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Hepatotoxicity of fenitrothion insecticide in adult rats. Cytological Aspects

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Indiscriminate application of fenitrothion has led to environmental pollution and severe health problems. Accordingly, this study was conducted to evaluate the hepatotoxic and histopathological changes induced by the pesticide fenitrothion (FNT) in the liver of adult Wistar rats. The study consisted of three different treatment groups. Each group was divided into two sub-groups containing an equal number of animals that received a daily treatment for two different experimental periods, of 14 and 28 days, as follows: G1, control group; G2, low-dose administration of FNT (1/60 of the LD50); and G3, high-dose administration of FNT (1/20 of the LD50). Results: Significant alterations in the histology of the liver were observed. Transmission electron microscopy examinations revealed histopathological damage to different organelles of the liver. Damage to the liver samples was confirmed by morphological and histological measures. FNT caused significant alterations in measures of the nucleus, mitochondria, and Kupffer cell nuclei compared with the control samples. Conclusion: FNT caused cytological changes in the liver of Wistar rats and the damage intensity was correlated with an increase in FNT dose and the duration of administration.

Keywords: Fenitrothion, liver, mitochondria, nucleus, Kupffer cell.

INTRODUCTION

Pesticides have a role in pest control and the prevention of the spread of infectious diseases, resulting in increased agricultural productivity. However, the excessive use of pesticides may be harmful to human and animal health through inhalation or the presence of residues in agricultural products (Rjeibi et al. 2016). Organophosphate insecticides are the most widely used group of pesticides and are very effective (Ojha et al. 2013). Despite their beneficial effects against undesired pests, exposure to organophosphate pesticides can cause severe environmental and health hazards to nontarget organisms, including humans (Tuzmen et al. 2008; Al-Attar 2015; Milošević et al. 2017).

Fenitrothion (FNT), [O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate] is a broad-spectrum organophosphorus insecticide frequently used for the control of insect pests and mites that affect cereals, rice, fruits, vegetables, stored grains, and cotton (Uygun et al. 2005; El-Demerdash, 2011; Abdel-Ghany et al. 2016). However, the health risks caused by FNT in humans has attracted the attention of many researchers, because humans may be exposed to FNT either directly or indirectly (Taib et al. 2013). Previous investigations have shown that FNT induced biochemical, morphological, and functional alterations in the brain, reproductive organs, kidney, and liver (Afshar et al. 2008, Taib et al. 2013, Abdel-Ghany et al. 2016).[8-10] FNT is rapidly and intensely absorbed in the digestive tract, and is characterized by preferential accumulation in blood and the liver (Afshar et al,

2008).

The liver is an essential part of many physiological processes. It is a critical organ in which biotransformation, detoxification, and the excretion of xenobiotics, including pesticides, occurs (Trefts et al. 2017). In addition, owing to the importance of blood supply and the high concentration of enzymes involved in the metabolic transformation of exogenous molecules and the production of metabolites, the liver may be injured by the accumulation of large amounts of toxic compounds (Jayusman *et al.,* 2014). Similarly, some previous studies have shown that exposure to FNT can lead to the disruption of liver enzymes, hepatocyte damage, leukocyte infiltration, necrosis, and moderate congestion of blood vessels (Afshar et al. 2008; Milošević et al. 2018).The present study aimed to evaluate the detailed histopathological effects of FNT on the hepatocytes of Wistar rats.

MATERIALS AND METHODS

Fenitrothion

Fenitrothion was purchased from Arabian farms in Jeddah (Saudi Arabia), (CAS no: 118-141-2324). FNT was used as the formulated product, which contains 40% fenitrothion in addition to 60% oil soap solute.

Animals

Rats were supplied by the King Fahd center for Scientific Research, King Abdel-Aziz University, Jeddah, Saudi Arabia. Adult male Wistar rats (*Rattus norvegicus*), 2 months of age, with an average weight of approximately 164.5 ± 1.225 g were used in this study. The rats were housed in plastic cages and were fed with industrial standard diet formulations that contained all essential nutrients and protein, salts, vitamins, fiber, and water. All rats were maintained in standard environmental conditions (12 h light/12 h dark cycle with a controlled temperature of 25°C–28°C) throughout the study period.

