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Histopathological and Biochemical Studies on The Effect of Green Tea Extract and vitamin C Against Fenitrothion Toxicity in Male Albino Rats.

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ABSTRACT

This study aimed to evaluate the effect of green tea extract (GTE) and vitamin C as antioxidants on the biochemical oxidative stress parameters and histopathological changes induced by fenitrothion (FNT) pesticide in liver and kidney of albino rats. Twenty-four rats were divided into four groups; group one (control), group two received 10 mg/kg b.wt. of FNT orally, group three received FNT daily plus green tea extract at a concentration of 3% w/v and group four received FNT daily plus vitamin C at a concentration of 2 g ascorbic acid (vitamin C) /1 L. The experiment was conducted for six weeks. Blood samples were taken for biochemical parameters. The histopathological studies were carried out on liver and kidney tissues at the end of the experiment. Damage in the tissue samples was observed and confirmed with elevation of oxidative stress marker malondialdehyde (MDA). Exposure of rats to fenitrothion induced significant increase of MDA and decrease in the values of SOD, GSH, CAT, TAC compared to control one in both serum and tissues samples. However, administration of green tea and vitamin C to FNT treated group decreased in the values of MDA and improved the values of SOD (superoxide dismutase), GSH (glutathione) and CAT (catalase) toward the control values in both serum and tissues but could not normalize it. Total antioxidant capacity (TAC) level showed a significant increase with FNT plus vitamin C and the FNT plus GTE treated groups compared to the FNT group. Green tea and vitamin C, probably due to their strong antioxidant properties could improve and partially counteracts the toxic effect of FNT on oxidative stress parameters and repair the destructive effects of its damage in rat's liver and kidney tissues especially with GTE treated groups.

1. INTRODUCTION

Fenitrothion as an organophosphate pesticide is widely used in agriculture, veterinary and industries. For centuries, pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors. They enter the body through inhalation of aerosols, dusts & vapor; ingestion of food additives and direct contact. They can damage vital organs with the liver being the most susceptible due to its role in transforming, metabolizing, and eliminating chemicals from the body. Studies have found that many pesticides are potential hepatotoxicants and cause structural and functional changes in mammalian and avian hepatocytes (Chen et al., 2017). Among common pesticides; organophosphorus compounds (OP) which are widely used in agriculture (for controlling a wide range of insects and other pests), medicine and industry. Organic insecticide poisoning remains one of the major issues in both developing and developed communities. A great proportion of acute poisoning cases are caused by exposure to pesticides, especially OP compounds. The mechanism of their toxicity is mainly by blocking of acetyl cholinesterase (AchE).

Once AchE has been inactivated, acetylcholine accumulates throughout the nervous system, resulting in overstimulation of muscarinic and nicotinic receptors. Toxicities of pesticides cause adverse effects on many organs and blood factors (Teimouri et al., 2006 and Kerem et al., 2007). Fenitrothion[o,o-dimethyl-o-(3-methyl-4-nitrophenyl) phosphorothiote] is one of the most widely used organophosphorus pesticides mainly used in agriculture for controlling chewing and sucking insects, it is also used for the control off lies, mosquitos and cockroaches in public health programs and/or indoor use (Afshar et al., 2008 and Abdel-Ghany et al., 2016). Previous researchers have found that various concentrations of FNT caused histopathological effects on the liver and kidneys of rats and immunosuppressive effects (Hayes & Laws, 1991; Afshar et al., 2008; Elhalwagy et al., 2008; and Budin, et al., 2011). After oral administration, fenitrothion (FNT) is rapidly and extensively absorbed from the mammalian intestinal tract, and then it is mainly distributed to the liver, blood, and carcass (Afshar et al., 2008). In fact, the toxicity of organophosphorus insecticides results in negative effects on many organs and systems such as the liver, kidney, nervous system, immune system and reproductive system (Elzoghby et al., 2014). Recently, there has been worldwide interest in the role of medicinal plants in complementary medicine. Tea, the dried leaves of the *Camellia sinensis* species of the aceae family, it is a popular beverage with an annual production of three billion kilograms worldwide. Green tea is a non-oxidized and non-fermented product that is made by drying fresh leaves (roasting) at high temperatures to inactivate the oxidizing enzymes. It contains several tea polyphenols - primarily green tea catechins that account for 30–40% of the extractable solids of dried green tea leaves (Yang and Landau, 2000 and Heikal et al., 2013). Green tea is a favorite beverage and possesses many pharmacological effects such as anti-mutagenic, anti-proliferative and anti-carcinogenic properties. It is a potent neuro-protective remedy in models of degenerative disorders (Al-Attar & Abu-Zeid, 2013 and Mehri et al., 2016). Reactive Oxygen Species (ROS) have been implicated in hepato and neurotoxicity induced by several organophosphorus and is associated with lipid peroxidation and phospholipids degradation. Oxidative stress occurs when the generation of reactive oxygen species in the body exceeds the ability of the body to neutralize and eliminate them. The susceptibility of liver tissues to this stress due to exposure to pesticides is a function of overall balance between the degree of oxidative stress and the antioxidant capacity (Khan et al., 2005). Therefore, there is growing need to exogenous sources of antioxidant. Antioxidants as vitamins have a various biological activities, so oxidative stress seems to be attenuated by non-enzymatic nutritional antioxidants such as vitamin E and C (Verma et al., 2009).Vitamin C (L-ascorbic acid, ascorbate) is a simple glucose-related carbohydrate with rather unique properties. The presence of an enediol group in the molecule confers electron lability, which makes it a member of an oxidation-reduction system with electron-donating or accepting properties. A loss of the first electron results in formation of the ascorbate free radical, which can be further oxidized by another electron loss to give dehydroascorbic acid. Both ascorbic acid and ascorbate free radical have a reducing potential low enough to react with most of the physiologically important radicals and oxidants (Buettner, 1993 and Magdy et al., 2016), enabling vitamin C to act as a powerful hydrosoluble antioxidant in body fluids, scavenging reactive oxygen and nitrogen species. Vitamin C also acts as a co-substrate for some hydroxylase and oxygenase enzymes, maintaining their active center metal ions in a reduced state for optimal enzyme activity (Djurašević et al., 2008 and Elzoghby et al., 2014). Although the neurotoxicity induced by FNT has been extensively studied, the effects on other vital organs as liver and kidney have not been fully investigated. Therefore, the present work was conducted to study the histopathological and biochemical oxidative stress effects of FNT toxicity on liver and kidney of albino rats and also aimed to evaluate and compare between the possible protective effect of green tea extract and ascorbic acid against these toxicity.

