Oxidative Stress Induced by Diazinon and the Alleviation Effect of Virgin Olive Oil in Male Albino Rats

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Abstract: Pesticides are widely used in order to enhance the food protection by controlling the unwanted insects and disease vectors. The organophosphate insecticide, diazinon (DZ) is widely used in agriculture and veterinary medicine. The present study was carried out to investigate the impact of repeated sublethal doses (36.5 mg/ kg bw / day) for two weeks of diazinon on some hematological and biochemical parameters and histopathological changes in the liver tissue of male albino rats and pinpoint the possible protective effect of olive oil against its adverse effect. DZ orally treated 3 doses /week over a period of two weeks, then two weeks without treatment as an observation period. Group I: Rats were served as control and given water. Group II: Rats were given virgin olive oil 3.3ml/kg bw. Group III: Rats were given DZ 36.5 mg/kg bw which represent 1/10 LD_{50} (LD_{50} was 365 mg/ kg bw). Group IV: Rats were given DZ 36.5 mg/kg bw plus virgin olive oil 3.3ml /kg bw. Results showed significant decrease in the body weight and increase in the relative liver weight of group III animals. Also, significant increase in the hematological parameters; white blood cells (WBC's) and hematocrite (Hct) value, while total thromocyte (platelet) count (PLT) was decreased at the first week after treatment, followed by significant decrease in WBC's count, hemoglobin (HGB) concentration and hematocrite (Hct) value, where the PLT count was increased at the second week after treatment. At the end of the experiment; WBC's count and Hct value were decreased, and the PLT count was increased. While there were no significant changes in the erythrocytes indices. Moreover, DZ reduced serum catalase (CAT), liver superoxide dismutase (SOD) activities and reduced glutathion (GSH) level in liver, while the lipid peroxidation (LPO) level in serum was increased, jointly serum acetyl cholinesterase (AChE) activity showed significant alteration. The effect of DZ on the previous parameters was confirmed by the histological changes of liver. In addition, olive oil supplementation showed significant reduction of DZ adverse effect which notarized its protective influence against the oxidative damage induced by DZ.

Keywords: Diazinon, Virgin olive oil, Relative organ weight, Hepatotoxicity, Hematological, Oxidative stress, Histopathological.

1. Introduction

Organophosphorus compounds (OPs) are one of the most common types of organic pollutants found in environmental and food products **(Tang *et al.,* 2009).** Diazinon (DZ) (diethoxy-[(2-isopropyl-6-methyl- 4-pyrimidinyl) oxy]-thioxophosphorane) is an OP compound used extensively to control flies, lice, insect pests of ornamental plants and food crops, as well as nematodes and soil insects in lawns and croplands **(Pena-Llopis 2005).** The main mechanism of action of DZ is acetylcholinesterase enzyme inhibition **(Kamanyire and Karalliedde, 2004).** However, it may induce imbalance in the free radicals production/elimination processes which altered the activities of the main antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) , and catalase (CAT) with consequent induction of cellular damage **(Roegge *et al.,* 2008; Cakici and Akat, 2013).** Additionally, several studies showed that DZ was capable of inducing histopathological, biochemical, hematological and physiological alterations **(Kalender *et al*., 2005; Cakici and Akat, 2013; El-Demerdash and Nasr, 2014; Abdel-Daim, 2016).** Previous studies showed that DZ-induced tissues injury depends on the increase of oxidative stress and cell death **(John *et al*., 2001; Pena-Llopis 2005)**, which can be directly induced by the parent pesticide or by toxic oxygenated metabolites. However, antioxidants can reduce the toxic effects of Diazinon.

In recent years, a considerable emphasis has been focused on the importance of the naturally available botanicals that can be consumed in an individual's everyday diet because of their antioxidant properties **(Nandakumar *et al*., 2008).** Plant-derived products are playing an essential role in the primary health care of about 80–85% of the world's population. Olive oil may have great health benefits including the reduction in coronary heart disease risk, the

effect on liver damage and oxidative stress, the prevention of some cancers and the modification of immune and inflammatory responses **(Keys, 1995; Boukhobza *et al*., 1988; Visioli *et al.*, 2002; Abd El-Fattah and Barakat 2013).** Virgin olive oil appears to be a functional food with various components such as monounsaturated fatty acids that may have nutritional benefits. It is also a good source of phytochemicals, including polyphenolic compounds **(Stark *et al.*, 2002; Lavelli, 2002).** Phenols have preventive effects against degenerative diseases mediated by the reactive oxygen species (ROS). It has been reported that the phenolic compounds are able to interact with the biological systems as bioactive molecules. They are particularly important inhibitors of lipid peroxidation **(Bonanome *et al*., 1992),** and are believed to be effective through their free radical scavenging and metal-chelating propertie **(Salah *et al*., 1995; Kandaswami *et al*., 1994).** Consequently, virgin olive oil could be of great interest in the prevention of various aspects of hepatodegenerative diseases. The present study was carried to investigate the protective effect of virgin olive oil against DZ- induced oxidative damage in rat.