Experimental design

Sixty adult Wistar rats (*Rattus norvegicus*) were divided into three groups containing 20 animals. Each group was divided into two sub-groups of 10 animals that received a daily treatment for two different experimental periods (14 and 28 days). The animals received the following treatments: Group G**1**, the control group, not given FNT. Group G**2**, comprised 20 rats divided into two equal subgroups of 10 rats, received a low dose of 40% FNT (1/60 of the LD50). Subgroups G2a and G2b received the treatment for 14 and 28 days, respectively. Group G3, comprised 20 rats divided into two equal subgroups of 10 rats, received a high dose of 40% FNT (1/20 of the LD50). Subgroups G3a and G3b received the treatment for 14 and 28 days, respectively. After the indicated treatment periods, the rats were sacrificed by cervical decapitation to avoid stress. The liver tissue was collected for histological analyses.

Histological study by transmission electron microscope

Transmission electron microscopy examinations were conducted to study the structural changes in cellular organelles at the end of each experimental period (14 and 28 days) for each experimental group. Sections with a thickness of 1 mm² from the liver tissue were placed in a solution of 3% buffered glutaraldehyde, cooled at 4°C for 24 h, washed in a solution of phosphate buffer (0.1 M, pH 7.4), and placed in osmium tetroxide (1%) at atmospheric room temperature. Then samples were washed again in phosphate buffer. The specimens were dehydrated in solutions with an ascending gradient of alcohol propylene oxide, transferred to a mixture of propylene oxide and epon, and embedded in a mixture of epon and oraldite. Ultrathin sections (0.5–1 µm) were prepared by using ultramicrotome and stained with uranyl acetate and lead citrate (Woods and Stirling , 2002). The sections were viewed and photographed by using an electron microscope (Philips-cm100) at the King Fahd Center for Medical Research, Jeddah, Saudi Arabia.

Morphological and histological measures

Analyzed different electron micrographs of the liver in rats the treatment and control groups by measuring internal organelles (nuclei, mitochondria, and Kupffer cells) using the scale recorded on the electron micrograph images and the electron microscope scale, and then calculated the median and deviation standard of the measurements, and compared the significance of the changes in measures in the treatment and control groups.

Statistical analysis

Data for all groups were expressed as the mean ± standard deviation (X±SD). The statistical analyses methods used were Student's *t*-test and

the Chi-square test (Fisher and Yates, 1948) and were computed by using SSPS 20.0 statistical software. The results were considered to be statistically significant, highly significant, and non-significant for P values less than 0.05, less than 0.001, and more than 0.05, respectively.

RESULTS AND DISCUSSION

Histological examination:

Normal hepatocyte architecture was observed in the control group with an intact nucleus, well-defined nuclear membrane, and normal chromatin structure (Fig. 1). Mitochondria were abundant and uniformly distributed in the hepatocytes. Pleats of endoplasmic reticulum were also prominent. The liver has a pivotal metabolic role to maintain the level of energy and the structural stability of the body. It is the first target organ of toxic substances, owing to its major role in the detoxification process (Afshar et al. 2008). Daily administration of low-dose FNT (1/60 LD50) for 14 days in group (G2a) induced some alterations in hepatocytes, including a loss of electron density in the cytosol of hepatocytes, atrophied and deformed mitochondria and peroxisomes, and an indistinct nuclear envelope and loss of electron density in the nucleoplasm (Fig.2). After the longer treatment period 28 days in group (G2b), low-dose FNT induced marked alterations in hepatocytes, including hepatic vacuolization, fragmented chromatin in the nuclei, and lymphocyte infiltration. Congestion of blood vessels, damaged blood sinusoids, and vacuolation of Kupffer cells were also noticed (Fig.2). Daily administration of high-dose FNT (1/20 LD50) for 14 days in group (G3a) induced marked alterations in hepatocytes, with damaged blood sinusoids and endothelium, dilated Disse space with degeneration of hepatocyte microvilli, clumped electron dense mitochondria with indistinct detail, and deformed desmosomes (Fig. 3). An increase in the period of high-dose treatment for 28 days in group (G3b) induced the most marked alteration in hepatocytes showing proliferated and hypertrophied smooth endoplasmic reticulum, deformed mitochondria, and dilated nuclear envelope with dense nucleoli. In addition, congestion in blood vessels, high dilatation of sinusoids, and hypertrophied Kupffer cells were observed G**3b** (Fig. 3). The high-dose FNT treatment for 28 days administered in this experiment caused histopathological damage in hepatocytes. These results agreed with other studies that described the hepatotoxicity of FNT (Afshar et al. 2008; Somia and Madiha, 2012; Milošević et al. 2018; Galal et al. 2019). In addition, the intensity of hepatic syndrome as correlated with the increase in dose and duration of FNT (Toś-Luty et al. 2003; AlJahdali et al. 2009; Galal et al. 2019).