2. MATERIALS AND METHODS

2.1. Animals: All ethical points regarding the treatment of laboratory animals were observed in this research. A total of twenty-four male albino rats (*Rattus norvegicus*) of (160-170 g) were purchased from AL-Zyade Experimental Animals Production Center, Giza, Egypt. They were clinically healthy and were acclimatized to the experimental conditions for two weeks before start of the experiment. During this period, the rats were housed in plastic cages with galvanized iron filter tops and placed in quiet room with natural ventilation and 12:12-hrs light–dark cycle. Clean food and water were 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.

2.2. Chemicals:

All chemicals and solvents used were of analytical grade

1. Fenitrothion (Sumithion 50®, 500 mg/mL) (O,O dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothiote was purchased from Kafr Elzayat Co. for Insecticide Ind., (Kafr Elzayat, Egypt). Fenitrothion 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 FNT was selected based on a previous study that used FNT at 10 mg/kg (Abdel-Ghany et al., 2016).
2. The green tea leaves: China green tea (Lipton Unilever brand) of commercially available market was purchased locally packaged by the Egypt National Native Product sources.
3. L-ascorbic acid was purchased from Pharmasuid Company, Egypt.
4. Kits for determination of biochemical oxidative stress parameters were purchased from Bio-Diagnostic Company, El-Dokki, Giza, Egypt.

Preparation Green Tea Extract (GTE): the green tea extract was freshly prepared daily as the method adopted by Hussein et al., 2014 by soaking 30 g of dried tealeaves in 1 L of boiling distilled water for 5 min. The solution was cooled to room temperature and filtered to make 3%w/v GTE (3g tealeaves /100 ml water) and administrated to the rats in water bottles instead of drinking water as their sole source of drinking water. The resulting clear solution is similar to tea consumed by human. Doses of antioxidant compounds (vitamin C and green tea) were chosen to be within the range levels reported in pamphlet to Laurence and Bacharach (1964).

2.3. Experimental design and animal grouping: after acclimatization for two weeks, the 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/k (Abdel-Ghany et al., 2016) for 42 day (6 weeks).

Group III: received FNT as in group II plus green tea extract in drinking water at a concentration of 3% w/v (Hussein et al., 2014).

Group IV: received FNT as in group II plus vitamin C in drinking water at a concentration of 2 g ascorbic acid/l water (El-Refaiyand Eissa 2013)

2.4. Collection of blood and tissue samples:

After recording the final body weights 24 h after the last treatment, rats were anaesthetized under light Diethyl Ether (DEE) and were prepared for collection of samples. Blood samples were collected from the medial canthus of the eye of each rat by a fine capillary glass tube, poured gently and carefully on the wall of labeled clean and dry glass centrifuge tubes without using anticoagulant in an inclined position and left for clotting at room temperature for 20 minutes, then put in the refrigerator to avoid glycolysis and for clot retraction then centrifuged at 3000 rpm for 10 minutes. The clean supernatant serum was aspirated by automatic pipette into labeled epindorff tubes and kept frozen at -20 until the time of analysis. Immediately after blood collection rats were rapidly sacrificed and tissue specimens from liver and kidney were collected. These tissue samples were fixed in 10% neutral buffered formalin solution for histopathological examination.

2.5.1. Biochemical analysis (redox state evaluation) serum MDA, SOD, GSH, CAT and TAC activities were measured by colorimetric techniques using commercially available kits (Bio-diagnostic, Egypt) by following kits instructions: malondialdehyde (MDA), lipid peroxidation and oxidative stress markers occurs as a result of lipid peroxidation in plasma and was measured after incubation at 95^{O}C with thiobarbituric acid in aerobic conditions (pH 3.4). The pink color produced by these reactions was measured spectrophotometrically at 532 nm to measure MDA levels (Ohkawa et al., 1979). Total reduced glutathione (GSH) was determined in erythrocytes by Beutler et al., (1963) the method based on the development of a yellow color when DTNB (5, 5- dithiobis (2-nitrobenzoic acid) is added to the supernatant of the precipitated RBCs containing sulfhydryl groups. Concerning to superoxide dismutase (SOD) activity, it was determined according to the method described by Marklund and Marklund, (1974) by assaying the auto oxidation and illumination of pyrogallol at 440 nm for 3 min 40. One unit of SOD activity was calculated as

the amount of protein that caused 50% pyrogallol autooxidation inhibition, SOD activity is expressed as U/l. Also CAT activity was measured according to the method described by Aebi (1984), by assaying the hydrolysis of H2O2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25 ◦C 39, the activity of CAT enzyme is expressed as U/l. On the other hand total antioxidant capacity (TAC) was measured by ferric reducing ability of plasma (FRAP) method. This method is based on the ability of plasma in reducing Fe^{3+} to Fe^{2+} in the presence of TPTZ. The reaction of Fe^{2+} and TPTZ gives a complex with blue color and maximum absorbance in 593 nm according to Benzie, (1996).

