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Effect of Green Tea extract against Intoxication with Fenitrothion Induced Some Clinicopathological Alteration in Thyroid Gland in Albino Rat.

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Abstract

This present investigation reviews the evidence for protective effects of green tea extract (60 mg / animal / day) dietary supplements against an organophosphorus insecticide fenitrothion (Fn) toxicity in male albino rats for five weeks and describes the proposed possible biochemical and histopathological alters in blood and thyroid gland samples. Rats were divided into 6 groups. The 1st group served as a control, 2nd and 3rd groups were treated with high (FnH) and low (FnL) dose as 20 and 10 mg Fn / kg b. w, respectively, 4th group (+ve control) received green tea extract (GT), and 5th and 6th group administrated high (GTFnH) and low dose (GTFnL) with green tea, respectively. The results revealed that a reduction in plasma glucose level post intoxication with FnL compared with FnH and slight improvement induced by green tea supplementations were observed. Compared with the control group, a significant increase was observed at both fenitrothion doses in total cholesterol and thyroxine (T_4) levels. Green tea supplementation counteracts the elevation of plasma total protein, and triglycerides induced as a result of fenitrothion intoxication. Prolonged supplementation of green tea extract (GTFn) declares significant improvement in induced significant reduction in hormonal plasma triiodothyronine (T_3) and thyroxine (T_4) concentration. Intoxication with both doses of fenitrothion (Fn) induced an increase in protein bands to be 12 bands versus (11 bands) compared with presence green tea and 3 new bands. On the other hand, Supplementation with green tea extract caused a reduction in the protein band number to be 11 bands versus 13 in the control group with 3 new protein bands.

According to the histopathological alters, both treatments (Fn groups) showed thyroid follicle congestion with areas of hemorrhage and rupture of some follicles. Meanwhile, supplementation with green tea extract showed an improvement in the intoxicated thyroid tissues.

Keywords: Fenitrothion, Green Tea, Biochemical Parameters, Thyroid Hormones, Rat.

Introduction

Although several studies have shown that pesticides exhibits low mammalian toxicity, biochemical and functional changes in animal tissues have been reported (**Briggs, 1992**). While, prolonged administration of these pesticides decreases the concentration of glucose, total protein and change in protein profile bands as reported by **Khan, *et. al*., (1990), El - Demerdash, *et. al*., (2001),** and **El - Zayat, *et. al*., (2005)**. So in the present study, we were studying subacute toxicity of fenitrothion (O, O-dimethyl - O - 4-nitrophenyl phosphorothioate) is an organophosphate (OP) insecticide and acaricide (**Worthing, 1987 and Meister, 1994**) which widely used for commercial greenhouse and outdoor use on ornamentals, including trees, to control a variety of insects, and mites (**EPA, 1995**).

Polyphenols constitute a large group of naturally occurring substances in the plant kingdom, which include the flavonoids. The plant phenolics are commonly present in fruits, vegetables, leaves, nuts, seeds, barks, roots and in other plant parts. These substances have considerable interest in the field of food chemistry, pharmacy and medicine due to a wide range of favorable biological effects including antioxidant properties (**Cook and Samman, 1996**). Evidence suggests that high intake of antioxidant nutrients from food sources offers health advantages (**Aruoma, 1998**).

Green tea (GT) is a favorite beverage and its extracts are popular components of dietary supplements. GT prepared from the leaves of *Camellia sinensis* L., is a beverage that is popular worldwide and possesses many pharmacological effects, such as anti-mutagenic, anti-proliferative, anti-carcinogenic properties. It is a potent neuro - protective remedy in models of degenerative disorders (**Choi, *et. al*., 2002**). GT intake is accompanied with a lower incidence of cancer, cardiovascular disease, and neurodegenerative disorders; hence green tea extract is included in multivitamins and other dietary supplements (**Abd El - Fattah and Ismail, 2015)**.

Many studies have shown that the polyphenolic fractions isolated from green tea inhibit oxidant stress and possess anti - inflammatory activity (**Chen, *et. al*., 2004).** They have been reported to prevent liver toxicity induced by a number of hepatotoxicants in animal models including: 2-nitropropane, carbon tetrachloride and acetaminophen. Also, dietary intake green tea has been shown to prevent fatty liver disease in both diets induced and genetic animal model (**Lambert, *et. al*., 2010)**.

Consequently, the present work aimed at studying the toxic effect of repeated application of fenitrothion insecticide on thyroid functions together with the possible protective role of green tea extract against fenitrothion induced changes. This was done using biochemical, and histological methods. The rate of gene expression manifested by protein pattern was also checked.

Materials and Methods

Tested Chemicals:

Fenitrothion insecticide: Sumithion (fenitrothion 50% EC) purchased from Kaffer Elzayat Co for Insecticide Ltd. Kaffr Elzayat, Egypt.

Green tea extract purchased from Human Changsha Yuanhang Biology Product Co., Ltd, China, contains 98 % polyphenols.

Tested Animals and Experimental Design

Sixty adult male albino rats (*Rattus norvegcious*) weighing between 160 - 180 g were used. The animals were housed in plastic cages, fed *ad - libitum* and acclimatized for two weeks before starting the experiment to adjust to the new environment.