Cytological measurements of fenitrothion on hepatocytes:

Cytological measurements in micrometers of hepatocyte organelles in control and FNT-treated adult male rats are shown in Table 1. The average diameter of the control nuclei was estimated at 5.223 ± 0.511 μm. Compared with the control group, a significant increase ($P<0.05$, $P<0.01$) in the average nuclear diameter was observed in the G_{2a} and G_{2b} groups, respectively (Table 1). However, a significant decrease ($P<0.05$, $P<0.01$) in the nuclear diameter of was observed in the G_{3a} and G_{3b}, respectively. This result confirmed the histological examination results that showed a large nucleus containing densely stained heterochromatin masses under the distinct nuclear envelope (Chomphuwiset et al. 2010, Trefts et al. 2017). These results could be explained by the swelling of the nuclear envelope, chromatin condensation, and the loss of electron density in the nucleoplasm. However, the treatment of adult rats with a high dose of FNT resulted in a significant decrease in the average nuclear diameter in the G**3a** and G**3b** groups, this was in agreement with our transmission electron microscopy study, which described damage to the hepatocyte nuclear membrane and the loss of nuclear integrity (Kumar et al. 1993; Jayusman et al. 2014).

In the hepatocytes (G1), the mitochondria appeared as numerous spheroid or ovoid organelles and there were also some elongated mitochondria. The average diameter of the spheroid mitochondria was estimated at 1.117 ± 0.14 μm and the dimensions of the ovoid mitochondria were estimated at 1.571 ± 0.20 μm (length) and 0.833 ± 0.05 μm (width) (Table 1). The statistical analysis illustrated a significant reduction in the average mitochondrial diameter in low-dose FNT treatment groups compared with the control ($P<0.05$). For the G_{2a} group, the average diameter of spheroid mitochondria was estimated at 0.755 ± 0.03 μm and the average dimensions of the ovoid mitochondria were estimated at 1.05 ± 0.37 μm (length) and 0.736 ± 0.052 μm (width) (Table 1).

