

The Potential Toxic Effects of Ginkgo Biloba Extract on Thyroid Gland of adult male and female Albino Rats: Light and Electron Microscopic study

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Abstract

Background: Ginkgo biloba extract (GBE) is an alcohol extract of leaves from the Ginkgo biloba tree. The extract is available in form of tablets or capsules and its main medical use to improve memory and brain function. In spite of widespread human exposure to relatively high doses over potentially long periods of time, there is few studies regarding the toxicity and carcinogenicity associated with GBE. **Aim:** to study the potential toxicity of GBE on thyroid gland. **Methods:** solutions containing GBE in corn oil were administered by peroral intubation to male and female rats five times a week for three months. Groups of 20 rats received 40 mg/kg of GBE, and another group received 500 mg/kg five times a week. Another group of rats given solutions of corn oil with no chemical added. Similar group of animal of rats were given nothing except food and water and served as the blank control groups. Thyroid hormones including thyroid stimulating hormone, total triiodothyronine (T₃), and total thyroxine (T₄) were measured during the study. At the end of the study, thyroid tissues were examined for every animal by light and electron microscope.

Results: In the small dose group, thyroid gland showed disorganized follicles of varying diameters with little amount of the colloid in some follicles while others demonstrated absent colloid with desquamated epithelial cells in their lumens. In the high dose group, thyroid gland was composed of very small follicles. Some follicles had no apparent lumina. Follicular cells were found in more than one layer (adenoma) with Infiltration of interfollicular spaces by fatty cells.

Conclusions: GBE is a complex mixture that induces only pathological changes in rat thyroid gland.

Keywords GBE, Thyroid,

Introduction

Ginkgo biloba extract (GBE) is an alcohol extract of leaves from the *Ginkgo biloba* tree. The ginkgo tree is also known as Maidenhair tree, and referred as living fossil being one of the oldest living trees, dating back approximately 200 million years and is native to China (Zheng 1992, Zhou and Zheng, 2003). Various parts of the *Ginkgo biloba* plant have been used for food or medicine (Kobayashi et al., 2011).

Ginkgo biloba leaves contain a complex mixture of chemical constituents, the main constituents in *Ginkgo biloba* leaves include terpenylactones, flavonol glycosides biflavones, proanthocyanidins, alkylphenols, phenolic acids, and polyphenols (van Beek and Montoro, 2009).

The exact formulation of ginkgo biloba extract (GBE) in the available pharmaceutical preparations may vary from manufacturer to manufacturer (Kressmann et al., 2002). The main current use of (GBE) is for the improvement of cognitive function, and the treatment of Tinnitus (NTP, 2013). Beside the well established therapeutic uses of GBE for improving cognitive functions and mild dementia in elderly, GBE

is used traditionally to relief heaviness of legs and cold sensation of extremities caused by minor circulatory disorders (EMA, 2014). However A meta-analysis study concluded that GBE is more effective for milder forms of cognitive impairment or at earlier stages of the dementing process (Jiang et al., 2013). Furthermore, another study showed that GBE has no benefit for age-related cognitive decline (Snitz et al., 2009).

GBE is usually give in a dose of 120-240 mg (in two to three daily doses) for treating dementia syndromes in oral liquid or solid form. For improving peripheral arterial occlusive disease, vertigo and tinnitus of vascular origin, 120–160 mg of native dry extract is given in two or three daily doses (Hilton et al., 2013).

The exact mechanism of action of GBE is not fully known. Data showed that human use of GBE caused reduction of blood viscosity, improvement of cerebral perfusion and reduction in platelet aggregation. In addition to, vasodilatation of blood vessels (EMA, 2014).

The therapeutic use of GBE as a neuroprotective agent is based on its functions as an antioxidant, a free-radical scavenger, a membrane stabilizer, and an inhibitor of platelet-activating factor via the terpeneginkgolide B (Ahlemeyer and Krieglstein, 1998). In addition to relaxation of endothelium by inhibiting guanosine monophosphate phosphodiesterase (WHO, 1999).

Despite the wide use of GBE, there is lack of sufficient knowledge regarding its potential to produce toxicity and carcinogenicity. Animal experiments of GBE mainly focused on demonstrating its anticarcinogenic and antioxidant properties without paying attention to its possible toxicity and carcinogenicity (Mahadevan and Park 2008). However, a recent study demonstrated that oral GBE intake resulted in increase in liver tumors in male and female mice and thyroid gland follicular cell tumors in male and female rats (Rider et al., 2014).

Material and Methods

Chemicals and reagents: GBE (capsules) was purchased from EIMC united pharmaceuticals, Egypt, Corn oil.

Oral LD50 of GBE was determined by an acute toxicity (LD50) study using the method of (Lorke, 1983). Oral LD50 of GBE for rats was 1000 mg/kg.

The experimental procedures were carried out after ethical approval according to the Guidelines of the National Institutes of Health for Animal Care followed within the Faculty of Medicine, Assiut University, according to referenced authority (Institute of Laboratory Animal Resources, 1996). A total of 40 healthy adult albino rats of 4–5 months old (with average weight 200 ± 50 g) were purchased from Faculty of Medicine's Animal House. The animals were maintained under temperature 22-25 °C, a 12 h light/dark cycle, ad libitum availability of pellet food and water.

