental groups (group II & III) when compared to the Group I (control group) and comparing MSG treated group (group II) with MSG and pomegranate peel extract treated group (Group III). The statistical analysis of data was carried out using Excel and statistical package for the social science software, version 11. In all statistical analysis $P<0.05$ was taken as the level of significance, $P\ value > 0.05$ was considered non-significant and $P\ value < 0.001$ was considered highly significant[24].

RESULTS

General observations

- No deaths were observed in rats during the experiment.
- Control subgroups (IA, and IB) showed similar histological, histochemical, and immune-histochemical results so; they were collectively named as control group.

Regarding body weight, Group II (MSG treated group) exhibited a highly significant increase in body weight (*P value*<0.001) as compared to Group I (control group). While group III (MSG and pomegranate peel treated group) showed non- significant change in body weight as compared to control, and a highly significant decrease (*P value*<0.001) as compared to Group II (MSG treated group) as seen in (Histogram 1 (H1)). All parameters were set in (Table 1).

I-Hormonal assay results

There was a highly significant decrease (P value<0.001) in the mean serum level of T3, T4 and TSH in Group II (MSG treated group) as compared with group I (control group). While, Group III (MSG and Pomegranate peel treated group) showed non-significant difference (*P value*>0.05) in the mean serum T3, T4 and TSH level as compared with group I (control group), and a highly significant increase (*P value*<0.001) in the same parameter as compared with group II (MSG treated group) as seen in (Histogram 2 (H2)).

II-Light microscopic results

Thyroid gland results

1-H. &E. stain

By H. & E. stain, control group (I) of thyroid gland sections consisted of follicles of variable sizes and

shapes. The lining epithelium of thyroid follicles ranged from simple squamous to simple cubical epithelium. The lumens of the follicles were occupied by homogenous acidophilic colloid material with peripheral vacuolation. The follicles were separated by thin connective tissue septa housed by plenty of blood vessels and Interfollicular cells. Interfollicular cells appeared with esinophilic cytoplasm and large rounded vesicular nuclei (Figures 1A,2A).

Thyroid sections of MSG treated group (group II) revealed marked histological alterations. The thyroid follicles became irregular, distorted, and degenerated. The follicular cells varied from cubical to columnar cells with oval nuclei. The cells had vacuolated cytoplasm. Their nuclei showed variable degree of degeneration (pyknosis and kariolysis). Some follicles were partially lined with multiple cell layers (stratification). Their lumens contained fewer colloid materials and detached follicular cells. Connective tissue spetae revealed widely separated collagen fibers & dilated blood vessels with blood and inflammatory cells inside. Intense inflammatory cells infiltrate was detected (Figures 1B,1C,2B). Interfollicular cells were seen with vacuolated cytoplasm and degenerated nuclei (Figure 2B).

Thyroid follicles of MSG & pomegranate peel treated group (group III) were regular and intact. The follicular epithelium varied from squamous with flat nuclei to cubical with rounded nuclei. The follicles lumen`s contained variable amounts of acidophilic colloid material. Interfollicular cells in between the thyroid follicles appeared with intact membrane and oval regular nuclei. The blood vessels appeared small with regular diameter (Figures 1D,2C).

2- Mallory's Trichome stain

Mallory`s Trichrome stained thyroid sections of control group(I) showed minimal collagen fibers deposition between thyroid follicles (Figure 3A). However, the amount of collagen fibers in MSG treated group (II), was highly significantly increased (*P value*<0.001) (Figures 3B,3H3). With the use of pomegranate in group III, minimal collagen fibers between thyroid follicles were seen like that of control (Figures 3C,3H3).

3- Periodic acid Schiff reaction (PAS)

PAS-stained control thyroid gland (group I) revealed strong +ve reaction in the colloids with faint reaction in marginal vacuoles inside thyroid follicles lumens. Also, strong PAS +ve reaction was detected within the follicle`s basement membrane (Figure 4A). In group II (MSG-treated group), a weak to moderate PAS positive reaction in the colloid was noticed and weak PAS reaction was seen in the distorted follicular basement membrane. This was confirmed morphometrically by highly significant decrease (*P value*<0.001) in the area percentage of PAS-positive reaction (Figures 4B,4H4). By using pomegranate peel in Group III, PAS moderate +ve reaction in the colloid within thyroid follicles was noticed. Also, moderate to strong PAS +ve reaction was detected in the follicular basement membrane. This was confirmed morphometrically by a highly significant increase (*P value*<0.001) in the mean area percentage of PAS-positive reaction as compared with group II (MSG treated group) (Figures 4C,4H4).

4. BAX immunostaining

The sections of thyroid gland of control group(I) showed - ve Immunostaining for BAX in follicular and interfollicular cells cytoplasm. (Figure 5A). In Group II (MSG treated group), a strong +ve immunoreaction for BAX in follicular and inter follicular cells in the form of brown cytoplasmic deposits was detected. This was confirmed morphometrically as a highly significant increase (*P value*<0.001) in its color intensity within group II (MSG treated group) as compared with group I (Figures 5B,5H5). Group III (MSG and pomegranate peel treated group) demonstrated moderate +ve immunoreaction for BAX in the follicular and interfollicular cells cytoplasm. This was confirmed morphometrically as a highly significant decrease (*P value*<0.001) in the color intensity of BAX immunoreaction as compared with group II (MSG treated group) (Figures 5C,5H5).

Pituitary gland results

1- H. & E. stain

Sections stained with H. & E. of pars distalis of anterior pituitary of control group (I) showed clusters of cells separated by blood capillaries. According to staining affinity there were two types of cells, chromophils and chromophobes. The chromophil cells were subdivided into acidophils and basophils. Basophils were fewer in number and larger in size in comparison with acidophils. They were oval, having granular basophilic cytoplasm, with vesicular nuclei. The basophils included thyrotrophs, gonadotrophs, and corticotrophs. The acidophils were more numerous and smaller in size than basophils. They were rounded, having highly acidophilic cytoplasm, provided with rounded vesicular nuclei. The acidophils included somatotrophs and mammotrophs. Chromophobes were small and numerous, possessed pale cytoplasm and relatively large nuclei (Figure 6A).

In MSG treated group (II) basophils appeared with pale vacuolated cytoplasm, their nuclei appeared dark and small. Degenerated necrotized basophils were present. The blood capillaries were dilated and congested with RBCs (Figures 6B,6C).

By using pomegranate peel in Group III, basophils showed basophilic cytoplasm provided with scattered fine granules and vesicular nuclei. Regular small blood capillaries were present in between different types of cells (Figure 6D).

2- Toluidine blue stain

Using semi-thin sections stained with toluidine blue of pars distalis of anterior pituitary of control (group I), the thyrotrophs were distinguished by being large angular

cells located in close relation to blood capillaries. The cells had fine secretory granules distributed in their cytoplasm, mainly at the periphery, their nuclei were rounded (Figure 7A).

Thyrotrophs with vacuolated cytoplasm and very few scattered granules were found in sections of pars distalis of anterior pituitary of MSG treated group (group II). There were nuclear changes as the nuclei appeared small, dark with irregular outlines. Dilated congested blood capillaries with RBCs inside were also noticed (Figures7B,7C).

In Group III, the thyrotrophs showed granular cytoplasm and vesicular nuclei, and were in position close to blood capillary, that became smaller in size in comparison with blood capillaries in group II (Figure 7D).

3- Anti-TSH immunostaining

Regarding the Immunostaining with anti TSH, dark brown positive cytoplasmic immunoreaction was detected in many branched cells (thyrotrophs) in the control (group I) (Figure 8A). While MSG treated group (group II) exhibited a very weak reaction for TSH immunostaining detected in scanty scattered thyrotrophs. This was confirmed morphometrically as a highly significant decrease (*P value*<0.001) in the mean number of thyrotrophs in group II (MSG treated group) as compared with control group as seen in (Table 1,Figures 8B,8H6). In Group III, moderate reaction for TSH Immunostaining in thyrotrophs cytoplasm was detected as their mean numbers showed highly significantly increase (*P value*<0.001) as compared with group II (MSG treated group) as seen in (Table 1,Figures 8C,8H6].

II-Electron microscopic results

Thyroid gland results

Ultra-structurally, the thyroid gland of control group (I) showed the follicular lining cells with almost oval euchromatic nuclei resting on clear basal lamina. Their cytoplasm exhibited numerous cisternae of rough endoplasmic reticulum, mitochondria with moderate electron dense colloidal droplets appeared in the apical part of the follicular cells. The apical border of the cells revealed numerous microvilli that project into the central lumen (Figures 9,10). Parafollicular or C-cell appeared within some follicles incorporated with the follicular cells resting on clear basal lamina. The cell had oval nucleus having clumps of peripheral heterochromatin surrounded with clear nuclear membrane. Its cytoplasm contained few tubular cisternae of rough endoplasmic reticulum, mitochondria, and smooth endoplasmic reticulum. The cell was separated from the lumen by a part of the cytoplasm of a flat follicular cell containing heterochromatic nucleus (Figure 10).

Sections of the thyroid gland of MSG treated rat group (II) showed disturbed normal architecture of thyroid gland. The follicular cells appeared distorted resting on irregular basal lamina. Their nuclei had irregular outlines with peripherally located heterochromatin, widening of nuclear pore complex is also noticed. Their cytoplasm showed disturbed, dilated cisternae of rough endoplasmic reticulum with degenerated mitochondria. Their apical border has few, short microvilli with areas of complete depletion (Figures 11,12). The parafollicular (C) cell had indented heterochromatic nucleus. Its cytoplasm showed disturbed, dilated cisternae of rough endoplasmic reticulum and degenerated mitochondria (Figure 11).