Animals were randomly divided into six experimental groups for each ten animals and treated daily via gastric tube for 28 days. The doses were dissolved in 1 ml distilled water and green tea extract was given one hour prior administration of fenitrothion as follows:

Group 1 (Control Group): Animals were given daily distilled water (1 ml / animal).

Group 2 (Green Tea, GT): rats were orally given 60 mg green tea extract / animal.

Group 3 (Fenitrothion High Dose, FnH): rats were orally given 20 mg fenitrothion / kg b.w.

Group 4 (Fenitrothion Low Dose, FnL): rats were orally given 10 mg fenitrothion / kg b.w.

Group 5 (Green Tea + Fenitrothion High Dose, GTFnH): rats were orally given green tea and 20 mg fenitrothion / kg b.w.

Group 6 (Green Tea + Fenitrothion Low Dose with (GTFnL): rats were orally given green tea extract 10 mg fenitrothion / kg b.w.

Sampling:

Blood has been collected from the retro - orbital plexus vein according to **Schermer (1967),** on heparinized tubes at the end of the experimental period (five weeks). Plasma samples were separated by centrifugation of blood samples at 3600 rpm for 15 minutes and left at - 20 ^{o}C for subsequent use. Animals were scarified and thyroid gland was dissected for histopathological studies.

Biochemical Assay:-

Plasma glucose concentration (**Darham and Trinder, 1972)** and total protein **Weichselbaum (1946)** were determined calorimetrically methods. Total cholesterol (**Richmond, 1973 and Allain, *et. al*., 1974**) and triglycerides (**Fossati and Prencipe, 1982)** were estimated by an enzymatic colorimetric method. Hormonal analysis of total Tri-iodothyronine, T_3, (**Larsen, 1972**) and Tetra-iodothyronine, Thyroxine - T_4, (**Britton, 1975)** concentrations were performed by radioimmunoassay (RIA) method using a calibration curve.

SDS – PAGE Electrophoresis of Plasma Protein Profile:

Assaying molecular change in plasma protein was identified by 12 % SDS - Polyacrylamide Gel Electrophoresis (SDS - PAGE) according to the method of **Laemmli (1970).**

Histological Study:

Histopathological examination of the thyroid gland was carried out according to **Drury and Wallington (1980).** Thyroid gland sections of rats were fixed at 10 % neutral buffered formalin (pH 7.2), dehydrated in ascending series of ethanol, cleared in methyl benzoate, embedded in paraffin wax, deparaffinized with xylene, cut at 5 - 7 µm thickness and stained with Haematoxylin and Eosin stain (H & E) for gross histological evaluation according to **Bancroft and Stevens (1977)** method that modified by **Kiernan (2001)** technique. The sections were examined and photographed on an Olympus light microscope (Olympus BX51, Tokyo, Japan) with attachment photograph machine (Olympus C-5050, Olympus Optical Co. Ltd., Japan).

Statistical Analysis:

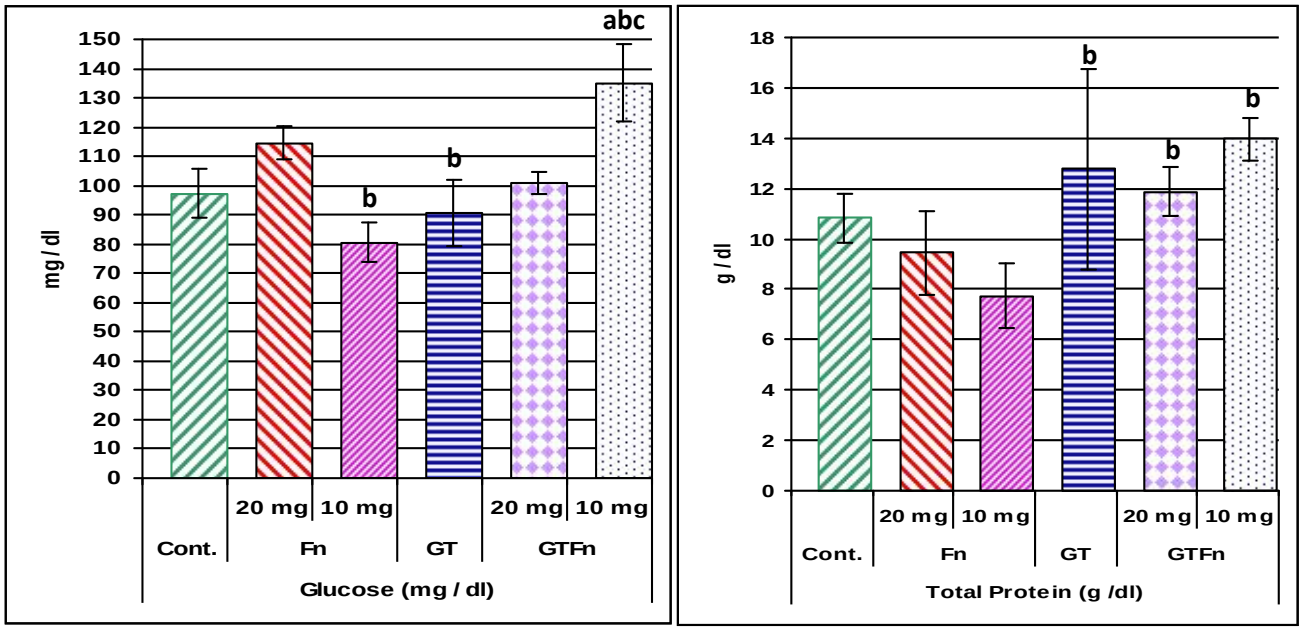
Ten replicates of each sample were used for statistical analysis. Data were reported as means ± SE and analyzed by standard statistical analysis, one - way analysis of variance (ANOVA) using package SPSS (Version 16). Least significant difference (LSD) test was conducted to identify differences among means. The statistical significance level was declared at $p \leq 0.05$.

