Original Article

Effects of Ginkgo Biloba Extract Administration on the Thyroid Gland of the Adult Male Albino Rat: A Histological, Histochemical and Morphometric Study

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ABSTRACT

Introduction: Ginkgo biloba extract (EGb) has been used for many years as a dietary supplement and as a herbal treatment for a wide range of diseases. However, it was found to be associated with adverse effects. Studies should be conducted to provide an information on its safety in medicine.

Aim of the Work: The goal of this study was to investigate the effects of two different doses of EGb on the thyroid gland of the adult male albino rat on the functional, histological, histochemical and morphometric levels.

Material and Methods: Thirty adult male albino rats each weighing (200-250 g) were categorized into three equal groups. Group 1(control, n=10) were given distilled water. Group 2 (low dose, n=10) was given (100 mg EGb /kg b.w.). Group 3 (high dose, n=10) was given (200 mg EGb /kg b.w.). All the treatment was given orally by daily gavage for 4 weeks. At the designated time of the study, blood samples were taken for determination of serum (T3, T4 and TSH). After anesthesia, rats were scarified and thyroid lobes were dissected and processed for light and electron microscopic examinations. Follicular epithelial height, follicular diameter and colloid area % means of all groups were registered and compared.

Results: EGb treated animals (groups 2 and 3) demonstrated a significant decreased serum levels of T3 and T4 and a significant increase of TSH levels when compared to the control group. The follicular cells height was significantly increased, while the follicular diameter and colloid area % were significantly decreased in groups (2&3) on comparing to control. The EGb-treated animals showed histological changes in the form of irregular follicular cells, follicular cell hypertrophy and hyperplasia, deeply stained follicular nuclei, dilated profiles of rER and Golgi apparatus, mitochondrial and lysosomal alterations, vacuolated cytoplasm and dilated interstitial blood capillaries. These changes were more pronounced in the group 3. While the follicular cells of the normally secretory active (control group) revealed a week basal lamina PAS reaction and a moderate colloid PAS reaction, the inactive follicles of groups 2 and 3 showed a moderate PAS reaction in the basal follicular lamina and a marked colloid PAS reaction. Our results demonstrated that EGb administration leads to a state of hypothyroidism.

Conclusion: Considerations should be taken in cases where the EGb is used as a necessary treatment.

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Key Words: Albino rat, ginko biloba extract (EGb), thyroid follicular cells.

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INTRODUCTION

A dietary supplement is a product that is intended to supplement the diet and contains a dietary ingredient as vitamins, minerals, herbs or any other botanicals ***U.S. Food and Drug Administration, (2011)***.

Ginkgo biloba (ginkgo) is one of the world's oldest living tree species. It is a unique tree with no close living relatives. The ginkgo tree has become popular as an ornamental tree and has been cultivated around the world ***Chan et al. (2007)***.

Various parts of the Ginkgo biloba plant have been used for food or medicine ***Kobayashi et al. (2011)***. Ginkgo seeds and leaves have been used in the Chinese and Japanese medicine for many thousand years ***Lin et al. (2014)***. The seeds were used to treat enuresis, asthma, cough, pyogenic skin infections and intestinal tract worm infections ***Van Beek & Montoro, (2009)***. On the other hand, gikgo leaves were applied to treat asthma, bronchitis, fatigue and memory loss ***Dunnick & Nyska, (2013)***. Ginkgo extract (EGb) is one of the most widely sold products

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in herbal dietary supplement in many countries ***Lindstrom et al. (2013)***.

The active chemical constituents of the plant includes the potent antioxidants flavonoids and terpene lactones. Other constituents are ginkgolic acids, glucose, rhamnose and d-glucaric acid ***Mahadevan &Park, (2008)***.

EGb is taken from the leaves of the Ginkgo biloba tree as a neuroprotective agent, an antioxidant, a free-radical scavenger, a membrane stabilizer and an inhibitor of platelet-activating promotes the brain circulation and treats Tinnitus ***National Toxicology Program, 2012 and Hilton et al. (2013)***.

EGb is typically taken by humans in the form of tablets or capsules at doses ranging from 120 to 240 mg/day for at least 6–8 weeks in order to observe and evaluate its efficacy and such dosages are not based on any scientific research ***Solaiman & El Gazaerly, (2015)***. Even though the long-term administration of GBE did not improve some conditions. Moreover, Gb products at the market exhibited a wide range of component concentrations ***Laws et al. (2012) and Vellas et al. (2012)***. Except for a few countries, EGb is not subjected to the same standards of premarket testing as drugs intended to treat, cure, or prevent diseases. As such, the extract does not need approval from the Food and Drug Administration (FDA) before it is marketed ***Ashar et al. (2007)***.

Previous animal studies as those by ***Koç et al. (1995)***, ***Mahadevan and Park (2008) and Dardano et al. (2012)*** focused on its therapeutic effects with little spotlights on its toxicity. They explored the antioxidant and anticancer potential of EGb.

Although EGb has long been used, there is not enough information to recommend its broad use and its long-term safety ***Rider et al. (2014)***. ***Nahin et al. (2009)*** recommended further investigations to define the clinical importance of the concomitant ginkgo use with medication, especially in elderly patients who consume multiple prescription drugs. Moreover, ***Miller (1998)*** noticed that herbal medicinal was being used by an increasing number of patients who typically do not advise their clinicians of concomitant use. He added that known or potential drug-herb interactions existed and should be screened for. ***Evans (2000)*** referred that ginkgo might alter bleeding time and should not be used concomitantly with warfarin sodium. ***Han et al. (2016)*** reported an allergic reaction case to ginkgo nut.

Zehra et al. (2010) and Rider et al. (2014) reported hepatotoxic effect and thyroid gland tumors in response to EGb intake. ***Solaiman and El Gazaerly (2015)*** studied the effect of EGb extract on the histological structure of the thyroid follicular cells on two different durations. They reported that the EGb effect on the thyroid follicular cells was with pronounced the longer term EGb administration. Nasal lesions ***Sells et al. (2007)*** and toxic effects on reproductive function in female ***Elmazoudy & Attia, (2012)*** were observed. In addition, a study by ***He et al. (2009)*** showed that the EGb damaged red blood cells by increasing cell fragility, changing the cellular morphology and inducing glutathione depletion. The results of selected National Toxicology Program (NTP) 2-year rodent studies for commonly used herbal medicines including ginkgo declared side effects from its usage ***National Toxicology Program, (2013)***. There was clear evidence for a carcinogenic activity in the liver of male and female mice where the incidences of hepatocellular adenoma, hepatocellular carcinoma were increased ***U.S. Food and Drug Administration, (2011)***.

Thus, this study aimed to spotlight on the effects of two different EGb oral doses in order to distinguish the histological, morphometrical and functional thyroid gland responses to each dose.

MATERIAL AND METHODS

1. Chemicals

In the present study ginkgo biloba leaf powder extract (EGb) was obtained in the form of gelatin capsules manufactured by EIMC United Pharmaceuticals (for EMA Pharm Pharmaceuticals, Egypt) (24% ginkgo flavones glycosides - 6% total ginkgolides; lactones).

2. Animals

The study was performed on a total number of 30 adult male albino rats; weighing 200–250 g each. The animals were housed in separate cages (five rats per cage) under standard laboratory and environmental conditions with free access to food (commercial rat food) and tap water. They were obtained from the Animal House of the Faculty of Medicine, Assiut University. Animal handling and treatment were carried out in accordance with The Laboratory Animal Protection Law proposed guidelines and protocols.