Ultrathin sections of the thyroid gland of MSG and Pomegranate peel treated rat group (III) revealed restoration of the normal structure of thyroid gland. The follicular cells had oval heterochromatic nuclei like that of the control. The cytoplasm showed multiple tubular cisternae of rough endoplasmic reticulum, some of them appeared slightly dilated and electron dense colloidal granules. The apical border showed microvilli like that of the control group projecting into the lumen (Figures 13,14). Parafollicular (C) cell appeared incorporated between the basal lamina and the follicular cells having heterochromatic nucleus. Well-developed mitochondria, tubular cisternae of rough endoplasmic reticulum, and multiple electron dense secretory granules were seen within their cytoplasm (Figure 14).

Pituitary gland results

Ultra-structurally, the thyrotrophs of the pars distalis of anterior pituitary gland of control group(I) appeared angular in shape with elongated cytoplasmic processes. The cells had oval euchromatic nucleus with prominent nucleolus. Their cytoplasm had multiple small spherical electron dense granules limited to the periphery. Well-developed elongated mitochondria and rough endoplasmic reticulum were also seen within the cytoplasm (Figures 15,16).

On the other hand, ultrathin sections of the pituitary gland of MSG treated group (II) showed marked distortion within the structure of thyrotrophs. Their nuclei appeared irregular fragmented heterochromatic ones. Their cytoplasm appeared vacuolated housed with degenerated mitochondria, dilated cisternae of rough endoplasmic reticulum, dilated Golgi apparatus and lysosomes (Figures 17,18). The number of electron dense secretory granules appeared highly significantly decreased (*P value*<0.001) within the cytoplasm as compared to the control group (Histogram 7 (H7), Table 1). Some of them were aggregated in groups in some sections (Figure 19).

Restoration of the normal structure of thyrotrophs detected on examination of ultrathin sections of the pituitary gland of MSG and Pomegranate peel treated group (III). Thyrotrophs appeared with euchromatic nucleus having regular smooth nuclear envelope. The cytoplasm had mitochondria and cisternae of rough endoplasmic reticulum more or less similar to control. The number of electron dense granules seen limited to the periphery of the cytoplasm exhibited a highly significant increase (*P value*<0.001) as compared with group II (MSG treated group) (Figures 20,Histogram 7 (H7), Table 1).

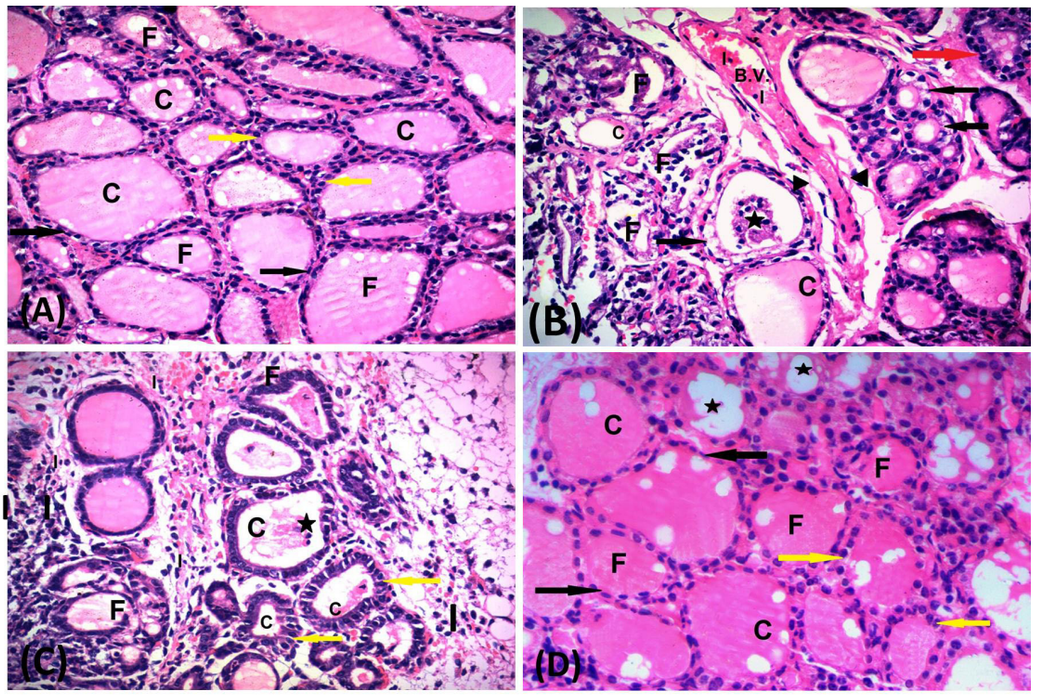


Fig. 1: A photomicrograph of a section of thyroid gland A) Group I (control group) showing follicles (F) with variable sizes, some of them are lined by squamous cells with flattened nuclei and basophilic cytoplasm (black arrow). Others are lined by cubical cells that show basophilic cytoplasm and rounded nuclei (yellow arrow). The follicles contain acidophilic colloid with peripheral vacuoles (C). B) Group II showing distorted and degenerated follicles (F) with apparent decrease in the colloid (C) inside. Some follicular cells possess vacuolated cytoplasm and degenerated nuclei (black arrow), others show stratification (red arrow). Detached follicular cells are seen within the follicle`s lumen (*). Connective tissue spetae reveals widely separated collagen fibers (►) & dilated blood vessels (B.V.) with blood and inflammatory cells inside (I). C) Group II showing irregular distorted follicles (F). Their lumens contain detached cells (*) and decreased or absent colloid (C). Some of the follicular cells appear tall with oval nuclei (yellow arrow). Intense inflammatory cells infiltrate (I) is detected. D) Group III showing regular thyroid follicles (F) which are filled with esinophilic colloid (C), with peripheral vacuoles. Follicular cells vary from squamous with flat nuclei (black arrow) to cubical with rounded nuclei (yellow arrow). Few distorted follicles with depleted colloid are still present (*). (H&E. x 200)

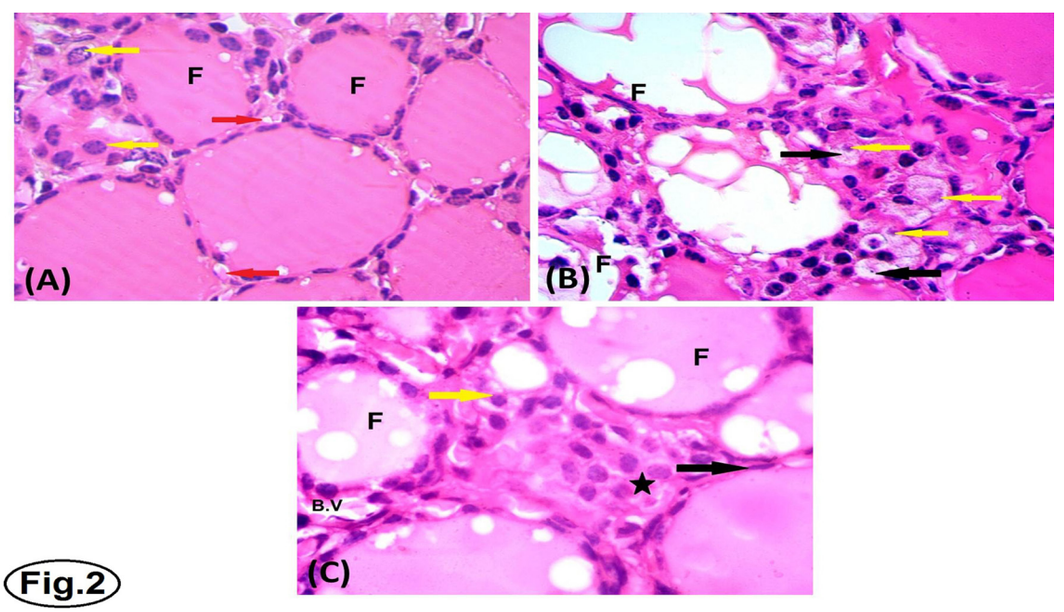


Fig.2

Fig. 2: A photomicrograph of a section of thyroid gland of A) Group I (control group) showing follicles (F) with regular outlines , separated by thin connective tissue containing blood vessels (red arrow) and Interfollicular cells. Interfollicular cells have esinophilic cytoplasm and large rounded vesicular nuclei (yellow arrow). B) Group II showing degenerated follicles (F). Interfollicular cells are seen with vacuolated cytoplasm (black arrow) and degenerated nuclei (yellow arrow). C) Group III showing regular intact thyroid follicles (F), which are lined by squamous cells with flattened nuclei (black arrow). Some follicular cells are cubical, and house rounded nuclei (yellow arrow). The Interfollicular cells in between the thyroid follicles shows homogenous cytoplasm and oval vesicular nuclei (*). The blood vessels appear slightly dilated (B.V.). (H& E. x 400)

