

Evaluation of the Use of Green Tea Extract and Vitamin C on Lung, Brain and Testis of Rats Exposed to Fenitrothion Toxicity

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Abstract: *Organophosphorus pesticides like Fenitrothion (FNT) are inevitably found in the environment. The common involving pathway green tea extract (GTE), ascorbic acid (Vit. C) and FNT share is oxidative stress and pathological alterations in vital organs. Therefore, we conducted this study to find the possible neutralizing or synergistic effects of FNT oxidative stress and pathological alterations responses in FNT induced toxicity by daily administrated dose of GTE and Vit. C. The experiment was conducted for six weeks. Four groups containing six male albino rats each were selected. The animals were sacrificed at the end of treatment period, then lung, brain and testes were isolated for assess tissues antioxidant enzymes and histopathology purpose. Rats in FNT group exhibit significant increase of oxidative stress (OS) marker malondialdehyde (MDA) and decrease in the values of superoxide dismutase (SOD), total glutathione (GSH), catalase (CAT) and total antioxidant capacity (TAC) compared to control one in tissues. GTE and Vit. C treated groups showed decreased in the values of MDA and improved the values of SOD, GSH, CAT toward the control values in tissues although the treatment could not normalize it. While TAC level showed a significant increase with fenitrothion plus vitamin C and the fenitrothion plus green tea treated groups compared to the FNT group ($p<0.05$). Fenitrothion caused histopathological changes in lung, brain and testes of male rats while GTE and Vit. C administration to FNT treated animals resulted in overall improvement in these organs damage, emphasizing its antioxidant role. In light of the available data, it can deduce that FNT induced lipid peroxidation, oxidative stress, lung, brain and testes damage and injury in male rats, and conjunction supplementation of GTE and Vit. C has resulted in pronounced ameliorating effect.*

Keywords: Antioxidant enzymes, Rats Lung, Brain, Testis, Fenitrothion toxicity, green tea, vit.C

1. Introduction

Organic insecticide poisoning remains one of the major health issues in both developing and developed communities and thousands of deaths occur every year, mostly in agriculture focused developing countries [1&2]. Fenitrothion [o, o-dimethyl-o-(3-methyl-4-nitrophenyl) phosphorothioate] is an OP insecticide used to control a variety of insects. It has been widely used throughout the world with applications in agriculture and horticulture for controlling insects in crops. FNT is known to cause inhibition of acetylcholinesterase (AChE) activity in the target tissues, induce oxidative stress affect metabolic pathways and cause multiple organ dysfunctions [3&4]. Oxidative stress is defined as a disruption of the prooxidant-antioxidant balance in favor of the former leading to potential damages. This damage occurs through production of reactive oxygen species (ROS), it can be generated by a variety of sources at the cellular level and includes alterations of cellular macromolecules such as lipids, proteins and DNA, lipids are probably the most susceptible [5]. To prevent this oxidative damage, organisms have an endogenous defense system (antioxidant enzymes) with constituents such as SOD, GSH and CAT [6]. Extensive data suggest that free radical formation and oxidative stress can be a major contributor to the toxicity of OPs, which are oxidants and impair enzymatic antioxidant defences [4]. Antioxidants have proved to be a good defence mechanism against free radical effects, which might be produced from contamination

with pesticide and other toxic substances. Cellular defence mechanism to oxidative damage is activated endogenously by antioxidant enzymes which convert the oxidised molecules to their reduced form. Also the endogenous defence mechanism against oxidative damage is complemented by antioxidants like vitamin C, vitamin E, carotenoids and flavonoids (e.g. polyphenols), mainly found in vegetables, fruits and green tea. Studies support a role for ROS in the mechanism of OP toxicity, excessive generation of ROS causes irreversible impairment of DNA and damage to lipids membrane during the production of malondialdehyde [7&8]. It has been reported that toxicity of OP results in deleterious effects on vital organs such as lung, liver, kidney, brain, testes and pancreas. The lung is the first vital organ that comes into contact with inhaled and ingested toxic substances. The main cause of death from acute organophosphate poisoning is attributed to acute lung injury, which is a result of the combined contributions of peripheral and central cholinergic effects. Lung injury is induced as a result of an increase in pulmonary capillary pressure (hemodynamic edema) and/or an increase in pulmonary capillary permeability [9&2]. On the other hand, neuronal necrosis has been observed in multiple cortical and subcortical regions in experimental rats exposed to OPs. Also, symptoms of chronic OPs toxicity vary between headache, sweating, Parkinson's, alterations in memory, and psychiatric or neuropsychological dysfunction [10&11]. Concerning testes, oxidative stress conditions may cause alterations in sperm cells due to the high levels of polyunsaturated fatty acid