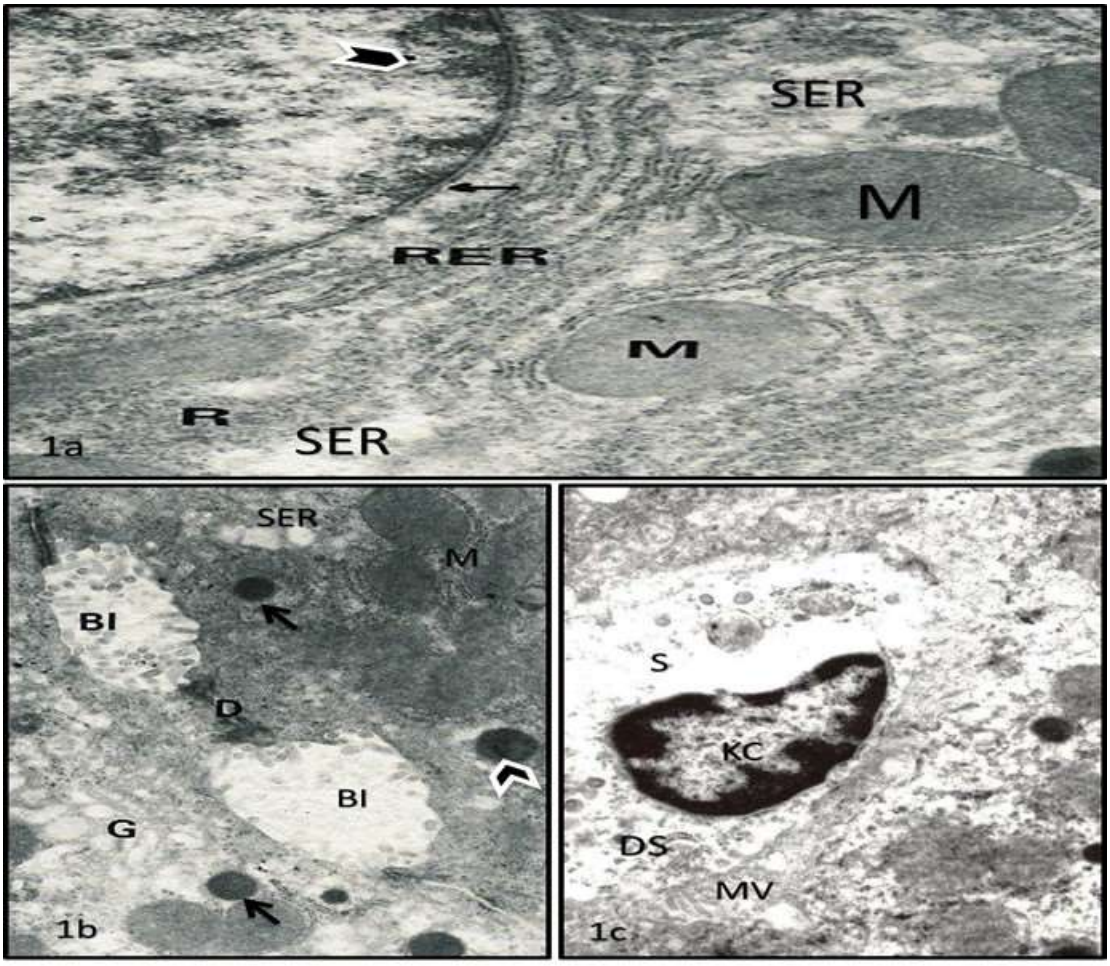

Figure 1 : E.M. ultrathin sections of control liver (G_1) of adult wister rats.

1a : Part of hepatocyte showing nucleus with distinct nuclear envelope (arrow) & small irregular clumps of heterochromatin adjacent to internal nuclear envelope (head arrow); parallel stacks of (RER); smooth endoplasmic reticulum (SER) ;mitochondria (M) and polyribosome (R); scale bar = 500 nm.

1b : Part of two adjacent hepatocytes. Note bile canaliculi (BI) with well-defined microvilli and surrounded by desmosomal junctions (D). Note also Golgi areas (G) electron, mitochondria (M), smooth endoplasmic reticulum (SER), dense peroxisomes(head arrow) and lysosome(arrows); scale bar = 2μm.

1c : Note sinusoidal lumen (S); kupffer cell (KC) with large nucleus contain densely stained heterochromatin masses under the nuclear envelope; microvilli (MV) of hepatocyte wall protruding into Disse space (DS); scale bar = 2 μm.