3. Experimental design

The rats were divided into three equal groups (10 rats each) and treated once a day, as follows:

(1) Group 1 (control group; n=10): received distilled water (1ml/rat) orally by daily gavage; as a vehicle for 4 weeks.

(2) Group 2 (low dose group; n=10): received EGb at a dose of 100 mg/kg; orally by daily gavage for 4 weeks. This dose is equivalent to the human therapeutic dose ***Zehra et al. (2010) and Solaiman & El Gazaerly, (2015***).

(3) Group 3 (high dose; n=10): received a dose of EGb 200 mg/kg; orally by daily gavage for 4 weeks ***Ashar et al. (2007)***.

Twenty-four hours after the last dose, rats were anaesthetized by intraperitoneal injection of 50 mg/kg sodium pentobarbital ***Liu et al. (2007)*** to obtain blood samples from eye balls by capillary tubes then they were sacrificed by decapitation.

4. Hormonal assay

Obtained blood samples were centrifuged at 3000 rpm for ten minutes. Sera were separated and stored at -20°C until performing the hormonal assay. Total serum T3 and T4 levels and TSH were measured by radioimmunoassay (RIA) using commercial kits ***Abdel-Dayem & Elgendy, (2009)***.

5. Histological study

After intracardiac perfusion with the fixative solution (2% gluteraldehyde in phosphate buffer), the thyroid glands were removed together with a portion of the adjoining trachea.

The right lobes of the thyroid gland of the animals were dissected, cut into small cubes (about 1mm3) and immediately fixed in 2% fresh gluteraldhyde at 4°C for 4 h. Thereafter, specimens were washed in 0.15 mol/l phosphate buffer, pH 7.4, for 2h (two changes), and then postfixed in 1% osmium tetroxide for 1h at 4°C. The specimens were dehydrated and embedded in epoxy resin. Semithin sections of 0.5µm thickness were cut using an ultramicrotome, stained with 1% toluidine blue, and examined under a light microscope. Ultrathin sections (50–80nm) were cut using the ultramicrotome and stained with uranyl acetate and lead citrate ***Hayat, (2000)***. The sections were examined by a transmission electron microscope (TEM) (JEOL JEM-100 CXII; Jeol, Tokyo, Japan) at the Assiut University Electron Microscopic Unit.

The left lobes of the thyroids were fixed in 3.7% phosphate-buffered formalin (pH =7.2), dehydrated through an ethanol series and xylol and embedded in paraffin. 5µm-thick paraffin sections stained with hematoxylin/eosin (H&E) and Periodic Acid Schiff (PAS) ***Bancroft, (2007)***, were analyzed on Leica DMLB light microscope (Wetzlar, Germany).

6. Morphometrical evaluation

With objective lenses of magnification 100 of a light microscope connected to a computer provided with the image j program the slides were examined ***Irving et al. (2007)***. The thyroid follicular cell height mean was determined from toluidine blue-stained semi-thin sections. The mean diameter of thyroid follicles, which was calculated as (max transverse diameter+max diameter perpendicular to the first one divided by 2) was measured in sections stained with H&E ***Rajab et al. (2015)***. Colloid area % was assessed in PAS stained sections ***Shady & Noor El-Deen, (2010)***. For each parameter, ten readings per animal in each group were recorded.

Statistical analysis

Data from the hormonal assay, follicular epithelial height, follicular diameter and colloid area % of all groups were expressed as means±SD. They were fed into the computer using statistical package for the social sciences (SPSS, version 16; IBM SPSS, Chicago, Illinois, USA) software package.

Statistical analysis was carried out using one-way analysis of variance and the post-hoc test (LCD) for pairwise comparison. The level of significance was set at a P value less than 0.05

RESULTS

I. Morphometrical Results

1. Hormonal assay

Animals of groups 2 and 3 (EGb treated groups) demonstrated a significant decreased serum levels of T3 and T4 and a significant increase of TSH levels when compared to the control group (Table 1).

2. Thyroid Morphometrical Results

The follicular cells height was significantly increased in both EGb treated groups (groups 2&3), while the follicular diameter and colloid area % were significantly decreased in both on comparing to control (Table 2 and Histogram 1).

3. Histological Results

1. Light microscopic results

The thyroid gland of control animals (group 1) consisted of thyroid follicles which appeared generally oval or rounded follicles lined by a single uniform layer of epithelium and filled with luminal acidophilic homogenous colloid. A bubbly or scalloping (knife chatters) appearance of the colloid was noticed. The follicles varied somewhat in size. The microfollicles were lined with cuboidal cells. They exhibited large vesicular rounded nuclei. Some follicles contained endocytotic vacuoles. The macrofollicles were lined with flattened or low cuboidal cells. Normal parafollicular were seen within the follicular basement membrane and individual or clustered between follicles The interstitium was consisting of strands of connective tissue that pervaded the gland creating a lobular structure and containing blood capillaries (Figures 1&4). Basal lamina of follicular cells showed weak PAS reaction while colloid showed a moderate reaction (Figure 9).

Histological examination of the thyroid gland of rats given low dose of EGb (group 2) revealed large follicles lined with irregular layer of follicular cells forming papillary projection into the follicular lumen. Follicles surrounded with irregular basal lamina were seen. Follicular cell hypertrophy and hyperplasia were noticed. The hypertrophy was characterized by enlargement of the follicular cells often assuming a tall columnar shape. Follicular hyperplasia was characterized by follicles lined with more than one layer of cells. Some follicular cells exhibited deeply stained nuclei or vacuolated cytoplasm. Separated cells from basal lamina were also observed. The luminal colloid appeared reduced in amount and contained desquamated cells. Some follicles revealed empty lumina. Dilated numerous blood capillaries were seen in the interstitium (Figures 2&5). Basal lamina of follicular cells showed a moderate PAS reaction while colloid showed a marked reaction (Figure 10).

Sections of EGb high dose treated rats (group 3) showed apparent histological alterations of the thyroid gland represented in a preponderance of the microfollicles over the macrofollicles and bizarre shaped follicles. Disorganized follicles with budding were seen. The basement membrane was disrupted in some views. Separated follicular cells from the basement membrane. Follicular cell hypertrophy and hyperplasia were noticed. Some areas showed closely packed follicles with narrow lumina. The proliferation of follicular cells was sometimes associated with papillary projections into the follicular lumens. Some follicles showed deeply stained shrunken irregular nuclei and vacuolated cytoplasm. The colloid contained exfoliated cells or even absent in most of the follicles. The interfollicular connective tissue showed infiltration by fibroblasts, mononuclear cells and mast cells. The parafollicular cells showed a normal appearance increase in number. Congested and dilated interfollicular blood capillaries were encountered (Figures 3&6-8). Basal lamina of follicular cells showed a moderate PAS reaction while the greatly decreased colloid content showed a marked PAS reaction (Figure 11).

2. Electron microscopical results

Electron microscopy revealed that thyroid follicles of the control group were lined with a single layer of cuboidal follicular cells and a colloid-filled lumen. The cells were situated on a thin basal lamina. A dense meshwork of capillaries encircled the thyroid follicles. The cells exhibited regularly dispersed apical microvilli. The cells had regular, euchromatic and basally located nuclei, well-developed rough endoplasmic reticulum (rER), mitochondria, Golgi complex, secretory vesicles, ribosomes and lysosomes. Intercellular junctional complexes were observed. C (parafollicular) cells having no direct access to the lumen separated from thyroid interstitium by follicular basal lamina with no colloid droplets, but with regular, euchromatic and centrally located nucleus (N) with a prominent nucleolus (n), mitochondria (M), ribosomes (r) and small argyrophil, dense core secretory granules were depicted (Figures 12-14).