in their plasma membrane [12]. In recent years, increasing attention has been paid to the application of nutritional antioxidants such as herbal products in diseases related to oxidative stress. The protective effects of herbal products have been attributed to their role as free radical scavengers and antioxidant defense regulators [13]. On the other hand, a large amount of synthetic and natural antioxidants have been revealed to induce beneficial effects on health and disease prevention. In fact, herbal medicines derived from plant extracts are being increasingly utilized as adjunct treatment options for a wide variety of clinical disease. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities. The health benefits of green tea have been extensively studied in the past few decades. Nowadays, tea is considered as a source of dietary constituents endowed with biological and pharmacological activities with potential benefits to human health. The increasing interest in the health properties of tea extract and its main catechin polyphenols have led to a significant rise in scientific investigation for prevention and therapy in several diseases. Furthermore, many previous investigators reported that green tea extract displays antioxidants and free radicals scavenger properties [14, 15, 16&17]. Also to overcome the oxidative damage caused by toxic compounds, other natural antioxidants have been studied as Vit C which is a very potent antioxidant and has a role in the redox mechanism of the cell by reacting with free radicals, therefore protecting biomembranes from oxidative damage [18]. Even though biochemical studies may give an idea of the pathological state of the animal, a clear picture of cytoarchitectural changes produced during the chemical intoxication can be traced by histopathological studies which give a useful insight into the tissue lesions to prove the external manifestations of the deleterious effects of toxic chemicals. These studies would help in assessing the extent of pollution in the ecosystem by the pollutants such as pesticides and offer an exceptional opportunity to detect the effect of pollutants in various organs and organ systems of an organism [19]. Although the toxicity induced by FNT has been extensively studied, the effects on the lung, brain and testis have not been fully investigated. Therefore, the present work was conducted to study the oxidative stress and histopathological effect of FNT on the lung, brain and testis tissues of albino rats and also aimed to evaluate and compare between the possible protective effect of GTE and Vit. C against the toxic effects induced by fenitrothion in male albino rats.

2. Materials and Methods

All ethical points regarding the treatment of laboratory animals were observed in this research. A total of twenty-four male albino rats of (160-170 g) were used. They were clinically healthy and were acclimatized to the experimental conditions for two weeks before start of the experiment. Clean food and water was given to rats *ad libitum* throughout the experimental period. All the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. All used chemicals and solvents were of analytical grade. FNT emulsion was freshly diluted in

distilled water to 10 mg/ml and orally administered at a dose of 1 ml/kg rat body weight which corresponds to 10 mg/kg. The dose of fenitrothion was selected based on a previous study that used fenitrothion at 10 mg/kg [11]. Green Tea Extract: the green tea extract was freshly prepared daily as the method adopted by [20]. L-ascorbic acid were purchased from Pharmasuid Company, Egypt. Kits for determination of biochemical parameters were purchased from Bio-Diagnostic Company (El-Dokki, Giza, Egypt).

Experimental design and animal grouping: Rats were randomly divided into four groups, with six animals in each group, as follow: Group I: Control -ve without any treatment, Group II: Control +ve received FNT at dose 10 mg/kg for 6 weeks, Group III: received FNT as in group II plus GTE in drinking water at a concentration of 3% w/v. and Group IV: received FNT as in group II plus Vit. C in drinking water at a concentration of 2 g ascorbic acid/L water [21].

Collection of tissue samples for Redox State Evaluation and histopathological examination: At the end of experiment rats were sacrificed and tissue specimens from lung, brain and testes were collected. Lung and brain was fixed in 10% neutral buffered formalin solution, while testes were fixed in Bouin's solution.

Redox State Evaluation: GSH concentration was determined in lung, brain and testes tissues homogenate by method described by [22], Glutathione peroxidase activity according to the method of [23], SOD activity according to [24], CAT activity was measured according to the method described by [25] and MAD was determined colorimetrically following the method described by [26]. On the other hand total antioxidant capacity (TAC) was measured according to [27].

Histopathological examination: The fixed specimens (formalin and Bouin's fixed tissues) were trimmed, washed, dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate and embedded in paraffin after having completed the routine follow-up steps. Sections at 3-5 μ sections were obtained from lung, brain and testes using microtome (LEICA RM 2135) and stained by hematoxylin and eosin (H & E) stain for light microscopically investigation according to [28]. Photos were taken using digital camera (LEICA DMLB Germany).

Statistical analysis: Values are presented as mean \pm standard error. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. All data were statistically analyzed using statistical software program SPSS (Statistical package for Social Sciences) Version 16 released on 2007. Statistical significance was considered at probability ($P \leq 0.05$).

3. Results and Discussion

Tissues oxidative stress biomarkers: Oxidative stress is one of the possible mechanisms resulted from OPs toxicity. Damage induced by oxidative stress primarily occurs through