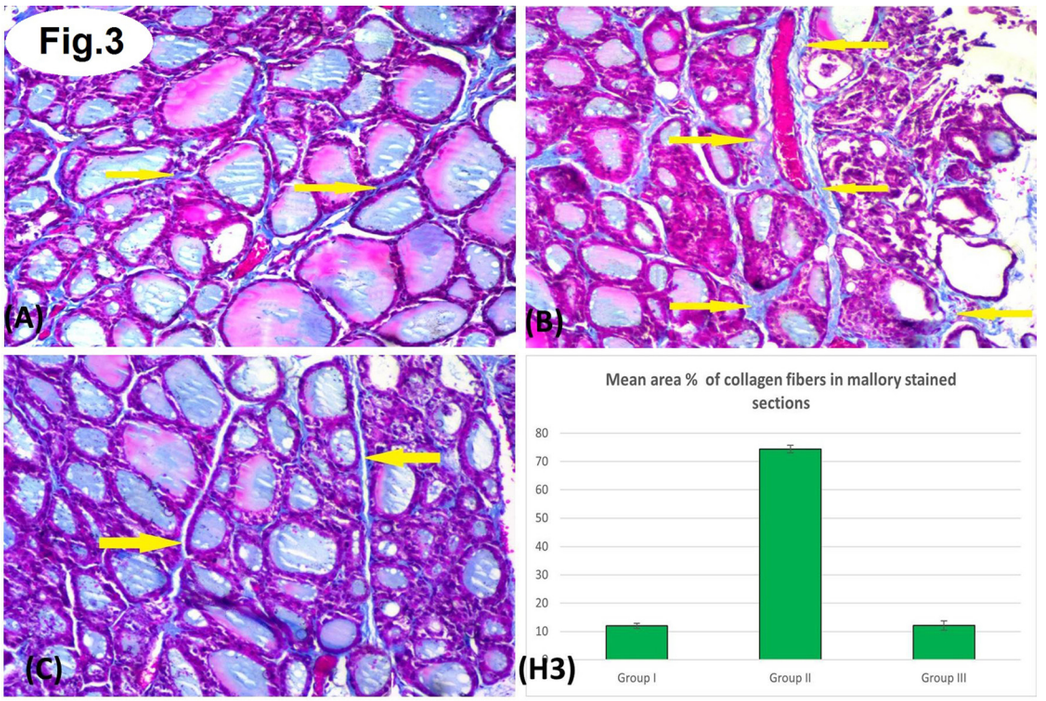


Fig. 3: A photomicrograph of a section of thyroid gland A) Group I (control group) showing minimal collagen fibers in between the thyroid follicles (yellow arrow). B) Group II showing apparent increase in the collagen fibers in the connective tissue in between the thyroid follicles (yellow arrow). C) Group III showing minimal collagen fibers between thyroid follicles (yellow arrow). (Mallory`s Trichrome x 100). H3) A histogram showing comparison between the mean area % of collagen fibers in different studied groups.

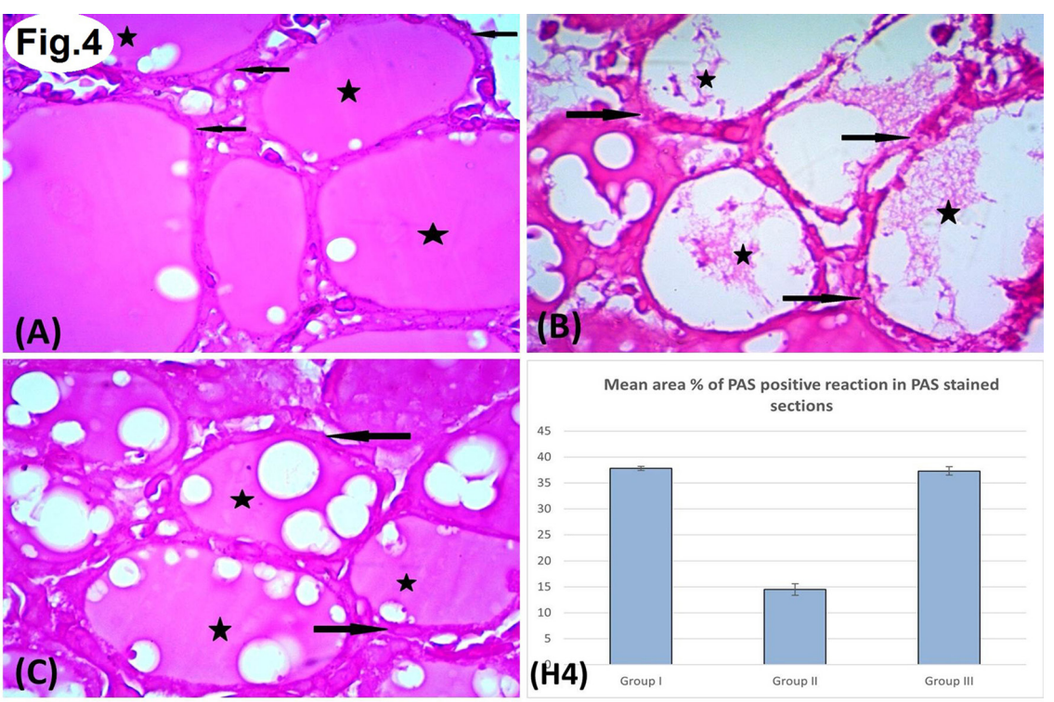


Fig.4: A photomicrograph of a section of thyroid gland A) Group I (control group) showing PAS strong +ve reaction in the colloids (*) with faint reaction in marginal vacuoles inside thyroid follicles. Strong PAS +ve reaction is seen within the follicle`s basement membrane (→). B) Group II showing weak PAS reaction in the colloid (*). Weak PAS reaction is noticed in the distorted follicular basement membrane (→).C) Group III showing moderate +ve reaction for PAS in the colloid inside thyroid follicles (*). Also, moderate PAS +ve reaction is found in the follicular basement membrane (→). (PASx400). H4) A histogram showing comparison between the mean area % of PAS positive reaction in different studied groups.

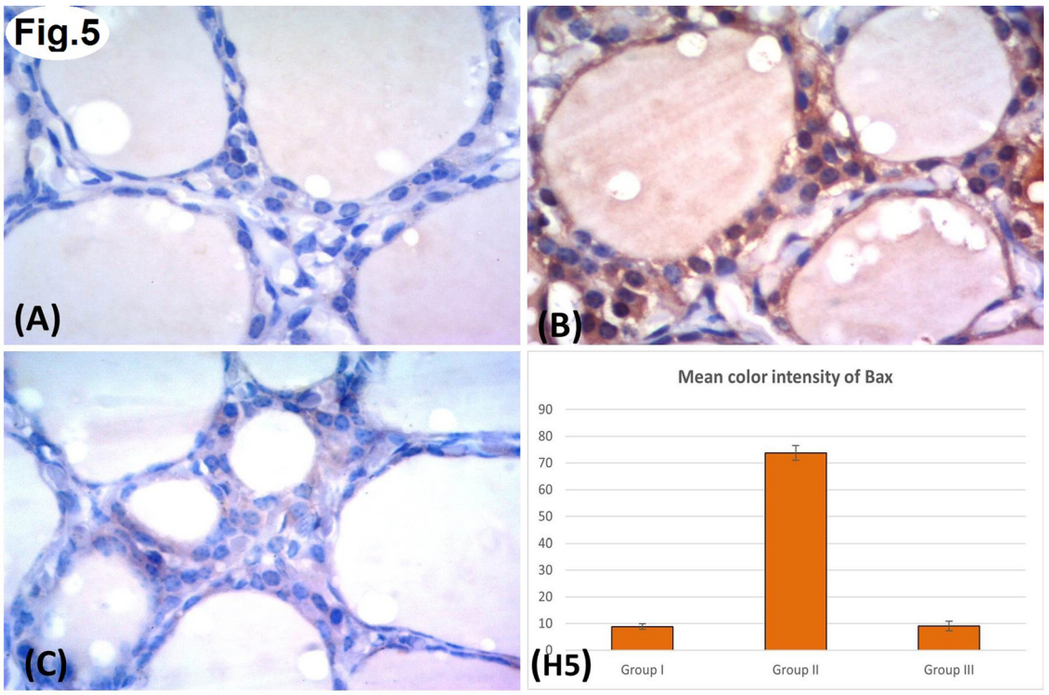


Fig.5: A photomicrograph of a section of thyroid gland A) Group I (control group) showing - ve Immunostaining for BAX in follicular and interfollicular cells cytoplasm. B) Group II showing strong +ve immunoreaction for BAX in follicular and inter follicular cells in the form of brown cytoplasmic deposits. C) Group III showing moderate +ve immunoreaction for BAX in the follicular and interfollicular cells cytoplasm (BAX x 400). H5) A histogram showing comparison between the mean color intensity of Bax immunostained in different studied groups.