Adult rats weighing 150-200 gram were maintained under optimal laboratory conditions. Feed and water were provided for ad libitum consumption. All groups were exposed to the main two stages of the experiment period as follows; the first 2 weeks were the pre-treatment period, followed by 90 days of treatment. The animals were divided into 4 groups. First group of animals served as the control group (10 rats received nothing except laboratory diet). The second group of 10 rats received the vehicle (Corn oil). The third group (20 rats) received 40 mg/kg (body weight) of GBE (low dose group), and the 4th group of 20 rats received 500 mg/kg (high dose group). The low dose (40 mg/kg) used as an equivalent to the usual therapeutic dose prescribed and ½ LD50 (400 mg/kg) was administered to the high dose group.

A solution of GBE dissolved in corn oil was administered five times a week by peroral intubation to rats for three months. All Animals were followed until the end of study duration, and all survivors were killed by neck dislocation under anesthesia with ether. After animals were sacrificed, a thorough necropsy were performed and sections of thyroid glands were taken

for histopathological study (Sheldon et al., 1969). Tissues were fixed in 10% neutral buffer formalin and processed for histopathological examination using routine paraffin embedding technique. Thyroid sections (5 mm thickness) were stained with hematoxylin and eosin (H&E) and examined by an optical microscope under 400 magnification (Pearse, 1979 ; Sheehan and Harapcbak, 1980). Semithin sections (0.5µm) were stained with toluidine blue and examined by an optical microscope under 1000 magnification. Ultrathin sections (80-90 nm) were prepared and examined at 100 kv by transmission electron microscopy (JEOL) (Bancroft & Cook, 1994). Thyroid hormones including thyroid stimulating hormone (TSH), total triiodothyronine (T3), and total thyroxine (T4) were measured at the start and at the end of the study. Blood samples were collected before scarification.

Results

Change in Body Weight

Body weight of each rat in all groups was measured at the start of the experiment, after 6 weeks and at the end of the experiment as shown in table (1). There was no significant difference in body weights between rats of groups administered GBE and control group.

Biochemical Results (thyroid hormones)

There were no significant changes in the levels of TSH, T3 and total T4 (table 2).

Examination of thyroid gland

A. **Gross picture:** Both groups low and high dose groups showed no significant increase in thyroid weights compared to the control group. Table (3)

B. **Microscopic picture:**

Light Microscopic examination:

1. Control group

The thyroid gland was composed of thyroid follicles, which appeared generally oval or rounded, lined with a single layer of cuboidal cells with vesicular nuclei and prominent nucleoli. The amount of colloid substance filling the lumina of the follicles was homogenous and varied from one follicular lumen to another. Thyroid stroma consisted of interlobular connective tissue, interfollicular cells and blood capillaries (Fig. 1 and 2).

2. Low dose group

The thyroid gland showed disorganization of follicles, many follicles were small. Some follicles showed little amount of the colloid and other follicles showed empty lumens with no colloid. Desquamated epithelial cells were noticed in lumens of some with disruption of their basement membranes. The follicular epithelium showed an apparent increase in thickness; and cells appeared to be enlarged with vacuolated cytoplasm and irregular shrunken nuclei. Widening of interfollicular spaces was also noticed with congestion and dilatation of the blood vessels. (Figs 3 and 4).

3. High dose group

Many small follicles were demonstrated. Some follicles showed no apparent lumens. Follicular cells were found in more than one layer. There was infiltration of interfollicular spaces by fatty cells. The

interfollicular blood vessels were dilated and congested. (Figs 5 and 6).

Electron microscopic Examination

1. Control group

Electron microscopic study of the thyroid follicles in control rats showed rounded follicles formed of cuboidal cells with rounded nuclei. The follicular cells were lying on a regular basement membrane and the follicles were surrounded with capillaries. The follicular cells demonstrated abundant rough endoplasmic reticulum, mitochondria apparatus and intracytoplasmic colloid vesicles. The apex of the cells near the lumen showed well-formed microvilli. (Fig. 7).

The parafollicular cells showed large nuclei, numerous variably sized secretory granules in the cytoplasm, clusters of ribosomes, and mitochondria. Adjacent blood capillaries could be observed. (Fig. 8).

2. Low dose group

Electron microscopic study of the thyroid follicles in rats of low dose group showed follicular

cells with atrophied disrupted apical microvilli, vacuolated cytoplasm, irregular shrunken nuclei, and large intracellular vacuoles. (Fig. 9).

Examination of parafollicular cells demonstrated euchromatic nuclei and nucleoli. The rough endoplasmic reticulum showed dilated cisternae. Few secretory granules were noticed. Interfollicular spaces showed congested blood capillaries. (Fig. 10).

3. High dose

Electron microscopic study of the thyroid follicles in rats of high dose group showed follicular cells that were columnar in shape with oval nuclei. Prominent intracytoplasmic colloid vesicles were noticed in addition to cytoplasmic vacuoles. Atrophied disrupted apical microvilli were seen. Irregularity of the nuclear membrane was noticed. (Fig. 11). Interfollicular spaces showed thick and dilated blood vessels with irregular nuclei of the lining endothelial cells (Fig. 12). Parafollicular cells showed reduced secretory granules, abnormal nuclei and vacuolated cytoplasm. (Fig. 13).