After EGb low dose-treatment, thyroid follicles were seen limiting a narrow irregular lumen with irregular and dispersed apical fuzzy-like microvilli and shortening or even loss of microvilli and containing desquamated cells. Irregularly-shaped follicular cells resting on an irregular basal lamina adjacent to dilated congested (engorged) blood capillaries and containing shrunken, irregular and hyperchromatic nuclei, dilated profiles of rER, dilated Golgi apparatus, vacuoles, electron dense mitochondria with disrupted cristae, lysosomes and secretory vesicles sometimes seen filling a large portion of the cytoplasm were noticed. Follicular cells with degenerated cytoplasm were also observed. More or less normal parafollicular

cell having no direct access to the follicular lumen with rounded euchromatic nucleus and clear cytoplasm revealing numerous small argyrophil, dense core secretory granules and mitochondria were observed (Figures 15-19).

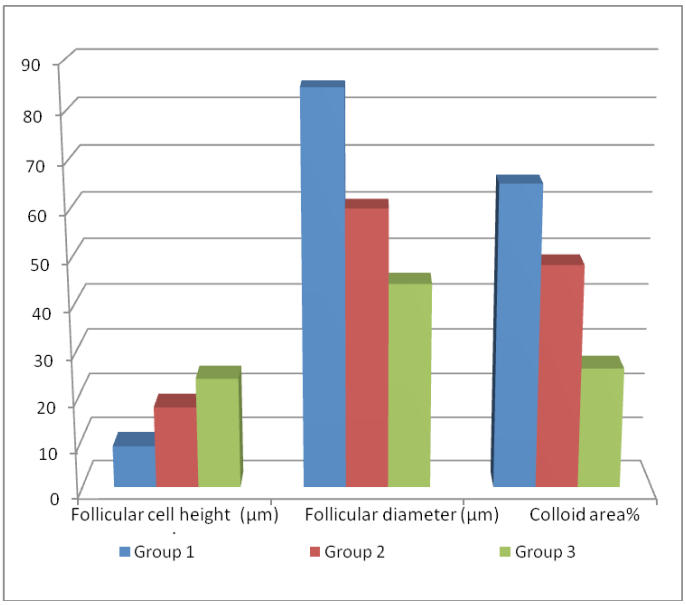
Transmission electron microscopic studies of the thyroid glands of group 3 rats showed morphological alterations. Follicular cells with fuzzy-like apical or abnormally absent microvilli and follicular lumen containing desquamated cells and/or papillary projection into the lumen. Follicular cells resting on irregular basal lamina and dilated congested blood capillaries encircled the thyroid follicles as well were depicted. Hyperchromatic, abnormal shaped and sometimes eccentric irregular nuclei with prominent nucleolus were seen. Vacuolated cytoplasm, colloid-filled secretory vesicles, prominent dilated profiles of rER, obviously dilated Golgi apparatuses, many lysosomes and electron dense, irregular disrupted or swollen mitochondria were encountered. On the other hand, more or less normal parafollicular cells having no direct access to the lumen, separated from the thyroid interstitium by follicular basal lamina and contained numerous small argyrophil, dense core secretory granules were noticed (Figures 20-25).

Table 1: T3, T4 and TSH serum means levels in the different groups

	Group 1 (control) n=10 Mean ± SD	Group 2 (low dose) n=10 Mean ± SD	Group 3 (high dose) n=10 Mean ± SD	*P value*		
				1 vs. 2	1 vs. 3	2 vs. 3
T3 ng/dL	54.36± 1.49	45.32± 0.79	39.88±1.19	0.000***	0.000***	0.000***
T4 ng/dL	66.60±1.10	59.44±0.85	49.73±1.12	0.000***	0.000***	0.000***
TSH µU/dL	34.28±1.08	36.30±1.60	46.02±1.13	0.000***	0.000***	0.000***

Table 2: Thyroid Morphometrical Results in the different groups

	Group 1 (control) n=10 Mean ± SD	Group 2 (low dose) n=10 Mean ± SD	Group 3 (high dose) n=10 Mean ± SD	*P value*		
				1 vs. 2	1 vs. 3	2 vs. 3
Follicular cells height (µm)	9.07±1.64	17.66±3.67	23.90±4.72	0.003**	0.000***	0.017*
Follicular diameter (µm)	84.60±12.85	60.00±11.59	44.22±5.66	0.003**	0.000***	0.035*
Colloid area %	65.10±3.88	48.19±12.80	26.11±14.24	0.035*	0.000***	0.009**



Histogram 1: Follicular cells height (µm), follicular diameter (µm) and Colloid area % in control (Group 1), EGb low dose treated (Group 2) and EGb high dose treated (Group 3) animals

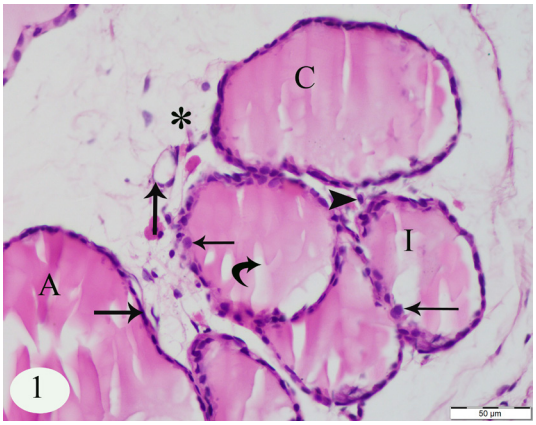


Fig. 1: A photomicrograph of control animals thyroid gland (group 1) showing oval and rounded thyroid follicles lined by a single uniform layer of epithelium and filled with luminal acidophilic homogenous colloid (C). A bubbly or scalloping (knife chatters) appearance of the colloid (curved arrow) was noticed. The follicles varied somewhat in size: the microfollicles (I) were lined with cuboidal cells exhibiting large vesicular rounded nuclei (←) and the macrofollicles (A) were lined with flattened or low cuboidal cells (→). The interstitium (*) was consisting of strands of connective tissue that pervaded the gland containing blood capillaries (↑) and normal parafollicular cells were seen clustered between follicles (arrowhead). H&E, ×400.

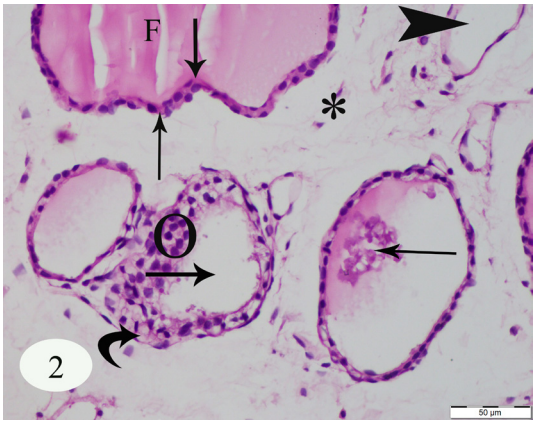


Fig. 2: A photomicrograph of EGb low dose treated rats thyroid gland (group 2) showing large follicle (F) lined with irregular layer of follicular cells (↑) forming papillary projection (↓) into the follicular lumen. Follicular cell hypertrophy (curved arrow) and hyperplasia (O) were noticed. The luminal colloid appeared reduced in amount and contained desquamated cells (←). Some follicles revealed empty lumina (→). Dilated blood capillaries (arrowhead) were seen in the interstitium (*). H&E, ×400.