Results

Biochemical Effects:

Figure (1) cleared that significant decrease in plasma glucose level at FnL was induced comparing with level at FnH. Slight improvement was induced by green tea extract and FnL which significant versus control and non supplemented groups. However, a significant increase was recorded in all supplemented groups versus intoxication group with tested insecticide at low dose (FnL) after the experimental period.

Fig. (1): Effect of green tea extract supplementation (60 mg / animal) on plasma glucose and protein contents of male albino rats intoxicated with fenitrothion (20 and 10 mg / Kg b.w).



All data are expressed as mean ± SE (10 rats) groups at p ≤ 0.05.

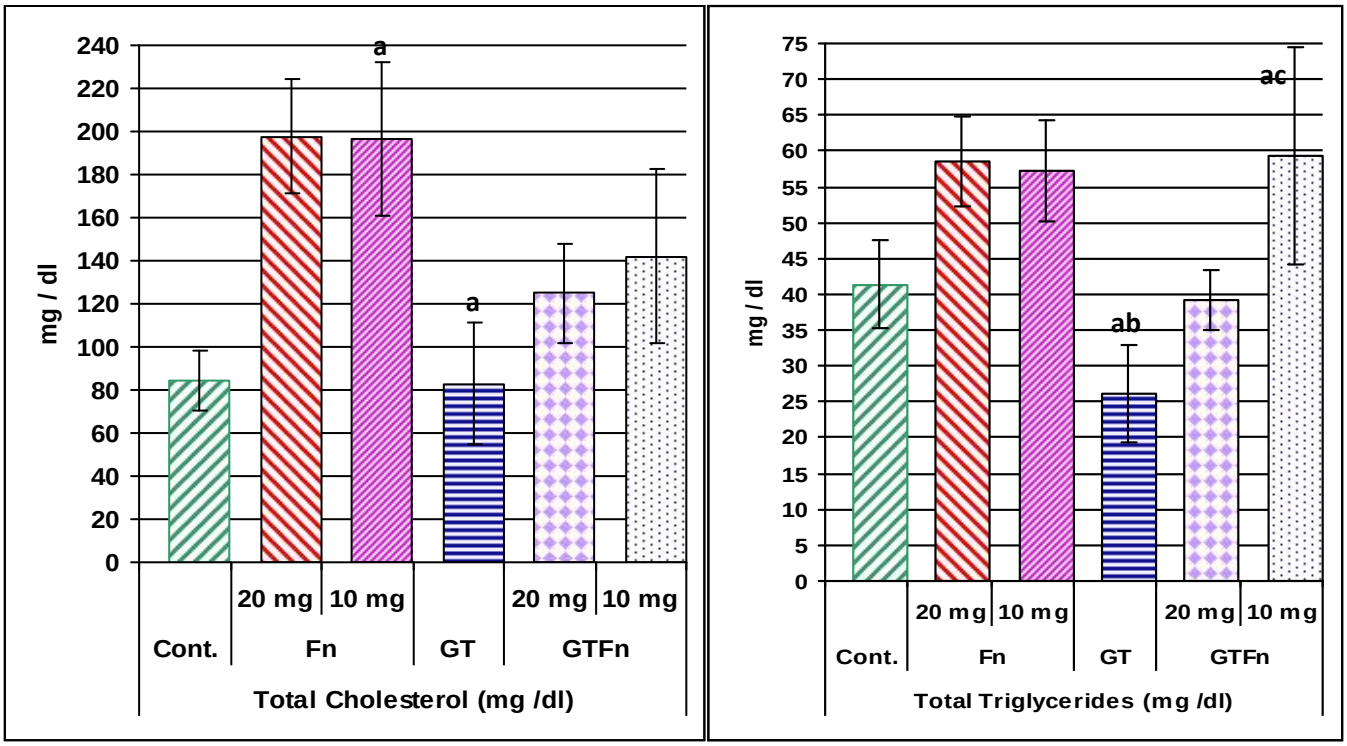
a Significant difference versus control at p ≤ 0.05

b Significant difference versus Fenitrothion treated

c Significant difference versus GT groups p ≤ 0.05

Subacute intoxication with fenitrothion insecticide at both doses (20 and 10 mg) induced a significant increase in plasma total cholesterol concentration of male albino rats compared with control group as shown in figure (2). On the other hand, supplementation with green tea extract induced significant decrease in plasma total triglycerides versus control and FnH groups. While, a significant increase in plasma total triglycerides was noticed at group supplementation with green tea extract and low dose of fenitrothion (Fig. 2)

Fig. (2): Effect of green tea extract supplementation (60 mg / animal) on plasma total cholesterol and triglyceride concentrations of male albino rats intoxicated with fenitrothion (20 and 10 mg/Kg b.w).