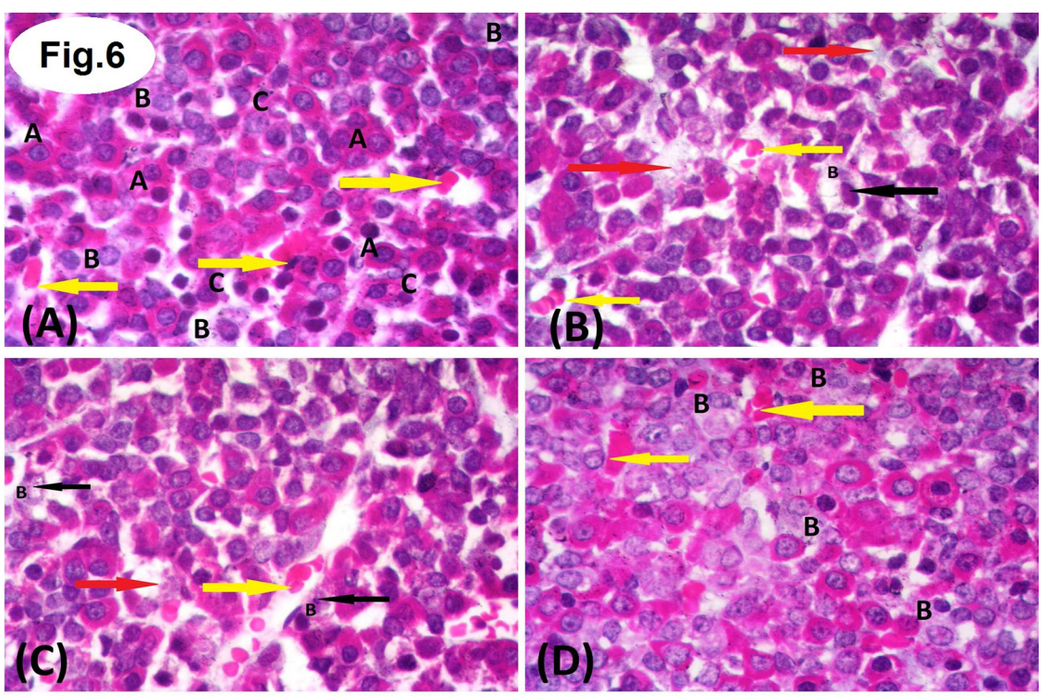


Fig.6: A photomicrograph of a section in the pars distalis of anterior pituitary gland A) Group I (control group), showing few basophils (B) that are noticed as large cells with basophilic granular cytoplasm, provided with large vesicular nuclei. Plenty of small sized cells with acidophilic cytoplasm and rounded nuclei are recognized as acidophils (A). Notice the chromophobes (C) and blood capillaries (yellow arrow). B&C Group II, showing basophils (B) with pale vacuolated cytoplasm, their nuclei appear dark and small (→). Degenerated necrotized basophils are present (red arrow). The blood capillaries are dilated and congested with RBCs (yellow arrow). D) Group III, showing basophils (B) with basophilic cytoplasm provided with scattered fine granules and vesicular nuclei. Regular small blood capillaries (yellow arrow) are present in between different types of cells (H&E. x 200).

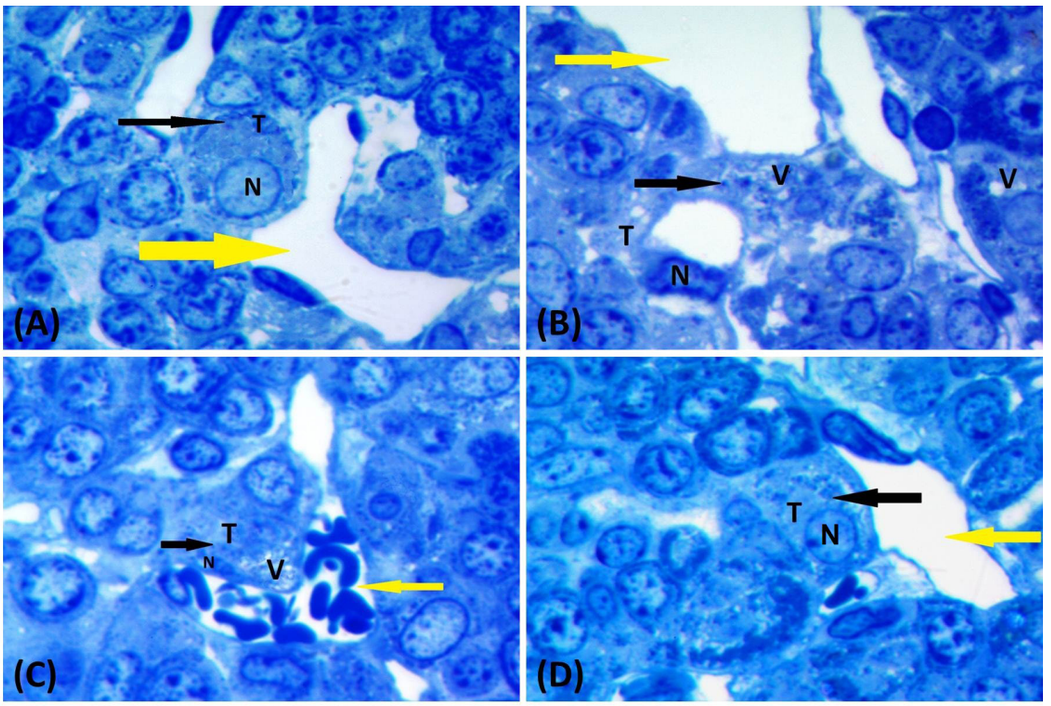


Fig.7: A photomicrograph of a section in the pars distalis of anterior pituitary gland showing A) Group I (control group), thyrotrophs (T) which is large angular cell present close by the blood capillaries (yellow arrow). The nucleus (N) appears regular and rounded. Notice the fine secretory granules which are distributed at the periphery of thyrotrophs cytoplasm (→). B&C Group II, thyrotrophs (T) with vacuolated cytoplasm (V), and very few granules (→) scattered. The nuclei appeared small, dark with irregular outlines (N). Dilated congested blood capillaries with RBCs inside are noticed (yellow arrow). D) Group III, thyrotrophs (T) with granular cytoplasm (→) and vesicular nuclei (N), in a position close to blood capillary (yellow arrow). (T.B. x 1000).

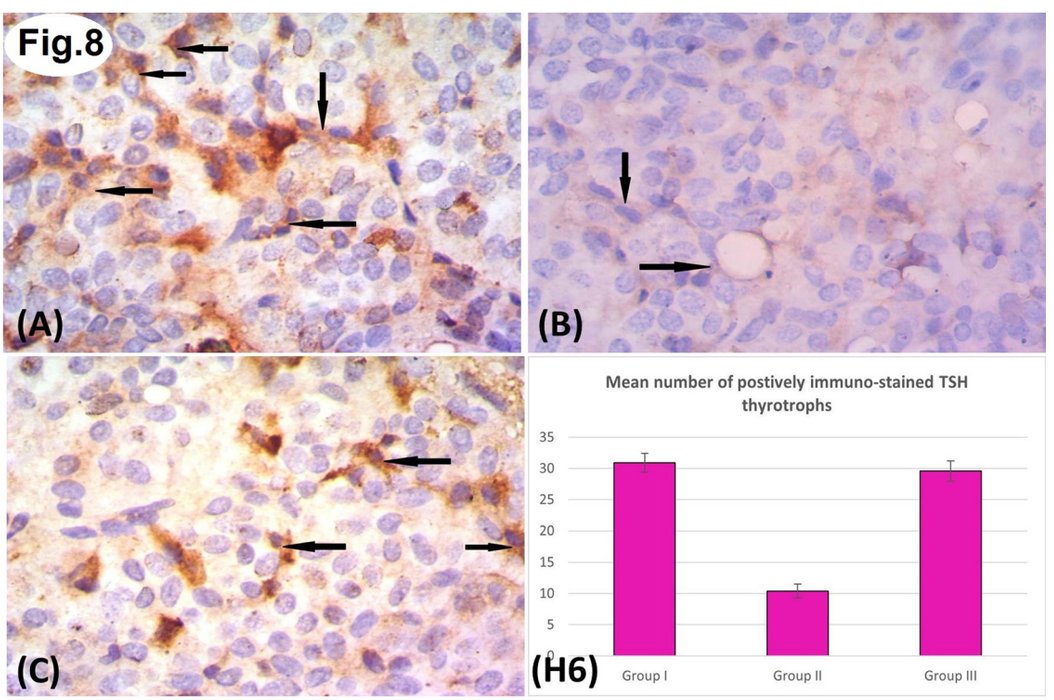


Fig.8: A photomicrograph of a section in the pars distalis of anterior pituitary gland showing A) Group I (control group), branched cells (thyrotrophs) with strong positive reaction for TSH immunostaining in the form of dark brown cytoplasmic coloration (→). B) Group II, very weak reaction for TSH Immunostaining in scanty scattered thyrotrophs present in the field (→). C) Group III, moderate positive reaction for TSH immunostaining in the cytoplasm of thyrotrophs in the form of brown color (→) (Anti TSH immunostaining x 400). H6) A histogram showing comparison between the mean number of positively immuno-stain TSH thyrotrophs in different studied groups.