production of ROS which involves stealing electrons from nucleic acids, lipids, and proteins, leading to the damage of cells and consequently disease phenomena [29&30]. The present study was designed to evaluate oxidative stress and histopathological effects induced by fenitrothion pesticide toxicity on lung, brain and testes of male albino rats and the possible protective effect of green tea extract and ascorbic acid against this toxicity. The results revealed that administration of fenitrothion for six weeks induced significant increase ($P < 0.05$) of malondialdehyde (oxidative stress marker) in lung, brain and testes tissues. One of the main lesion mechanisms is lipoperoxidation (oxidation of the lipid layer of cellular membrane), this is a destructive, self-perpetuating chain reaction, releasing malonaldehyde as the end product [5]. Meanwhile ROS generated by fenitrothion significantly reduced the levels of cellular antioxidants so a significant decrease ($P < 0.05$) in the values of antioxidant parameters SOD, GSH, CAT and TAC compared to control one in tissue samples. On the other hand administration of green tea extract and vitamin C to FNT treated group as shown in Table1, there were significant increase in the same parameters associated with significant decrease in MDA in fenitrothion plus green tea extract and the FNT plus vitamin C treated groups when compared with fenitrothion group, the highest value for MDA (56.01 ± 1.38 nmol/g tissue) was recorded in lung tissue compared with control group, this may be due to that lung tissue continuously and increasingly hazard of facing reactive derivatives of oxygen with the exogenous or endogenous sources and it is the first organ to come in contact with inhaled toxic chemicals especially organophosphate insecticides, which can cause adverse effects in various organs such as the lungs [31]. In the same context, according to antioxidant parameters the lowest value of SOD and TAC (517.4 ± 8.19 & 0.31 ± 0.07 U/g tissue) were recorded in lung tissue fenitrothion group compared with control group respectively. This finding agree with the later author who stated that lung is the first organ to come in contact with inhaled toxic chemicals especially organophosphate which can cause adverse effects in various organs. On the other hand glutathione, in the reduced form (GSH), acts as one of the major detoxifiers in the body and the lowest value of it (20.62 ± 0.75 U/g tissue) was found in brain tissue compared with control group. Brain is extremely susceptible to oxidative stress, oxidative damage and accumulative evidence is indicating that free radicals and other reactive oxygen species play an important role in neurodegeneration and association cognitive decline in aging and Alzheimers disease [32]. The present study is in agreement with [29] who found that GSH activity was significantly decreased in the brain tissues of rats exposed to OP chlorpyrifos. Similarly, fenitrothion caused a significant decrease in CAT content and the lowest value (0.99 ± 0.04 U/g tissues) was recorded in testes tissue. The toxic effect of organophosphorus pesticides exposure is well established; several authors stated that Ops may induce oxidative stress through their "redox-cycling" activity, where they generate superoxide anions and hydrogen peroxide or through ROS generation via changes in normal antioxidant homeostasis that results in depletion of antioxidants. In another words, oxidative stress can be described as an imbalance between the

body antioxidant defense system and the production of free radicals [33, 34, 30, 31&35]. These authors mentioned that there are so many hydrophobic pesticides, like malathion, dichlorvos and fenitrothion that bind strongly to biological membranes, particularly phospholipid bilayers of cells, and are capable of damaging the membranes by induction of lipid peroxidation. If the latter overcome the former, or by any other reason antioxidant defense falls into decreased state a condition of oxidative stress will be developed, that might cause long-term problems. Biochemical changes in tissues of exposed animals reflect the degree of hazards induced as a result of pesticide exposure so, the vital role of lung in facing ROS in daily life and its susceptibility to the variety of diseases cannot be denied. Moreover, testes oxidative stress plays an important role in the pathogenesis of male infertility as a result of exposure to environmental contaminants [13]. Oxidative stress, which takes place due to the overproduction of ROS and reduction in the antioxidant level, is suppressed by the antioxidant defence mechanism, as SOD and CAT. On the other hand, SOD accelerates the dismutation of H_2O_2 as it prevents further degeneration of free radicals also CAT helps in the removal of H_2O_2 formed during the reaction of SOD [36&37]. In our study, we found a significant decrease in the antioxidant levels of CAT and SOD in the testes of FNT treated rats when compared with the control group. Similar finding were recorded by [12]. It is worthwhile to mention that similar effects of OPs were previously reported by [31] in lung, [29] in brain and [13] in testes. Our results are consistent with [38] who explained that, the brain is particularly vulnerable to oxidative injury because of its high rate of oxygen consumption, intense production of reactive radicals and high levels of transition metals, such as iron, which can catalyze the production of reactive radicals. Moreover, neuronal membranes are rich in polyunsaturated fatty acids, which are a source for lipid peroxidation reactions. It is worth mentioned that, there is a growing interest in the role and usage of natural dietary antioxidants as a strategy to amelioration of the various health disorders [39]. Green tea (*Camellia sinensis* L.) is a beverage that is popular worldwide and possesses many pharmacological effects, it is generally made while polyphenol ingredient of green leaf does not begin to wilt and oxidize. The major GT polyphenol belongs to the family of catechins. Its main constituents have considerable effects on treatment of cancer, obesity, diabetes, arthritis and cardiovascular diseases, by functioning as anti-viral and anti-carcinogenic and neuroprotective properties. Green tea extract could prohibit reproductive toxicity [40]. This study revealed that there were significant increase in SOD, GSH, CAT and TAC associated with significant decrease in MDA in FNT plus GET and the FNT plus vit. C treated groups when compared with FNT group after administration of GET and vit. C to FNT treated group as shown in Table1. By regarding these results it seems reasonable to say that in treatment via overproduction of antioxidants, body is capable of defending against ROS. Presumably, as reviewed in publications of other researchers, as reported by [41] & [42] who found that green tea polyphenols have been shown to possess potent antioxidant activity and that is several folds higher than that of Vitamin C and E. However, antioxidants as vitamins can

prevent the excess formation of free radicals or inhibit their reaction with biological sites [43&40]. On the other hand, activities of these enzymes were recovered and oxidative stress caused by FNT was suppressed by the action of GTE and Vit C. The increase in endogenous antioxidants could be due to GTE and Vit C abilities to reduce free radical accumulation [44&18]. Also [45] demonstrated that protective

effects of green tea and its constituents were mainly attributed to their anti-oxidative, radical scavenging, chelating, anti-apoptotic properties and modulating inflammatory responses. These data are consistent with our previous observation in liver and kidney tissues of rats administered green tea extract and vitamin C against FNT treated group.