All data are expressed as mean ± SE (10 rats) groups at $p \leq 0.05$.

a Significant difference versus control at $p \leq 0.05$

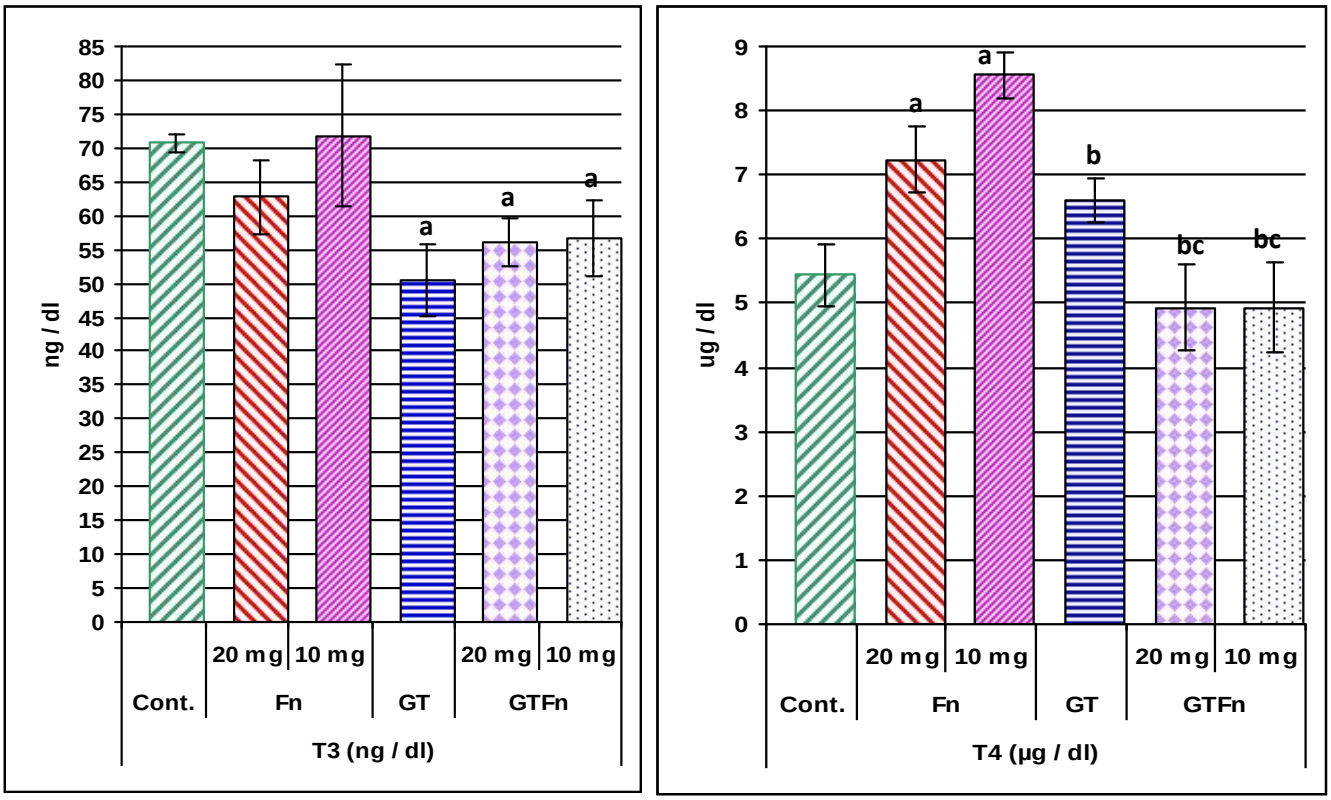
b Significant difference versus Fenitrothion treated

c Significant difference versus GT groups $p \leq 0.05$

Hormonal Effects:

Data presented in figure (3) declared that green tea supplementation to fenitrothion - intoxicated groups have shown, exhibited a significant decrease in plasma triiodothyonine (T_3) versus control group and in thyroxine (T_4) versus Fn groups. On the other hand, intoxication with fenitrothion at both tested doses has shown a significant increase in plasma T_4 versus the control group at $p \leq 0.05$ at the end of the experimental period.

Fig. (3): Effect of green tea extract supplementation (60 mg / animal) on plasma thyroid hormones (T_3 and T_4) levels of male albino rats intoxicated with fenitrothion (20 and 10 mg / Kg b.w).



All data are expressed as mean ± SE (10 rats) groups at p ≤ 0.05.

a Significant difference versus control at p ≤ 0.05

b Significant difference versus Fenitrothion treated

c Significant difference versus GT groups p ≤ 0.05

Electrophoretic Protein Pattern Changes:

Tabulated data in table (1) cleared that, reduction in protein bands was demonstrated in animals intoxicated with both the tested doses in the presence or absence of the green tea supplementation for a testing period to be 12 bands versus 13 bands in the control group (Fig 4).