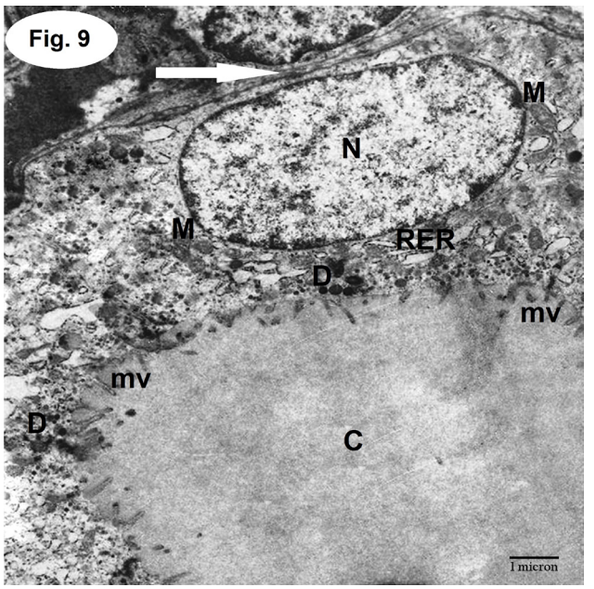


Fig.9: An electron micrograph of ultrathin section in the thyroid gland of (group I) showing follicular cell with oval euchromatic nucleus (N) resting on clear basal lamina (arrow). Their cytoplasm shows mitochondria (M), cisternae of rough endoplasmic reticulum (RER) and electron dense cytoplasmic colloid droplets (D). Its apical border shows microvilli (mv) projecting into the colloid (C). (TEM×17500)

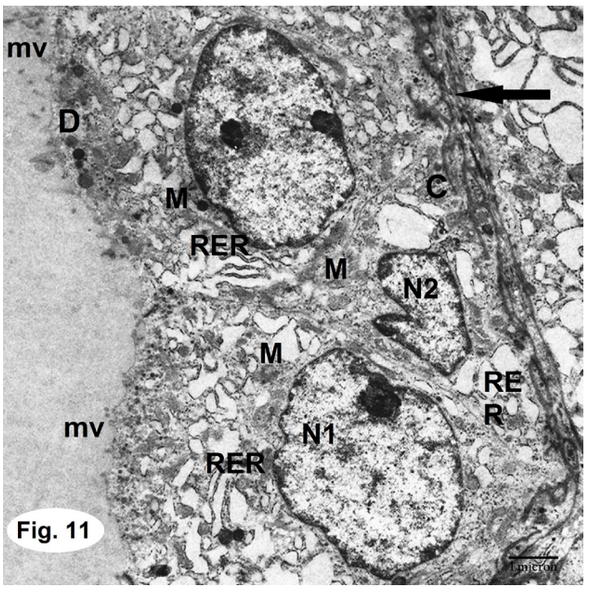


Fig.11: An electron micrograph of ultrathin section in the thyroid gland of (group II) showing two adjacent thyroid follicular cells with one parafollicular (C) cell in-between resting on irregular basal lamina (arrow). The follicular cells have irregular nuclei (N1). Their cytoplasm shows disturbed, dilated cisternae of rough endoplasmic reticulum (RER), degenerated mitochondria (M) and few electron dense cytoplasmic colloid droplets (D). Their apical border has scarce, short microvilli (mv). The parafollicular (C) cell has indented heterochromatic nucleus (N2). The cytoplasm shows disturbed, dilated cisternae of rough endoplasmic reticulum (RER), degenerated mitochondria (M).(TEM×17500)

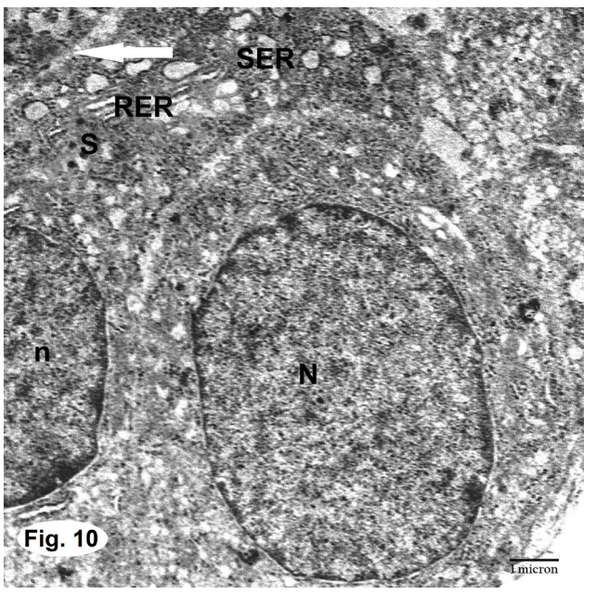


Fig.10: An electron micrograph of ultrathin section in the thyroid gland of (group I) showing parafollicular or C-cell rests on clear basal lamina (arrow). The cell has oval nucleus (n) having clumps of peripheral heterochromatin and surrounded with clear nuclear membrane. Its cytoplasm contains few tubular cisternae of rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), and electron dense secretory granules (S). The cell is separated from the luminal colloid by a part of the cytoplasm of follicular cell containing euchromatic nucleus (N).(TEM×17500)

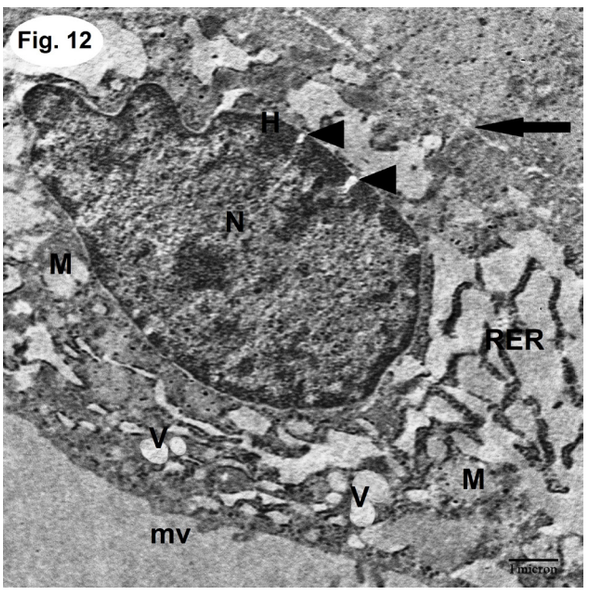


Fig.12: An electron micrograph of ultrathin section in the thyroid gland of (group II) showing follicular cell resting on basal lamina (arrow). Its nucleus (N) appears indented having peripherally located heterochromatin (H) with widening of nuclear pore complex (arrow heads). The cytoplasm shows marked dilated cisternae of rough endoplasmic reticulum (RER), degenerated mitochondria (M). Its apical border has scarce microvilli (mv) with areas of complete depletion. Notice: presence of vacuoles (v) within the cytoplasm (TEM×17500)

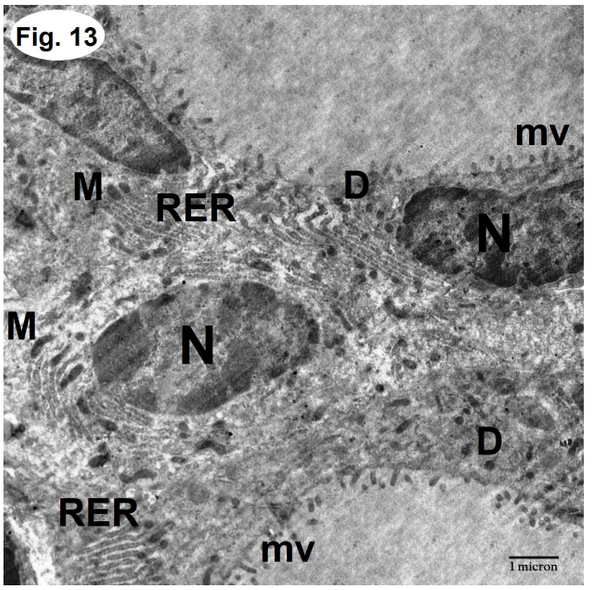


Fig.13: An electron micrograph of ultrathin section in the thyroid gland of (group III) showing three adjacent follicular cells with oval heterochromatic nuclei (N). The cytoplasm shows multiple tubular cisternae of rough endoplasmic reticulum (RER), mitochondria (M) and electron dense colloidal droplets (D). The apical border shows microvilli (mv) more or less like that of the control group projecting into the lumen. (TEM×17500)

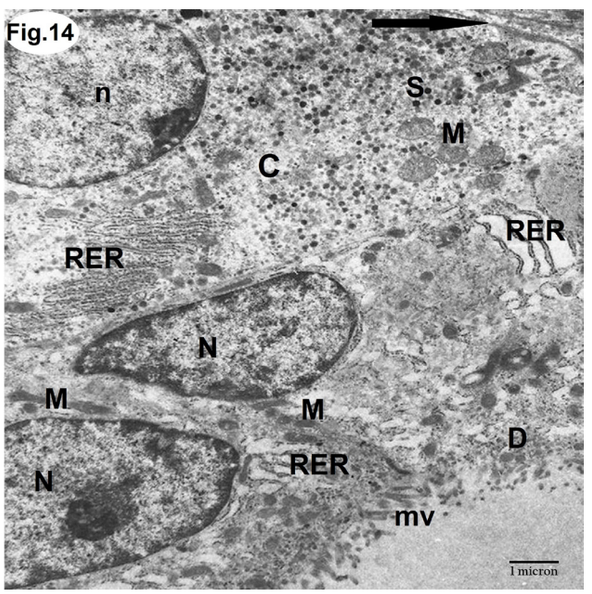


Fig.14: An electron micrograph of ultrathin section in the thyroid gland of (group III) showing two adjacent follicular cells with oval euchromatic nuclei (N). The cytoplasm houses mitochondria (M), electron dense colloidal droplets (D), and few moderately dilated tubular cisternae of rough endoplasmic reticulum (RER). The apical border shows microvilli (mv) more or less similar to control group projecting into the lumen. The parafollicular (C) cell is noticed with rounded nucleus (n) resting on clear basal lamina (arrow). Its cytoplasm shows multiple cisternae of rough endoplasmic reticulum (RER), mitochondria with clear cristae (M), and multiple electron dense secretory granules (S). (TEM X 17500)