Table (1): Statistical Analysis of Body Weights in Different Groups at 3 Interval during Experiment Period by the One Anova Followed By Post Hoc Test.

Groups	At beginning of the study Mean \pm SD (grams)	P. Value	After one 6 week Mean \pm SD (grams)	P. Value	After 12 weeks (end of study) Mean \pm SD (grams)	P. Value
Group 1 (control)	172.6 \pm 21.08	-----	204.33 \pm 4.50	----	224.33 \pm 5.32	----
Group 1 (corn oil)	173.70 \pm 15.05	.875	202.90 \pm 3.38	.589	226.67 \pm 5.48	.285
Group 2 (Low dose)	176.10 \pm 14.47	.616	198.11 \pm 7.24	.024	223.00 \pm 4.90	.540
Group 3 (high dose)	173.90 \pm 10.91	.852	203.40 \pm .48	.725	223.67 \pm 3.81	.308

Values in each column followed by * are significantly different (≤ 0.05), and that followed by ** (≤ 0.001) are highly significantly different.

Table (2): Statistical Analysis of T3, T4 and TSH Levels at the Start and at the End of the Experiment of Each Group Compared By the One Anova Followed By Post Hoc Test

Groups	T3 At beginning of the study (Mean \pm SD)		T3 at end of study (Mean \pm SD)		T4 At beginning of the study (Mean \pm SD)		T4 at end of study (Mean \pm SD)		TSH At beginning of the study (Mean \pm SD)		TSH At end of the study (Mean \pm SD)	
	Mean \pm SD	P. Value	Mean \pm SD	P. Value	Mean \pm SD	P. Value	Mean \pm SD	P. Value	Mean \pm SD	P. Value	Mean \pm SD	P. Value
Group 1 (Blank)	0.61 \pm 0.22	-----	0.62 \pm 0.21	----	3.5 \pm 0.20	----	3.6 \pm 0.17	-----	13.29 \pm 0.27	----	13.38 \pm 0.23	---
Group 1 (corn oil)	0.62 \pm 0.32	.570	0.65 \pm 0.60	.215	3.6 \pm 0.15	.408	3.5 \pm 0.23	0.800	13.03 \pm 0.44	0.095	13.16 \pm 0.23	0.093
Group 2 (Low dose)	0.64 \pm .31	.272	0.66 \pm 0.62	.851	3.5 \pm 0.17	.939	3.5 \pm 0.18	0.480	13.19 \pm 0.47	0.481	13.24 \pm 0.42	0.266
Group 3 (high dose)	0.63 \pm .28	.509	0.66 \pm 0.61	.652	3.6 \pm 0.15	.664	3.6 \pm 0.09	0.496	13.44 \pm 0.28	0.489	13.44 \pm 0.30	0.735

Values in each column followed by * are significantly different (≤ 0.05), and that followed by ** (≤ 0.001) are highly significantly different.

Table (3): Statistical Analysis of Weights of Thyroid Glands in Different Groups of Rats by the One Anova Followed By Post Hoc Test.

Groups	Weight of thyroid gland in grams (mean±SE)	Sig.
Group 1 (control)	8.25 ± 0.82	-----
Group 1 (corn oil)	8.27 ± 0.73	.947
Group 2 (Low dose)	8.22 ± 0.59	.920
Group 3 (high dose)	8.32 ± 0.43	.815

Values in each column followed by * are significantly different (≤ 0.05), and that followed by ** (≤ 0.001) are highly significantly different.

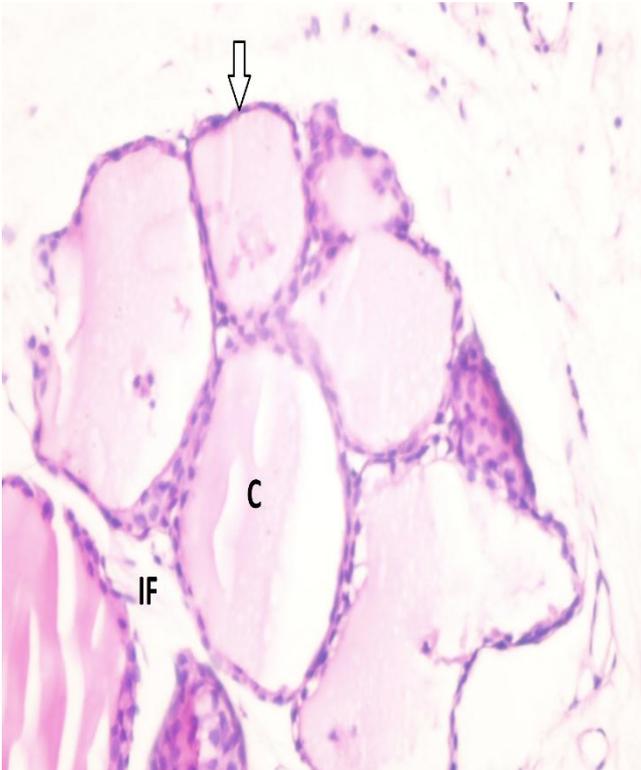


Figure 1: A photomicrograph of T.S section in thyroid of adult albino rat of control group showing normal thyroid follicles lined with single layer of epithelial cells (arrow). The follicles filled with homogeneous colloid (C). Notice: interfollicular tissue (IF) (H&E x400).