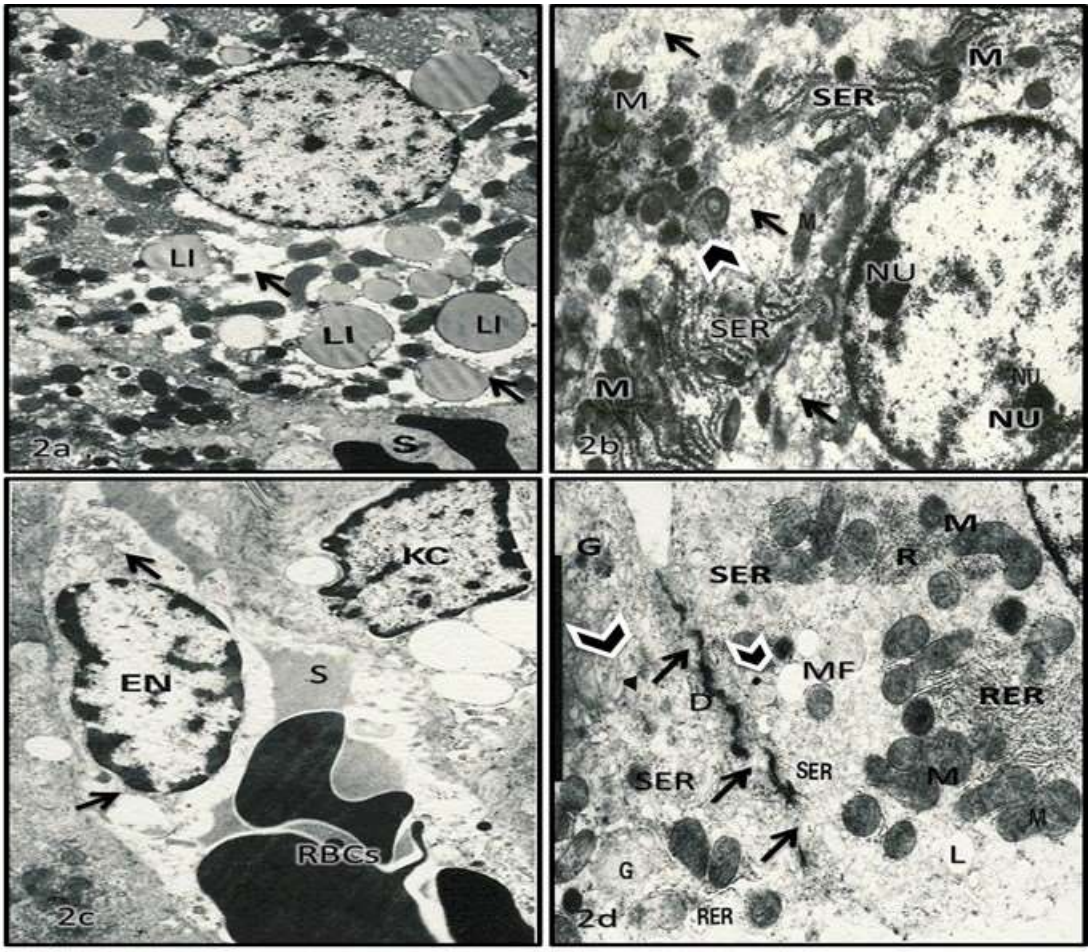

Figure 2 : E.M. Ultrathin sections of liver from adult wister rat (G_2) treated with low dose of fenitrothion (uranyl acetate-lead citrate stained sections).

2a : Part of hepatocyte (G_{2a}). Note digested lipid droplets inside the liposome (LI); lose electron density of hepatocyte cytosol (arrows); sinusoid with RBCs (S); scale bar = 2μm.

2b : Part of hepatocyte (G_{2a}). Notice atrophied and deformed mitochondria (M)& peroxisomes(head arrow); lysis of cytosol (arrows); nucleus with indistinct nuclear envelope and lose of electron density of nucleoplasm; marginal nucleoli with segregated components (NU); scale bar = 2 μm.

2c: Section of treated liver (G_{2b}) showing damaged blood sinusoid. Note endothelial cell (EN) with damaged organelles (arrows); vacuolated kupffer cell (KC) & deformed RBCs; scale bar = 1μm.

2d : Part of hepatocyte (G_{2b}). Showing pleomorphic mitochondria(M) with slipper form & osmiophilic cristae; proliferated (SER); polyribosomes (R); deformed and proliferated desmosomes(D); autophagic vacuoles (head arrow); atrophied Golgi(G); Fragmented RER; myelin figure (MF); proliferated bile canaliculi (arrows); scale bar = 2μm.