Table 1: Tissue oxidative stress biomarker and antioxidant parameters in control and treated groups

Group		G I	G II	G III	G IV
Analyzed parameter		Control	Fenitrothion	(Fenitrothion + green tea)	(Fenitrothion + vitamin C)
MDA nmol/g tissue	lung	31.41 ± 1.71	56.01 ^a ± 1.38	38.24 ^b ± 1.26	33.24 ^b ± 1.47
	brain	38.24 ± 1.26	53.52 ^a ± 0.51	41.87 ^b ± 0.51	39.74 ^b ± 0.47
	testes	32.68 ± 0.61	49.68 ^a ± 0.37	35.24 ^b ± 1.47	33.62 ^b ± 0.34
SOD U/ g tissue	lung	595.4 ± 8.15	517.4 ^a ± 8.19	545.2 ^b ± 2.41	553.1 ^b ± 9.86
	brain	684.8 ± 14.15	635.0 ^a ± 7.05	655.2 ^b ± 4.46	611.8 ^b ± 8.12
	testes	695.3 ± 6.82	614.0 ^a ± 9.45	647.0 ^b ± 8.14	634.0 ^b ± 7.01
GSH mg/g tissue	lung	35.71 ± 0.24	28.64 ^a ± 0.31	32.71 ^b ± 2.31	33.82 ^b ± 2.41
	brain	27.62 ± 0.75	20.62 ^a ± 0.75	24.62 ^b ± 2.75	23.62 ^b ± 3.14
	testes	37.30 ± 0.73	29.64 ^a ± 0.81	34.53 ^b ± 1.32	32.71 ^b ± 2.11
CAT U/g tissue	lung	1.98 ± 0.11	1.09 ^a ± 0.64	1.41 ^b ± 0.14	1.58 ^b ± 0.18
	brain	1.55 ± 0.14	1.19 ^a ± 0.04	1.09 ^b ± 0.02	1.09 ^b ± 0.02
	testes	1.51 ± 0.05	0.99 ^a ± 0.04	1.19 ^b ± 0.02	1.18 ^b ± 0.27
TAC (mmol/g)	lung	0.51 ± 0.03	0.31 ^a ± 0.07	0.40 ^b ± 0.11	0.46 ^b ± 0.03
	brain	0.59 ± 0.03	0.36 ^a ± 0.05	0.47 ^b ± 0.08	0.48 ^b ± 0.01
	testes	0.50 ± 0.03	0.32 ^a ± 0.03	0.41 ^b ± 0.02	0.43 ^b ± 0.03

MDA; Malondialdehyde, SOD; Superoxide dismutase, GSH; Reduced glutathione, CAT; Catalase, TAC; Total antioxidant capacity.

Values are expressed as mean ± standard error. Values with superscript a indicate statistical significant different from group I at (P < 0.05), values with superscript b indicate statistical significant different from group II at (P < 0.05).

Lung histopathology: Histopathological studies would help in assessing the extent of pollution in the ecosystem by the pollutants such as pesticides and offer an exceptional opportunity to detect the effect of pollutants in various organs and organ systems of an organism [46&47]. Histopathological findings of the present study suggested that exposure to FNT exhibited several changes in the lung tissue as shown in Figure 1A, the lung of control rats is showing normal histology, it shows a terminal bronchiole with the epithelium and the underlying strip of smooth muscle in addition to the respiratory bronchiole, alveolar ducts, alveolar sacs and numerous alveoli separated by alveolar septum. On the other hand this architecture was altered as shown with FNT group in Figure 1 B1 by the observation of congestion, desquamated epithelium in bronchi and bronchioles (thick arrows), peribronchial and perivascular edema (stars). Also thickening of interalveolar septa by inflammatory cells, edema and congestion (thin arrows). Meanwhile, Figure 1B2 showing areas of inflammatory cells infiltration in the interalveolar septa (arrow). Concerning to FNT plus green tea group, they

