

Effect of Metalaxyl on the thyroid gland in adult female albino rats and the possible protective role of ginger (Histological and immunohistochemical study)

Original
Article

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ABSTRACT

Introduction: Metalaxyl is a widely used fungicide, with high residual level in crops that lead to dangerous human health problems.

Aim of work: Study effect of metalaxyl on the thyroid gland structure & function and evaluate the efficacy of ginger supplementation.

Materials and Methods: Thirty female albino rats were classified into three equal groups: Group I: (control group): Subdivided into two equal subgroups 1a: given distilled water by gastric tube for 8 weeks. 1b: given ginger (100mg/kg/day) by gastric tube for 8 weeks. Group II: given metalaxyl 3 times per week (130 mg/kg/day) by gastric tube for 8 weeks. Group III: given the same dose of metalaxyl given to group II concomitant with ginger by gastric tube for 8 weeks. At end of the experiment, rats were anaesthetized, and blood samples collected for measurement of T3, T4 and TSH. Thyroid gland biopsies were processed for light and electron microscope examination. Immunohistochemical study was done for detection of PCNA. Results were analyzed morphometrically and statistically.

Results: Metalaxyl-treated rats showed numerous interfollicular cells, vacuolated colloid, an increase height of follicular epithelium and many follicles exhibited stratification with vacuolated cytoplasm, dilatations of endoplasmic reticulum and irregular dark nuclei. Positive immunoreaction was detected in numerous nuclei of follicular cells of group II and significant decrease in T3, T4 with highly increase in TSH. Group III showed a considerable degree of improvement of thyroid architecture.

Conclusion: Metalaxyl affects the thyroid follicular structure and function. Concomitant administration of ginger was partially efficient in protecting it.

Key Words: Ginger, metalaxyl, PCNA, thyroid.

Revised: 13 November 2022, **Accepted:** 9 December 2022.

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ISSN: 2536-9172, December 2022, Vol. 6, No. 2

INTRODUCTION

Pesticides have an important role in the effort to increase food production in today's agriculture but, they cause environmental hazards because of their toxicity and sometimes high persistence representing one of the problems of world-wide importance. Presence of these toxic chemicals was recorded in water, air, house dust and in the tissues of occupationally and non-occupationally exposed people, particularly in adipose tissue, blood and urine^[1,2].

Metalaxyl is a benzenoid fungicide belonging to the most widely known member of the amide group used to control soil-born fungal diseases of fruits, cotton, soybean, peanuts, tobacco, and grasses. Metalaxyl (methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-DL-alaninate), an important acylanilide fungicide with residual and systemic activity, is used to protect crops against diseases caused by oomycetous fungi^[3].

Available laboratory and field studies indicate that metalaxyl is stable under normal environmental pH values. It is also photolytically stable in water and soil when exposed to natural sunlight. Its tolerance to a wide range of pH, light, and temperature leads to its continued use in agriculture. However, health problems come from their high residual level in agriculture crops especially vegetables cultivated under greenhouse conditions^[4].

Metalaxyl formulations include granules, powders, dusts, and emulsifiable concentrates. Application may be by foliar or soil incorporation, surface spraying and seed treatment. Metalaxyl registered products either contain metalaxyl as the sole active ingredient or are combined with other active ingredients (e.g., captan, mancozeb, copper compounds, carboxin)^[5].

Cocarcinogenic potential of metalaxyl was assessed in albino mice and induced histopathological and biochemical alterations in liver, kidney and testis of albino rat^[6,7].

It was reported that the oxidative stress is the principal mechanism of metalaxyl-induced toxicity^[8]. Also, Sultana *et al.*^[9] recorded a significant increase of L-MDA level in erythrocyte lysate after 30th day of dermal treatment with metalaxyl compared to normal rat group.

Over several years, herbal plants were considered to be effective medicinal factor and many drugs have been prepared from expected sources, based on their use in habitual medicine. Medicinal plants are important to the health of individuals and communities. Herbal medicines supply the health needs of almost 80% of the world's population^[10].

Ginger is one of medicinal plants with a strong antioxidant effect, antiarthritic, antiplatelet, antitumor, anti-inflammatory, antiviral, and antihepatotoxic properties. Its antioxidant activity has been attributed to its major active phenolic ingredients (e.g., zingerone, gingerdiol, zingibrene, gingerols and shogaols). In addition, the administration of ginger has been shown to improve oxidative stress by decreasing lipid peroxidation and protein oxidation as free radical-generating sources and elevating the levels of enzymes implicated in the antioxidant defense system^[11,12].

In Egypt, the uncontrolled usage of metalaxyl as one of the widely used plant fungicides carries many health hazards. Many studies have described the effect of metalaxyl on many organs as liver, kidney and testis, but no available literatures were found to demonstrate the effect of metalaxyl on the thyroid gland. Therefore, the aim of this study was to demonstrate the effect of metalaxyl on the histological structure and function of the thyroid gland of adult female albino rats. In addition, to evaluate the possible role of ginger supplementation against these changes.

MATERIALS AND METHODS

Thirty adult female albino rats aged 3-6 months were used in this study, their weight ranged from 200-250 gm. They were apparently healthy. The animals were supplied by the Laboratory Animal Unit, Faculty of Medicine, Zagazig University. They were housed in the animal house in controlled conditions at room temperature. They were allowed standard balanced diet and water ad-libitum. All animals were cared in accordance with the guidelines for animal research issued by the National Institute of Health, and approved by Animal Ethics Committee, Zagazig University.

These animals were classified into three equal groups (10 animals each): Group I: (control group): Which were further subdivided into two equal subgroups. Subgroup 1a: Were given distilled water by gastric tube for 8 weeks. Subgroup 1b: were given ginger in a dose of (100mg/kg body weight /day) by gastric tube for continuous 8 weeks.

Group II: Animals of this group were given metalaxyl by gastric tube in a dose of (130 mg/ kg body weight) three times per week for continuous 8 weeks^[13]. Group III: were given the same dose of metalaxyl given to animals of group II (130 mg/ kg body weight) three times per week concomitant with ginger in a dose of (100mg/kg body weight /day) orally by gastric tube for 8 weeks^[14].

Metalaxyl was supplied from Central Agricultural Pesticides Laboratory, Egypt. It was provided in a white powder form. For every 10 rats 0.5 gm was dissolved in 15 ml distilled water.