2.5.2. Tissue analysis: MDA concentration was determined in liver and kidney tissue homogenate by method described by (Ohkawa et al., 1979), GSH concentration was determined in liver and kidney tissue homogenate by method described by Beutler et al. (1963). Superoxide dismutase activity (SOD) in liver and kidney tissue homogenates was determined according to the method of Nishikimi et al. (1972). Catalase (CAT) and total antioxidant capacity (TAC) activities in liver and kidney tissue were measured according to the method described by Aebi (1984) and Benzie, (1996) respectively, the activity of CAT is expressed as u/g tissue by colorimetric techniques using commercially available kits (Bio-diagnostic., Egypt).

2.6. Histopathological examination: the fixed specimens (formalin 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 liver and kidney using microtome (LEICA RM 2135) and stained by hematoxylin and eosin (H & E) stain for light microscopically investigation according to Bancroft and Gamble, (2008) Photos were taken using digital camera (LEICA DMLB Germany).

Statistical analysis: values are presented as mean ± 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

3.1. Bichemical oxidative stress parameters:

Pesticides residues remain in the environment for varying length of time, these residues represent a chronic hazard to man and animals. The obtained results revealed that exposure of male rats to fenitrothion for six weeks induced significant increase ($P< 0.05$) of malondialdehyde (MDA) (oxidative stress marker) associated with significant decrease ($P< 0.05$) in the values of antioxidant parameters (SOD, GSH, CAT & TAC) compared to control one in both serum and tissue samples. On the other hand administration of green tea extract and vitamin C to FNT treated group as shown in Table 1&2 there were significant increase in SOD, GSH, CAT and TAC associated with significant decrease in MDA in fenitrothion plus green tea extract and the fenitrothion plus vitamin C treated groups when compared with fenitrothion group. Attempts have been made to select protective agents and drugs which reduce overt toxicity to the exposed individuals. In recent years there is wide spread concern over exposure to low levels of organophosphrous insecticides in the diet over a long period of time and there are reports which suggested that OP insecticides' manifest their toxic by enhanced production of reactive oxygen species (ROS) which is a major cellular source of oxidative stress (Abdollahi et al., 2004) and (Cemek et al., 2010). Results of numerous studies suggest the protective effects of green tea against various poisons and medicines (El Daly 2011, Hussein et al., 2014, Chen et al., 2017 and Mehri et al., 2016 and Sharifzadeh et al., 2017). Elevation levels of the mentioned antioxidant enzymes following GTE administration may result from the antioxidant properties of the extract and from the resultant prevention of intracellular enzymes from leaking out of cells due to cellular stabilization or regeneration (Elhalwagy et al., 2008). Green tea (Camellia sinensis) supplementation to diabetic rats improves serum and hepatic oxidative stress markers, also dietary supplementation with green tea catechins can improve total antioxidant capacity (TAC) and decrease malondialdehyde (MDA) concentration (Haidari et al., 2013). Polyphenols, especially catechins are among the main soluble constituents of green tea extract, they are strong scavengers of superoxide, hydrogen peroxide, and nitric acid which are obtained from various chemical materials. Moreover, green tea catechins prevent lipid peroxidation by chemical materials in the liver and kidney of animals and have the antioxidant properties of urate, beta-carotene, vitamin C and vitamin E in protecting cells (Sano et al., 1995 and Chen et al.,

