

## Ameliorative Effect of Ginger Extract and Selenium in the Thyroid Gland Toxicity Induced by Chlorpyrifos in Male Albino Rats

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### ABSTRACT

**Background:** Chlorpyrifos CPF is one of the organophosphate insecticides that were interfered with the body's hormones. Thyroid hormones are necessary to maintained normal metabolism, growth and development and the disturbance of their levels leads to adverse medical conditions. Ginger extract and selenium are effective anti-oxidants. **Aim of the work:** This study examined effect of CPF on the thyroid gland structure and function and the ameliorative effect of ginger extract and selenium. **Material and Methods:** forty rats were categorized into four groups. Control group I, group II: rats were given CPF at a dose of 6.7 mg/kg orally five days / week for six weeks, group III: rats were given CPF and ginger extract at a dose of 750 mg/kg orally, five days / week for six weeks. Group IV: rats were given CPF and sodium selenite 10 µg/Kg orally five days / week for six weeks, blood samples were taken for hormonal essay. Thyroid gland specimens were prepared for the histological and immunohistochemical studies. **Results:** chlorpyrifos induced functional toxic effect and destruction of the thyroid gland histological structures. There was a significant decrease in area % of the colloid and a significant increase in mean number of PCNA positive nuclei in comparison with the control group. Ginger extract and selenium eliminated the damage effect of CPF on the thyroid gland function and histological structure. **Conclusion:** CPF had adverse effects on the thyroid structure and function that could be eliminated by administration of ginger and selenium.

**Keyword:** chlorpyrifos, selenium, ginger extract, thyroid gland.

### INTRODUCTION

Organophosphates insecticides produce environmental pollution and accumulate in the food products and water that leads to acute and chronic poisoning in human and animals<sup>(1)</sup>. Chlorpyrifis one of the widely used organophosphates insecticides in agriculture<sup>(2)</sup>.

CPF produced reactive oxygen species and oxidative stress that distressing normal cellular differentiation and development<sup>(3)</sup> and elevated malonaldehyde (MDA) level that, considered as a marker of lipid peroxidation resulting from interaction of reactive oxygen species and cellular membrane that produced membrane damage by changing membrane characteristics<sup>(4)</sup>. CPF inhibits acetylcholinesterase activity that leads to over stimulation of cholinergic transmission mediated by nicotinic and muscarinic receptors, in tissues of the different organs. Chemicals including CPF are considered as a potential thyroid gland disrupter<sup>(5)</sup>.

Ginger (*Zingiber officinale*), has an effective protective role against oxidative stress and it is used as anti-emetic; it contains flavonoids and polyphenolic constituents that have antioxidant properties<sup>(6)</sup>.

Selenium is a micronutrient that has essential role in the preservation of cellular homeostasis, cellular metabolism and immune-endocrine function<sup>(7)</sup>. Sufficient dietary intake of Selenium from the diet as it found in animal meats, seafood, vegetables cabbage, broccoli, garlic, onion, and animal products are essential to maintain normal thyroid function<sup>(8)</sup>. For this reason, this study aimed to examine the changes in

the thyroid gland function and structure induced by CPF and to detect the ameliorative effect of ginger extract and selenium.

### MATERIAL AND METHODS

#### The experimental animals:

Forty adult male Albino rats were used in this experimental study about 200 - 250 g body weight and obtained from Faculty of Veterinary Medicine, Benha University then housed in cages at homogeneous temperature of 25°C. and were given standard diet.

#### Ethical approval

The study was conducted in accordance with the ethical policies approval by committee recommendations of Faculty of Medicine, Benha University.

#### Drugs:

**Chlorpyrifos:** chlorpyrifos powder was purchased from El-Watanya Company, Egypt. It was dissolved in corn oil.

**Ginger extract:** tablets 400 mg was purchased from MEPACO, Arab Company, Cairo, Egypt and dissolved in the distilled water.

**Selenium:** sodium selenite powder was obtained from Sigma Chemical Company- Egypt and dissolved in the distilled water.

Rats were equally categorized into four groups as follow:

**Group I (Control group):** ten rats were given normal diet and water and subdivided equally into:



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Subgroup a: five rats were kept for six weeks without medications.