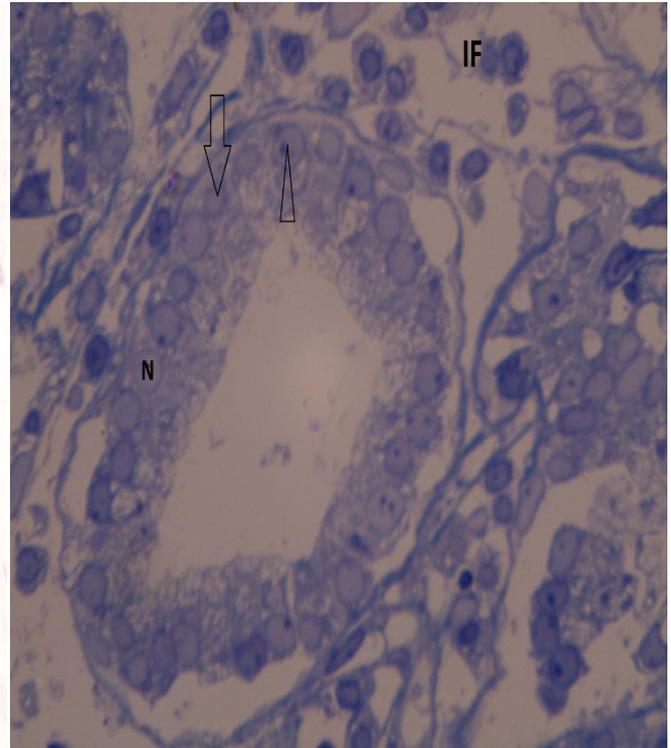


Figure 2: A photomicrograph of T.S section in thyroid of adult albino rat of control group showing normal thyroid follicles lined with single layer of cuboidal epithelial cells (arrow) with vesicular nuclei (N) and prominent nucleoli (arrow head). Notice the interfollicular cells (IFc) (Toluidine blue X1000).

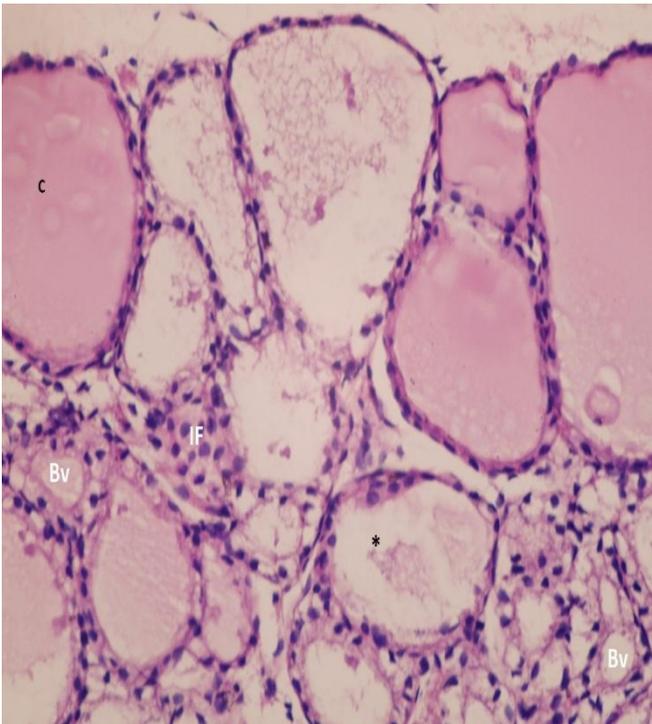


Figure 3: A photomicrograph of T.S section in thyroid of adult albino rat of the low dose group showing thyroid follicles of varying size. Some follicles demonstrate absent colloid (*). Notice the increased interfollicular tissue (IF) and dilated blood vessels (BV) (H&E x400).

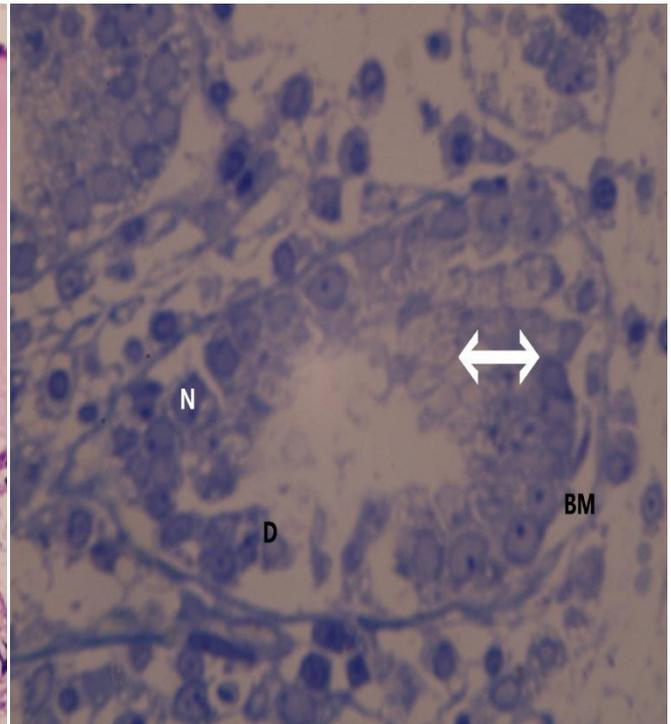


Figure 4: A photomicrograph of a semithin section in thyroid of adult albino rat of low dose group showing increased thickness of epithelial cells lining thyroid follicles (↔), with some cells having shrunken dark nuclei (N). Notice the desquamated cells (D) and the disrupted basement membrane (BM) (Toluidine blue X1000).