Ginger was purchased from MEPACO-MEDIFOOD, (Enshas, Sharkeya, Egypt), in the form of 30 tablets that each contained 400 mg ginger. For every 10 rats 1 tablet was dissolved in 16 ml distilled water.

At the end of the experimental period, rats were anaesthetized using ether inhalation and blood samples were collected from orbital vein for measurement of serum total T3, total T4 and thyroid stimulating hormone (TSH). Thyroid glands were dissected carefully and prepared for the following:

Light microscopic examination:

For light microscopic study, specimens were fixed in 10% buffered formalin, dehydrated, embedded in paraffin, and 5 μ m thick paraffin sections were cut and stained with hematoxylin and eosin stains for routine histological examination^[15].

Immunohistochemical reaction was carried out on sections of the thyroid gland using streptavidin system with antibody against Proliferating Cell Nuclear Antigen (PCNA). The primary antibody was a mouse monoclonal antibody; the kits were obtained from Dako Life Trade, Egypt (clone PC10, code no M0879). The universal kit used was biotinylated secondary antibodies. The immune reaction was visualized with 0.05% diaminobenzidine and the slides were counter stained with Mayer's hematoxylin before mounting. For negative control, primary antibody was omitted and replaced with phosphate buffer saline (PBS). Positive results for the PCNA immunoreactions were indicated by brown coloration in the nuclei of cells^[16].

For electron microscopic study, small pieces of 1 mm³ of the thyroid gland were excised, fixed in 2% gluteraldehyde buffered with 0.1 M phosphate buffer at pH 7.4 for 2h at 4°C, and post-fixed in 1% osmium tetroxide. They were then dehydrated with ascending grades of ethanol and placed in propylene oxide at room temperature, followed by impregnation in a mixture of propylene oxide and resin (1: 1). The specimens were embedded in an EM bed-812 resin in BEEM capsules at 60°C for 24h^[17]. Semithin sections were prepared and stained with toluidine blue for examination. Ultrathin sections were cut and

double stained with uranyl acetate and lead citrate and were examined with a JEOL transmission electron microscope, Department of Histology and Cell Biology, Faculty of Medicine, Zagazig University, Egypt.

Image Analysis and Morphometric Study

H&E and immunohistochemical stained sections were morphometrically analyzed using Leica Qwin 500 Image Analyzer Computer System (England), at Pathology Department, Faculty of Dentist, Cairo University. Four parameters were measured: height of follicular epithelium, count of microfollicles (small follicular clusters of 6–12 follicular cells in a ring with or without a small amount of central colloid), area percent of colloid, and area percent of PCNA immunoreactions. Ten non overlapping fields from a slide of each rat in the different groups were taken.

Biochemical (Hormonal) Analysis:

At the time of sacrifice, blood from each animal was rapidly collected from orbital vein, transferred to centrifuge tubes without anticoagulant, and serum was separated by low-speed centrifugation (1500 xg, 15 min). The serum samples were stored at -20°C until the analysis time. Serum thyroid hormones [total T3, total T4 and thyroid stimulating hormone (TSH)] levels were measured by in vitro diagnostic radioimmunoassay with the immunolite 2000 analyzer for the quantitative measurement^[18]. Mean serumTT3 ranged from 38.96-41.04 µg/dl, mean serumTT4 ranged from 3.1-5.3 µg/dl and mean serum TSH ranged from 0.114-0.126 µIU/ml.

STATISTICAL ANALYSIS

The data obtained (serum total T3, serum total T4, serum TSH, height of follicular epithelium, count of microfollicles, area percent of colloid and area percent of PCNA immunoreactions) for all groups were expressed as means (\bar{X}) and standard deviations (SD) and subjected to statistical analysis using one-way analysis of variance (ANOVA) for comparison between the different groups (more than two groups). Followed by least significant difference test (LSD), for comparison between different groups to find the statistical difference between groups when ANOVA was statistically significant. (*P* value <0.05 was considered statistically significant)^[19]. All statistical analyses were done using the Statistical Package for the Social Sciences (SPSS) version 19 packages.

RESULTS

Histological results:

No histological difference between the two control subgroups Therefore, we considered them as one group.

H&E-stained sections of the control thyroid revealed normal morphology. It was surrounded by connective tissue capsules and divided into lobules by connective tissue trabeculae. Thyroid parenchyma was composed of multiple follicles of variable sizes and shapes, where large follicles were present, especially in the periphery of the gland (Fig. 1a). The thyroid follicles were lined by cuboidal follicular cells with rounded nuclei. Its lumen was filled with acidophilic colloids that had peripheral small vacuoles. Interfollicular cells were detected in between the follicles (Fig. 1b). Metalaxyl treated group revealed numerous interfollicular cells, and relatively few follicles containing colloid. Some of these follicles were large while the others were small with little colloid content. This colloid showed vacuolations. Numerous blood vessels were detected. (Fig. 1c) Metalaxyl and ginger treated group displayed that most of thyroid follicles were lined by single cell layer with flattening of their nuclei. Some colloid vacuolations were still observed (Fig. 1d)

Immunohistochemical stained sections revealed positive PCNA immunoreactions in few nuclei of follicular cells in both control group and metalaxyl and ginger treated groups (Fig. 2a, 2c). While metalaxyl treated thyroid exhibited positive PCNA immuno-reactions in numerous nuclei of follicular and interfollicular cells. (Fig. 2b)

Toluidine blue stained semi thin sections of control group showed closely packed thyroid follicles that were lined by a single layer of follicular cells and separated by delicate connective tissue contained blood capillaries. Also, pale parafollicular cells were observed (Fig. 3a). Thyroid gland of metalaxyl treated rat showed some follicles with stratification of epithelial lining on one side. Other follicles appeared fused with loss of connective tissue in between. Follicular cells showed vacuolated cytoplasm. Degranulated mast cells and disorganized follicular cells with dark stained nuclei and no follicles formation were also observed (Fig. 3b, 3c, 3d). Whereas semi thin sections of metalaxyl and ginger treated group displayed lining of thyroid follicles by a single layer of follicular cells and some cytoplasmic vacuoles were still noticed (Fig. 3e).