Subgroup b: five rats were given 0.5 ml corn oil orally five days / week for six weeks, a solvent for chlorpyrifos.

**Group II (CPF group):** rats were given CPF dissolved in 0.5 ml corn oil at a dose of 6.7 mg/kg b.w. five days / week for six weeks<sup>(9)</sup>

**Group III (ginger extract-treated group):** rats were given CPF and ginger extract at a dose of 750 mg/kg b.w. five days / week for six weeks<sup>(10)</sup>

**Group IV (selenium-treated group):** rats were given CPF and sodium selenite at a dose of 10 µg/Kg b.w five days / week for six weeks<sup>(11)</sup>

Blood samples were taken from the retro orbital venous plexuses for hormonal assay, then rats were anaesthetized by ether inhalation. An incision was made in the skin of the neck then trachea was exposed and thyroid gland was taken out then prepared for the histopathological and immunohistochemical examination.

**Hormonal assay:**

The collected blood was placed in tubes with heparin, blood samples were centrifuged at 2000 rpm for about 15 minutes, then plasma stored at -20 °C until analysis. Levels of tri-iodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) were analyzed in plasma <sup>(12)</sup>.

**The histological study**

Samples of the thyroid gland were put in 10% formalin for 24 hours then dehydrated in ethanol and embedded in paraffin then xylene. The blocks were sectioned and stained with Haematoxylin and Eosin stain for detection the histopathological changes and Periodic acid-Schiff (PAS) stain to determine the colloid changes <sup>(13)</sup>.

**PCNA immuno-histochemical stain:**

PCNA proliferating cell nuclear antigen is considered as an indicator for cell proliferation. It is a rabbit polyclonal antibody (catalogue number ab15497, Abcam, Cambridge, UK). The sections were boiling for 10 minutes in 10 mmol/l citrate buffers for antigen retrieval then were left in room temperature for 20

minutes. After that they were incubated with the primary antibody for one hour and then completed by using the ultraviolet detection system. Mayer's hematoxylin was used in counterstaining. Small intestine was used as a positive control sections. Positive reaction was appeared as brown nuclear coloration <sup>(14)</sup>.

**Morphometric study**

Area percent of colloid in PAS stained sections at a magnification of X400.

Numbers of PCNA positive nuclei in PCNA immunostained sections at a magnification of X400. The measurements were done in ten non overlapping sections for each specimen by using image analysis program "Leica Quin 500 " software image analyzer

**Statistical Analysis**

Data were expressed as mean± SD. p≤0.05 was significant tested by using one-way analysis of variance (ANOVA) and post hoc multiple comparisons by using SPSS software (v.16; Chicago, USA).

**RESULTS**

Hormonal analysis: (Table 1)

Hormonal assay of CPF group showed a significant reduction in serum T3, T4 levels and significant elevation in serum TSH levels in comparison with the control group this indicated the functional damaging effect of CPF on the thyroid gland hormones and TSH hormone level. The main value of serum T3 & T4 levels in ginger extract-treated group showed a significant elevation and a significant reduction in serum TSH level as compared to CPF group. Moreover, these levels in selenium-treated group showed a significant elevation in T3& T4 levels and a significant reduction in TSH level in comparison with CPF and ginger extract-treated group this result indicated the powerful effect of selenium in elimination of CPF functional toxic effect on the thyroid gland than ginger extract.

**Table 1: hormonal assay the mean values ± SD of T3, T4 and TSH in all groups**

Groups	T3( µg/ml)	T4 (ng/ ml)	TSH (IU/ml)
Control group	3.6±0.05	3.28±0.057	1.36±0.019
Chlorpyrifos group	2.6±0.14a	2.18±0.035a	3.12±0.033a
ginger extract -treated group	2.98±0.09 a,b	2.69±0.035 a,b	2.95±0.037a,b
Selenium -treated Group	3.38±0.07 a,b,c	3.12±0.034 a,b,c	1.71±0.033 a,b,c

Significant <sup>a</sup>p< 0.05 vs. control group. <sup>b</sup>p< 0.05 vs. Chlorpyrifos group. <sup>c</sup>p<0.05 vs. ginger group.