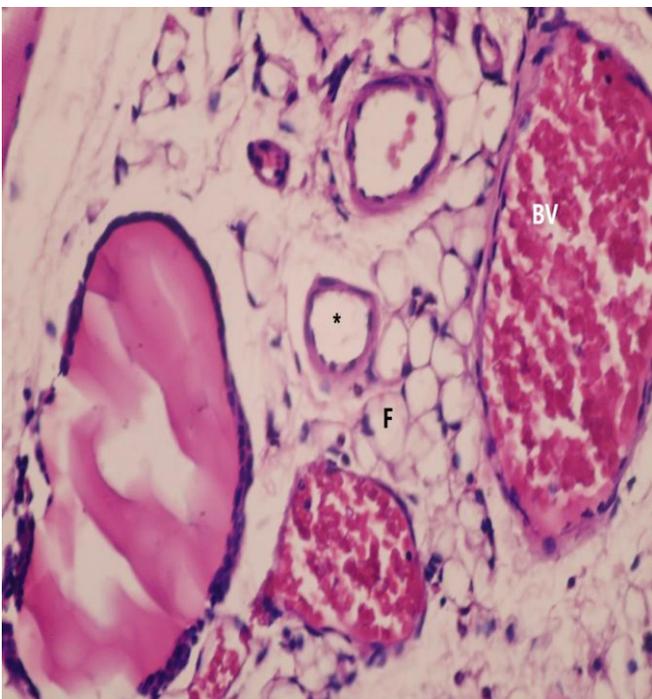


Figure 5: A photomicrograph of T.S section in thyroid of adult albino rat of the high dose group showing thyroid follicles of varying size. Many follicles are small in size and demonstrate absent colloid (*). Notice the congested dilated blood vessels (BV) and multiple fat (F) cells in interfollicular spaces (H&E x400).

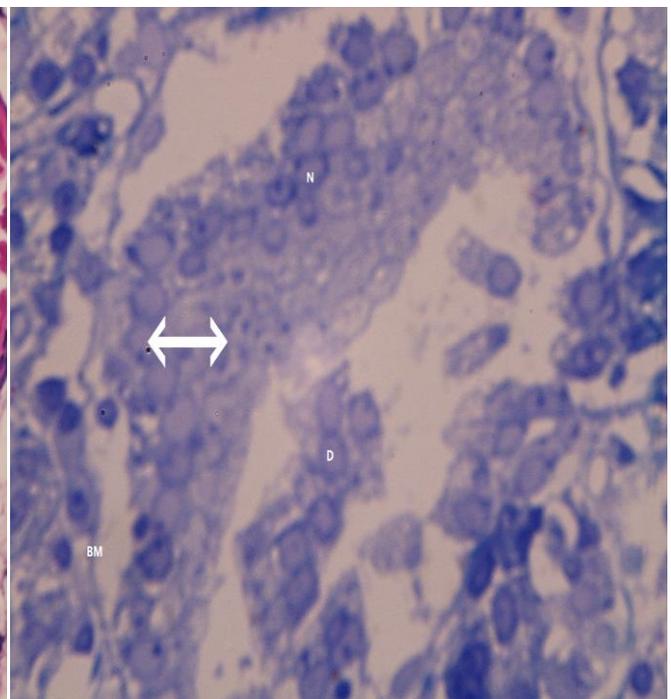


Figure 6: A photomicrograph of a semithin section in thyroid of adult albino rat of high dose group showing increased thickness of epithelial cells lining thyroid follicles (↔) with some cells having shrunken dark nuclei (N). Notice the desquamated cells (D) and the disrupted basement membrane (BM) (Toluidine blue X1000).

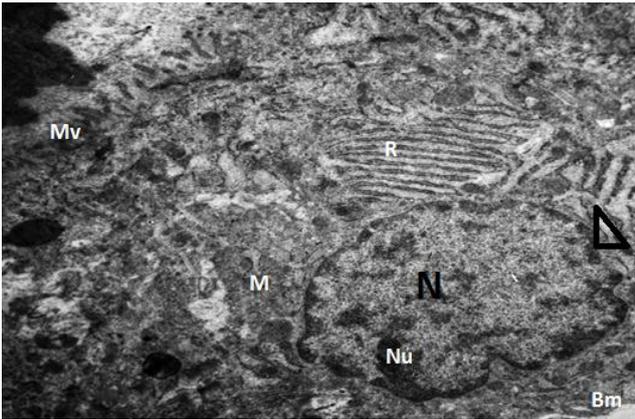


Figure 7: A transmission electron micrograph of thyroid of adult male albino rat of the control group showing part of the follicle line by cuboidal cells (F) having rounded euchromatic nuclei (N) and nucleoli (Nu) with peripheral clumps of heterochromatin (arrow head), showing, rough endoplasmic reticulum (R), Microvilli (Mv), Golgi apparatus (G), basement membrane (bm) x 7200.