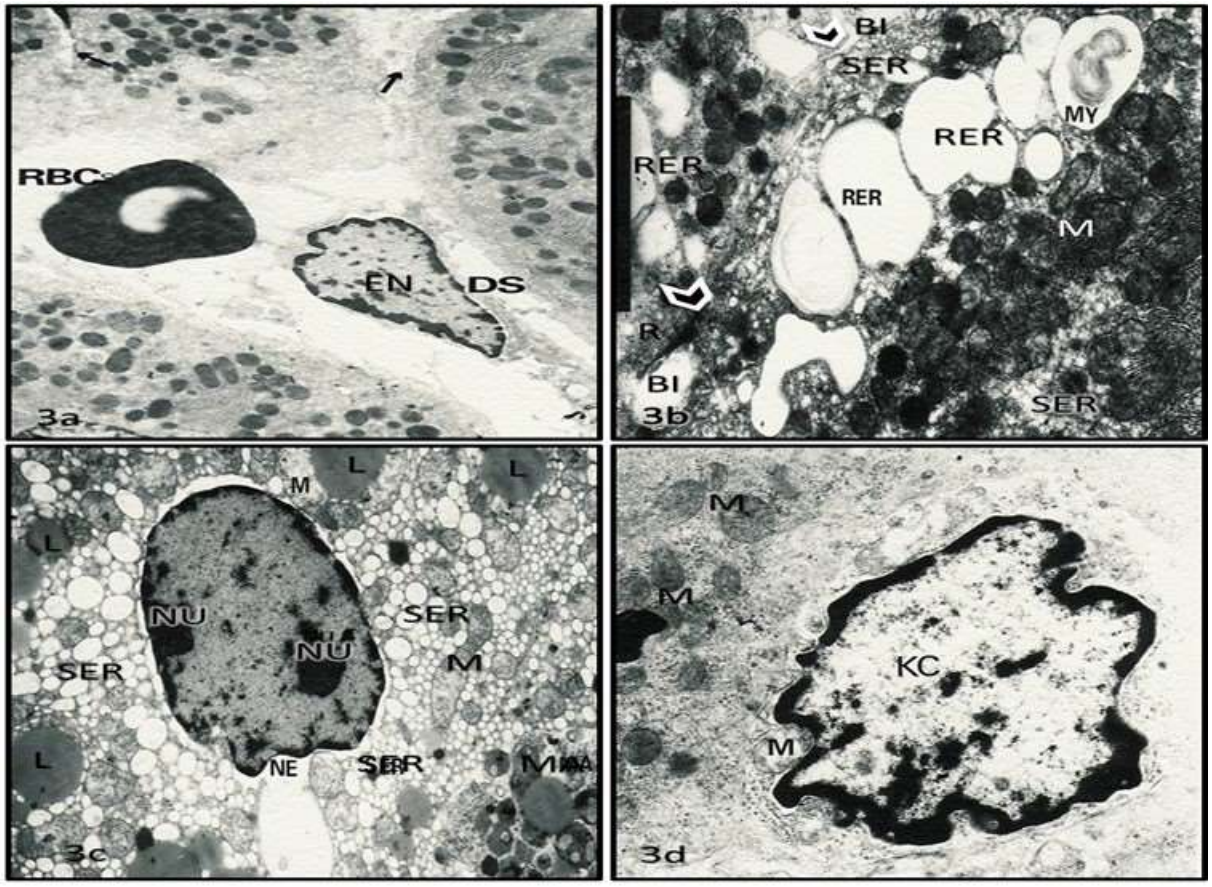

Figure 3 : E.M. ultrathin sections of liver from adult wister rats treated with high dose of fenitrothion (G_3). (uranyl acetate-lead citrate stained sections)

3a : Section of treated liver (G_{3a}). Showing damaged blood sinusoids with deformed RBCs; damaged endothelium (EN). Note also dilated Disse space (DS) with damaged hepatocyte microvilli; widen inter cellular spaces (arrow); scale bar = 2 μm.

3b : Section of treated liver (G_{3a}). Showing clumped electron dense mitochondria with indistinct detail; dilated (RER); myelinosomes (MY); bile canaliculus (BI) with swollen microvilli surrounded by deformed desmosomes (arrows). scale bar = 2 μm.

3c : Section of treated liver (G_{3b}). Showing proliferated and hypertrophied (SER); lipid droplets (L); deformed mitochondria (M); with low electron dense matrix and damage or osmiophilic cristae. Note; electron dense nucleus with irregular surface; dense nucleoli (NU); dilated nuclear envelope (NE); macrophage (MA) filled with phagolysosome & lipid droplets; Scale bar = 2 μm.

3d : Section of treated liver (G_{3b}). Showing hypertrophied kupffer cell (KC) with damaged mitochondria (M) and nucleus with irregular surface and low electron dense matrix. scale bar = 2 μm.