**Histological and immunohistochemical results**

**The control group**

H and E-stained section from the thyroid gland showed regular follicles of various sizes. The follicular walls lined by single layer of flat cells and cubical cells. The follicular lumen full of homogenous colloid and there were few interfollicular cells (Fig. 1). PAS stained section showed strong PAS reaction in the colloid and normal blood vessel (Fig.2). Immunohistochemical stained section showed positive PCNA immunoreaction in the form of brown nuclear deposits in few follicles (Fig. 3).

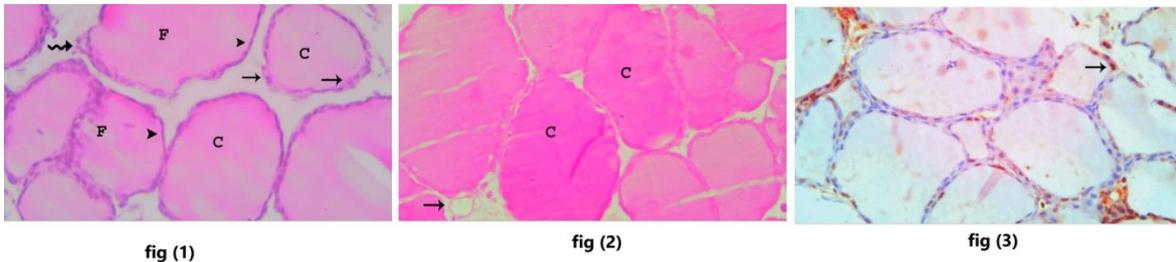


Fig 1: a photomicrograph of the thyroid gland section from the control group showing regular follicles of various sizes (F); follicular walls lined by single layer of flat cells (arrow heads) and cubical cells (arrows) follicular lumen full of homogenous colloid (C) and few interfollicular cells (wavy arrow). (H& E X 400)  
Fig 2: a photomicrograph of the thyroid gland section from the control group showing strong PAS reaction in the colloid (C ) and normal blood vessel (arrow). (PAS X 400)

Fig 3: a photomicrograph of the thyroid gland section from the control group showing positive PCNA immunoreactions in the form of brown nuclear deposits (arrow) in few follicles.(PCNA immune-staining X 400)

#### **Chlorpyrifos group**

H and E-stained section from the thyroid gland showed irregular follicle with desquamated cells in the lumen, other follicles with no colloid, fused follicles, other follicle with obliterated lumen and numerous interfollicular cells (fig. 4) PAS stained section showed negative PAS reaction in the colloid and vacuolated follicular cells (Fig. 5). Immunohistochemical stained section showed numerous positive PCNA immunoreactions in all the follicles (Fig. 6).

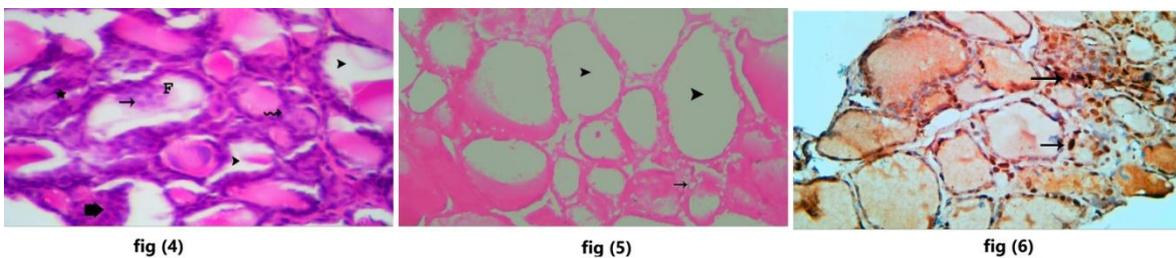


Fig 4: a photomicrograph of the thyroid gland section from chlorpyrifos group showing irregular follicle (F) and desquamated cells in the lumen (arrow), other follicles with no colloid (arrow heads), fused follicle (wavy arrow) follicle with obliterated lumen (thick arrow) and numerous interfollicular cells (star).(H& E X 400)

Fig 5: a photomicrograph of the thyroid gland section from chlorpyrifos group showing negative PAS reaction in the colloid (arrow heads) and vacuolated follicular cells (arrow).(PAS X 400)