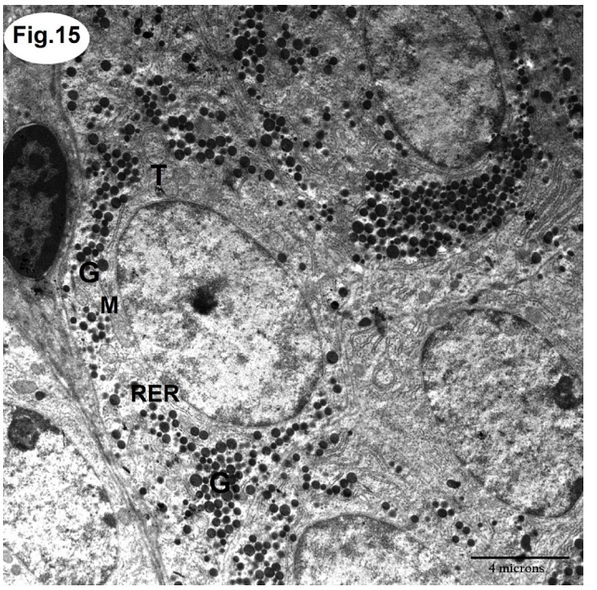


Fig.15: An electron micrograph of ultrathin section of pars distalis of anterior pituitary gland of (group I) showing angular shaped thyrotrophic cells (T) with elongated cytoplasmic processes. The nucleus (N) appears oval euchromatic with prominent nucleolus. The cytoplasm has multiple small spherical electron dense granules limited to the periphery (G). Well-developed mitochondria (M) and rough endoplasmic reticulum (RER) is seen within the cytoplasm. (TEM×8000)

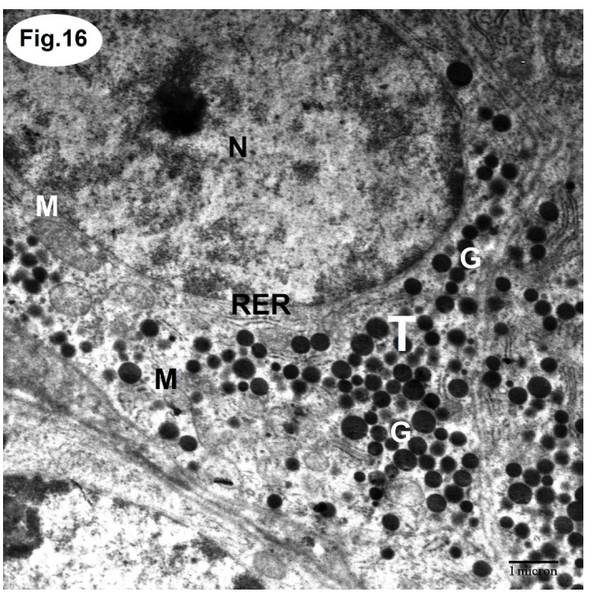


Fig.16: Higher magnification of the previous electron micrograph showing part of thyrotrophic cell (T) within the anterior pituitary of group I having euchromatic nucleus (N) with prominent nucleolus. The cytoplasm has multiple electron dense granules (G), well developed elongated mitochondria (M) and tubular cisternae of rough endoplasmic reticulum (RER). (TEM×17500)

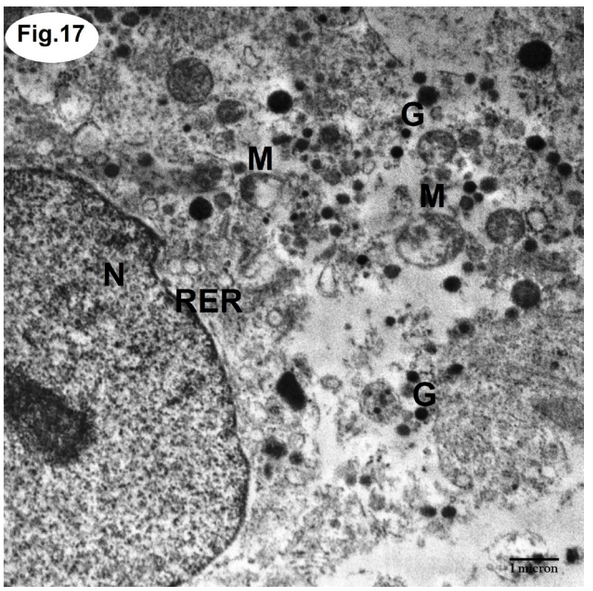


Fig.17: An electron micrograph of ultrathin section of pars distalis of anterior pituitary gland of (group II) showing thyrotrophic cell with irregular nucleus (N). The cytoplasm appears rarefied having few electron dense granules (G), degenerated mitochondria (M), and dilated cisternae of rough endoplasmic reticulum (RER) are also seen. (TEM×17500)

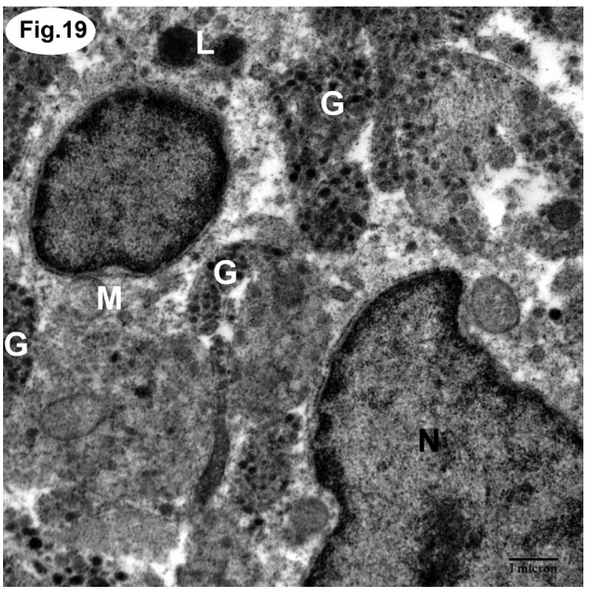


Fig.19: An electron micrograph of ultrathin section of pars distalis of anterior pituitary gland of (group II) showing two adjacent thyrotrophs with irregular heterochromatic nuclei (N). Their cytoplasm appears rarefied with aggregation of electron dense granules (G) in groups, degenerated mitochondria (M) and lysosomes (L). (TEM×17500)

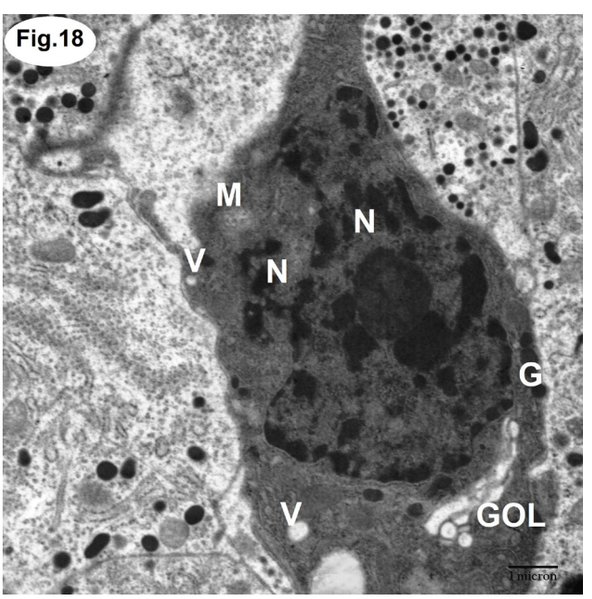


Fig.18: An electron micrograph of ultrathin section of pars distalis of anterior pituitary gland of (group II) showing thyrotrophic cell having irregular heterochromatic fragmented nucleus (N). The cytoplasm shows few electron dense cytoplasmic granules (G), dilated Golgi apparatus (GOL), and degenerated mitochondria (M). Small vacuoles (V) are seen within the cytoplasm. (TEM×17500)

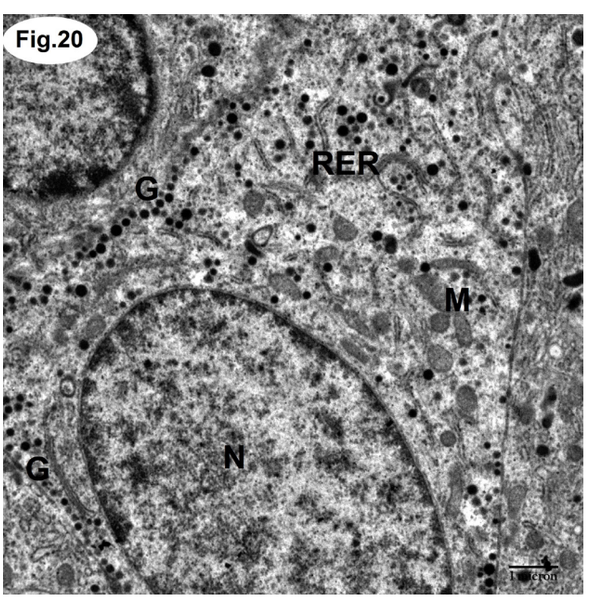
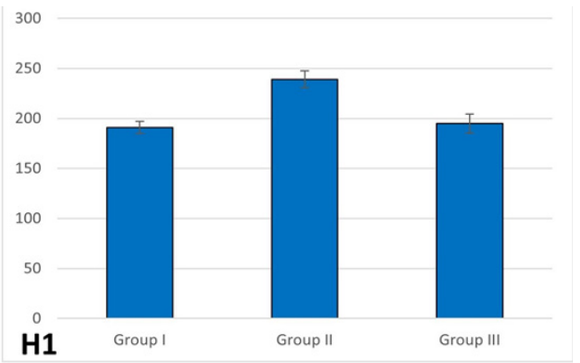