Materials and Methods

2.1. Chemicals

Diazinon (Diazomax 60 % EC) was purchased from Egyptchem international for agrochemicals, while the virgin olive oil extracted by traditional method was obtained from Borg El Arab city (Alexandria). All other chemicals used were of analytical grade purchased from Sigma and Merck Chemical Companies.

2.2. Experimental design

Male albino rats weighing 150 -160 g were purchased from Faculty of Medicine; Alexandria University. The animals were allowed to acclimatize for two week before the initiation of the experiment under laboratory conditions (12 h light / 12 h dark, 22– 26 °C., 40–70% humidity) in stainless steel cages and provided with commercial diet and water *ad libitum*. Animals were divided into four groups , each of five rats, and orally treated 3 doses /week over a period of 2 weeks and then left for another 2 weeks without treatment for observation as the following: Group I: Rats were served as control and given water. Group II: Rats were given virgin olive oil 3.3ml/kg bw. Group III: Rats were given DZ 36.5 mg/kg bw of its oral LD_{50} which represent 1/10 LD_{50} (LD_{50} was 365 mg/kg bw; according to Department of Mammalian Toxicology, Pesticide Central Laboratory, Agriculture Research Center). Group IV: Rats were given DZ 36.5 mg/kg bw plus virgin olive oil 3.3ml /kg bw. DZ and olive oil were orally administrated to animals by esophageal intubation. The body weights of control and treated animals were recorded weekly.

2.3. Blood sample collection

Blood samples were withdrawn weekly from the animals from the retro-orbital plexus vein into either tube containing EDTA or non-heparinzed tube. Non-coagulated blood samples were analyzed for peripheral blood cell indicators such as white blood cells (WBC's) count, red blood cells (RBC's), haemoglobin content (Hb), Hematocrite(Hct %) and total thromocyte (platelet) count (PLT) as described by **Dacie and Lewis(1991)**. Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. Serum was separated by centrifugation at 3000 rpm for 15 minutes and kept at -20 ^{o}C until conducting the biochemical analysis.

At the end of the experiment the animals were sacrificed and liver was dissected, rinsed in saline solution (0.9% NaCl), weighted individually in all rats and the relative organ weight was calculated (organ weight: body weight). A specimen of the liver was fixed immediately in 10% buffered formalin for histological study.

2.4. Biochemical studies

Liver was minced and homogenized (10% w/v) in 50mM phosphate pH 7.5 and 1 mM EDTA using a polytron homogenizer, centrifuged at 8,000 g for 30 min at 4^{o}C and the resultant supernatant was used for enzyme assay.

2.4.1. Reduced glutathione (GSH) content

GSH content as a nonenzymatic antioxidant was determined in liver homogenate according to the method of **Beutler *et al.*, (1963)**. The method based on the reduction of 5, 5' dithiobis (2- nitrobenzoic acid) (DTNB) with glutathione to produce a yellow compound measured at 405 nm, and the reduced chromogen is directly proportional to GSH concentration. The amount of GSH was expressed as mg glutathione oxidized/g tissue.

2.4.2. Lipid peroxidation (LPO)

LPO was carried out in serum following the procedure of **Ohkawa *et al.* (1979)** using thiobarbituric acid (TBA). Malondialdehyde (MDA) formed as an end product of peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA reacts with TBA to generate a colored product that absorbs at 532 nm. The level of lipid peroxides was expressed as nmoles of MDA released/ ml.

2.4.3. Superoxide dismutase (SOD) activity

SOD as enzymatic antioxidant was measured in liver homogenate spectrophotometrically by the method of **Nishikimi *et al*., (1972)** at 560 nm. One unit of the enzyme activity is defined as the amount which produced 50% inhibition of phenazine methosulphat under the stan-

dard assay conditions. SOD activities were expressed as Units/mg tissue.