Table 1: Cytological measurements (μm) of liver organelles, for control and fenitrothion treated adult male rats at the end of each experimental period.

Organelles		Nucleus			Spheroid - mitochondria			Ovoid –mitochondria						Kupffer nucleus		
Days		G_1	G_2	G_3	G_1	G_2	G_3	G_1		G_2		G_3		G_1	G_2	G_3
								length	Width	length	Width	length	Width			
14	Avg.	5.223	6.051	4.521	1.117	0.755	0.502	1.571	0.833	1.051	0.736	0.641	0.603	2.97	2.98	3.83
	± SD	0.511	2.413	0.724	0.14	0.03	0.05	0.20	0.05	0.37	0.052	0.05	0.049	0.05	0.03	0.04
	P	-	*	*	-	*	**	-	-	*	*	**	**	-	-	**
28	Avg.	5.433	7.001	2.570	1.221	0.543	0.375	1.592	0.891	0.688	0.241	0.377	0.330	3.25	2.01	6.296
	± SD	0.464	3.13	1.725	0.17	0.062	0.046	0.31	0.03	0.141	0.04	0.099	0.078	0.06	0.16	1.346
	P	-	**	**	-	**	**	-	-	**	**	**	**	-	**	**

Significant levels: - $p > 0.05$ not significant - $P < 0.05$ * significant; $p < 0.01$ ** highly significant. T-student t-test; ± SD: standard deviatio

For the G_{2b} group, the average diameters of the spheroid mitochondria were 0.543 ± 0.0621 μm and the average dimensions of the ovoid mitochondria were estimated at 0.688 ± 0.1411 μm (length) and 0.241 ± 0.04 μm (width). The statistical analysis confirmed a strongly significant decrease ($P<0.01$) in the mitochondrial dimensions in the G3 group compared with the control group (Table 1). The average diameters of the atrophied mitochondria (G_{3b}) showed a strongly significant decrease ($P<0.01$) in comparison with the G_{3a} and control group, from 0.641 ± 0.05 μm in length and 0.603 ± 0.049 μm in width, 0.377 ± 0.099 μm in length and 0.330 ± 0.078 μm in width, respectively (Table 1). This matched the transmission electron microscopy results, which described severe damage to mitochondria, such as atrophied and deformed mitochondria, and pleomorphic mitochondria, in different groups (G2 and G3) and conformity in previous studies. (Jayusman *et al.,* 2014).

Kupffer cells are present on the blood sinusoid wall neighboring the endothelial cavities (G1). They are characterized by microvilli processes and contain large ovoid or triangular nuclei, including highly pigmented chromatin masses that stick to the inner nuclear envelope, which has an average dimension of 2.97 ± 0.05 μm (Table 1). It was noted in group (G2) damaged in the Kupffer cells with necrosis, the average size of the nuclei was 2.01 ± 0.16 μm, (Table 1).The average dimension of the Kupffer cell nuclei reached 6.2963 ± 1.3462 μm, which was a strongly significant ($P<0.001$) increase in diameter compared with the G_{3a} group and control group (Table 1). These findings were in line with the study of El-Aziz Tahoun *et al.,* (2018), which investigated the histopathological changes in Kupffer cells of the liver after the oral administration of FNT. Changes in the organelle liver weight is a known sensitive indicator of exposure to potentially toxic chemicals (El-Sheikh and Galal, 2015).This result may be the consequence of the increased degradation of lipids and protein due the toxicity of treatment (Mansour and Mossa, 2010). However, inflammatory changes have been found in the liver of FNT-intoxicated rats (Afshar *et al.,* 2008, Somia and Madiha 2012, Galal *et al.,* 2019).

CONCLUSION

From these results and observations, it was concluded that fenitrothion induced hepatotoxicity in rats, causing significant histopathological changes in the liver tissue. In addition, the intensity of liver toxicity was correlated with an increase in dose and duration time in rats that were administered a daily injection of low (1/60 of the LD50) and high (1/20 of the LD50) FNT doses for 2 and 4 weeks, respectively. Further research is needed to determine the most effective doses of FNT, as well as to identify the molecular mechanisms and mode of action of this pesticide that induces adverse effects on non-target organisms, including humans..

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

ADA fully contributed in all aspects of the study.

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