Electron microscopic examination of ultrathin sections of thyroid glands of control rats revealed follicular cells contained euchromatic nuclei. Their cytoplasm had numerous paralleled cisternae of rough endoplasmic reticulum, Golgi apparatus, and dense lysosomal granules. Their apical border showed microvilli projected into lumens (Fig. 4a). Parafollicular cells were located in the periphery of follicular epithelium and within the follicle basal lamina. They were separated from the luminal colloid by follicular cell cytoplasm. Parafollicular cells had indented euchromatic nuclei, many small electron-dense secretory granules, and few cisternae of endoplasmic reticulum (Fig. 4b). While thyroid follicular cells of metalaxyl treated rats appeared with intended heterochromatic

nuclei, numerous disrupted cisternae of rough endoplasmic reticulum, and numerous vacuoles with nearly loss of most of microvilli (Fig. 5a). Other follicular cells were arranged in layers, with variable forms of nuclei. Marked dilated rough endoplasmic reticulum were observed. Multiple blood vessels were detected (Fig. 5b). Parafollicular cells were found with rounded euchromatic nuclei and numerous small, low density secretory granules. They are separated from the luminal colloid by follicular cell cytoplasm. Intrafollicular infiltrated cells were also detected (Fig. 5c).

Ultrastructurally, Metalaxyl and ginger treated group showed some follicular cells appeared with euchromatic, and others with small electron dense apoptotic nuclei. Mild dilatation of the rough endoplasmic reticulum, and apical microvilli projected into the lumen. Intrafollicular infiltrated cells were still recorded (Fig. 6a). Some parafollicular cells appeared with euchromatic nuclei, and numerous small low density secretory granules, while others had more dense secretory granules (Fig. 6b).

Morphometric Results:

Statistical analysis of height of follicular epithelium revealed a highly significant increase ($p. value < 0.001$) in height of follicular epithelium in metalaxyl treated group compared to the control group. On the other hand, there was a non-significant increase in metalaxyl and ginger treated group compared to the control group (Table 1).

Statistical analysis of count of microfollicles revealed a significant increase ($p. value < 0.05$) in count of microfollicles in metalaxyl treated group compared to

the control group. On the other hand, there was a non-significant increase in metalaxyl and ginger treated group compared to the control group (Table 2).

Statistical analysis of the area % of colloid revealed a highly significant decrease ($p. value < 0.001$) in area % of colloid in metalaxyl treated group compared to the control group. On the other hand, there was a non-significant decrease in metalaxyl and ginger treated group compared to the control group (Table 3).

Whereas area % of PCNA immunoreactions revealed a highly significant increase ($p. value < 0.001$) in area % of immune reaction in metalaxyl treated group compared to the control group. On the other hand, there was a non-significant increase in metalaxyl and ginger treated group compared to the control group (Table 4).

Biochemical (Hormonal) Results:

Statistical analysis of both serum T3 and T4 levels revealed a significant decrease ($p. value < 0.05$) in their levels in Metalaxyl treated group compared to the control group. On the other hand, there was a non-significant decrease in Metalaxyl and ginger treated group compared to the control group (Table 5, 6).

On the other hand, statistical analysis of serum TSH level revealed a highly significant increase ($p. value < 0.001$) in its serum levels in metalaxyl treated group compared to the control group. While there was a non-significant increase in metalaxyl and ginger treated group compared to the control group (Table 7).

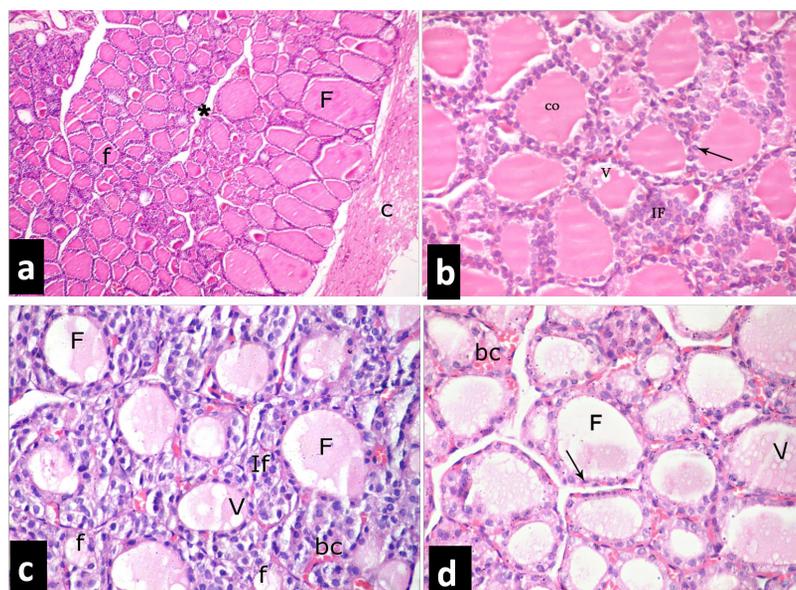


Fig. 1 a,b: H&E-stained sections of the control thyroid showing the covering connective tissue capsule(C) and divided into lobules by connective tissue trabeculae (*). Thyroid parenchyma appear with multiple follicles of variable sizes and shapes (f). Some follicles appear larger especially in the periphery of the gland (F). the follicular walls lined by cuboidal (arrow) follicular cells with rounded nuclei. The follicular lumens are filled with acidophilic colloids (CO) that show peripheral small vacuoles (V). An apparent interfollicular cells (If) can be noticed in between follicles. c: Metalaxyl treated group showing some large thyroid follicles (F), and many small follicles (microfollicles) (f) with little colloid content. Many vacuoles are seen in colloid (V). Numerous interfollicular cells (If), and several blood vessels (bc) are also seen. d: Metalaxyl and ginger treated thyroid showing most follicles (F) are lined by a single cell layer with flattening of most of their nuclei (arrow). Vacuolation of colloid (V) many blood vessels (bc) are also noticed, (H&E x100 (a),400).