were showing somewhat normal picture Figure 1C. In addition FNT plus vitamin C group, they were showing somewhat normal picture except some fibrosis around bronchi (thin arrows) Figure 1D. These finding which in line with several studies provide information on lung effects in animals following oral exposure to OPs, as damage to pulmonary tissue, edema was serious, an increased hemorrhage within the alveoli, necrosis and alveolar edema [2, 48 & 49]. Oral administration of 20mg/kg bw of FNT resulted in several morphological changes in rats' lung, destruction of the bronchiole lining at the terminal bronchiole and swollen of alveolar cells. A slightly disruption of alveolar walls as well as necrosis of cells is seen although lung is not the vital organ which come in contact through ingestion route, these results could be due to penetration of FNT and its metabolites as stated by [50]. In addition, the lung is the first vital organ that comes into contact with inhaled and ingested toxic substances; some studies have reported that organophosphate compounds give rise to pulmonary impairments in mice and rabbits, such as alveolar congestion, hemorrhage, neutrophil infiltration, emphysematous changes [51 & 49]. Our finding were in accordance with those obtained by the later author, who found that treatment with vitamin C to dimethoate applied rats showed amelioration in the lung toxicity when compared with only dimethoate treated group. Histopathological alterations in lung tissue such as emphysema, hemorrhages and hemosiderin depositits were noticed after 30-day exposure of adult rats to dimethoate at dose of 0.2 g/L however; cotreatment with selenium (0.5 mg/kg) or vitamin E (100 mg/kg) to the diet of

dimethoate administered rats alleviated the histological impairments of lung [52]. Also [53] were determined that rats treated with chlorpyrifos ethyl lead to remarkable changes in the histomorphology of the lung. These were infiltration of mononuclear cells, hyperplasia of type II pneumocyte, and thickened and increased connective tissue. However, treatment with vitamin C, vitamin E, and melatonin considerably reduces the toxic effect of chlorpyrifos ethyl on lung tissue in rats. Furthermore, marked histopathological alterations were observed in the lung of fenitrothion treated groups including slight, mild, moderate, severe hypertrophied vascular smooth

muscles and thickened intra-alveolar septa, and severe hypertrophied vascular smooth muscles, interstitial hemorrhages with narrow alveolar spaces and intense thickened septa with perivascular leukocytic aggregation. These findings are supported by previous studies [8, 54, 11&55]. Lung histopathological findings of the present study suggested that co treatment with GTE partially improved these alterations, while co treatment with Vit C exhibited approximately full protection and more protective role and markedly reduced tissues damage induced by fenitrothion.