Fig.20: An electron micrograph of ultrathin section of pars distalis of anterior pituitary gland of (group III) showing thyrotrophic cell with euchromatic nucleus (N) having regular smooth nuclear envelope. The cytoplasm has mitochondria (M) and cisternae of rough endoplasmic reticulum (RER) more or less similar to control. Multiple spherical electron dense granules (G) limited to the periphery of the cytoplasm are noticed. (TEM×17500)

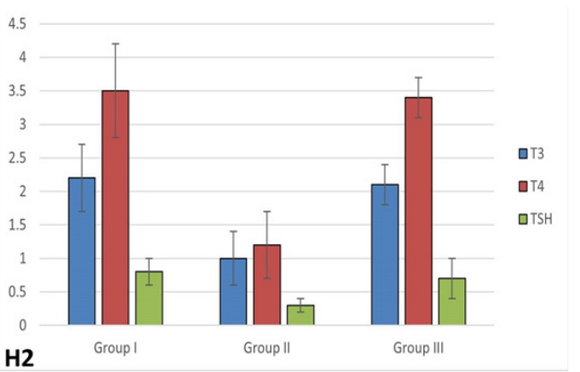
Table 1: Effect of different treatments on biochemical and histological parameters of thyroid and pituitary glands

	Group I	Group II	Group III	*P-value*
	Mean ±SD	Mean ±SD	Mean ±SD	
Body weight (g.)	190.9±6.0	238.9±8.5	194.8±9.6	P1=0.000 P2=0.309 P2=0.000
Serum T3(ng/ml)	2.2±0.5	1.0±0.4	2.1±0.3	P1= 0.000 P2= 0.351 P3= 0.000
Serum T4(ng/ml)	3.5±0.7	1.2±0.5	3.4±0.3	P1= 0.000 P2= 0.074 P3= 0.000
Serum TSH(µu/ml)	0.8±0.2	0.3±0.1	0.7±0.3	P1=0.000 P2=0.142 P3=0.000
Area% of collagen fibers	12.0±0.9	74.4±1.3	12.1±1.6	P1=0.000 P2=0.416 P3=0.000
Area% of PAS positive reaction	37.8±0.4	14.5±1.1	37.3±0.8	P1=0.000 P2=0.068 P3=0.000
Mean color intensity of Bax	8.9±1.0	73.8±2.8	9.1±1.8	P1=0.000 P2=0.295 P3=0.000
Mean no. of positively immune-stained TSH thyrotrophs/field	30.9±1.5	10.4±1.1	29.6±1.6	P1=0.000 P2=0.082 P3=0.000
Mean no. of electron dense granules within the cytoplasm of thyrotropes/field	167±9.5	66.3±7.5	160.3±6.7	P1=0.000 P2=0.086 P3=0.000

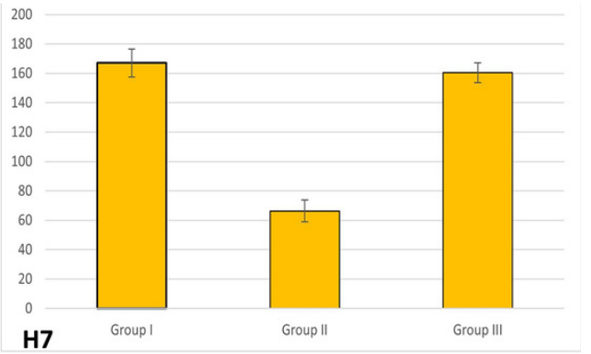
P1: comparison was done between MSG treated group (group II) and control group (group I).
P2: comparison was done between MSG and Pomegranate peel treated group (group III) and control group (group I).
P3: comparison was done between MSG and Pomegranate peel treated group (group III) and MSG treated group (group II).
P value > 0.05 = Non-significant
P value < 0.05 = Significant
P value < 0.001 = Highly significant.



Histogram 1: Mean body weight in grams



Histogram 2: Mean serum level of T3, T4&TSH



Histogram 7: Mean number of electron dense granules within the cytoplasm of thyrotropes

DISCUSSION

MSG is a well-known, widely used food additive and flavor enhancer[25]. Daily intake of it can alter pituitary-adrenal axis and induce elevated corticosterone and ACTH serum levels[25,26], induce hepatotoxicity, nephrotoxicity[27], endocrine disorders[10], dysregulate hypothalamic–pituitary gonadal axis[28] and increased risk of obesity and diabetes[29].

As a result of previous possible adverse effects, the present work suggests the possible use of natural material to achieve relieve from MSG effect, hence Pomegranate was used in the current study. From all parts of pomegranate, peel was chosen due to its high content of antioxidants as previously stated by some authors[30]. Recent studies reported the neuroprotective and anti-inflammatory effect of Pomegranate on animal models[31,32]. So, the current study was designed to evaluate the histological, histochemical and immunohistochemical changes within the pituitary thyroid axis with the use of MSG and the possible protective role of Pomegranate peel extract powder in male albino rats.

In the current study, male rats were used in line with the previous published research denoting that, male rats are more sensitive and vulnerable to thyroid toxicant as compared to female rats due to the higher TSH levels in male rats[33]. However, feminine sex hormones and estrogen may modify thyroid diseases risk[34].

In the present study, administration of MSG induced highly significant increase in mean body weight as compared to control group (*P value*<0.001). The finding in the present work run in harmony with the work of some scientists[10] who observed obesity of rats treated with MSG. This increase in body weight might be due to hypothalamic arcuate nucleus lesion with hindered insulin and leptin signals in brain followed by hyperinsulinemia and hyperleptinemia as previously explained by some researchers[35]. Leptin is an appetite regulating hormone control appetite, regulate energy and body weight[36]. MSG intake may induce injury in the intra-hypothalamic arcuate-para-ventricular nuclear axis (ARCN) which plays main role in food-uptake regulation by NPY (neuropeptide – Y). Declining in neurons secreting NPY as a squeal to ARCN damage can explain the hyperphagia associated with MSG intake[5].

MSG intake induced biochemical changes in the form of a significant reduction in the mean serum level of T3, T4 and TSH (*P value*<0.001) as compared to control group. Due to a negative feedback mechanism regulating hypothalamic-pituitary-thyroid axis, reduction in T4 lead to increased serum TSH, but in the present manuscript, decreased T3 and T4 associated with reduction in TSH, consistent with the results reported by some scientists[37]. Declining in thyroid hormones may be related to enhanced production of ROS (reactive oxygen species) and free radicals in the hypothalamic –pituitary axis leading to their aging. Hypothalamic-hypophyseal-thyroid axis had a vital role in thyroid function[38]. Finally, endocrine deficiency and functional loss occurs resulting from the previous axis aging[39].

Microscopic examination of the thyroid gland specimens after MSG intake showed irregular thyroid follicles having distorted shape. Follicular epithelial cells lining varying from cubical to columnar with oval nuclei and decline in colloid content were also, reported after MSG treatment. Connective tissue septa between the follicles appeared widely separated with dilated congested blood vessels and inflammatory cellular infiltration. Our findings run in accordance with other researchers[5,10] who observed irregular thyroid follicles with disruption of their basement membrane after administration of higher doses of MSG.

Also, some of the follicles in the present work were partially lined with multiple cell layers (stratification) with their lumens contained less colloid material and detached follicular cells with cytoplasmic vacuolation and degenerated nuclei. This observation was partially in agreement with the findings detected by some authors[10] who denoted follicular cells hypertrophy and hyperplasia after MSG intake in rat model.

By ultrastructure examination of thyroid sections of MSG treated group, the follicular cells had few, short microvilli with areas of complete depletion. The follicular cells appeared distorted resting on irregular basal lamina. Their nuclei appeared indented with peripherally located heterochromatin and widening of nuclear pore complex. Their cytoplasm showed disturbed, dilated cisternae of rough endoplasmic reticulum with degenerated mitochondria. The parafollicular (C) cell had segmented heterochromatic nucleus with Its cytoplasm showed disturbed, dilated cisternae of rough endoplasmic reticulum, degenerated mitochondria, and lysosomes. These findings were in line with the studies constructed by some scientists[5,40]. They observed that follicular cells had areas of small or total loss of microvilli, contained pyknotic or heterochromatic nuclei with asymmetric nuclear membrane after different doses of MSG.

These degenerative changes observed with MSG treatment might be due to oxidative stress caused by

MSG. MSG can generate oxygen-derived free radicals and reactive oxygen species (ROS). These previous substances are harmful for biological systems reacting with DNA, lipids, and proteins resulting in cellular damage as stated by some authors[41].

Other researchers explained the possible causes of cellular damages associated with MSG treatment. Some referred them to existence of glutamate receptors in endocrine system, and hypothalamus[42,43]. Furthermore, glutamate receptors had an essential role in pathogenesis of MSG induced disorders. Glutamate disturbs the activity of voltage-gated potassium channels leading to enhanced intracellular calcium content. Rising calcium influx triggered copious uptake of calcium by mitochondria that may lead to initiate cell death by mechanisms such as the delivering of proapoptotic factors and enhanced generation of reactive oxygen species as previously stated by some researchers[44]. Other researchers also, denoted MSG neurotoxicity on hypothalamic-pituitary-gonadal system function[45]. Other investigators resorted MSG adverse effects due to decline of ascorbic acid level which induce oxidative injury in rat various organs[46,47].