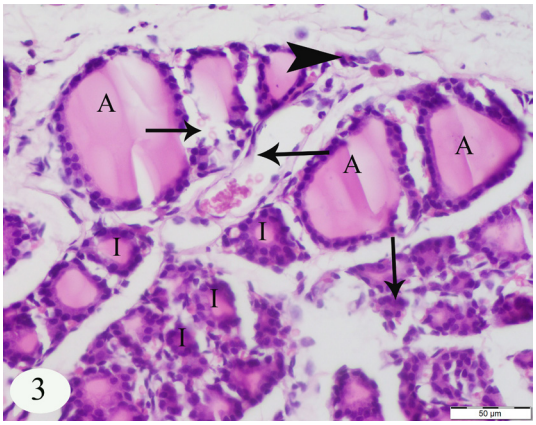


Fig. 3: A photomicrograph of EGb high dose treated rats thyroid gland (group 3) showing follicles of varying diameters with a preponderance of the microfollicles (I) over the macrofollicles (A). The basement membrane was disrupted (→) in some views. The interfollicular connective tissue showed infiltration by mononuclear cells (arrowhead). Parafollicular cells (↓) were noticed. Congested and dilated interfollicular blood capillaries were encountred (←). H&E, ×400

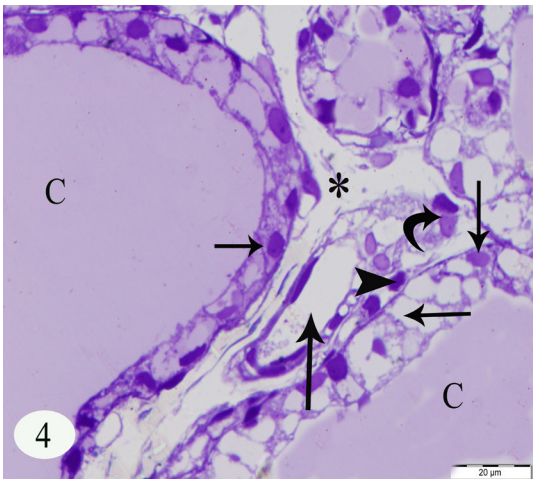


Fig. 4: A photomicrograph of control rats thyroid gland (group 1) showing (group1) thyroid follicles filled with luminal homogenous colloid (C). The follicles were lined with cuboidal cells with large vesicular rounded nuclei (→). Some follicles contained endocytotic vacuoles (←). Normal parafollicular cells were seen within the follicular basement membrane (↓) and individual (arrowhead) or clustered (curved arrow) between follicles. The interstitium was consisting of strands of connective tissue pervaded the gland (*) and containing blood capillaries (↑). Touilidine blue, ×1000

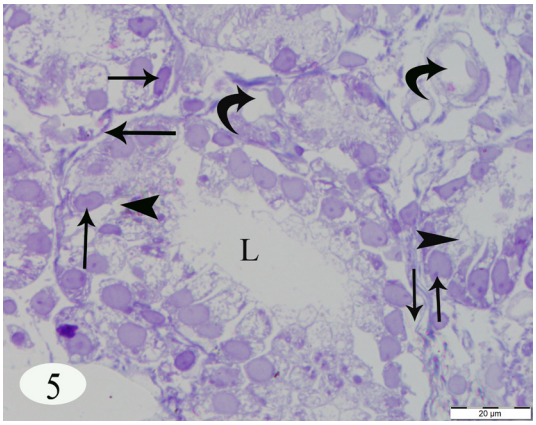


Fig. 5: A photomicrograph of EGb low dose treated rats thyroid gland (group 2) showing follicles surrounded with irregular basal lamina (←). Other follicles were encased by hypertrophied (↑) and vacuolated (arrowhead) cytoplasm. Some follicular cells exhibited deeply stained nuclei (→). Separated cells from basal lamina (↓), empty lumen (L) and numerous blood capillaries (curved arrows) were noticed. Touilidine blue, ×1000

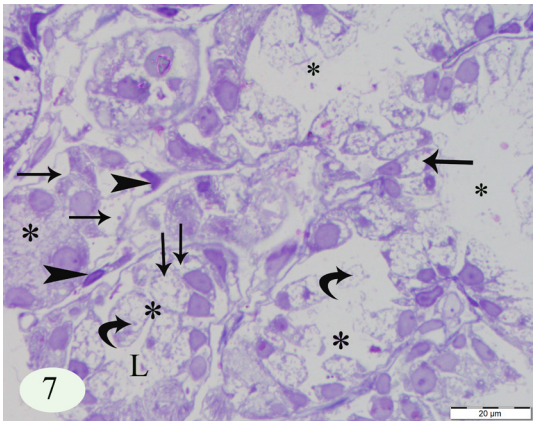


Fig. 7: A photomicrograph of EGb high dose treated rats thyroid gland (group 3) showing disorganized bizarre-shaped closely packed follicles (*), with narrow lumina (L), separated follicular cells from the basement membrane (→). Follicular cell hypertrophy (←) and hyperplasia (↓↓) was noticed. Deeply stained shrunken follicular nuclei (arrowhead) were seen. The follicular lumen contained exfoliated cells (curved arrow). Touilidine blue, ×1000

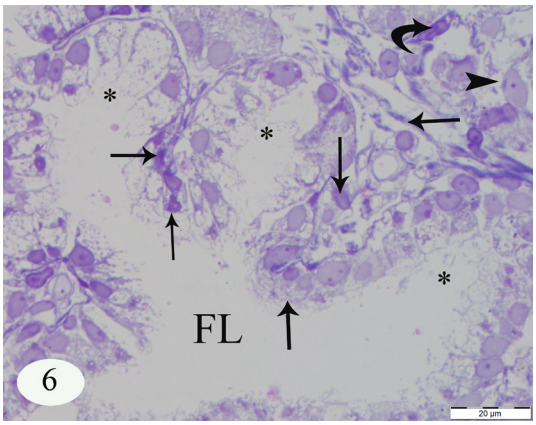


Fig. 6: A photomicrograph of EGb high dose treated rats thyroid gland (group 3) showing disorganized follicle with multiple budding (*). The proliferation of follicular cells was associated with papillary projections (↑) into the follicular lumen (FL). Other follicles showed deeply stained nuclei (→) and vacuolated cytoplasm (arrowhead). The interfollicular connective tissue showed infiltration by fibroblasts (←), mononuclear cells (↓) and mast cells (curved arrow). Touilidine blue, ×1000