Table (1): Electrophoretic Protein Pattern Changes of Plasma Male Rats after Treatment With Fenitrothion (20 and 10 mg / Kg b.w) Presence or Absence of Green Tea Supplementation Extract (60 mg / Animal) For 28 Days.

M. W (KDa)	% of Amount					
	Cont	FnH	FnL	GT	GTFnH	GTFnL
* 198	4.71					
# 191			5.37			
188	5.63	7.35		5.31	6.26	4.37
# 177		4.45	5.04	4.17	3.79	6.24
168	7.86	5.00	3.43	4.65	4.10	6.08
# ↑ 155		4.17	5.96			
* 136	4.90					
# 127				5.26		
# 121		7.27	5.11		8.81	4.45
108	4.61		4.71	4.68	4.98	3.47
97	7.14	7.09	3.55	4.71	3.04	
# 86		5.26	6.58		8.48	4.17
↑ 76	5.47	5.55				
66	6.64		3.43	6.50		3.91
# 59					10.20	
57	6.45			13.40		12.50
# ↑ 54		21.60	38.50			
↓ 49	26.10			14.40		
# ↑ 48					28.20	30.50
# ↑ 41		5.38		13.90		
35	9.87	18.90	12.70			
# 33				15.70	12.50	17.00
↑ 17	6.59	7.74				
14	3.81		5.55	7.11	9.47	7.19
Sum	*99.78*	*99.76*	*99.93*	*99.79*	*99.84*	*99.88*
Total No.	*13*	*12*	*12*	*12*	*11*	*11*

*** Disappear # Appear ↑ Increase Amount ↓ Decrease Amount**

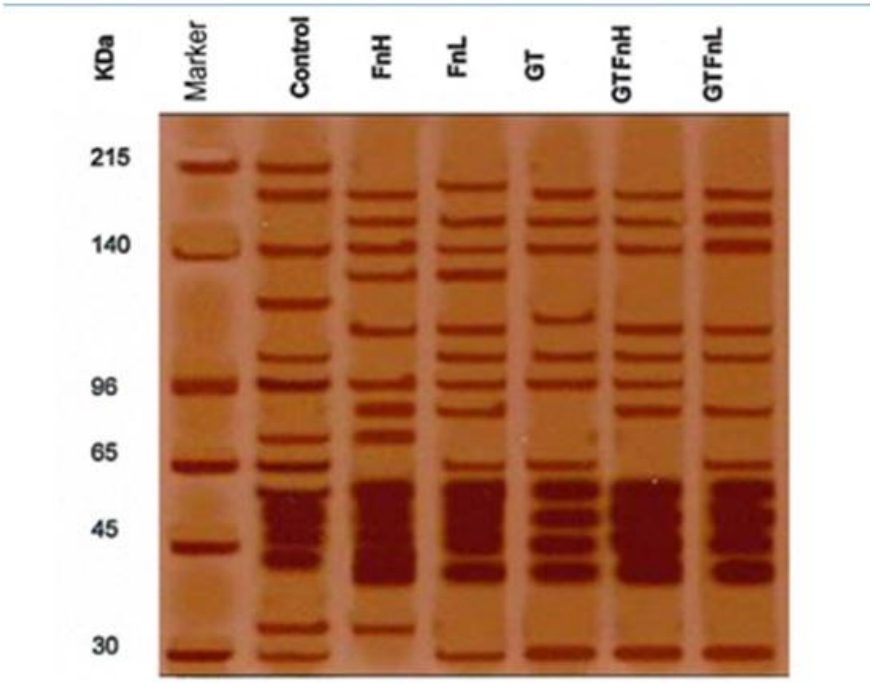


Fig (4): Plasma protein electropphoretic patterns of male albino rats after fenitrothion intoxication with or without green tea supplementation at 28 days. Individuals lanes reflect pooeld sample of individual groups.
FnH: High dose of fenitrothion (20mg)
FnL: Low dose of fenitrothion (10mg)
GT: Green tea extract (60mg/animal)
GTFnH: Green tea extract and High dose of fenitrothion
GTFnL: Green tea extract and Low dose of fenitrothion

The electrophoretic protein pattern changes (appear, disappear, or increase / decrease amount) in animals intoxicated with both the tested doses in the presence or absence of the green tea supplementations can summarized as follows:

1- Bands (198 and 136 KDa) were appearing in the control group only, where band 177 KDa was disappeared in the control group.

2- Bands 191 (FnL), 155 and 54 (both Fn), 127 (GT), 59 (GTFnH), 49 (Cont. and GT), 48 (both GTFn), 41 (FnH and GT), 33 (GT and both GTFn) and 17 (Cont. and FnH) KDa were appeared.

3- Bands 188 (FnL), 121 and 86 (Cont. and GT), 108 (FnH), 97 (GTFnH), 66 (FnH and GTFnH) and 14 (FnL) KDa were disappeared.

4- Band that molecular weight 168 KDa was appeared at different amount in all treatment groups.

5- Bands 54 (both Fn), 49 (Cont.). 48 (both GTFn) and 33 (GT) KDa were recording the highest percentage of the amount.