2017). Also oxidative stress seems to be attenuated by non-enzymatic nutritional antioxidants such as vitamin C (Ahmed et al., 2000, Djurašević et al., 2008 and Magdy et al., 2016). Our results confirm the protective effects of vitamin C against FNT -induced changes in both enzymatic and non-enzymatic antioxidants due to either direct scavenging of ROS or induction of antioxidant enzymes. It has been reported that vitamin C ameliorates OP pesticide-induced hematological and biochemical alterations in humans and animals. Vitamin C is readily available, cheap and relatively non-toxic antioxidant possesses great benefit in the amelioration of toxic effects (Aly et al., 2010, Uchendu et al., 2012 and Magdy et al., 2016). On the other hand the activity of SOD is elevated in the liver of rats fed vitamin C. It is known that vitamin C increases hepatic activity and accompanying superoxide anion radical production, which suggests a possible pro-oxidant effect of ascorbate in the liver (Djuraševićet al., 2008). It worth mentioned that MDA is an end product of polyunsaturated fatty acid oxygenation; it is also a reliable and commonly used as a marker of the overall lipid peroxidation level and the presence of oxidative stress. Also it is one of the most frequently used indicators of lipid peroxidation and liver damage (Rahimi et al., 2011). our present study demonstrate that, the activity of the MDA in fenitrothion exposed group increased approximately two fold compared with that of the control one, these finding is in accordance with Gultekin et al. (2001), Abdollahi et al., (2004) and Ranjbar et al. (2005). In consistence, SOD is the first line of defense against ROS and is active in catalyzing detoxification of superoxide radical. The hydrogen peroxide generated in this reaction is restored to water in the presence of CAT and GPX (Nazifi et al., 2010). Taking into consideration serum total antioxidant capacity (TAC), it is a biochemical parameter suitable for evaluating the overall antioxidant status of serum and body fluids resulting from antioxidant intake and/ or production, and their consumption by normal or increased levels of ROS production. When the oxidant/antioxidant balance is tilted towards oxidants and oxidative stress arises (Kayar et al., 2015). SOD, GPx and CATenzymes are work together to eliminate active oxygen species. In this respect, SOD accelerates the dismutation of H_2O_2, also termed as a primary defense, as it prevents further generation of free radicals whereas, GPx, responsible for enzymatic defense against hydrogen peroxide, it is strictly linked with the concentration of GSH because it catalyses the reaction between glutathione and hydrogen peroxide, resulting in the formation of glutathione disulphide and CAT helps in the removal of H_2O_2 formed during the reaction catalyzed by SOD (Heikal et al., 2013 and Elzoghby et al., 2014). Our results are in accordance with those of Khorsandi et al. (2010) who were found that injecting rats with green tea extract could improve liver failure caused by taking acetaminophen due to the strong antioxidant effects of the GTE. So in this study, elevation of SOD, GSH, CAT and TAC and reduction of MAD levels in groups treated with FNT and GTE may be attributed to antioxidant activity of green tea extract, it has antioxidant and free-radical-scavenging properties as mentioned by Crespy et al. (2004), Elhalwagy et al., (2008) and Mehri et al., (2016). The maximum level of lipid peroxidation was observed in fenitrothion exposed group. In the same time, antioxidant enzyme activities showed the oboist trend in the same group. So the results of the present study indicated that there was a negative relationship between antioxidant enzyme activities and lipid peroxidation content (Table 1&2). The susceptibility of cells to oxidative stress is a function of the overall balance between the degree of oxidative stress and the antioxidant defense capability. Decreased concentrations of SOD, GSH, CAT and TAC activities may reflect oxidative stress in fenitrothion exposed group. While in groups treated by fenitrothion plus vitamin C or fenitrothion plus green tea there were a significant decrease in MDA values in comparison with fenitrothion group may be due to the protective effect of vitamin C over fenitrothion toxicity is likely to be mediated via the inhibition of free radical generation and enhancement of free radical scavenging activity and green tea prevents the loss of lipophilic antioxidant α-tocopherol by repairing tocopheryl radicals and protection of the hydrophilic antioxidant ascorbate. These findings are in agreement with those of Skryzdlewska et al. (2002), Suna et al. (2010) and Magdy et al. (2016). Therefore, it may decrease the concentration of lipid free radicals and terminate initiation and propagation of lipid peroxidation (Guo et al., 1999 and Mehri et al., 2016). From these results we can noticed that, treatment with green tea extract and vitamin C could reduce the values of the oxidative stress parameters and increase the values of the antioxidant parameters towards normal values and taking into consideration that results of green tea treatment were better than the results of vitamin C treatment.

Table 1: Serum oxidative stress marker and antioxidant parameters in control and treated groups.

T / Group / Analyzed parameter	G I Control	GII fenitrothion	G III (fenitrothion + green tea)	G IV (fenitrothion + vitamin C)
MDA nmol/L	4.8± 0.22	7.44[a]± 0.36	4.49[b] ± 0.19	4.28[b] ± 0.14
SOD U /L	74.1 ± 1.85	32.9[a]± 2.81	69.4[b] ± 2.24	68.3[b] ± 1.73
GSH mg/dl	11.89±0.52	6.83[a]±0.49	10.23[b]±4.89	9.18[b]±2.35
CAT U/L	0.222 ± 0.069	0.449[a]± 0.06	0.302[b]± 0.09	0.376[b]± 0.05
TAC (mmol/L)	1.21±0.55	0.78 [a]± 0.09	1.02[b]±0.01	1.00[b]±0.11

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 1 at ($P < 0.05$). Values with superscript [b] indicate statistical significant different from group II at ($P < 0.05$).

Table 2: Tissue oxidative stress marker and antioxidant parameters in control and treated groups.

Group / Analyzed parameter		G I Control	GII fenitrothion	G III (fenitrothion + green tea)	G IV (fenitrothion + vitamin C)
MDA nmol/g tissue	**liver**	30.01 ± 1.71	63.51[a] ± 1.78	45.04[b]± 1.45	51.02 [b]± 1.35
	kidney	29.15 ± 1.14	40.79[a]± 1.31	33.77[b] ± 0.41	37.77 [b]± 0.56
SOD U/g tissue	**liver**	640.6 ± 10.1	572.0 [a]± 2.12	611.4[b]± 10.70	630.2[b]± 9.90
	kidney	616.0 ±9.85	544.8 [a]±5.79	605.0[b]±11.04	600.1[b] ± 7.75
GSH mg/g tissue	**liver**	48.19 ± 1.79	32.10 [a]± 1.32	45.8[b]± 1.73	42.74[b]± 1.34
	kidney	36.40 ± 1.17	22.46 [a]± 4.15	36.40[b] ± 3.17	36.40[b] ± 4.17
CAT U/g tissue	**liver**	1.17± 0.03	0.89[a] ± 0.04	1.09[b] ± 0.02	1.00[b] ± 0.02
	kidney	1.80± 0.03	1.04[a]± 0.65	1.68[b]± 0.23	1.64[b]± 0.09
TAC (mmol/g)	**liver**	.0.49± 0.12	0.31[a]± 0.04	0.40[b]± 0.03	0.42[b]± 0.06
	kidney	0.57 ± 0.03	0.34[a]± 0.09	0.44[b]± 0.09	0.43[b]± 0.03