Fig 6: a photomicrograph of the thyroid gland section from chlorpyrifos group showing numerous positive PCNA immunoreactions (arrow) in all the follicles. (PCNA immunostaining X 400)

#### **Ginger extract-treated group**

H and E-stained sections from the thyroid gland showed normal follicles full of colloid, other follicles contained scanty colloid, there was a follicle with obliterated lumen and another follicle with desquamated cells in the lumen, there were apparent many interfollicular cells and congested blood vessels (Fig. 7). PAS stained section showed some follicles with strong PAS reaction, others with negative PAS reaction and numerous vacuolated follicular cells (Fig. 8). Immuno-histochemical stained section showed numerous positive PCNA immunoreactions in most of the follicles (Fig. 9).

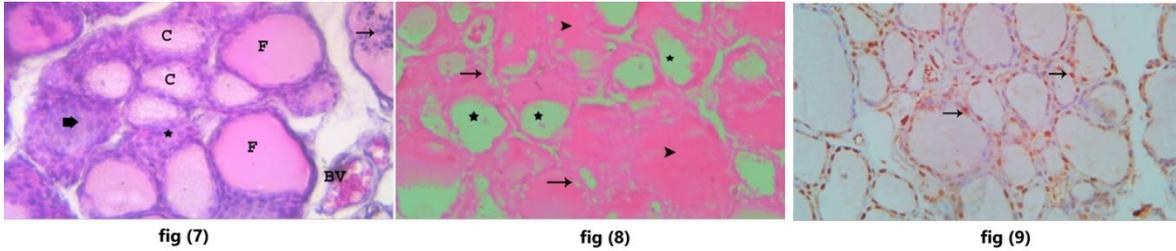


fig (7)

fig (8)

fig (9)

Fig 7: a photomicrograph of the thyroid gland section from ginger extract-treated group showing normal follicles full of colloid (F), other follicles contained scanty colloid (C), follicle with obliterated lumen (thick arrow), follicle with desquamated cells in the lumen (arrow), there were many interfollicular cells (star) and congested blood vessels (BV) (H& E X 400)

Fig 8: a photomicrograph of the thyroid gland section from ginger extract-treated group showing some follicles with strong PAS reaction (arrow head), others with negative PAS reaction (star) and numerous vacuolated follicular cells (arrows). (PAS X 400)

Fig 9: a photomicrograph of the thyroid gland section from ginger extract-treated group showing numerous positive PCNA immunoreactions (arrow) in most of the follicles. (PCNA immunostaining X 400)

**Selenium-treated group**

H and E-stained section from the thyroid gland showed normal follicles of various sizes lined by single layer of cells, but there was a follicle with double layers of cells, there were few interfollicular cells and normal blood vessel (Fig. 10). PAS stained sections showed strong PAS reaction in many follicles and few follicles with negative PAS reaction (Fig. 11). Immuno-histochemical stained section showed few positive PCNA immunoreactions in multiple follicles (Fig. 12).

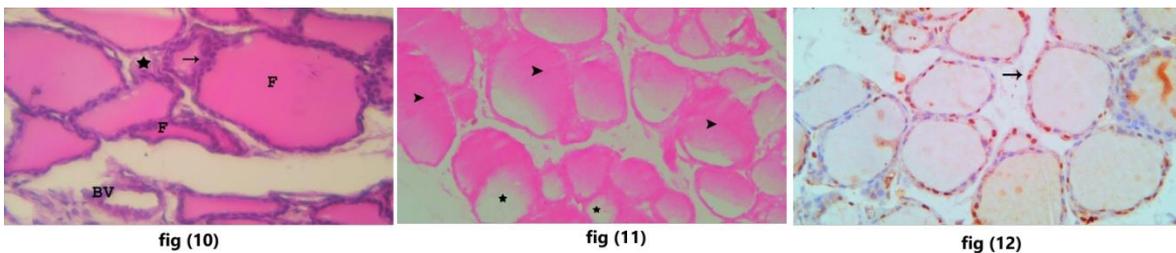


fig (10)

fig (11)

fig (12)