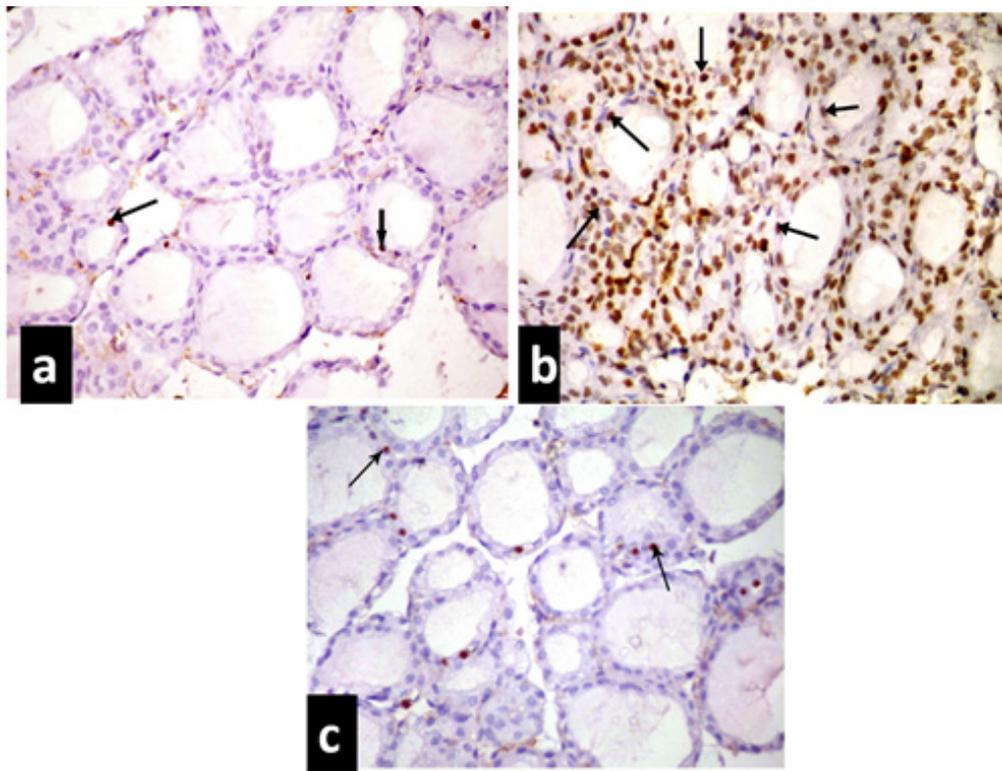


Fig. 2 a: Immunohistochemical stained sections of the control thyroid showing positive proliferating cell nuclear antigen (PCNA) immunoreactions in few nuclei of follicular cells (arrow). **b:** Metalaxyl treated thyroid reveals positive PCNA immuno-reactions in many nuclei of follicular and interfollicular cells (arrow). **c:** Metalaxyl and ginger treated group shows positive PCNA immuno-reactions in few nuclei of follicular cells. (Immunoperoxidase for PCNA, X 400).

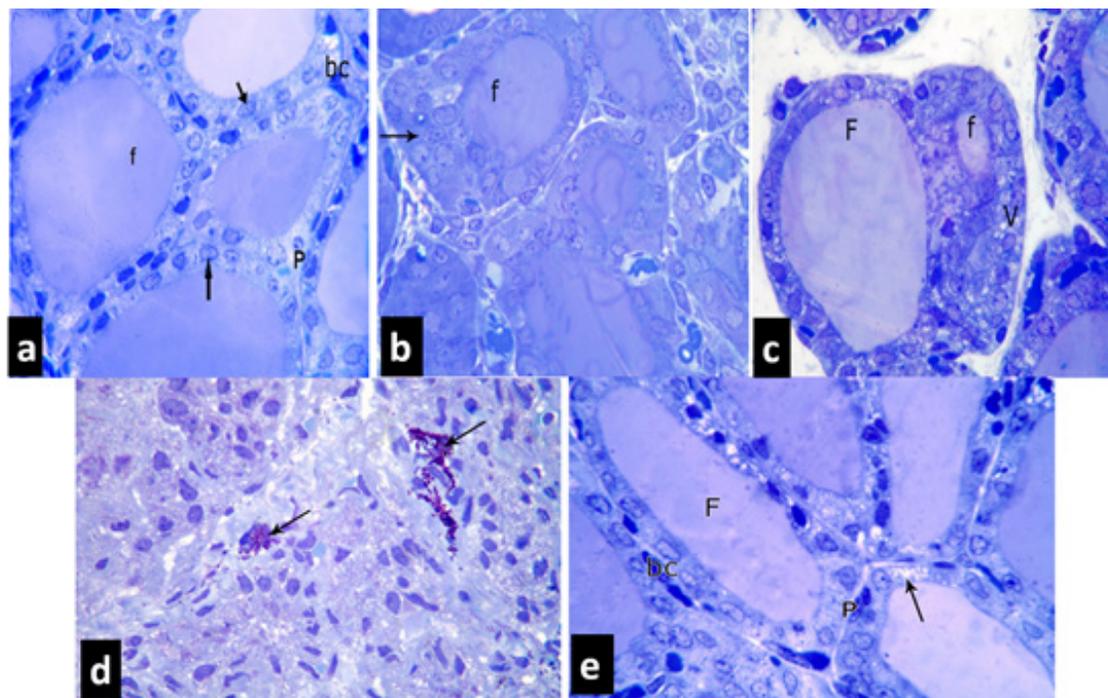


Fig. 3 a: Semithin sections of the control group stained with toluidine blue showing closely packed thyroid follicles (f) lined by single layer of follicular cells (arrow) and separated by delicate connective tissue containing blood capillaries (bc). Also, pale parafoallicular cells (P) can be detected. **b, c, d:** Thyroid gland of metalaxyl treated rat exhibit stratification of epithelial lining of the follicle (f) on one side (arrow). fused follicles (F), (f) appear with loss of connective tissue in between. Follicular cells show cytoplasmic vacuolation (V). degranulated mast cells (arrow) among disorganized follicular cells with dark stained nuclei are noticed with no follicular architecture. **e:** Metalaxyl and ginger treated rats showing thyroid follicles (F) appear lined by one cell layer. Some cytoplasmic vacuoles (arrow) are still present. Blood capillaries (bc) and parafoallicular cells (P) are noticed. (Toluidine blue x 1000).