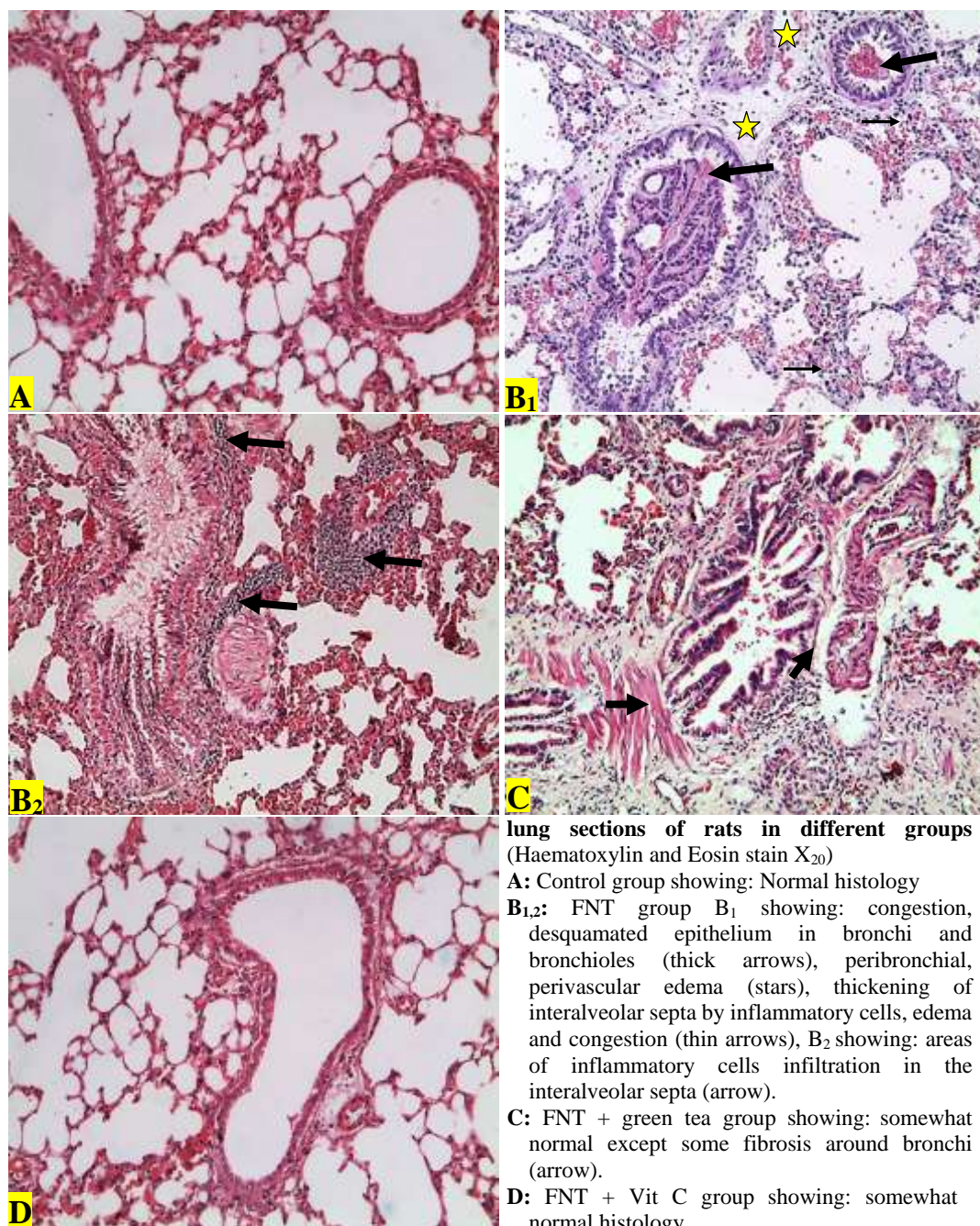
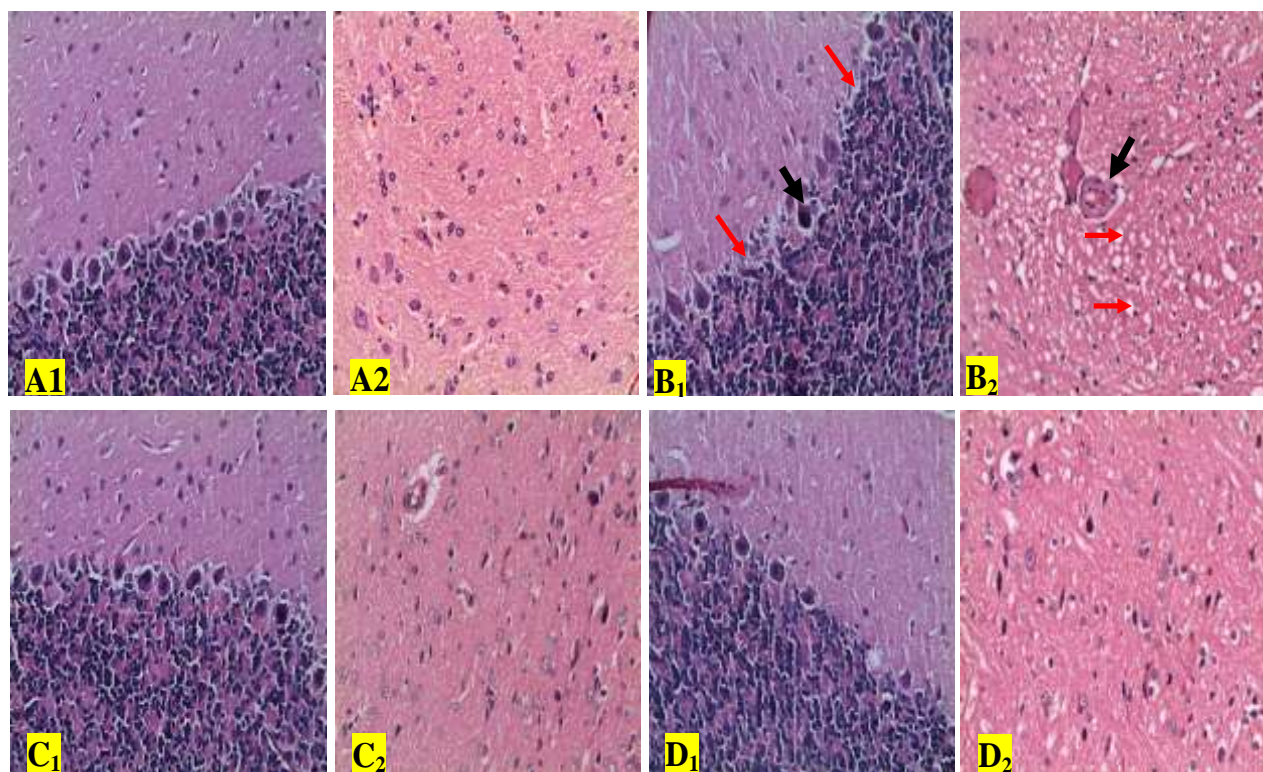


Figure 1: Lung sections of rat in different groups.

Brain histopathology: After oral administration of FNT, histopathological changes were observed in the brain of all treatment rats groups compared with control group as shown in Figure 2A, control group showing: normal histology of cerebellum (A₁) and cerebrum (A₂). On the other hand Figure 2B FNT group B₁ cerebellum showing: loss of most neurons of purkinji cell layer (red arrows) and necrosis of remaining neurons (black arrow), B₂ cerebrum showing: demyelination (red arrows) and vascular congestion (black arrow). Concerning FNT + GTE group in Figure 2 C, it is show normal histology of cerebellum (C₁) and cerebrum (C₂). But according to Figure 2 D, FNT + Vit C group showing nearly normal histology cerebellum (D₁) and cerebrum (D₂). Cerebral blood vessels are vital in supplying brain in both human and animals. Any anomaly by rupture or interruption of blood flow may lead to fatal consequences, dichlorvos as an Op caused change in the histoarchitecture of the cerebral blood vessels through the formation perivascular oedema and apoptosis of the endotheliocytes [56]. In the previous study, administration