Fig 10: a photomicrograph of the thyroid gland section from selenium-treated group showing normal follicles of various sizes lined by single layer of cells (F), follicle with double layers of cells (arrow) there are few interfollicular cells (star) and normal blood vessel (BV). (H& E X 400)

Fig 11: a photomicrograph the thyroid gland section from selenium-treated group showing strong PAS reaction in many follicles (arrow head) and few follicles with negative PAS reaction (star). (PAS X 400)

Fig 12: a photomicrograph of the thyroid gland section from selenium-treated group showing few positive PCNA immunoreaction (arrow) in multiple follicles. (PCNA immune-staining X 400)

**Morphometric results**

The mean area percent of colloid in PAS stained thyroid sections represented in table 2. In group II there was a significant decrease in area % of colloid in PAS stained sections in comparison with the control group and a significant increase in area % of colloid in PAS stained sections in group III and IV in comparison with CPF group. Mean area % in group IV was elevated than group III.

The mean number of PCNA positive nuclei in PCNA immune-stained thyroid sections was represented in table 3. Group II showed a significant increase in mean number of PCNA positive nuclei in group II in comparison with the control group and a significant decrease in mean numbers of PCNA positive nuclei in group III and IV in comparison with CPF group. The mean value was decreased in group IV than group III.

**Table 2: table showing mean values of area percent of colloid ± SD in all groups**

Main % ± SD	Group I	Group II	Group III	Group IV
MA	64.06±4.3	9.1±1.3	40.8±7.04	50.5 ±7.7
Significance ≤ 0.05	With group II	With groups I,III& IV	With groups II	With groups II

**Table 3: table showing mean values of numbers of PCNA positive nuclei ± SD in all groups**

Main % ± SD	Group I	Group II	Group III	Group I
MA	6.3 ±1.3	28.3±2.6	11.36±3.8	9.96 ±3.3
Significance ≤ 0.05	With group II	With groups I,III& IV	With groups II	With groups II

## DISCUSSION

Chlorpyrifos is one of the widely used chemicals in insecticide in agricultural and industry fields. Chlorpyrifos produced oxidative stress that induced pathological damage in tissues<sup>(15)</sup>. Ginger has flavonoids and polyphenolic components that used in medicinal purposes as antibacterial, antioxidant, anti-inflammatory, antidiabetic and analgesic properties<sup>(16)</sup>. Selenium plays an essential role in antioxidant activity, defense mechanism in the thyroid gland tissue, through oxygen free radicals elimination in the thyroid hormones synthesis and has an important role in the thyroid hormones metabolism<sup>(17)</sup>.

This study aimed to detect the effect of CPF on the thyroid gland function and structure and the ameliorative effect of ginger extract and selenium.

In this study chlorpyrifos induced functional toxic effect on the thyroid gland as the hormonal assay in CPF group revealed a significant reduction in serum T3, T4 levels and a significant elevation in serum TSH levels in comparison with the control group. These results were confirmed with the histopathological findings that showed destruction of the thyroid gland follicles as there were irregular follicles with desquamated cells in the lumen, other follicles with no colloid, fused follicles, other follicles with obliterated lumen and numerous inter follicular cells. Moreover, a significant decrease in area % of colloid and a significant increase in mean number of PCNA positive nuclei in comparison with the control group. In the same line **Shady and El-Deen**<sup>(18)</sup> stated that chlorpyrifos induced oxidative damage on the thyroid gland and reduction of the thyroid hormone level. Also **Kassab and El-Aasr** found that chlorpyrifos induced follicular cell degeneration, collagen fibers deposition and decreased colloid secretions<sup>(19)</sup>. Similarly, another study on the effect of chlorpyrifos on female wistar rats concluded that short term daily intake of low and high doses of chlorpyrifos induced a significant thyroid hormone level disrupting effects<sup>(20)</sup>. Other authors found that chlorpyrifos reduced antioxidant enzyme activities and glutathione (GSH) in the thyroid gland tissues. Moreover; it elevated the level of malondialdehyde<sup>(9)</sup>. In the same line **Abed and Alkalby**<sup>(21)</sup> found a destruction of thyroid follicles with infiltration of macrophages and a significant elevation in serum level TSH and a significant

reduction in (T4) and (T3) hormones compared to the control group. The chlorpyrifos toxic effects on the thyroid gland tissue may be due to lipid peroxidation that associated with stimulation of stellate cells for collagen synthesis in between thyroid follicles<sup>(18)</sup>. Another study revealed that the toxic effect of chlorpyrifos may be due to decreased the iodine binding proteins<sup>(22)</sup> and **Wang et al.** stated that organophosphate exposure associated with changes in the thyroid gland function in pregnant women<sup>(23)</sup>.