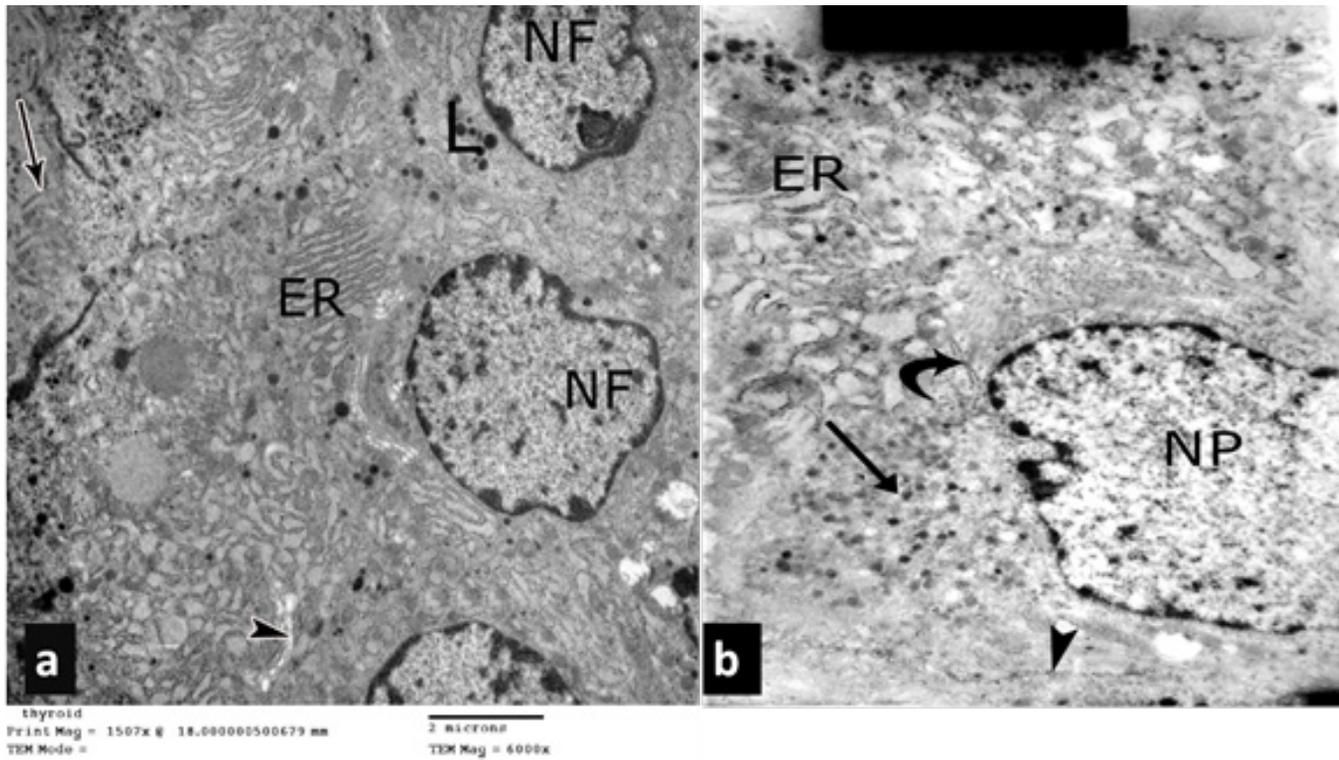


Fig. 4 a: Ultrathin sections of a control rat showing follicular cells reveal euchromatic nucleus (NF) Their cytoplasm has numerous paralleled cisternae of rough endoplasmic reticulum (ER), Golgi apparatus (arrowhead), and dense lysosomal granules (L) Their apical borders show microvilli (arrow) projecting into the colloid. **b:** Parafollicular cell of control thyroid resting on the basal lamina (arrowhead) has indented euchromatic nuclei (NP). It is separated from the luminal colloid by a part of the cytoplasm of follicular cells containing rough endoplasmic reticulum (ER). Its cytoplasm contains many small electron-dense secretory granules (arrow), and a few cisternae of endoplasmic reticulum (curved arrow). (Mic. Mag X 6000).