of FNT for 42 days revealed a significant gradual decrease in blood brain barrier integrity as evidenced by the increase in Evans blue extravasation in brain compared with control group, leading to neurological damage [11]. In addition, the brain DNA damage is demonstrated by the increase in damage index and damage frequency parameters, the administration of crude aqueous GTE has a protective role against the brain DNA damage following the Dimethoate pesticide toxicity in rats [43]. Meanwhile that may be due to brain accessible phytochemicals potentially defend against oxidative damage. A large amount of synthetic and natural antioxidants have been revealed to induce beneficial effects on human health and disease prevention as mentioned by [29]. On the other hand in brain, ischemic changes were seen in cerebrum and the nucleus of the brain cells showed changes in nuclear shape and chromatin condensation, which are characteristic of apoptotic cell of animals was orally administered Dimethoate at doses 0.6, 6, and 30 mg/kg for 30 days in these rats. [57].



Brain sections of rats in different groups (Haematoxylin and Eosin stain X₂₀)

A: Control group showing: normal histology of cerebellum (A₁) and cerebrum (A₂)

B: FNT group B₁ cerebellum showing: loss of most neurons of purkinji cell layer (red arrows) and necrosis of remaining neurons (black arrow), B₂ cerebrum showing: demyelination (red arrows) and vascular congestion (black arrow)

C: FNT + green tea group showing: normal histology of cerebellum (C₁) and cerebrum (C₂)

D: FNT + Vit C group showing: nearly normal histology cerebellum (D₁) and cerebrum (D₂).

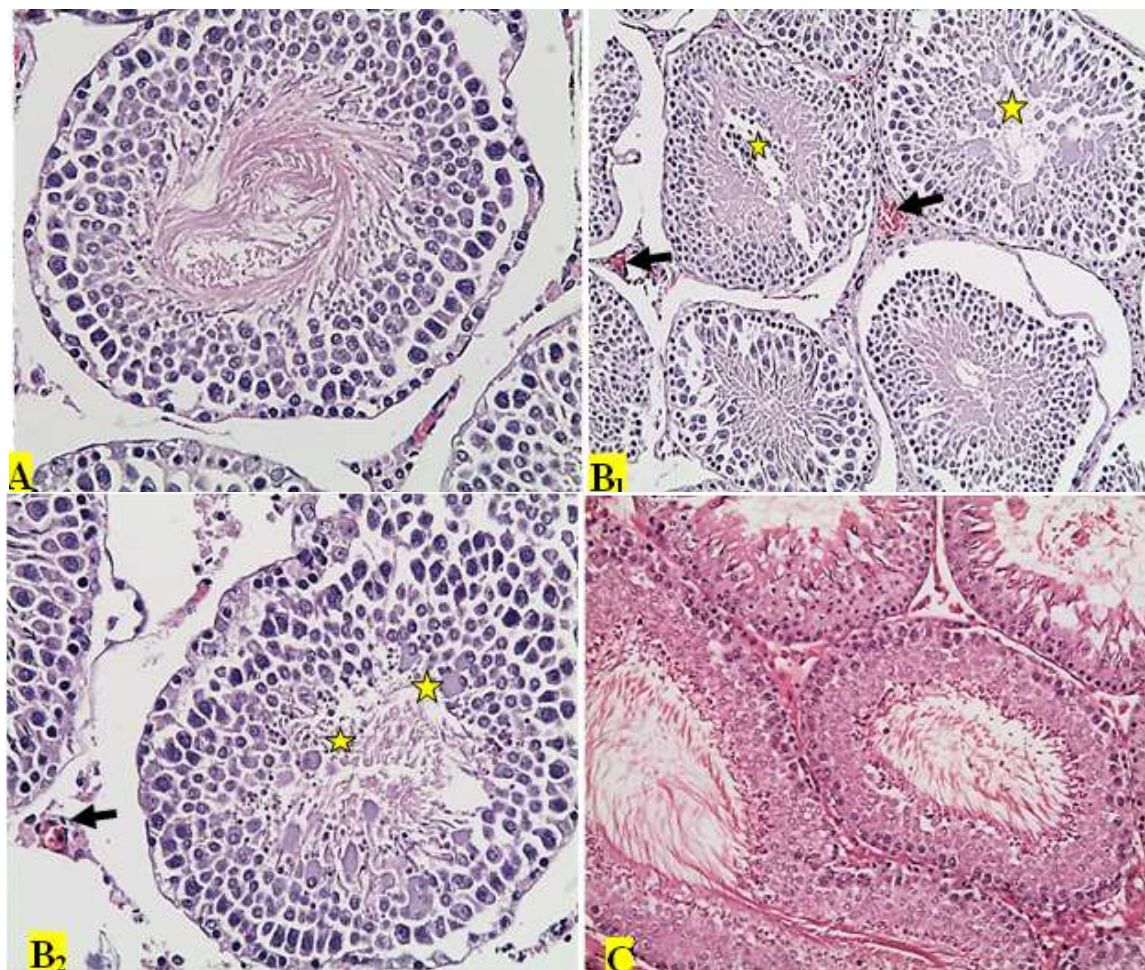
Figure 2: Brain sections of rats in different groups