2.4.4. Catalase (CAT) activity

CAT activity was determined in serum according to the method of **Aebi (1984),** and the rate of H_2O_2 decomposition was monitored at 510 nm. One unit of CAT activity is defined as the amount of enzymes required to decompose 1 µM of hydrogen peroxide/ min. CAT activity was expressed as U/ L.

2.4.5. Acetyl Cholinesterase (AChE) activity

AChE activity was measured at 412 nm in serum according to the method of **Ellman *et al.,* (1961),** using acetylthiocholine iodide as a substrate. One unit of AChE activity was expressed as µmoles acetylcholine hydrolyzed/min/ml.

2.5. Estimation of protein

Protein content was determined by the Biuret method, according to **Gornall *et al., (*1949)**.

2.6. Histopathological studies

Fixed livers were dehydrated by standard procedures, embedded in paraffin, sections approximately 5 µm thick were cut, stained with haematoxylin and eosin (H&E) stains and then examined by light microscope **(Drury, 1980).**

2.7. Statistical analysis

All data were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test to determine significance between different groups. The criterion for statistical significance was set at $p<0.05$.

3. Results and Discussion

3.1. Body weight and relative organ weight

In toxicological studies, body, organ and relative organ weights are important criteria for evaluation of toxicity **(Heikal *et al.,* 2011)**. In the present study, the animals' administrated diazinon showed that the body weights were significantly decreased ($p < 0.05$) and significantly increased ($p < 0.05$) in the relative weights of liver comparing to control animals. When DZ was combined with olive oil, the body weight was higher than the group treated with DZ only, while olive oil alone did not cause any change in body weight, which might be attributed to decreased appetite resulted in decreased food and water intake. Moreover, the treatments could affect lipid and protein degradation as a result of the direct effects of organophosphate compound **(Mansour and Mossa 2010).** The results were confirmed with the findings of **Mansour and Mossa 2011 and Mossa *et al.,* 2012.**

Table 1. Body Weight Pattern of Rats Orally Administrated to Diazinon (36.5 mg/kg bw), Olive Oil (3.3 ml/kg bw) and Their Combination.

Treatments	Zero week	One week	Two weeks	Four Weeks
Control	159 ± 0.54^{b}	175.4 ± 5.94^{b}	189 ± 1.22^{c}	207 ± 2.40^{b}
Olive oil	152 ± 2.12^{a}	172.2 ± 4.08^{b}	189 ± 4.47^{c}	203 ± 3.36^{b}
DZ	150 ± 0.45^{a}	151.8 ± 9.78^{a}	152 ± 6.45^{a}	175 ± 3.53^{a}
DZ + Olive oil	152 ± 2.17^{a}	156.2 ± 6.37^{a}	173 ± 2.38^{b}	198 ± 3.85^{b}

Values are expressed as means (5 rats) ± standard deviation (SD)
Values in column with different letters are significantly different at ($p < 0.05$).

Table 2: Body Weight and Relative Liver Weights of Male Rats after Two Weeks of Last Treatment with Diazinon (36.5 mg/kg bw), olive oil (3.3 ml/kg bw) and Their Combination.

Treatments	Final body weight (g)	Liver weight (g)	Relative liver weight (%)	% Change [a]
Control	207 ± 2.40^{b}	5.56 ± 0.01^{c}	2.69	___
Olive oil	203 ± 3.36^{b}	5.12 ± 0.005^{a}	2.53	5.95
DZ	175 ± 3.53^{a}	5.28 ± 0.002^{b}	3.02	-12.27
Diazinon + olive oil	197.8 ± 3.85^{b}	5.7 ± 0.1^{d}	2.88	-7.1

Values are expressed as means (5 rats) ± standard deviation (SD)
Values in column with different letters are significantly different at ($p < 0.05$).
[a] Percentage of increase (-) or decrease in treatment weights compared to control.

3.2. Hematological studies

Measurement of blood biochemical parameters is used as important diagnostic tool for the detection of abnormalities in the liver and other tissues **(Banaee *et al.,* 2011).** WBCs are important cells in the immune system, because of their main defensive function. The WBCs respond immediately to the change in medium due to toxicant. The effects of diazinon, olive oil and their combination on the peripheral blood cell indicators of male albino rats in Table 3.It was found that after one week of DZ treatment, WBCs counts and Hct value were increased, while PLT count was decreased. Two weeks after DZ treatment, WBCs, HGB and Hct levels were decreased whereas PLT count was increased when compared with control values. Four weeks after dosing rats with DZ, WBCs and Hct levels were decreased, while the count of PLT increased. On the other hand, hen rats were co-adminstered with DZ plus olive oil, the levels of WBCs were restored to be close to control values at all the tested times, while Hct contents were close to control values one