Histopathological Effects:

Examination of thyroid gland sections of the control group showed normal thyroid follicles with cuboidal cells with their basophilic cytoplasm, the central part of the follicle shows the acidophilic colloid secretion (Plate 1). In the animal group treated with green tea extract (GT, 60 mg / animal / day) normal sinusoids full of colloidal secretion (Plate 2) was also appeared.

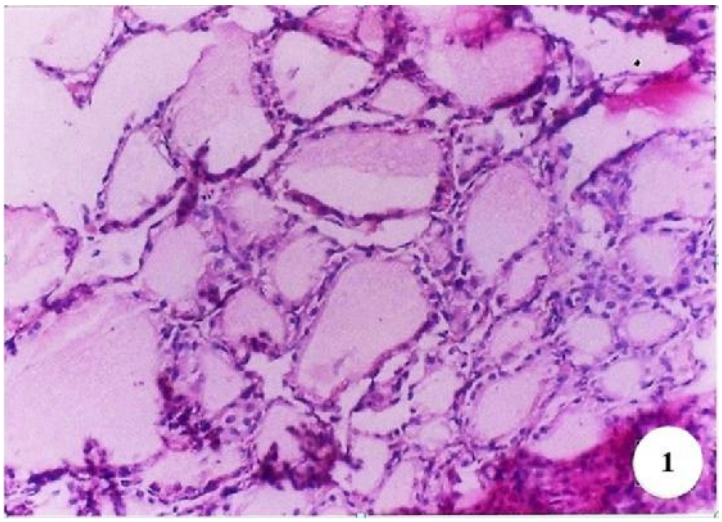


Plate (1): Showing normal thyroid gland from rat of control group with their thyroid follicles lined by low cuboidal cells with basophilic cytoplasm. (H & E, X 40)

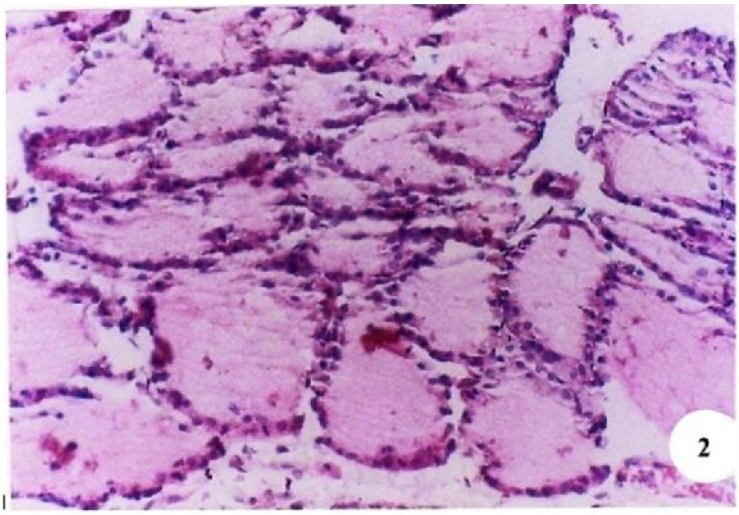


Plate (2): Showing normal thyroid gland from rat of supplementation green tea extract (60 mg / animal / day) showing with normal sinusoids full of colloidal secretion. (H & E, X 40)

On the other hand, Animals intoxicated with fenitrothion at both the tested doses (FnH and FnL) the thyroid follicles showed congestion with hemorrhage in between the ruptured follicles. The cuboidal epithelium appeared flattened and mononuclear infiltration was present (Plate 3 and 4).

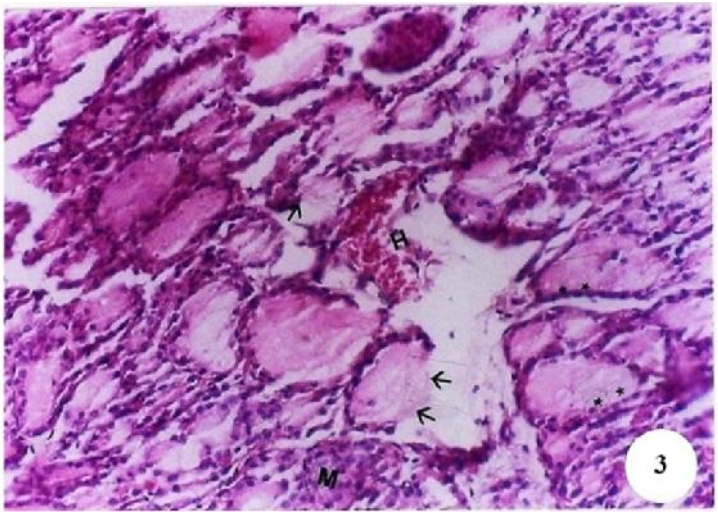


Plate (3): Light photomicrograph of thyroid gland from rats intoxication with 20 mg fenitrothion (FnH) showing the thyroid follicles congested with areas of hemorrhage and necrosis (H), rupture of some follicles (arrows), flattened cuboidal epithelium (*) and mononuclear cellular infiltration (M). (H & E, X 40)