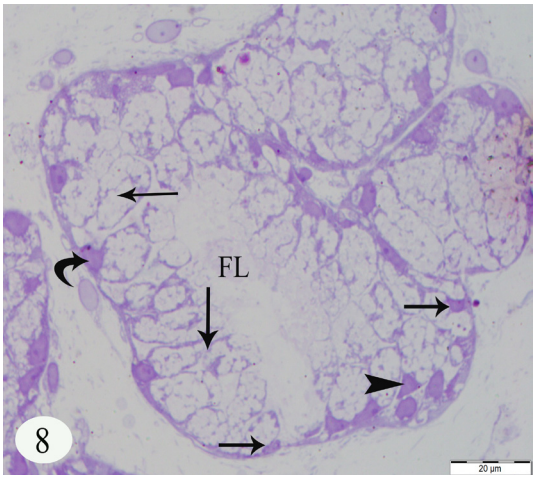


Fig. 8: A photomicrograph of EGb high dose treated rats thyroid gland (group 3) showing bizarre-shaped follicle. Follicular cell hypertrophy (↓), deeply stained (curved arrow), irregular (arrowhead) and shrunken (→) follicular nuclei, vacuolated cytoplasm (←) and absent luminal colloid (FL) were noticed. Touilidine blue, ×1000

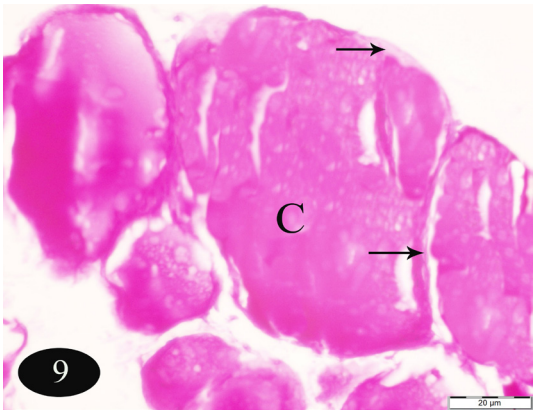


Fig. 9: A photomicrograph of thyroid gland of a control group (group 1) showing a moderate PAS reaction in the colloid (C) and a weak reaction in the basement membrane (→). PAS, ×1000

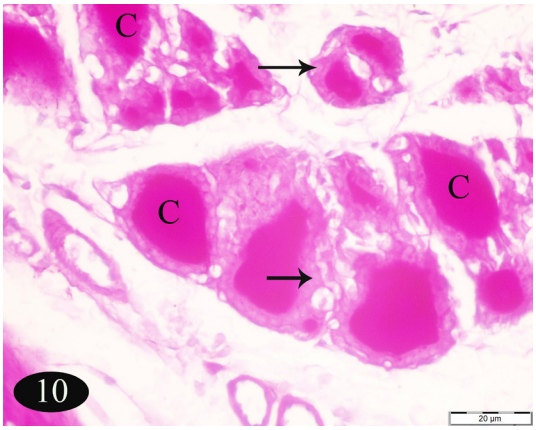


Fig. 10: A photomicrograph of thyroid gland of EGb low dose treated rats (group 2) showing a marked PAS reaction in the reduced-amount colloid (C) and a moderate reaction in the basement membrane (→). PAS, ×1000

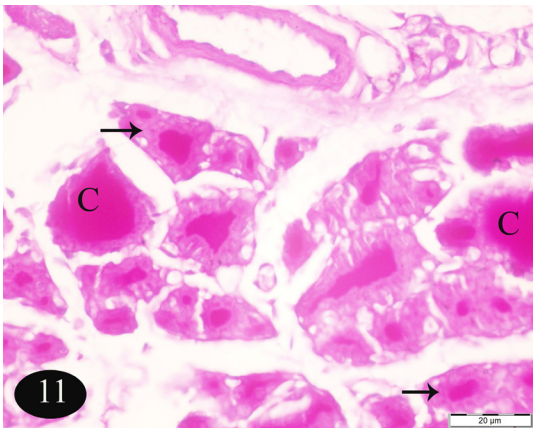


Fig. 11: A photomicrograph of thyroid gland of EGb high dose treated rats (group 3) showing marked PAS reaction in the greatly reduced-amount colloid (C) and a moderate reaction in the basement membrane (→). PAS, ×1000

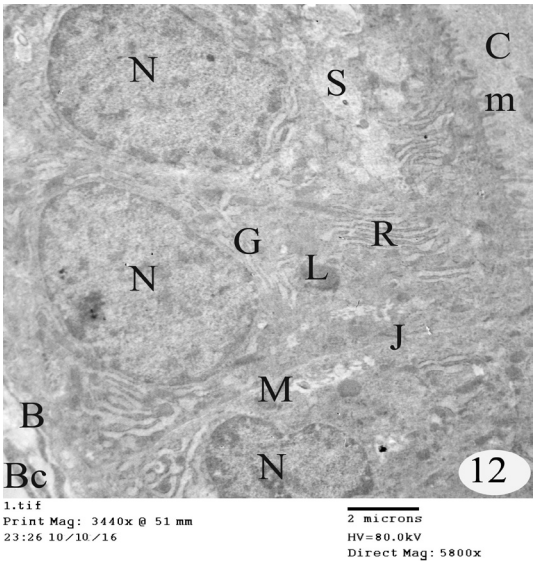


Fig. 12: An electron micrograph of thyroid follicles of control group (group 1) showing a single layer of cuboidal follicular cells and a colloid-filled lumen (C). The cells were situated on a thin basal lamina (B). A dense meshwork of capillaries (Bc) encircled the thyroid follicles. The cells exhibited regularly dispersed apical microvilli (m). The cells had regular and euchromatic nuclei (N), parallel cisterns of well-developed rough endoplasmic reticulum (R), mitochondria (M), Golgi complex (G), secretory vesicles (S) and lysosomes (L). Intercellular junctional complexes (J) were observed. ×5800

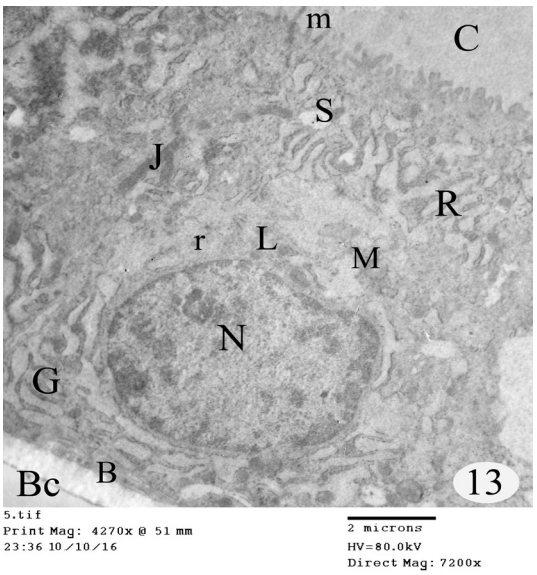


Fig. 13: An electron micrograph of thyroid follicles of control group (group 1) showing cuboidal follicular cell situated on a thin basal lamina (B) and a colloid-filled lumen (C). Blood capillaries (Bc) encircled the thyroid follicles. The cells exhibited regularly dispersed apical microvilli (m). The cell contained regular, euchromatic and basally located nucleus (N), well-developed rER (R), mitochondria (M), Golgi complex (G), secretory vesicles (S), ribosomes (r) and lysosomes (L). Intercellular junctional complex (J) was observed. ×7200