(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 1 at ($P < 0.05$). Values with superscript[b] indicate statistical significant different from groupII at ($P < 0.05$).

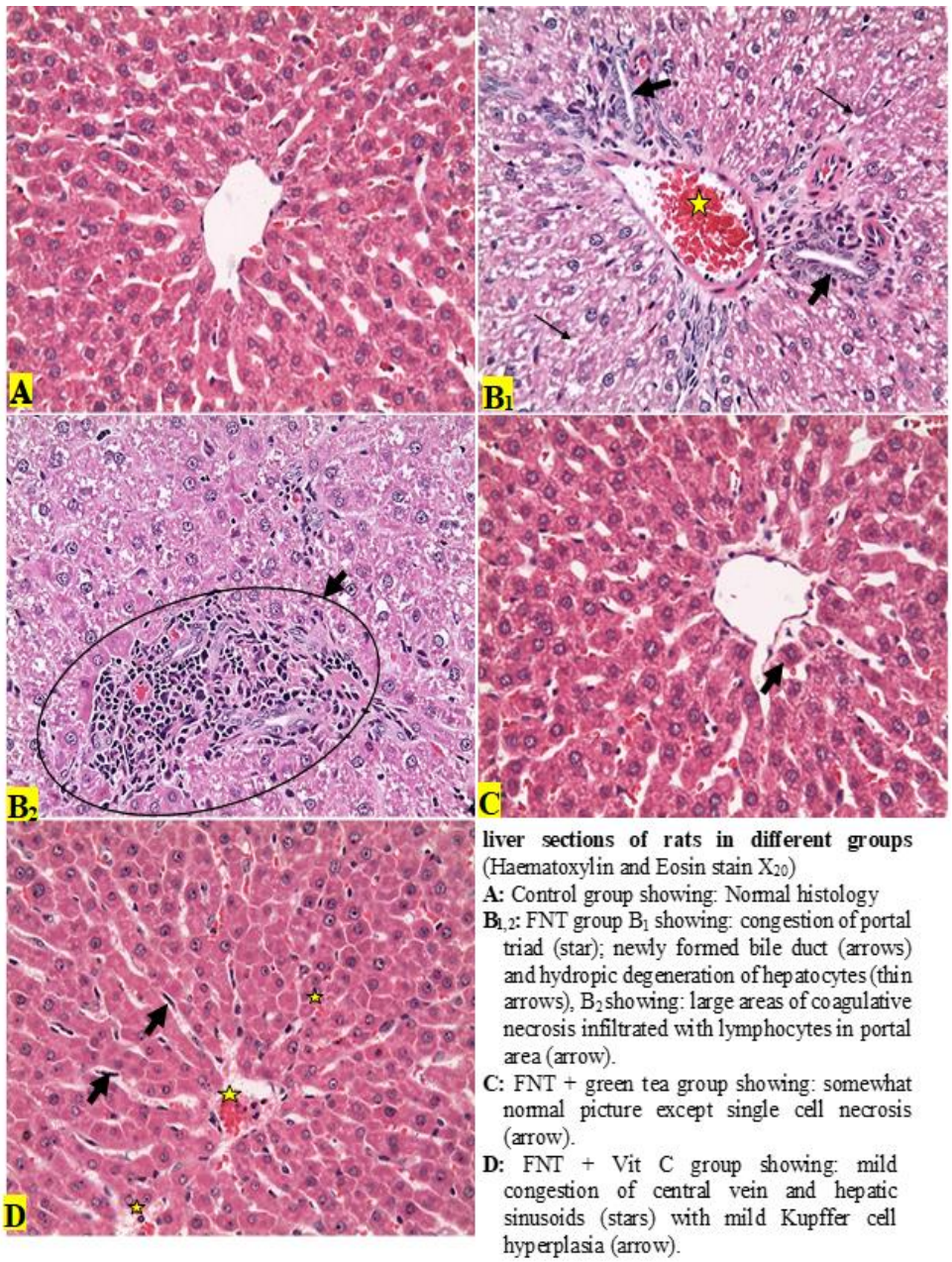
3.2. The histopathological changes in the liver