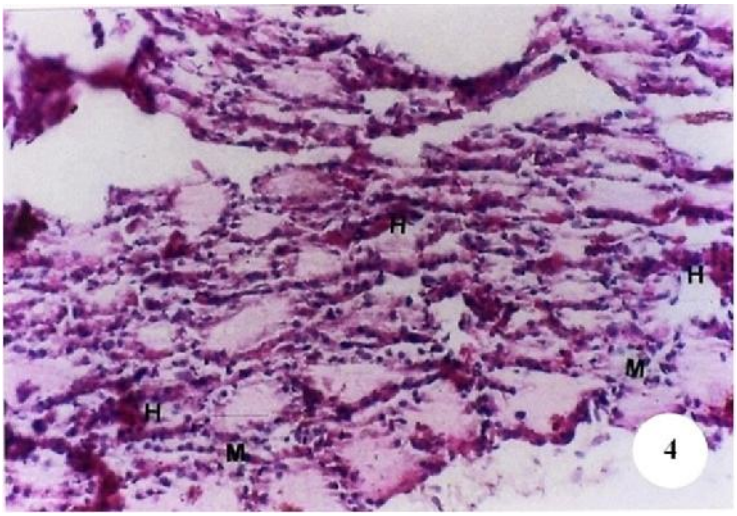


Plate (4): Light micrograph of thyroid gland from rats intoxication with 10 mg fenitrothion (FnL) showing congested thyroid gland with spots of hemorrhage (H), infiltration with mononuclear inflammatory cells was noticed (M). (H & E, X 40)

Meanwhile, supplementation with green tea extract to the intoxicated rats with both doses of fenitrothion (GTFnH and GTFnL) showed an improvement in thyroid tissues. The examined cells appeared normal in size and their lining showed a double layer of nuclei, proliferation or infiltration of cells was also noticed. Congestion and some hemorrhagic spots were still seen between the follicles (Plate 5 and 6).

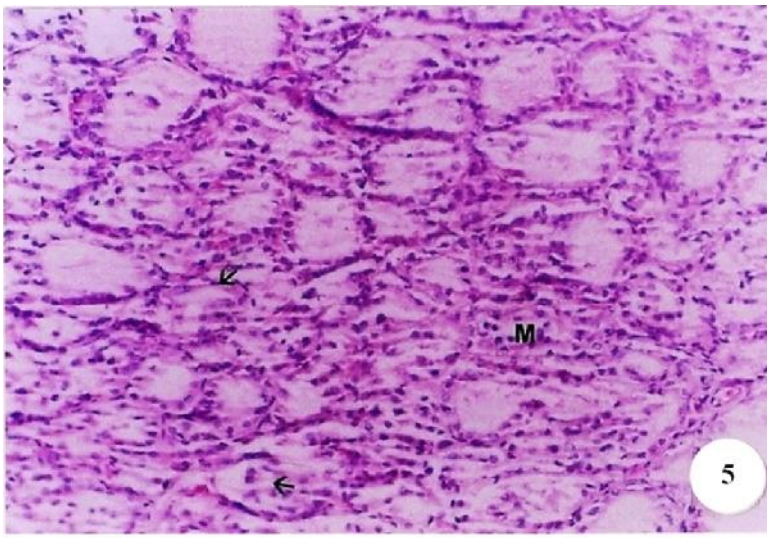


Plate (5): Light photomicrograph of thyroid gland from rat supplementation with green tea extract to intoxication with high dose of fenitrothion (GTFnH) showing some collapsed follicles with scattered hemorrhagic areas (arrows), proliferation and massive mononuclear inflammatory cells infiltration was marked (M). (H & E, X 40)

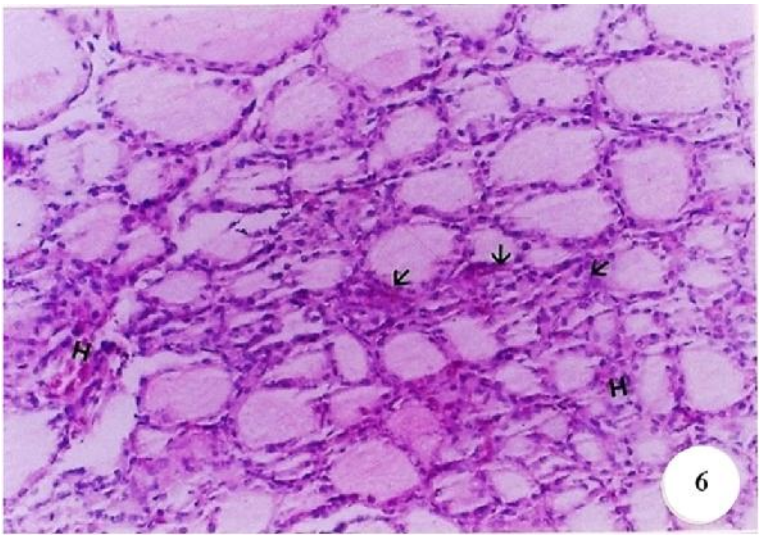


Plate (6): Light photomicrograph of thyroid gland from rat supplementation with green tea extract to intoxication with low dose of fenitrothion (GTFnL) showing double layer of nuclei in the lining of some thyroid follicles (arrows), few areas of hemorrhage were noticed between the follicles. (H & E, X 40)

Discussion

Naturally occurring antioxidants in leafy vegetables, fruits and seeds such as ascorbic acid, vitamin E and phenolic compounds have the ability to reduce oxidative damage associated with many diseases, including: cancer, cardiovascular diseases (**Middleton, *et. al.*, 2000)**, cataract (**Gupta, *et. al.*, 2009)**, arthritis and diabetes (**Kannappan and Anuradha, 2009**). The present study aimed to evaluate Fn effects on the thyroid gland and the possible ameliorating role of green tea extract treatment in adult male albino rats as a one polyphenol plant.