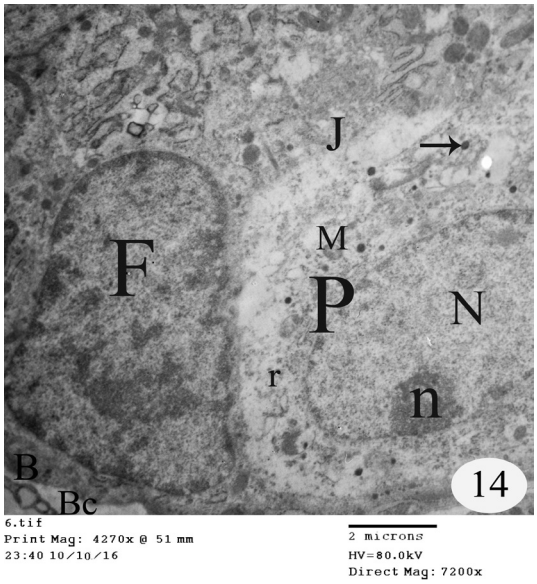


Fig. 14: An electron micrograph of thyroid follicles of control group (group 1) showing parafollicular cell (P) having regular, euchromatic and centrally located nucleus (N) with a prominent nucleolus (n), mitochondria (M), ribosomes (r) and small argyrophil, dense core secretory granules (→). Notice the no colloid droplets parafollicular cell was separated from thyroid interstitium blood capillaries (Bc) by a thin basal follicular lamina (B). An adjacent follicular cell (F) and intercellular junctional complexes (J) were depicted. ×7200

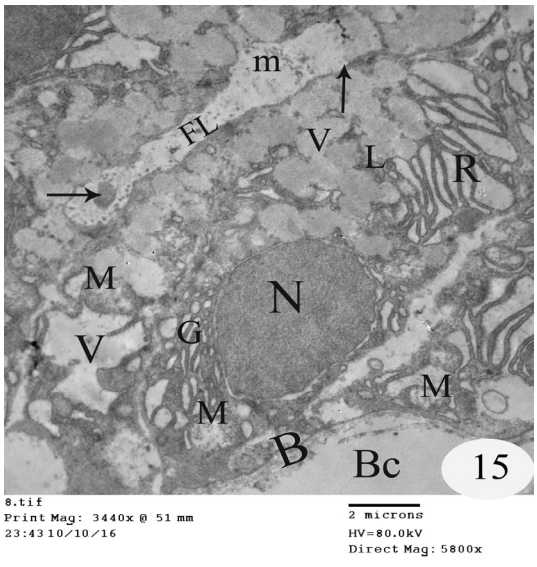


Fig. 15: An electron micrograph of EGb low dose treated rats thyroid gland (group 2) showing a thyroid follicle limiting a narrow irregular lumen (FL) with irregular and fuzzy-like microvilli (m) and shortening or even loss of microvilli (↑). Desquamated cells (→) were noticed in the lumen. Hyperchromatic nucleus (N), dilated profiles of rER (R), Golgi apparatus (G), mitochondria with disrupted cristae (M), lysosomes (L) and vesicles filling a large portion of the cytoplasm (V) were noticed. An irregular basal lamina (B) adjacent to a dilated blood capillary (Bc) was depicted. ×5800

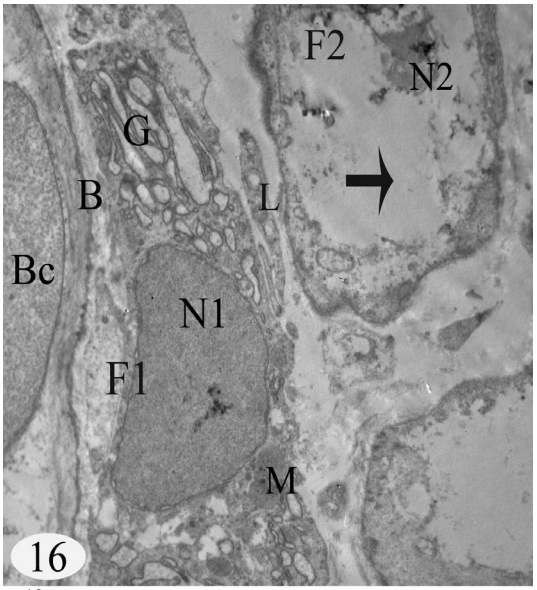


Fig. 16: An electron micrograph of EGb low dose treated rats thyroid gland (group 2) showing follicular cell (F1) with hyperchromatic nucleus (N1), dilated Golgi (G), lysosomes (L) and electron dense mitochondria (M). Follicular cell (F2) with irregularly shaped, hyperchromatic and shrunken nucleus (N2) and degenerated cytoplasm (→) was observed. Irregular basal lamina (B) and congested blood capillary (Bc) were seen. ×5800

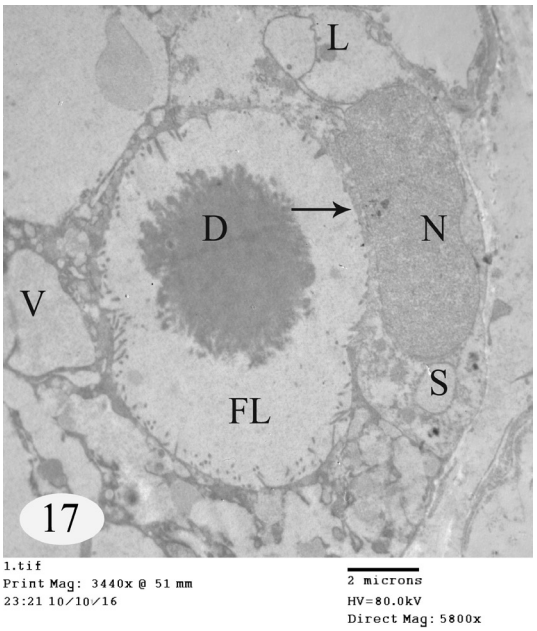


Fig. 17: An electron micrograph of EGb low dose treated rats (group 2) thyroid gland showing follicular lumen (FL) containing desquamated cells (D), loss of microvilli (→). Irregular follicular nucleus (N), vacuoles (V), lysosomes (L) and secretory vesicles were seen. ×5800

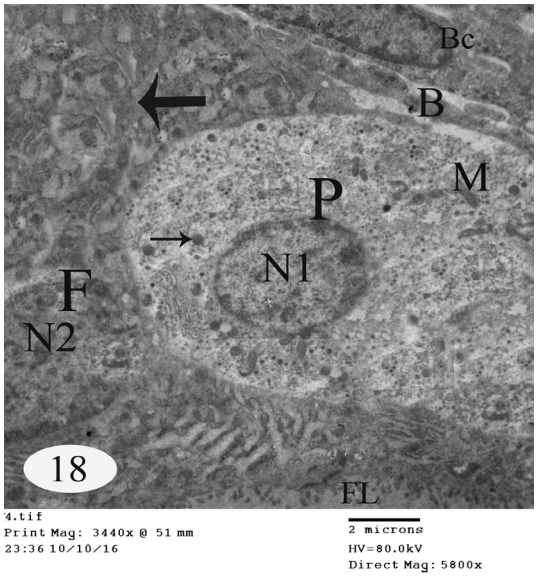


Fig. 18: An electron micrograph of EGb low dose treated rats thyroid gland (group 2) showing follicular cell lumen (FL) with dispersed apical microvilli. An engorged blood capillary encircled the thyroid follicles (Bc). More or less normal parafollicular cell (P) having no direct access to the follicular lumen (FL) with rounded euchromatic nucleus (N1) and clear cytoplasm revealing numerous small argyrophil, dense core secretory granules (→) and mitochondria (M). An adjacent follicular cell (F) with hyperchromatic nucleus (N2) and electron dense cytoplasm (←) was noticed. ×5800