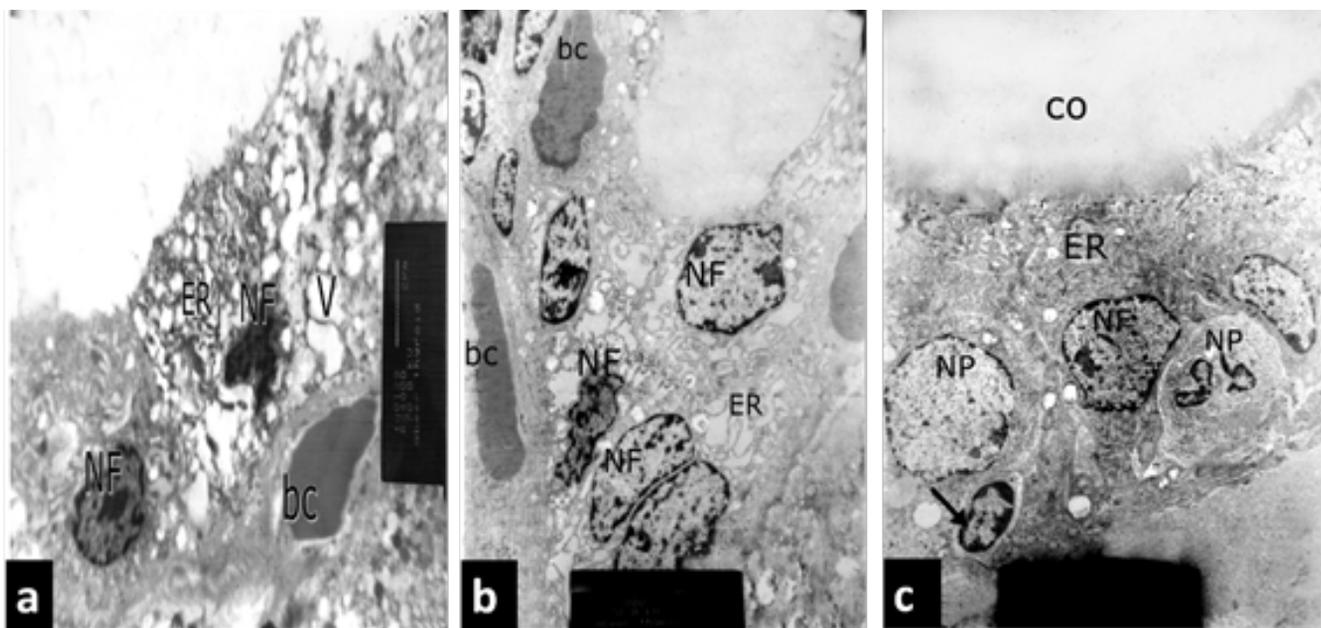


Fig. 5 a: Ultra-thin section of metalaxyl -treated rat showing follicular cells possess intended heterochromatic nuclei (NF). Numerous disrupted cisternae of rough endoplasmic reticulum (ER) and numerous cytoplasmic vacuoles (V) can be seen with nearly loss of most of microvilli. **b:** Follicular cells of the same group appear arranged in layers. Their nuclei have variable forms (NF), their cytoplasm have marked dilated rough endoplasmic reticulum (ER). Numerous blood vessels (bc) can be noticed. **c:** Metalaxyl -treated parafollicular cells exhibit rounded euchromatic nuclei (NP) and small low density secretory granules. They are separated from the luminal colloid (CO) by a part of the follicular cell cytoplasm showing dilated rough endoplasmic reticulum(ER) with heterochromatic nucleus (NF). Intrafollicular infiltrated cell (arrow) is also seen. (Mic.Mag X 4000(a) 3000 (b&c)).

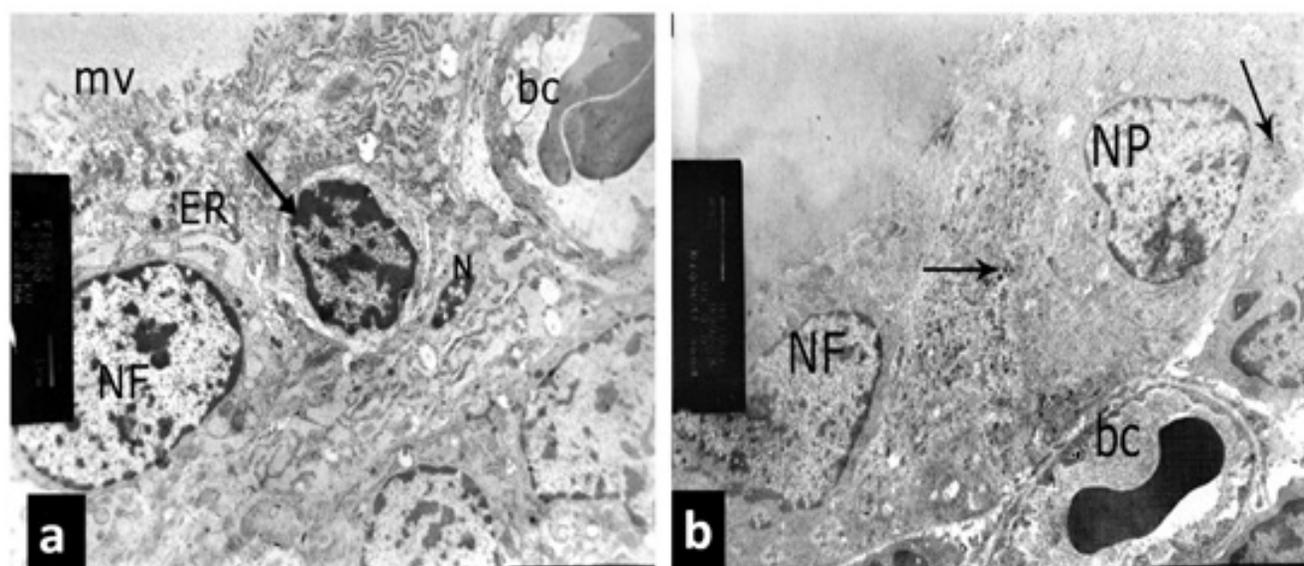


Fig. 6 a: Ultra-thin section of metalaxyl and ginger -treated thyroid follicular cell reveals euchromatic nucleus (NF) and apoptotic one (N), with mild dilatation of the rough endoplasmic reticulum (ER). Apical microvilli (mv) appear extending into the lumen. A blood vessel (bc) and intrafollicular infiltrated cell (arrow) are also seen. **b:** The same group showing follicular cell with euchromatic nucleus (NF), and two para-follicular cells appear one with euchromatic nucleus (NP), and numerous small low density secretory granules (arrow). The cytoplasmic profile of adjacent para-follicular cell contains more dense secretory granules (arrow). Interfollicular blood capillary is noticed (bc). (Mic. Mag X5000(a), 4000(b).

Table 1: -Comparison between mean values of epithelial height in different studied groups.

| Epithelial height (μm) | Mean \pm SD | Range | <i>P. value</i> |
|-------------------------------------|-----------------|------------|-----------------|
| Control a | 5.35 \pm 1.28 | 2.79-7.91 | |
| Control b | 5.45 \pm 1.12 | 3.21-7.69 | 0.854 |
| Metalaxyl treated | 9.4 \pm 1.33 | 6.74-12.06 | <0.001** |
| Metalaxyl and ginger treated | 6.1 \pm 1.32 | 3.46-8.74 | 0.212 |

* Significant
F=22.84 *P. value*= <0.001**

Table 2: -Comparison between mean values of count of microfollicles (ten non overlapping fields from each slide) in different studied groups.

| Count of microfollicles | Mean \pm SD | Range | <i>P. value</i> |
|------------------------------|----------------|-----------|-----------------|
| Control a | 0.4 \pm 0.1 | 0.2-0.6 | |
| Control b | 0.38 \pm 0.1 | 0.18-0.58 | 0.659 |
| Metalaxyl treated | 2.2 \pm 0.8 | 0.6-3.8 | 0.026* |
| Metalaxyl and ginger treated | 0.5 \pm 0.2 | 0.1 -0.9 | 0.171 |

* Significant
F=43.82 *P. value*= <0.001**

Table 3: -Comparison between mean values of area percentage of colloid in different studied groups.

| Area percentage of colloid | Mean \pm SD | Range | <i>P. value</i> |
|------------------------------|---------------|-------------|-----------------|
| Control a | 56.55 \pm 4 | 48.55-64.55 | |
| Control b | 57.11 \pm 3 | 51.11-63.11 | 0.727 |
| Metalaxyl treated | 11.06 \pm 2 | 7.06-15.06 | <0.001** |
| Metalaxyl and ginger treated | 48.16 \pm 9 | 30.16-63.16 | 0.127 |

*Significant
F=490.75 *P. value*= <0.001**

Table 4: -Comparison between mean values of area percentage of PCNA in the different studied groups.