The recorded inflammatory cellular infiltration in the present study might be due to lipid peroxidation occurring with MSG intake that in turn, may trigger and sustain an inflammatory response as previously reported in a former study[48]. Furthermore, related inflammatory cellular infiltration observed in MSG-treated rats may be due to glutamate that can encourage inflammation with enhanced production of tumor necrosis factor-α, that represent the chief proinflammatory cytokine as documented by some scientists[49].

Dilated congested blood vessels are being due to suppression of prostaglandins synthesis, as these compounds are expected to be involved in blood flow regulation. This result agreed with the work of some researchers[50] who demonstrated congestion of testicular blood vessels following MSG treatment.

Mallory`s Trichrome -stained thyroid sections revealed increased collagen fibers in between the thyroid follicles that were confirmed by a highly significant increase in the mean area percentage of collagen fibers in MSG treated group (*P value*<0.001) as compared with control group. The previous finding was in accordance with the results conducted by some authors[7]. They attributed the enhanced collagen synthesis to the transformation of fibroblasts within the connective tissue septate in-between the follicles to the more synthetic myofibroblasts induced by ROS overproduction. They also, documented that lipid peroxidation resulted in increased collagen fibers thickness among thyroid interfollicular spaces. Moreover, some authors explained this finding to be due to consecutive epithelial cells damage and regeneration with new fibers secretion resulting in collagen fibers overproduction[51].

In the current study, there was decline in PAS positive reaction after MSG treatment both in distorted follicular basement membrane and colloid content as proved morphometrically by highly significant decrease in the mean area percentage of PAS-positive reaction (*P value*<0.001) as compared to control group. The present study findings were in harmony with the work of some researchers[10,52]. The glycogen depletion in follicular basement membrane may be due to either defective glycogen synthesis following follicular degeneration or increased glycogenolysis.

In the present study, MSG induced a strong positive immunoreaction for BAX in follicular and inter follicular cells that was confirmed by a highly significant increase in area percentage of BAX-positive immunoreaction (*P value*<0.001) as compared with control group. This result indicates raised apoptosis and decline cellular proliferation as previously documented by some authors[15]. This could clarify that Bax activation plays an essential role in the mechanism of MSG induced tissue damages. Moreover, some authors explained the occurrence of apoptosis due to DNA damage as a consequence of enhanced ROS production[53].

On examination of pars distalis of anterior pituitary In MSG treated group showed basophilic thyrotrophs with pale vacuolated cytoplasm and dark, small nuclei present with dilated and congested blood capillaries. Their cytoplasm had dilated cisternae of rough endoplasmic reticulum, dilated Golgi apparatus and lysosomes with few separate and aggregated electron dense secretory granules. The current study findings agreed with some authors[54,55]. They explained alterations in thyrotrophs due to decreased thyroid hormones levels which usually associated with altered anterior pituitary glycoprotein hormones. On the other hand, the present work results were in contradiction with some researchers, who demonstrated no observable histopathologic effect of MSG administration for three weeks on anterior pituitary gland in adult rat models[56]. They attributed their findings may be due short duration of MSG uptake.

The reported dilated cisternae of rough endoplasmic reticulum, dilated Golgi apparatus and Few secretory granules that was confirmed morphometrically in pituitary thyrotrophs after MSG intake could be proof of disrupted protein synthesis induced by disturbed stimulation of TSH receptors in thyrocytes. RER dilation could be due to protein retention resulting from impaired processing, folding, or transporting to the target sites[57]. The disturbance in protein production may induce apoptosis with depletion of essential proteins essential for cellular homeostasis resulting in cellular degeneration[58].

In the present study, there was a very weak immunoreaction for TSH immunostaining in MSG treated group as revealed by a highly significant decrease in the mean number of positively immune-stained TSH thyrotrophs in this group (*P value*<0.001) as compared with control group. This also, was supported in the current manuscript by reduction in TSH level after MSG uptake. These

findings may be justified by long standings thyrotrophs stimulation as a result of reduction of thyroid hormone secretion by the injured thyroid, which induce cellular exhaustion as previously stated by some researchers[58]. Moreover, the similar findings were documented in the pituitary glands of experimentally produced hypothyroid rats[51,59] and untreated patients suffering from primary hypothyroidism[60]. Most of the metabolic processes in the body are controlled by hypothalamic-pituitary-thyroid axis as it constitutes essential part of the endocrine system. Thyroid, hypothalamus, and pituitary glands are all strongly linked by feedbacks mechanisms between thyroid hormones, TSH and TRH[61]. In the previous axis, synthesis, and release of TSH in pituitary gland is mainly regulated by thyroid hormones which are disrupted due to MSG uptake.

In this work, the results revealed that, supplementation of the rats with Pomegranate peel extract in concomitant with MSG, animal body weight was reduced to normal level while, increased biochemical parameters such as T3, T4, TSH towards normal as proved morphometrically by highly significant increase in their value as compared to MSG intake group. Previous studies explained that, intake of pomegranate reduces hunger sense and body weight and inhibit hyperlipidemia and obesity[62]. These effects may be due to pancreatic lipase inhibition and reduction of caloric intake[63].

Marked improvements of the thyroid and pituitary sections of rats received Pomegranate in concomitant with MSG, regarding histological, ultrastructural and immunohistochemical changes resulting from MSG treatment. This denotes the beneficial effect of Pomegranate peel extract in improving MSG adverse effects due to its properties as a rich source of natural antioxidants, immunomodulatory, anticancer, anti-inflammatory, anti-atherosclerosis and anti-microbial[64]. Pomegranate exerts anti-inflammatory activities by decreasing lipoxygenase and cyclooxygenase enzymes activities. Cyclooxygenase induced arachidonic acid degradation into prostaglandins that plays the main role in inflammation[65]. Moreover, previous study referred the anti-inflammatory property of Pomegranate to suppression of neutrophil activation and reducing lipid peroxidation[66].

Pomegranate peel extract suppresses the oxidative stress, hence, declines apoptosis and fibrosis in injured tissues. It contains phenolic substances which can inhibit lipid peroxidation by free radicles scavenging or antioxidant enzymes activation such as glutathione peroxidase, glutathione reductase and sodium oxide dismutase[67]. Polyphenol compounds as ellagic acid, anthocyanins, hydrolysable tannins documented to be strongly correlated with anti-inflammatory and antioxidant properties of pomegranate peel extract[68]. Other scientists come to the conclusion that; Pomegranate can protect catalase antioxidant enzyme against toxic chemicals effect[69].

Furthermore, the neuroprotective effect of pomegranate peel extract against Alzheimer and Parkinson diseases due to its contents of anti-inflammatory substances as previously documented by some researchers[70]. Other researchers added that, the effectiveness of pomegranate peel extract might be due to suppression of oxidative stress, α-synuclein aggregation, allowing neuronal survival and enhance mitochondrial aldehyde dehydrogenase activity[71]. Additionally, pomegranate reduces neuroinflammatory mediators as IL-6, NO, TNF-α[72].

CONCLUSION

MSG induces both thyroid and pituitary gland injury. Supplementation with pomegranate peel extract could be protective against MSG adverse effects. It can be used as a dietary supplement for the prevention of both thyroid and pituitary injury and diseases.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة بالمجهر الضوئي و الإلكتروني للتأثير الوقائي المحتمل لمستخلص قشور الرمان علي الضرر المستحدث ب احادي جلوتامات الصوديوم علي محور الغدة النخامية- الدرقية في ذكور الجرذان البيضاء البالغة

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الخلفية والهدف من البحث: احادي جلوتامات الصوديوم هي إحدى الإضافات الغذائية الجديدة. أصبح استخدام مضادات الأكسدة الطبيعية في الوقت الحاضر كمستخلص قشر الرمان استراتيجية علاجية جذابة للحد من مخاطر الإصابة بالأمراض.

صممت هذه الدراسة لتقييم آثار مادة احادي جلوتامات الصوديوم على محور الغدة النخامية- الدرقية والدور الوقائي المحتمل لمستخلص قشر الرمان.

المواد والطرق المستخدمة: تم تقسيم اربعين من ذكور الجرذان البيضاء البالغة إلى ثلاث مجموعات. المجموعة الأولى: (االضابطة) التي تم تقسيمها إلى مجموعتين فرعيتين١A و١ B. المجموعة الثانية (المعالجة ب احادي جلوتامات الصوديوم) تلقت احادي جلوتامات الصوديوم بجرعة (٦ مجم / جم / يوم). المجموعة الثالثة (المعالجة ب احادي جلوتامات الصوديوم ومستخلص قشر الرمان) تلقت احادي جلوتامات الصوديوم & مستخلص قشر الرمان بنفس جرعة المجموعة الفرعية ١ B والمجموعة الثانية. في نهاية التجربة ، تم استخراج الغدة النخامية والغدة الدرقية ومعالجتها لإجراء دراسات نسيجية وهستومناعية ودراسات بالمجهر الإلكتروني.

النتائج: تسبب احادي جلوتامات الصوديوم في تشويه حويصلات الغدة الدرقية و وظهور مؤشرات لتدهور تركيب خلاياها علي مستوي المجهر الضوئي و الإلكتروني.. بينما ظهرت خلايا basophiles و thyrotrophs في الغدة النخامية متضررة. ومع استخدام مستخلص قشر الرمان تحسنت هذه التغيرات بشكل واضح.

الاستنتاج: أظهرت هذه الدراسة أن مستخلص قشر الرمان يحسن التغيرات النسيجية والكيميائية الضارة التي يسببها احادي جلوتامات الصوديوم على محور الغدة النخامية-الدرقية.