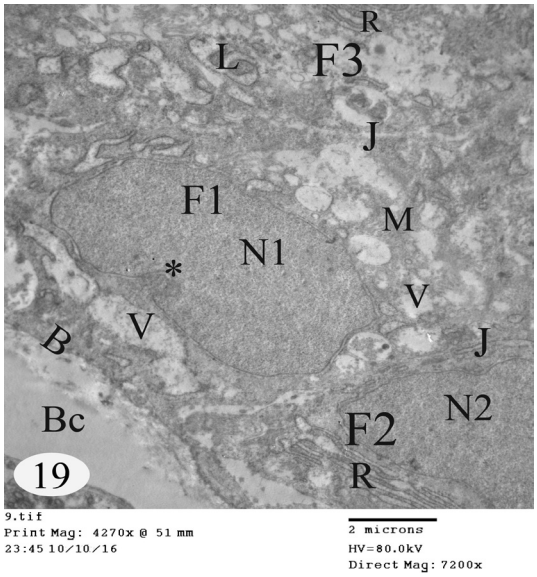


Fig. 19: An electron micrograph of EGb low dose treated rats thyroid gland (group 2) showing follicular cells resting on irregular basal lamina (B) adjacent to blood capillary (Bc). Follicular cell (F1) having an irregularly outlined nucleus (*) with condensed chromatin (N1), vacuoles (V) and mitochondria (M), follicular cell (F2) containing condensed chromatin (N2) and cisterns of rough endoplasmic reticulum (R) and follicular cell (F3) containing rER (R) and lysosomes (L). ×7200

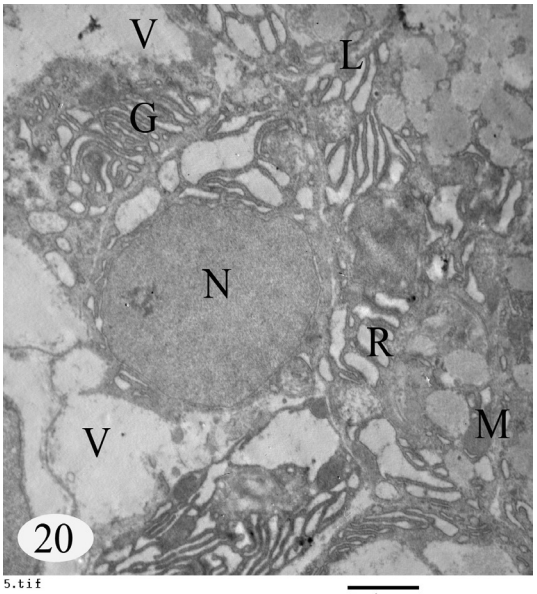


Fig. 20: An electron micrograph of EGb high dose treated rats thyroid gland (group 3) showing hyperchromatic nucleus (N), vacuolated cytoplasm (V), an obviously dilated profiles of rER (R), dilated Golgi apparatus (G), lysosomes (L) and electron dense mitochondria (M). ×5800

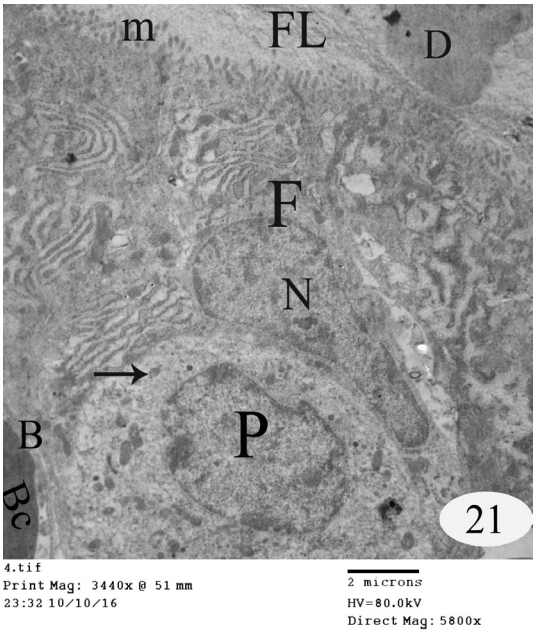


Fig. 21: An electron micrograph of EGb high dose treated rats thyroid gland (group 3) showing follicular cell (F) with abnormal shaped nucleus (N), fuzzy-like apical microvilli (m) and a follicular lumen (FL) containing desquamated cells (D). More or less normal parafollicular cell (P) having no direct access to the lumen, separated from the thyroid interstitium by follicular basal lamina (B) and contained numerous small argyrophil, dense core secretory granules (→). An engorged blood capillary (Bc) encircled the thyroid follicles. ×5800

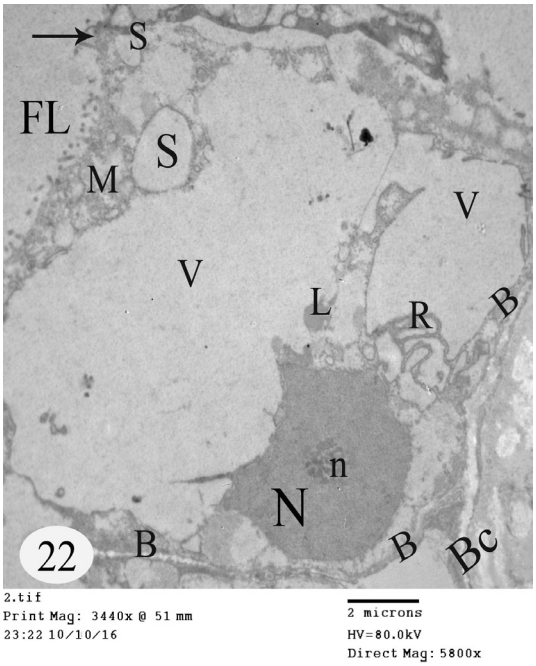


Fig. 22: An electron micrograph of EGb high dose treated rats thyroid gland (group 3) showing follicular cell having irregular, hyperchromatic and eccentric nucleus (N) with prominent nucleolus (n), papillary projection (→) into the lumen (FL), colloid filled secretory vesicles (S) , vacuolated cytoplasm (V), lysosomes (L), a prominent dilated rER (R) and swollen disrupted mitochondria (M). An irregular basal lamina (B) and dilated blood capillary (Bc) were encountered. ×5800

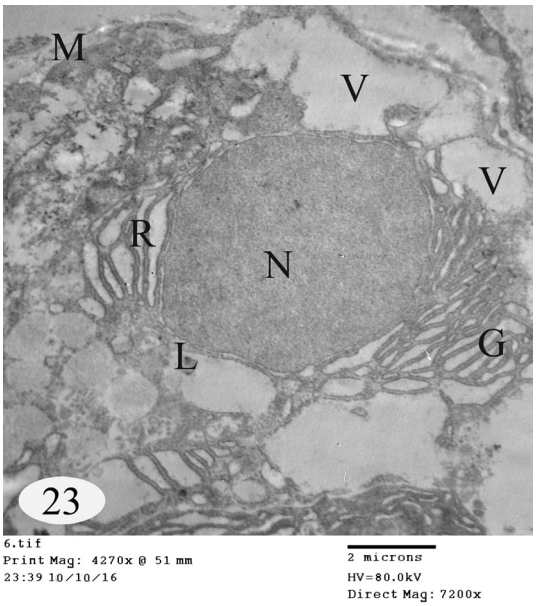


Fig. 23: An electron micrograph of EGb high dose treated rats thyroid gland (group 3) showing hyperchromatic nucleus (N), vacuolated cytoplasm (V), a prominent dilated rER (R), dilated Gologi apparatus (G), lysosomes (L) and electron dense mitochondria (M). ×7200