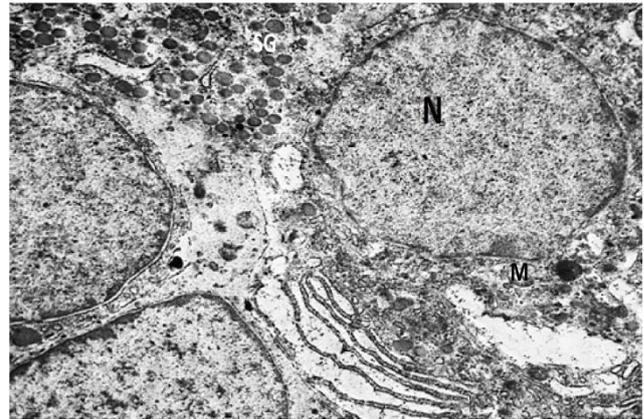


Figure 8: A transmission electron micrograph of thyroid of adult male albino rat of the control group showing part of the follicle. Parafollicular cell with regular nucleus (N), secretory granules (SG), mitochondria (M), x 7200.

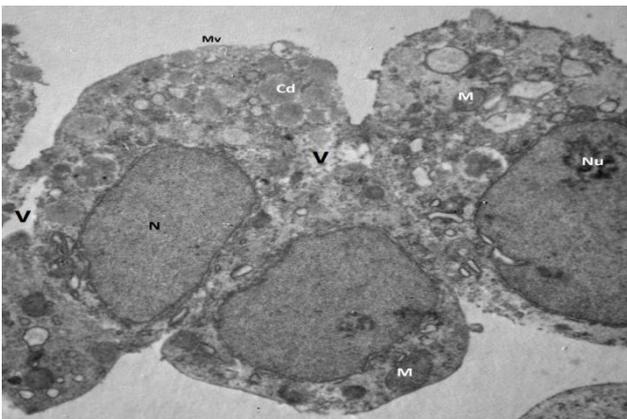


Figure 9: A transmission electron micrograph of an ultrathin section in thyroid of adult albino rat of the low dose group showing follicular cell with irregular nuclei (N), eccentric nucleoli (Nu), intracellular vacuoles (V), and electron dense mitochondria (M). Notice the atrophied microvilli (Mv) x 7200.

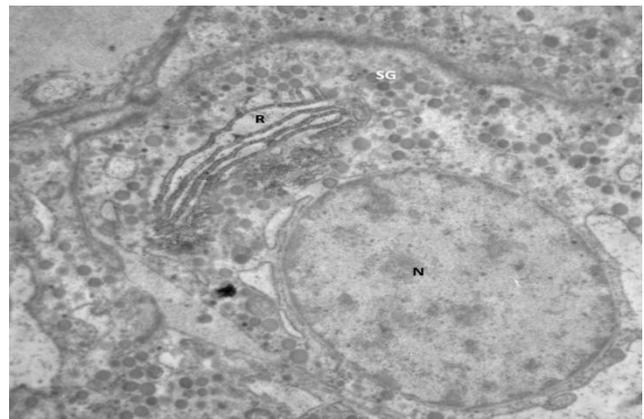


Figure 10: A transmission electron micrograph of an ultrathin section in thyroid of adult albino rat of the low dose group showing parafollicular cell having rounded euchromatic nuclei (N), dilated cisternae in rough endoplasmic reticulum (R) and decreased secretory granules (SG) x 7200.

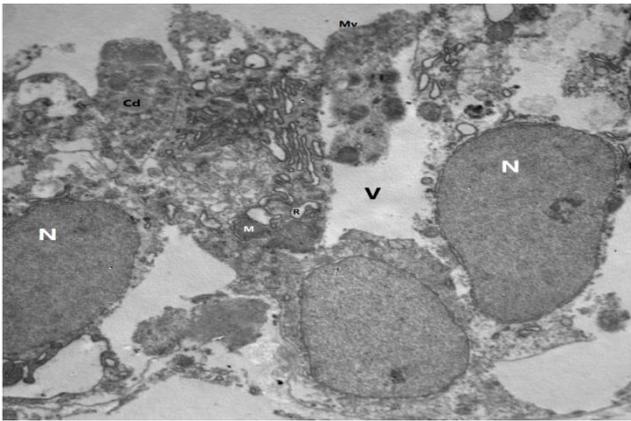


Figure 11: A transmission electron micrograph of an ultrathin section in thyroid of adult albino rat of the high dose group showing follicular cells having oval shrunken nuclei (N), vacuolated cytoplasm (V), prominent colloid droplets (C), disrupted microvilli (Mv), and electron dense mitochondria (M) x 5800.