In this study, histopathological changes were observed in liver and kidney of fenitrothion treated group, fenitrothion and vitamin C treated group and fenitrothion and green tea extract treated group. After oral administration of FNT for six weeks, histopathological changes were observed in liver and kidney tissues of rats in all treatment groups compared with control group. According to liver tissues the hepatocytes and other cells of the liver in control group were normal and systematically arranged. The central vein lies at the centre of the lobule surrounded by the hepatocytes with strongly eosinophilic granulated cytoplasm, and distinct nuclei. In addition, between the strands of hepatocytes the hepatic sinusoids are exhibited as shown in Fig.1 (Liver A) Our histopathological study revealed that the liver of the fenitrothion administrated rat showed congestion of portal vessels, newly formed bile duct and hydropic degeneration of hepatocyte Fig.1 (Liver B1). Also large areas of congulative necrosis infiltrated with lymphocytes in portal area were noticed Fig.1 (Liver B2). Concerning to FNT plus green tea group, they were showing somewhat normal picture except single cell necrosis Fig.1 (Liver C). In addition FNT plus vitamin c group showing mild congestion of central vein and hepatic sinusoids with mild kuppfer cell hyperplasia Fig.1(Liver D).The liver is well-known

target organ of the toxic impact regarding its function in biotransformation and excretion of xenobiotics. After entering uptake, liver is the first organ to be exposed by portal circulation. Hepatotoxicity is the toxicity to liver, bile duct, and gall bladder. The liver is particularly susceptible to xenobiotics due to a large blood supply and its role in metabolism (Roganovic-Zafirova and Jordanova, 1998). So in this study, after oral administration of FNT, histopathological changes were observed in the liver of rats in all treatment groups compared with control group. These findining which in line with several studies provide information on hepatic effects in animals following oral exposure to OP, as liver congestion and hemorrhage vacuolation of the hepatocytes, necrosis, portal mononuclear cell infiltration, diffuse hydropic degeneration (Al-Jahdali et al., 2007, Elzoghby et al. 2014 and Raoofi et al., (2016).

Histopathological changes in the liver of rats after exposure to FNT were reported by Al-Jahdali et al (2007) who found blood congestion, fatty degeneration, hepatocyte swelling, and necrosis. The liver syndrome's intensity correlated with the increase in dose and duration time in rats which was daily injected for two and four weeks with 80 and 100 mg/kg of body weight FNT, respectively. Our results are agreed with Kerem, et al. (2007) who reported that there was congestion of blood vessels at mild dose of fenthion (25 or 50 mg/kg) but in high-dose fenthion groups (75 or 100 mg/kg) there was mild injury such as hepatocyte swelling and vacuolization and sometimes moderate central lobular injury. Also albino mice treated with carbosulfan for 10, 20, and 30 days showed dilated central vein and sinusoid between hypertrophied hepatocytes with pyknotic nuclei, vacuoles, and hyalinization (Ksheerasagar and Kaliwal, 2006) also in studying the changes of liver rats which poisoned by malathion insecticide certain observations were recorded, degenerative changes in the cytoplasm of hepatocytes, extensive hepatocyte necrosis, degenerative changes, proliferation and activation of Kupffer cells (in aggregated or scattered forms), and infiltration and proliferation of inflammatory cells around the portal and central vein spaces of the centrilobular region and in the sinusoidal space. (Elzoghby et al., 2014 and Raoofi et al., 2016).On the other hand, results of numerous studies suggests the protective effects of GTE and Vit. C against various pathological changes as a result of OP toxicity as Elhalwagy et al., (2008) who studying the ameliorative effect of daily administrated dose of GTE and found that dilated congested central vein and dilated congested blood sinusoids in between the liver cords with intense mononuclear inflammatory cellular infiltration. On the other dimensionless damage was clearly noticed in liver with green tea supplementation especially with the group of intoxicated with low dose fenitrothion and green tea. The liver architecture was preserved with less hemorrhage and less cell degeneration; however, inflammatory cells were noticed. It worth mentioned that Raoofi et al., (2016) reported that green tea extract was able to protect rat liver against toxicity resulting from malathion intake probably because of the extract's antioxidant properties and due to the polyphenols that it contains. Protective effects of green tea extract against malathion were dose-dependent and in his research, it was found that the 200 mg/kg dose was more effective in improving hepatic failure caused by malathion. So GTE improve FNT insecticide toxicity and protects phospholipids from better peroxidation and prevents changes in biochemical parameters and morphologic changes and it protects membranes from peroxidation of lipids associated with ethanol consumption in rat liver by decreasing oxidative stress, so it reduces oxidative damage by its antioxidant properties (Elhalwagy et al., 2008 and El Daly, 2011).

On the other hand, many researchers have been interested in the protective effects of vitamin C against various pathological changes as a result of OP pesticides as Magdy et al. (2016) who mentioned that there were histopathological changes observed in liver of abamectin treated group and abamectin and vitamin C treated group,marked edema and dilation of Disse's space were noticed in abamect in treated rats but in abamectin and vit. C treated animals, most of the hepatocytes appeared to somewhat normal but associated with dilation of the blood vessels and conclude that vitamin C act as antioxidants to some extent, Djurašević et al., (2008) who confirmed that the additional intake of ascorbate improves the liver's antioxidative defense in a dose-dependent manner and Elzoghby et al., (2014) who studying the changes of liver rats which poisoned by malathion insecticide and revealed that the malathion plus vitamin C treated group exhibited histopathological changes in liver and kidney tissues.



liver sections of rats in different groups (Haematoxylin and Eosin stain X_{20})

A: Control group showing: Normal histology

$B_{1,2}$: FNT group B_1 showing: congestion of portal triad (star); newly formed bile duct (arrows) and hydropic degeneration of hepatocytes (thin arrows), B_2 showing: large areas of coagulative necrosis infiltrated with lymphocytes in portal area (arrow).

C: FNT + green tea group showing: somewhat normal picture except single cell necrosis (arrow).