The results of the current study revealed that intoxication with fenitrothion induced significant decrease in glucose level at FnL group. These findings can explain by **Kannappan and Anuradha (2009)** who demonstrated that plant polyphenols have been known to exert anti - diabetic action and promote insulin action.

Well, there is no remarkable effect on total plasma protein in animals intoxicated with tested fenitrothion doses, while green tea supplementation in intoxicated animals, elevated plasma protein level significantly. The scientific support is strongest for the protection of DNA from oxidative damage after black or green tea consumption (**Rietveld and Wiseman, 2003**). Also, **Meister (1989)** reported that glutathione (GSH) involved in protein and DNA biosynthesis, which declare that the effect of green tea supplementation on GSH can explain the elevation in proteins in the same groups.

As well as, the present study revealed that intoxication with high and low fenitrothion doses (FnH and FnL) elevated plasma total cholesterol levels of male rats. In addition, there is no remarkable effect on total plasma triglycerides in animals intoxicated with both fenitrothion doses. These results are inconsistent with that previously reported by **Roy, *et. al*. (2004) and Al - Sahhaf (2006).** They recorded a significant increase in cholesterol in liver, kidney, brain and serum of animals treated with fenitrothion, they speculated that treatment with fenitrothion increased tissue lipogenesis, and probably this has been achieved through acceleration of acetyl - COA to be the precursor of cholesterol biosynthesis.

In studies of animals fed high-fat or high cholesterol diets, tea catechins reduced serum cholesterol levels in rats, mice and hamsters (**Valsa, *et. al*., 1995; Ikeda, *et. al*., 1995 and Mastumoto, *et. al*., 1998)**. These findings are in agreement with the current results where green tea extract supplementation to fenitrothion intoxicated rats induced pronounced improvement in each of plasma cholesterol and triglyceride levels to be more or less near to the control level. Catechins are considered to lower cholesterol by a mechanism that suppresses cholesterol absorption in the intestine (**Chan, *et. al*., 1999 and Valsa, *et. al*., 1998**).

Concerning the effect of Fenitrothion - intoxication on thyroid hormones; plasma triiodothyronine (T_3) and thyroxine (T_4) of male albino rats, the present study has shown a slight reduction in T_3 and T_4 in supplementation green tea groups (GT, GTFnH and GTFnL), while, a significant increase in T_4 was recorded with intoxication groups with both tested fenitrothion doses (FnH and FnL) for the experimental period. However, these findings run parallel with that previously reported by **Ozmen and Akay (1993) and El - Halwagy (2000).** They reported that thyroid hormones did not alter significantly during intoxication with Malathion and Monocrotophos, respectively, whereas, a significant increase in T_4 was in agreement with **El - Zayat, *et. al*. (2005).** They reported a significant increase in plasma T_4 in rats treated with Deltamethrim insecticide.

Regarding the effect of fenitrothion intoxication on plasma protein pattern, Fn-intoxication with both doses induced the same alteration in the plasma protein pattern as compared to the control group. These alterations were manifested by a decrease or increase in the number of total separated bands and percentage of amount as well as the appearance or disappearance of individual bands. These results are supported with the previous findings recorded by **El - Demerdash, *et. al*. (2001)** and **El - Zayat, *et. al*. (2005).** They recorded changes in protein patterns in serum and tissues of rats after intoxication with Glyphosat and Deltamethrin. This effect may be due to the oxidation of protein as one of the oxidative damage effects induced by pesticides, whereas these findings reduced partially after supplementation with green tea extract, but it needs more time to declare the effect of large doses of green tea for a more pronounced effect.

These findings were confirmed by the histopathological changes in the thyroid gland that exhibited congestion and changes in the glandular epithelium with inflammatory cell infiltration that led to leakage of thyroxine hormone in plasma. These results agree with the previous studies reported by **Tamura, *et. al*. (2001),** who reported that acute and chronic exposure to fenitrothion induced ultrastructural changes in the thyroid gland with rupture and congestion of some follicles, hemorrhage in some area and mononuclear infiltration.

Fenitrothion had the highest affinity to the androgen receptor as mentioned by **Okubo, *et. al.* (2004).** These findings might be the underlying cause of T_3 and T_4 level changes reported in this study.

Conclusion

It can be pointed out that the concurrent administration of green tea extract (60 mg / animal / day) with Fn - intoxication at both doses has demonstrated the deteriorating effect on all the investigated parameters was improved by the thyroid structural state and the hormonal level of restoration of the follicular sizes, and increased T_3 and T_4 plasma levels. Also, this

confirmed by some biochemical parameters and appearance and disappearance of different bands was detected in the protein profile.

Finally, supplementation with green tea extract has a good effect, but it needs more time to restore the biochemical parameters to be near the control level.

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