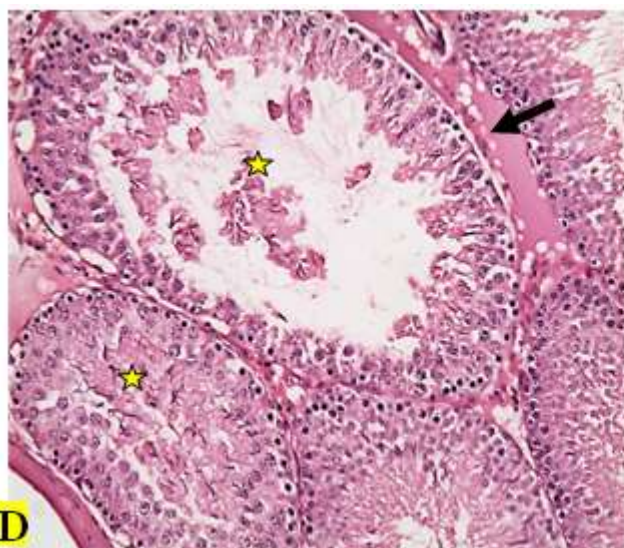
Testes histopathology: The current study indicates reproductive toxicity of FNT on rats treated with this pesticide during experimental period. Control group showed normal appearance of seminiferous tubules (Figure 3A). The

seminiferous tubule consists of normal somatic and spermatogenic cells and is surrounded by peritubularmyoid cells, Figure 3 B1 FNT group showed morphological alterations compared with the control one. Pathological

changes included the presence of congestion of interstitial blood vessels (arrows), desquamated epithelial cells and tissue debris in the lumen of seminiferous tubules (stars), B2 showing high power however, Figure 3C FNT+ green tea group showed somewhat normal histology. But concerning to Figure 3 D FNT+Vit.C group, it showed desquamated epithelial cells in the lumen of seminiferous tubules (stars) and edema in the interstitial tissue (arrow). Our results were concordant with other reports by [12] who found that FNT caused pathological changes in seminiferous tubules following a 28 day exposure and increased the formation of vacuoles and number of mitochondria in the testes. Vacuoles can be described as an early stage of damage induced by any toxicants. FNT at a dose of 20 mg/kg bw has a detrimental effect on the sperm and testes of rats. Also [58] & [59] found that FNT induces impairments of the seminiferous tubules structure and spermatogenesis in the rats and therefore damages of the male reproductive system. In the same context [13] stated that FNT acts on the endocannabinoid signaling system in male reproductive organs, causing spermatotoxicity and testicular damage in experimental animals. The later author found that focal testicular degeneration, congestion and extensive interstitial oedema were noticed in FNT treated rats. However, the interstitial oedema was represented by vacuolated eosinophilic fluid. In line with previous studies,

our results showed that GTE and vit.C improved the FNT induced toxicity. Study performed by [40], variable degrees of degenerative changes and necrosis in the seminiferous tubules up to total cellular destruction after chronic exposure for 30 days of male rats to methomyl were reported. Our findings are generally consistent with the studies by [46] who concluded that Ops, deltamethrin is severely toxic in male albino mice, which may be equally harmful to the male reproductive health. All of these changes resulted in reduction in male fertility. It should be noted that administration of FNT combined with GTE and FNT combined with vit.C had a recovery effect on the histological picture of testes. It means that vitamins C and green tea extract as antioxidants remove the toxic effects of fenitrothion on the histological structure of rat testes. These findings are supported by previous studies [60, 42, 61& 62]. Our aforementioned results (tissues oxidative stress) were corroborated with the histopathological studies of lung, testes and brain. FNT administration induced significant deleterious on oxidative stress and histopathological alterations. On the other hand in brain and testes samples, Co treatment with Vit C partially improved these alterations, while co treatment with GTE exhibited approximately full protection and more protective role and markedly reduced tissues damage induced by fenitrothion.





Testes of rats in different groups (Haematoxylin and Eosin stain A, B₂ X₂₀, B₁, C, D X₁₀)
A: Control group showing: Normal appearance of seminiferous tubules.
B₁: FNT group B₁ showing: congestion, of interstitial blood vessels (arrows), desquamated epithelial cells and tissue debris in the lumen of seminiferous tubules (stars), B₂ showing: high power.
C: FNT + green tea group showing: somewhat normal histology.
D: FNT + Vit C group showing: desquamated epithelial cells in the lumen of seminiferous tubules (star) and edema in the interstitial tissue (arrow).

4. Conclusion

The data obtained in this study imply that several changes in lung, brain and testes redox status via generation of oxidative stress. Additionally, morphological alternation could be seen in FNT group. The findings seem to indicate that FNT caused damage and injury to these tissue organs and suggesting that FNT may cause toxicity through oral route. The present work also highlights the protective role of GTE and Vit. C, they are effective against various adverse effects induced by fenitrothion in rats by attenuating the oxidative stress through scavenging of free radicals, or by enhancing the activity of antioxidants and due to the polyphenols that GTE contains.

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