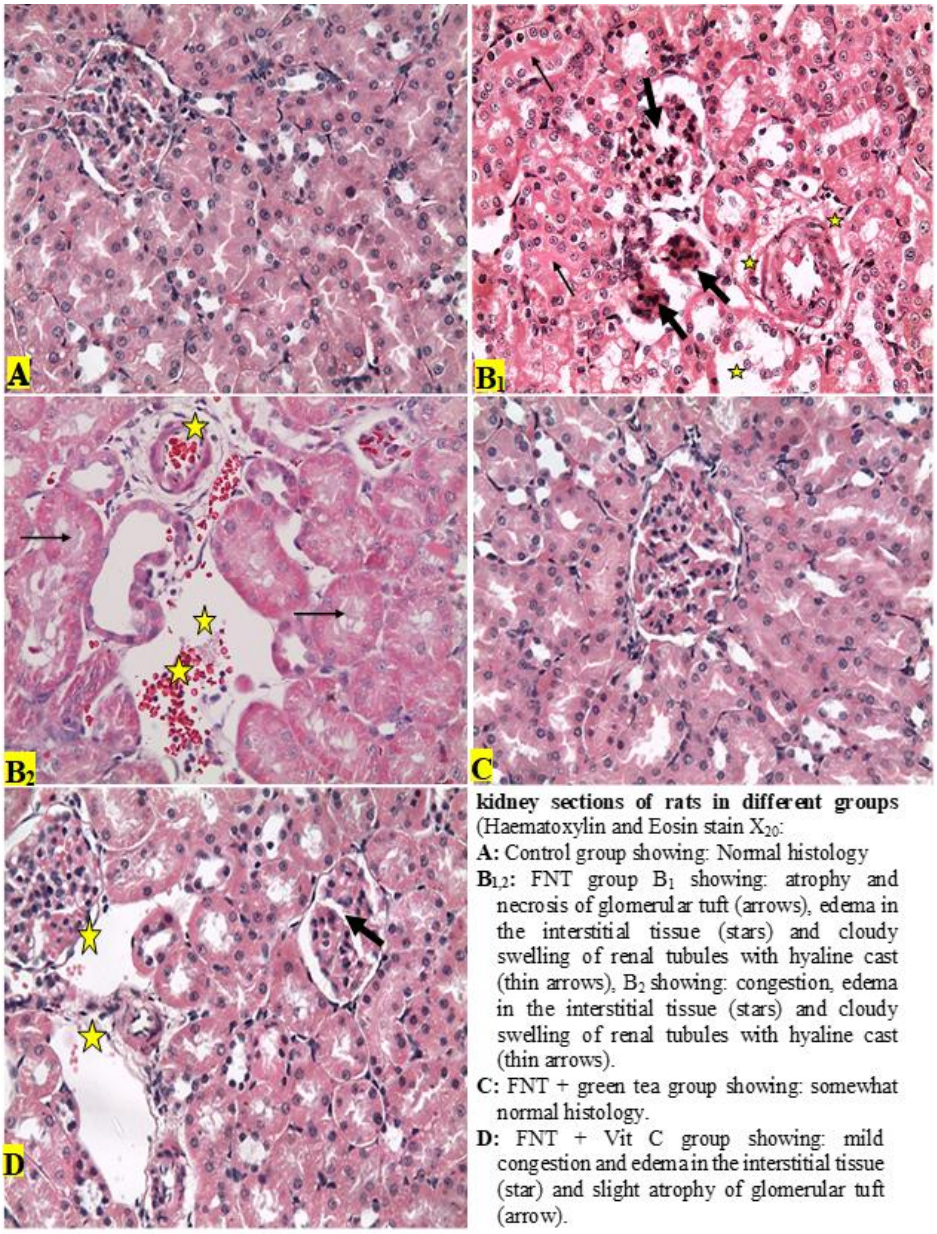
D: FNT + Vit C group showing: mild congestion of central vein and hepatic sinusoids (stars) with mild Kupffer cell hyperplasia (arrow).

Fig. 2. Liver sections of rat in different groups

Histopathological Alterations in kidney

Kidney is one of the targets organs of experimental animals attacked by OP compounds (Mansour and Mossa, 2010). In our study histopathological examination of the kidney sections in the control group showed normal kidney histology, a renal corpuscle and renal tubules, proximal convoluted tubules and distal convoluted tubules further, the glomerulus, urinary space and Bowman's capsule were noticed as shown in Fig.2 (Kidney A). But after oral administration of FNT for six weeks kidney FNT treated group was showed atrophy and necrosis of glomerular tuft, edema in the interstitial tissue and cloudy swelling of renal tubules with hyaline cast (thin arrows) as shown in Fig.2 (Kidney B1). On the other hand congestion, edema in the interstitial tissue (stars) and cloudy swelling of renal tubules with hyaline cast (thin arrows) as shown in Fig.2 (Kidney B2). It is worth