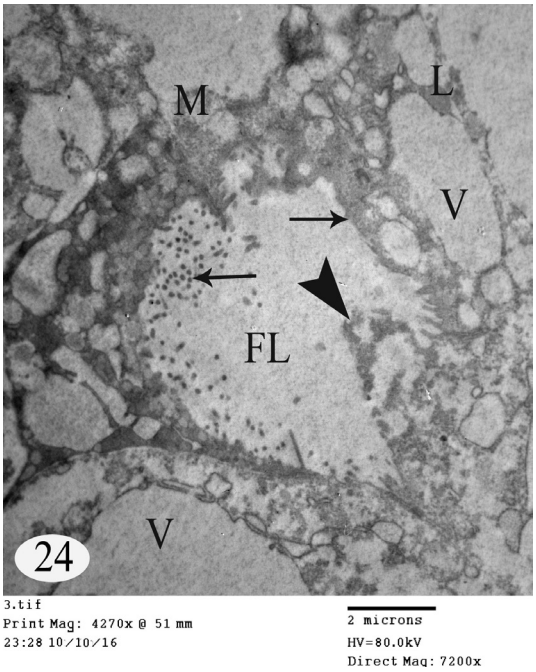


Fig. 24: An electron micrograph of EGb high dose treated rats thyroid gland (group 3) showing papillary projection of the follicular cell (arrowhead) into the lumen (FL), irregular microvilli (←), abnormally absent microvilli (→), vacuolated cytoplasm (V), many lysosomes (L) and swollen disrupted mitochondria (M). ×7200

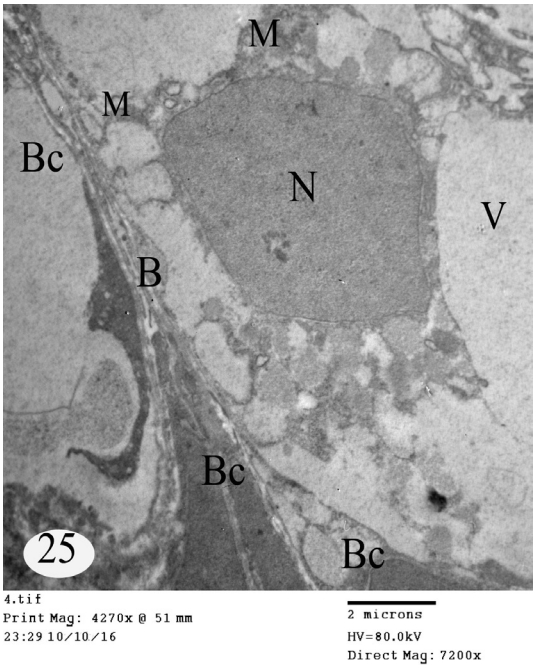


Fig. 25: An electron micrograph of EGb high dose treated rats thyroid gland (group 3) showing follicular cell resting on irregular basal lamina (B), hyperchromatic nucleus (N), irregular disrupted mitochondria (M), vacuolated cytoplasm (V) and dilated congested blood capillary (Bc) as well. ×7200

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CONFLICT OF INTERESTS

There are no conflicts of Interest.

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تأثيرات تناول مستخلص جنكوبيلوبا على الغدة الدرقية للفأر الأبيض الذكر البالغ : دراسة هستولوجية، هستوكيميائية ومورفومترية

رينيه رفعت بشرى
قسم التشريح الآدمى وعلم الأجنة- كلية الطب – جامعة أسيوط

ملخص البحث

المقدمة: يستخدم مستخلص الجنكوبيلوبا منذ العديد من السنوات كمكمل غذائى وعلاج عشبى لمدى واسع من الأمراض. ومع ذلك وجد أنه مرتبط بتأثيرات سلبية. لذلك وجب إجراء دراسات للحصول على معلومات عن سلامة استخدامه فى مجال الطب.

الهدف من الدراسة : فحص التأثير الناتج عن تناول جرعتين مختلفتين من مستخلص الجنكوبيلوبا على الغدة الدرقية لذكور الفئران البيضاء البالغة على المستوى الوظيفى والهستولوجى والهستوكيميائى والمورفومترى.

المواد المستخدمة وطرق الفحص: لقد استخدم ثلاثون من ذكور الفئران البيضاء البالغة و التى تزن (200 – 250 جم) و قسمت إلى ثلاث مجموعات متساوية. المجموعة الأولى (المجموعة الضابطة ،عشر فئران) أعطيت ماء مقطر. المجموعة الثانية (الجرعة المنخفضة ،عشر فئران) أعطيت 100مجم من مستخلص جنكوبيلوبا /كجم من وزن الجسم. المجموعة الثالثة (الجرعة المرتفعة ،عشر فئران) وقد أعطيت 200مجم مستخلص جنكوبيلوبا /كجم من وزن الجسم. كل العلاج تم إعطاؤه يوميا بواسطة أنبوب المعدة و لمدة أربعة أسابيع وفى الميعاد المحدد من الدراسة أخذت عينات الدم لتحديد نسب هرمونات ثلاثى يودو ثيرونين و الثيروكسين والهرمون المنبه للدرق. بعد التخدير تمت التضحية بالفئران وأخذت فصوص الغدة الدرقية و تمت معالجتها و فحصها بالميكروسكوب الضوئى و الالكترونى. ثم تم تسجيل و مقارنة ارتفاع الخلايا الجريبية و الاقطار الجريبية و النسبة المئوية لمساحة المادة الغروانية فى كل المجموعات.

النتائج: أظهرت الفئران المعالجة بمستخلص الجنكوبيلوبا نقصًا معنويًا فى مستوى هرمونىَ ثلاثى يودو ثيرونين و الثيروكسين مع زيادة معنوية فى مستوى الهرمون المنبه للدرق عندما قورنت بالمجموعة الضابطة. و لقد اظهرت الأقطار الجريبية و النسبة المئوية لمساحة المادة الغروانية نقصًا معنويًا فى المجموعتين الثانية و الثالثة عندما قورنت بالمجموعة الضابطة. هذا و قد أظهرت الحيوانات المعالجة بمستخلص الجنكوبيلوبا تغيرات هستولوجية فى صورة عدم انتظام و تضخم و فرط تنسج الخلايا الجريبية و زيادة صبغة أنويتها مع اتساع كل من : الشبكة الهيولية الباطنة الخشنة و جولجى و تغيرات فى الميتوكوندريا و الليسوسومات و تجوفات فى السيتوبلازم و اتساع فى الشعيرات الدموية. و لقد لوحظ أن التغيرات كانت أكثر وضوحا فى المجموعة الثالثة. بالنسبة إلى الخلايا الجريبية للمجموعة الضابطة ذات الإفراز الطبيعى أظهرت الصفائح القاعدية تفاعلا ضعيفا بينما أظهرت المادة الغروانية تفاعلا متوسطا لملون شيف . أما الخلايا الجريبية للمجموعتين الخاملتين الثانية و الثالثة فلقد أظهرت الصفائح القاعدية تفاعلا متوسطا بينما أظهرت المادة الغروانية تفاعلا قويا لصبغة (Schiff).

الخلاصة: تناول مستخلص الجنكوبيلوبا يؤدى إلى قصور فى إفراز الغدة الدرقية.