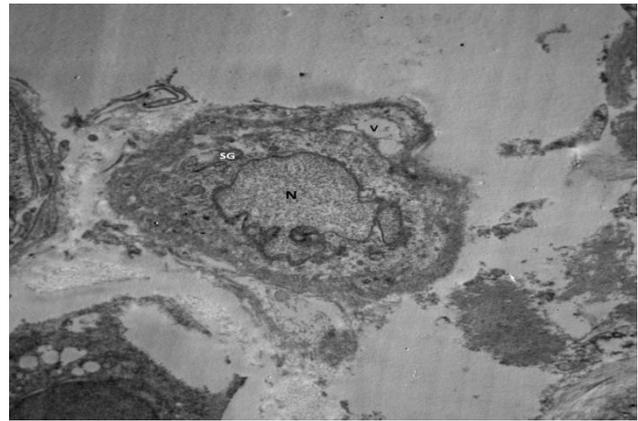


Figure 12: A transmission electron micrograph of an ultrathin section in thyroid of adult albino rat of the high dose group showing parafollicular cell having irregular nucleus (N), vacuolated cytoplasm (V) and decreased secretory granules (SG). x 5800.

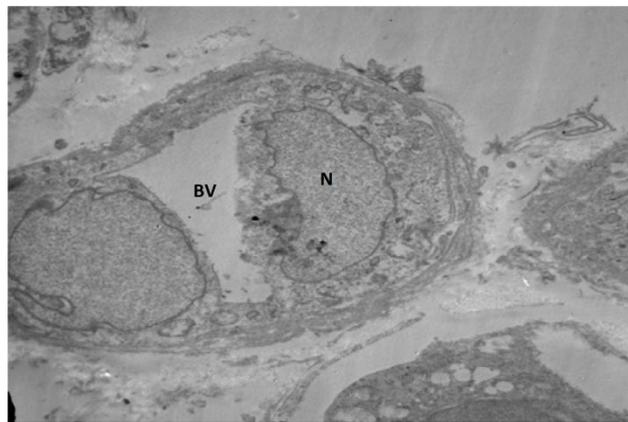


Figure 13: A transmission electron micrograph of thyroid of adult male albino rat of the high dose group showing thick blood vessel (BV) lined endothelial cells with irregular nuclei (N) x 7200.

Discussion

Herbal supplements like *Ginkgo biloba* extract are used extensively as alternatives to medical preparations. Those supplements are not regulated as drugs and usually there is no definitive evidence regarding their efficacy and safety (Bagchi, 2014). There is a great debate concerning safety of GBE use in human. Although a human study demonstrated that GBE may protect from oxidative and genotoxic damage due to treatment with radioactive iodine in thyroid cancer patients (Dardano et al., 2012), GBE was classified as being possibly carcinogenic to humans (2B group) by the International Agency for Research on Cancer (IARC, 2016). The results of animal studies demonstrated that GBE could induce pathological changes in the hepatic, thyroid, and nasal tissues of mice and rats. The most obvious carcinogenic responses were the increase in liver tumors in mice and thyroid gland follicular cell tumors in rats (Rider et al., 2014).

Despite some researcher did not recommend the use of GBE for preventing dementia. GBE is still used widely (DeKosky et al., 2008) (Charembon and Jaisin, 2015). GBE is usually given in a dose of 120-

240 mg for treating dementia (in two to three daily doses) in oral liquid or solid form (Hilton et al., 2013). The dose of the available GBE capsules available in the Egyptian market may reach up to 260 mg. Despite the 260 mg capsules are often prescribed to be taken once per day, the patients may administer the capsules three times per day. Thus, the dose consumed daily may reach up to 780 mg. This can be understood by availability of GBE as an over the counter preparation and being generally recognized as a safe herbal supplement.

In the current study, solutions containing GBE in corn oil were administered through an oral tube (lavage) to male and female rats five times a week for three months. Group of 20 rats received GBE in a dose of 40 mg/kg, and another group of rats received 500 mg/kg. At the end of the study, thyroid tissues of rats of each group were examined for by light and electron microscope and compared to those of the control group. According to Nair and Jacob (2016), the rat dose based on body surface area equivalent to the administration of 120 GBE capsule two times per day for a 60 kg human

subject was calculated to be 38.7 mg/kg. In the current study, we used the low dose (40 mg/kg) as an equivalent to the usual therapeutic dose prescribed and ½ LD50 (400 mg/kg) was administered to the high dose group.

The results of this study revealed that oral administration of 40 mg/kg or 500 mg/kg of GBE for 3 months to rats did not result in any significant change of body weights compared to the control group. A chronic study showed that administration of 300 mg/kg of GBE resulted in decrease in the mean body weights of rats at by (10% or more) than those of the controls after week 93, and administration of 1000 mg/kg resulted in decrease by (10% or more) after week 89 (NTP, 2013). Thus the effect of GBE on body weight may need a prolonged periods of administration that may exceed 18 months.

The current result showed that GBE had no significant effect on thyroid hormones (TSH, T3 and T4). According to NTP (2013), a 2 years study on rats showed that GBE administration resulted in dose-dependent increase in TSH level in male rats at week 14, at all dosed groups (100, 300 and 1000 mg/kg) and the 1000 mg/kg female group compared to those of the vehicle controls. There were no statistically significant changes in the levels of T3 or total T4. The synthesis of T3 and T4 hormones is dependent on the availability of iodine and thyroglobulin (Jameson and Weetman, 2008). TSH regulates thyroid function and is secreted by the anterior pituitary gland. In a human subjects study, it was concluded that TSH may not be involved in the etiology of thyroid carcinoma by its overstimulation (Rinaldi et al., 2014). Another human study demonstrated a significant increased risk of papillary thyroid cancer with TSH below the normal level among women and with TSH above the normal level among men (Huang et al., 2017). Thus, it is not always useful to use of TSH level for screening or early detection of thyroid tumors.