noting that more degeneration of cells of renal tubules in the kidney of rats exposed to fenitrothion. However according to FNT plus green tea group showing somewhat normal histology in Fig.2 (Kidney C) but FNT treated group plus vitamin c showing mild congestion and edema in the interstitial tissue and slight atrophy of glomerular tuft (arrows) in Fig.2 (Kidney D). Kidney is the organ whose primary function is the maintenance of water, electrolyte and acid-base haemostasis, other functions includes the excretion and elimination of many toxic waste products among others (Young et al., 2006). Histologically, nephrons are the functional units of the kidney and are composed of renal corpuscles (glomeruli and renal corpuscle) and renal tubules. They are involved in osmoregulation and excretion through the process of ultrafiltration, selective re-absorbtion and secretion of some of the excretory substances directly from the blood into the glomerular filtrate (Mescher, 2010). Therefore, injury to the kidney will affect the function of metabolic toxic waste excretion which may affect general bodily functions if renal failure should set in (Owoeye et al., 2014). Many authors found that many changes such as blood congestion in between the tubules and cellular degeneration, necrosis of the renal tissues of rats exposed to OP (Piramanayagam and Manohar 2002; Khogali et al., 2005 and Elzoghby et al. 2014).The toxicity of OP insecticides results in negative effects on many organs and systems such as the liver, kidney, nervous system, immune system and reproductive system (Mansour and Mossa, 2011). In other wise Elzoghby et al. (2014) showed that rats groups treated by malathion plus vitamin c or malathio plus green tea showed an improvement may due to inhibition of lipid peroxidation that cause damage of cell wall, cell lyses and necrosis (Morcos, 1997). These observations are agree with our finding and those of Ayo et al. (2006); Eraslan et al., (2007); Ambali et al. (2010) and Owoeye et al. (2014) who reported that vitamin c is an effective antioxidant in various biological systems. The reduction may be due to the protective effects of vitamin c, this have been reported previously by other investigators (Aly et al., 2010, Elzoghby et al., 2014 and Magdy et al., 2016) and green tea decreased renal degeneration and caused disappearance of cortical necrosis in kidney tissues. This protection may be attributed to phosphorylation and activation of endothelial nitric oxide syntheses in endothelial cells through modulation of protein kinase C. Signaling pathway by green tea resulting in endothelial dependant vasorelaxtion (Lorenz et al., 2004). Histopathological findings of this research agree with Afshar et al. (2008) who were stated that after oral administration of FNT, histopathological changes were observed in the liver and kidney tissues of rats.The kidney is highly susceptible to toxicants for two reasons: a high volume of blood flows through it and it filtrate large amounts of toxins which can concentrate in the kidney tubules. Nephrotoxicity is toxic to the kidney and it can result in systemic toxicity causing decreased ability to excrete body wastes, inability to maintain body fluid &electrolyte balance, and decreased synthesis of essential hormones as erythropoietin (Finn, 1977). Our results are agree with that obtained by Khogali et al., (2005) who found that many changes such as blood congestion in between the tubules and cellular degeneration, necrosis of the renal tissues in the kidney of mice treated with 60 mg/kg dimethoate pesticide. Also Elzoghby et al., (2014) reported that histopathological picture in rats kidney malathion-treated group showed swelling and vacuolization in the lining endothelium of the glomerular tuft associated with degeneration in the lining epithelium of the tubules. Wherever in groups treated by malathion plus vitamin C or malathion plus green tea there were a significant decrease of them when compared with malathion group. So from these observations we can notice that histopathological findings of this research conform to its biochemical outcomes. Fenitrothion administration induced significant deleterious biochemical oxidative stress and histopathological alterations, co-treatment with vit. C partially improved these alterations. While co-treatment with green tea extract exhibited full protection and more protective role and markedly reduced tissues damage induced by fenitrothion , these findings are in accordance with those of (Magdy et al., 2016) who was concluded that OP abamectin increased the catabolism of the biochemicals to meet the enhanced energy demand of animals under stress or their reduced synthesis due to impaired tissue function, whereas vitamins C and E by their antioxidant action may protect the kidney tissue against the oxidative stress of abamectin on the kidney tissues. It is worth noting that many studies have been reported that the potential antioxidant of green tea that is far greater than of vitamin C (Wiseman, 1997, Rice-Evans, 1999, El Daly, 2011 & Elzoghby et al., 2014).



kidney sections of rats in different groups (Haematoxylin and Eosin stain X_{20}:

A: Control group showing: Normal histology

$B_{1,2}$: FNT group B_1 showing: atrophy and necrosis of glomerular tuft (arrows), edema in the interstitial tissue (stars) and cloudy swelling of renal tubules with hyaline cast (thin arrows), B_2 showing: congestion, edema in the interstitial tissue (stars) and cloudy swelling of renal tubules with hyaline cast (thin arrows).

C: FNT + green tea group showing: somewhat normal histology.

D: FNT + Vit C group showing: mild congestion and edema in the interstitial tissue (star) and slight atrophy of glomerular tuft (arrow).

Fig. 2. Kidney sections of rat in different groups

CONCLUSION:

From these results and observations, it can be concluded that fenitrothion had toxic effects on biochemical oxidative stress parameters which correlates well with the histopathological changes of liver and kidney tissues. However supplementations with antioxidant green tea extract and vitamin C are able to improve these hepatic and renal oxidative stress and that probably because of their antioxidant properties and due to the polyphenols which attenuating the oxidative stress through scavenging of free radicals, or by enhancing the activity of antioxidants. Moreover green tea extract and vitamin C had ameliorated the toxic effect on the histological

changes in liver and kidney tissues. Co-treatment with vit. C partially improved these alterations, while co-treatment with green tea extract exhibited full protection and more protective role and markedly reduced tissues damage induced by fenitrothion insecticide.

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