| Area percentage of PCNA | Mean \pm SD | Range | <i>P. value</i> |
|------------------------------|----------------|-------------|-----------------|
| Control a | 3.08 \pm 0.2 | 2.68-3.48 | |
| Control b | 3.2 \pm 0.3 | 2.6-3.8 | 0.304 |
| Metalaxyl treated | 26.05 \pm 3 | 20.05-32.05 | <0.001** |
| Metalaxyl and ginger treated | 4.1 \pm 1 | 3.1 -5 | 0.109 |

*Significant

F=546.12

P. value= <0.001****Table 5:** - Comparison between mean values of serum levels of total T3 in μ g/dl \square SD in the different studied groups.

| Serum levels of total T3 | mean \pm SD | Range | <i>P. value</i> |
|------------------------------|------------------|-------------|-----------------|
| Control a | 40 \pm 0.52 | 38.96-41.04 | |
| Control b | 41.2 \pm 0.3 | 40-42.8 | 0.415 |
| Metalaxyl treated | 25.45 \pm 1.22 | 23.6-26.94 | 0.017* |
| Metalaxyl and ginger treated | 35 \pm 0.56 | 33.88-36.12 | 0.116 |

*Significant

F (ANOVA) =949.33

P. value= <0.001****Table 6:** -Comparison between mean values of serum levels of total T4 μ g/dl \square SD in the different groups.

| Serum levels of total T4 | Mean \pm SD | Range | <i>P. value</i> |
|------------------------------|-----------------|-----------|-----------------|
| Control a | 4.16 \pm 0.58 | 3.1-5.3 | |
| Control b | 4.22 \pm 0.4 | 3.42-5.02 | 0.79 |
| Metalaxyl treated | 2.73 \pm 0.15 | 2.43-3.03 | 0.021* |
| Metalaxyl and ginger treated | 3.88 \pm 0.51 | 2.86-4.9 | 0.264 |

*Significant

F =24.75

P. value=<0.001****Table 7:** -Comparison between mean values of serum levels of TSH μ U/ml \square SD in the different studied groups.

| Serum levels of TSH | Mean \pm SD | Range | <i>P. value</i> |
|------------------------------|------------------|-------------|-----------------|
| Control a | 0.12 \pm 0.003 | 0.114-0.126 | |
| Control b | 0.13 \pm 0.004 | 0.122-0.318 | 0.534 |
| Metalaxyl treated | 0.25 \pm 0.02 | 0.21-0.29 | <0.001** |
| Metalaxyl and ginger treated | 0.15 \pm 0.01 | 0.13-0.17 | 0.114 |

*Significant

F=94.89

P. value= <0.001**

DISCUSSION

Metalaxyl is a systemic benzenoid fungicide, widely used as a foliar spray for tropical and subtropical crops in a wide range of fruit, and vegetable crops. Residues of metalaxyl were detected in incurred grape and wine samples^[20].

In this study, adult female albino rat was chosen as an experimental model. It was reported that nearly all changes found in human thyroid treated with goitrogens were found to be similar to the rat thyroid model. Regarding the incidence of thyroid diseases among the general population, it has been shown that females are much more likely to develop thyroid diseases than males^[21,22].

Biochemical assay of hormones in metalaxyl treated rats showed a significant decrease in serum T3 and T4. However, there was a highly significant increase in serum TSH in comparison to the control animals. These results were in accordance with those reported by Al-Amoudi^[23] and Ksheerasagar & Kaliwal^[24] who found that hypothyroidism is the most common abnormality and are associated with the use of benomyl, mancozeb, metalaxyl and paraquat in addition to the class of organochlorines that includes aldrin, DDT, heptachlor, lindane, and chlordane.

Low T3 or T4 exerts negative feedback on the pituitary. It releases more TSH to stimulate the thyroid gland, which in turn accelerates the production of the thyroid hormone. TSH is a major growth factor for thyroid. Thus, the

gland under TSH undergoes enlargement, hyperplasia, neovascularization, and morphological alterations in the thyroid gland cells^[25, 26].

Thyroid glands of metalaxyl treated rats stained with haematoxylin and eosin (H&E) revealed numerous interfollicular cells, and relatively few follicles containing colloid. Some of these follicles are large while the others are small (microfollicles) with little colloid content. These results were confirmed in Toluidine blue stained semi thin sections which showed small follicles with little colloid, this was also confirmed by morphometric and statistical analysis which revealed a highly significant decrease in area percent of colloid and significant increase in the count of microfollicles in comparison with the control group. These findings were in agreement with Alsen *et al.*^[27] who attributed these results to an increased TSH level, which was responsible for the proliferative activity of follicular cells.

Furthermore, metalaxyl -treated thyroid showed many follicles lined by multiple layers of cells (stratification). These could be attributed to an increased TSH level, which was responsible for the proliferative activity of follicular cells. Also, metalaxyl itself has cocarcinogenic potential. It can cause uncontrolled proliferation of the cells^[6, 28].

Similar effects have been reported with fungicide mancozeb which has direct action on thyroid by inhibiting thyroxin synthesis and accelerates its deiodination and causes increased pituitary TSH levels, which causes the hypertrophy and hyperplasia of thyroid epithelium^[29]. Also, Patil and Dhurvey^[30] confirmed a significant increase in the height of epithelium in hypothyroidism.

In the current study, examination of metalaxyl treated thyroid follicles showed many vacuoles in colloid. These vacuolations were attributed to an increased endocytotic activity to release the stored hormones as a compensatory mechanism to metalaxyl-mediated suppressive effects on follicular cells. Waugh^[31] hypothesized that these vacuolations were under the influence of increased TSH.

In this work, many follicles were lined by multiple layers of cells with vacuolated cytoplasm. as increased TSH level causes hypertrophy and hyperplasia of thyroid epithelium. Such hyperplasia was further confirmed by a significant increase in the number of PCNA positive follicular cell nuclei. PCNA is directly correlated with the proliferative state of various tissues. Bolisetty and Jaimes^[32] and Liu *et al.*^[33] attributed these cytoplasmic vacuoles to free radicals that facilitate the release of lysosomal enzymes into the cytosol with subsequent oxidation of the protein architecture of the cells causing their fragmentation.