In our study, light microscopic examination of low and high dose groups revealed dose dependent pathological changes, thyroid tissue revealed hypertrophy and hyperplasia of the epithelial cells of thyroid follicles with abnormal nuclear features. Thyroid follicles also showed depletion of colloid. Interfollicular tissue showed fatty infiltration with congestion of blood vessels. These findings are in agreement with Rider et al., (2014) who described follicular cell hypertrophy in rats in 13-week study (GBE was administered at doses of 0, 62.5, 125, 250, 500, and 1,000 mg/kg for rats and 0, 125, 250, 500, 1,000, or 2,000 mg/kg for mice), thyroid follicular cells hypertrophy was observed in rats in a 2-year study in which, GBE was administered at doses of 0, 100, 300, and 1,000 mg/kg (rats) or doses of 0, 200, 400, and 2000 mg/kg (mice). He also described increase in follicular cells adenomas in male rats and mice and single case of follicular cell carcinomas in female rats.

In the present study, results of the electron microscopic examination of thyroid glands of low and high dose groups demonstrated changes indicating cell damage. The follicular cells of GBE administered rats

showed dilated rough endoplasmic reticulum, atrophic microvilli, and vacuolated cytoplasm.

The safety of GBE use in human is defended by the claim that experimental animals (mouse and rat) were administered high doses of GBE (several hundred to several thousand folds the humans under therapeutic use) (Heinonen and Gaus, 2015). The current study demonstrated that oral administration of GBE to rats in a dose (40 mg/kg) equivalent to the human therapeutic dose, also demonstrated histopathological changes in thyroid tissue.

The method of production of GBE is variable according to different manufacturers, so the constituents of the GBE are varying regarding amount and quality. This variation may be a factor in different results regarding studies of GBE safety (Ude et al., 2013).

In conclusion, oral administration of GBE caused histopathological changes in thyroid of rats. Although it was postulated that the effects of GBE on thyroid gland to be caused by rodent specific mechanisms not relevant for humans, there is a need for more studies to be conducted to assure the safest dose in human and to study the safety of other GBE products available in market and demonstrate its effects on human. It is also recommended for physicians prescribing GBE therapy to evaluate patients carefully during treatment i.e. thyroid gland examination, thyroid ultrasound and serum TSH, T4, and free T3 concentrations.

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الملخص العربي

الآثار السمية المحتملة لمستخلص الجنكوبيلوبا على الغدة الدرقية لذكور وإناث الفئران البيضاء: دراسة ميكروسكوبية

ضوئية وإلكترونية

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مستخلص الجنينكو ثنائي الفلقة هو مستخرج كحولي من أوراق شجرة الجنينكو ثنائي الفلقة ويوجد في صورة أقراص وكبسولات تستخدم لتحسين الذاكرة ووظائف المخ، بالرغم من الاستخدام الواسع بجرعات كبيرة نسبياً لفترات طويلة، فإن عدد قليل من الدراسات كان بخصوص السمية وإمكانية حدوث السرطان. غرض البحث هو دراسة السمية المحتملة لمستخلص الجنينكو ثنائي الفلقة على الغدة الدرقية للجرذان. تم تقسيم الجرذان إلى أربع مجموعات المجموعة الأولى لم تتلق أي علاج وكانت المجموعة الضابطة السالبة ومجموعة تم إعطائها المذيب (زيت الذرة). المجموعة الثالثة تم إعطائها مستخلص الجنينكو ثنائي الفلقة بجرعة ٤٠ مجم/كجم والمجموعة الرابعة تم إعطائها المستخلص بجرعة ٥٠٠ مجم/كجم. تم إعطاء مستخلص الجنينكو ثنائي الفلقة مذاب في زيت الذرة عن طريق أنبوبة فمية للذكور وإناث الجرذان خمسة أيام بالأسبوع لمدة ثلاثة أشهر. في نهاية مدة الدراسة تم قتل الجرذان وأخذت الغدة الدرقية حيث تم تجهيز العينات وتم فحصها بالمجهريين الضوئي والإلكتروني لبيان مدى تضررها. كما تم قياس مستويات هرمونات T3 , T4 , TSH أثناء فترة الدراسة وبنهايتها. أوضحت نتائج الدراسة أن المجموعة التي تلقت الجرعة الصغيرة من مستخلص الجنينكو ثنائي الفلقة أظهرت عدم انتظام وعدم تساوي الجريبات الدرقية والتي كان معظمها صغيرة وقلة المادة الغروية في بعض الجريبات وعدم ظهورها في البعض الآخر مع تقشر الخلايا الجريبية. أما المجموعة التي تلقت الجرعة الكبيرة من مستخلص الجنينكو ثنائي الفلقة فتكون النسيج الدرقي من جريبات صغيرة مع عدم ظهور تجويف بعض الجريبات مع اصطفااف الخلايا الجريبية في طبقات متعددة. وأظهر فحص النسيج بين الجريبات انتشار الخلايا الدهنية. أوضحت الدراسة أن مستخلص الجنينكو ثنائي الفلقة هو خليط مركب يسبب تغيرات باثولوجية في الغدة الدرقية للجرذان.

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