Ginger could regulate the gene expressions and increased antioxidant enzymes including glutathione, superoxide dismutase and glutathione peroxidase and increased the phase II detoxification enzymes. Ginger and its active ingredient as 6-gingerol and 6-shogaol could be used in different human diseases<sup>(24)</sup>. In this study, the main value of serum T3 & T4 levels in ginger extract-treated group showed a significant increase and significant decrease in serum TSH level in comparison with that of CPF group. These results were confirmed by moderate improvement in the histopathological changes induced by CPF as there were some normal follicles full of colloid, other follicles with obliterated lumen and desquamated cells in the lumen and congested blood vessels. There was a significant increase in area % of colloid and a significant decrease in mean number of PCNA positive nuclei compared to CPF group. Similarly, **Al-Amodie** stated that ginger extract improved the histochemical and histological changes of the thyroid gland induced by insecticide Lambda-cyhalothrin. Furthermore; it increased T3 and T4 and decreased TSH hormones levels, decreased MDA level the lipid peroxidation marker and increased the antioxidant enzymes<sup>(25)</sup>. Another study revealed the ameliorative role of ginger extract on the insecticide mancozeb toxicity of the thyroid gland in rats<sup>(26)</sup>. **Yousef et al.** suggested that administration of ginger extract was a valuable routine for protection in opposition to the damaging effect of cypermethrin in the thyroid gland structure<sup>(10)</sup>. Other study proved that the antioxidative, anti-inflammatory and antiapoptotic functions were the most effective role of ginger in protection against chlorpyrifos toxicity in the brain and reproductive organs of rats<sup>(27)</sup>. Ginger extract has an effective antioxidant activity in elimination the bisphenol induced thyroid toxicity through the activation of the

Nrf-2/HO-1 gene expressions and initiation the production of thyroid hormones<sup>(28)</sup>. Thyroid gland considered the uppermost tissue contained the highest concentration of selenium which is included the seleno-proteins. Those have an important role in production and metabolism of thyroid hormones<sup>(29)</sup>. In selenium-treated group T3 & T4 levels were significantly increased and TSH level was significantly decreased compared to CPF, this result indicated the powerful effect of selenium in elimination of CPF functional toxic effect on the thyroid gland. These results were confirmed by the histological findings as there was marked improvement in the thyroid gland structures with a significant increase in area % of colloid and a significant decrease in mean numbers of PCNA positive nuclei in compared with CPF group. This is in agreement with results of **Lamfon**<sup>(11)</sup> who found that selenium normalized the thyroid tissue architecture and improved the thyroid hormones levels, by its ability for ameliorating the antioxidative defense mechanism of the rats.

Researches revealed that selenium has antioxidant role against the oxidative stress that overloaded reactive nitrogen species and reactive oxygen species. It contained selenoproteins and glutathione peroxidase enzyme<sup>(30)</sup>. It participates in conversion of thyroxine to triiodothyronine in biosynthesis of thyroid hormone<sup>(31)</sup>.

Another authors studied the ameliorative role of selenium on the thyroid toxicity and revealed that selenium improved the histological and immunohistochemical changes induced by bisphenol toxicity<sup>(32)</sup>. **Ibrahim et al.** recommended nutritional intake of selenium and zinc for the treatment of hypothyroidism disorders<sup>(33)</sup>. A recent study added the role of selenium in improving the testicular histopathological toxicity by increasing glutathione, glutathione peroxidase, catalase, superoxide dismutase activities in rats<sup>(34)</sup>. In the same line, combination of ginger and selenium improved the oxidative stress in rats<sup>(35)</sup>. And the same study recommended administration of ginger and selenium in patients suffering from the medical oxidative stress condition.

## CONCLUSION

CPF had adverse effects on the thyroid structure and function that could be eliminated by administration of ginger and selenium.

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