In the present work, the metalaxyl -treated group showed congested, dilated blood capillaries. This could be attributed to a high level of TSH. This finding agreed with

Shady and Noor^[34] who reported increased vascularization of the gland after chlorpyrifos exposure. While Ramsden^[35] attributed this vascularization to growth factors and other vasoactive factors produced in the thyroids that were potent angiogenic proteins. This hyperemia is an attempt for removal of the toxicant. Also, mast cells that were observed frequently in the interstitium were considered to release growth factors that modulate folliculogenesis and angiogenesis with subsequent dilatation and congestion of blood vessels^[36]. Numerous mast cells that were detected in metalaxyl treated group may be attributed to Low thyroid hormone levels. as documented recently by Baccari *et al.*^[37] who found that hypothyroidism resulted in a significant increase in both number of mast cells and histamine content in skin and lacrimal gland of rats, Besides, an increase in the percentage of degranulating mast cells.

Degenerative changes such as fusion of follicles with loss of connective tissue in between and expansion or dilatations of endoplasmic reticulum with loss of their lamellar arrangement were observed in the current study because of glandular overstimulation. These cistern patterns were also observed in the follicular cells of experimentally induced goitre by a low-iodine diet and after cypermethrin administration^[38, 39]. Abdul-Hamid and Salah^[40] referred this dilatation to the retention of aberrant protein within the cisternae and added that disruption in protein production might prevent the synthesis of apoptosis inhibitors or the loss of essential proteins involved in cellular homeostasis leading to cellular degeneration.

Apoptotic follicular cells were observed in metalaxyl treated rats as evidenced by small dark nuclei and vacuolated cytoplasm, ultrastructurally by intended heterochromatic nuclei. These observations were in consistence with previous investigators who found that metalaxyl induces apoptosis with bax expression in the hepatocytes^[10]. Also, it has been reported that environmental stressors (metals and pesticides) can induce apoptotic cell death through oxidative damage, ROS production, and lipid peroxidation^[41, 42].

According to Hassa *et al.*^[43] fungicides induced damage is closely associated with increase in lipid peroxidation and the decrease in the antioxidant enzymes. Also other researchers reported that the oxidative stress is the principle manifestations of metalaxyl-induced toxicity. Through an increase in the production of reactive oxygen species (ROS) which react with macromolecules such as lipid, protein, and DNA had led to cell dysfunction and damage. Reaction of ROS with lipid membrane, rich in polyunsaturated fatty acids causes lipid peroxidation with production of malondialdehyde (MDA) which is lipid peroxidation marker^[8, 23].

Ultrastructurally, secretory granules of C cells were numerous, but small, with reduced electron density. Evidence of hyperactivity was found in C cells such as

follicular cells. These findings were also in agreement with Mohamed and Rateb^[44] who reported that a hyperactive thyroid showed the presence of enlarged C cells distributed either in small groups or even singly. Some authors^[45] attributed that to the high level of TSH, leading to hyperplasia and hypertrophy of C cells. These observations might point to the possibility of a relationship between the activity of C cells and the functional state of the thyroid gland.

Follicular cells of metalaxyl treated group showed lack of microvilli. Nakazawa *et al.*^[46] reported that poor thyroid hormones synthesis may be the main cause of lack of microvilli which disturb the transport of colloid between follicular lumen and follicular cells.

Mononuclear cellular infiltration, mostly plasma cells were observed among follicular cells in the thyroid follicles of rats treated with metalaxyl. Morshed *et al.*^[47] suggested contribution of immune response in activation of follicular cells. Plasma cells could secrete immunoglobulins whose effects resembled those of TSH as have been shown in Graves' disease.

Immunohistochemical stained sections in metalaxyl treated group showed positive (PCNA) immunoreactions in many nuclei of follicular cells which was confirmed by morphometric and statistical analysis which revealed a highly significant increase in the area percent of PCNA in comparison to control group. This was in agreement with others who confirmed that expression of proliferating cell nuclear antigen (PCNA) was used to determine the supposed hyperplastic character of morphological changes^[48].

In this study, we have registered significant signs of improved histological structure of thyroid gland, and enhanced level of thyroid hormones in animals treated with ginger together with metalaxyl. This indicates that ginger diminishes the adverse effects of metalaxyl on thyroid gland and thyroid hormone metabolism. As, ginger has a strong antioxidant property and may either mitigate or prevent generation of free radicals. Moreover, ginger and some of its constituents are effective against cytokines synthesized and secreted at inflammation sites. It is considered a safe herbal medicine with only few and insignificant adverse/side effects^[49]. Also, ginger improve disruption in thyroid hormones synthesis and metabolism induced by bisphenol A^[50].

Mitochondria are natural targets of phytochemical antioxidant protection. Ginger could protect through a number of mitochondrial actions including up-regulation of specific anti-reactive oxygen species proteins, prevention of mtDNA damage, stimulation of replication, inhibition of membrane-active lipases, and protection of the electron transport chain. Ginger also inhibits NO

synthesis in activated macrophages and prevents oxidation and nitration reactions induced by peroxynitrite^[51,52].

It was found that, ginger extract ameliorated metalaxyl induced nephrotoxicity. This effect is mediated by either preventing metalaxyl-induced decline of antioxidant defense system or by its direct free radicals scavenging activity^[53].

Also, other researchers, reported that rats treated with ginger (24 mg/ml/rat) daily for six weeks showed an improvement in percentage of leucocyte DNA fragmentation. So, the antioxidant characters of ginger could protect DNA and other important molecules from oxidation and damage and can improve liver function. Ginger exhibited DNA protection, blocked lipid peroxidation & decrease apoptosis and free radical scavenging^[54,55].

In agreement with the previous data, ginger extract and its effective components can be used as a suitable agent for controlling and preventing Lambda-cyhalothrin thyroid toxicity and genotoxicity-induced DNA damage^[56]. Moreover, the aqueous ginger extract had benefits for both smokers and non-smokers. smokers presented a significantly higher mean lymphocyte and RBC counts, and hemoglobin concentration; and a significantly lower mean neutrophil count, and IgM and thyroid stimulating hormone concentrations^[57].

CONCLUSION AND RECOMMENDATION

Metalaxyl seriously affect the histological structure of the thyroid gland with subsequent decreased thyroid hormones. So, use of it should be extremely limited and be under many precautions. Concomitant administration of ginger was partially efficient in ameliorating these effects. Additional studies should investigate the mechanisms of action of the natural components of ginger and its antioxidant effects.

CONFLICT OF INTEREST

There are no conflicts of interest.

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