and four weeks. The increase in WBCs may be due to the activation of defense mechanism in treated animals **(Kalender *et al.,* 2006),** while olive oil overcome these toxic effects without decrease in hemoglobin **(Mourad, 2005).** However, non- significant differences were observed in MCV, MCH and MCHC levels. While MCH is a marker for hemoglobin in erythrocytes and the present study showed that, RBC counts and hemoglobin concentration introduced the same non significant changes in DZ -treated rats, so that, no significant change was observed in MCH counts. These results are in concordance with those of **(Kalender *et al.,* 2006; Hariri *et al.,* 2011 and Yassa *et al.,* 2011).** Our results demonstrated that, the administration of olive oil had a curative protective role

Table 3 : Effect of Diazinon, Olive Oil and Their Combination on The Peripheral Blood Cell Indicators of Male Albino Rats.

Parameters	One week				Two Weeks				Four Weeks			
	C	O	D	DO	C	O	D	DO	C	O	D	DO
WBCs (10^3/ ul)	11.08^{a} ±1.70	12.12^{a} ±1.04	14.52^{b} ±1.06	12.34^{a} ±1.39	15.78^{a} ±1.31	18.46^{a} ±4.64	12.52^{a} ±2.74	14.68^{a} ±5.25	12.54^{b} ±1.03	12.40^{b} ±0.51	9.06^{a} ±0.49	12.56^{b} ±0.27
RBCs (10^6/ ul)	5.64^{a} ±0.48	6.24^{a} ±0.77	5.70^{a} ±1.12	6.24^{a} ±0.40	6.64^{a} ±0.92	7.10^{a} ±0.55	6.72^{a} ±0.59	7.18^{a} ±0.38	7.66^{a} ±0.30	7.78^{a} ±0.34	7.52^{a} ±0.38	7.48^{a} ±0.56
HGB (g/dl)	12.56^{a} ±0.15	12.46^{a} ±0.27	12.40^{a} ±0.32	12.98^{b} ±0.15	13.82^{a} ±0.28	12.00^{a} ±1.27	11.98^{a} ±1.07	12.28^{a} ±1.44	14.06^{a} ±1.01	13.28^{a} ±0.40	13.38^{a} ±0.64	12.92^{a} ±0.86
Hct (%)	42.36^{a} ±0.40	46.72^{b} ±4.46	44.00^{ab} ±0.53	43.44^{ab} ±1.16	46.90^{a} ±2.42	43.26^{a} ±2.93	45.36^{a} ±2.79	44.43^{a} ±4.14	46.64^{b} ±2.08	45.54^{b} ±2.05	42.98^{a} ±2.8	44.08^{ab} ±1.46
PLT (10^3/ ul)	1279^{a} ±293.52	1280^{a} ±186.06	1190^{a} ±184.32	1402^{a} ±203.22	744.0^{a} ±33.61	746.8^{a} ±8.16	756.0^{a} ±31.28	800.4^{b} ±17.93	555.0^{a} ±40.02	571.6^{a} ±48.37	631.2^{b} ±56.19	537.8^{a} ±21.02

C: Control, **O**: olive oil (3.3 ml/kg bw), **D**: Diazinon (36.5 mg/kg bw) and their combination
Values are expressed as means (5 rats) ± standard deviation (SD).
Values in row with different letters are significantly different at ($p \leq 0.05$).

Table 4 : Effect of Diazinon, Olive Oil and Their Combination on The Erythrocytes Indices of Male Albino Rats.

Parameters	One week				Two Weeks				Four Weeks			
	C	O	D	DO	C	O	D	DO	C	O	D	DO
MCV	72.72^{a} ±3.35	72.82^{a} ±13.42	74.76^{a} ±11.67	68.34^{a} ±2.46	65.56^{a} ±2.61	64.00^{a} ±3.37	66.72^{a} ±2.06	62.66^{a} ±2.94	60.68^{a} ±1.23	57.26^{a} ±2.08	58.66^{a} ±1.85	57.44^{a} ±4.01
MCH	21.26^{a} ±0.89	20.42^{a} ±1.33	20.78^{a} ±2.27	20.78^{a} ±0.86	18.90^{a} ±0.93	18.22^{a} ±1.22	17.90^{a} ±1.15	17.34^{a} ±1.55	18.46^{a} ±0.54	17.38^{a} ±1.17	17.56^{a} ±1.05	16.98^{a} ±1.29
MCHC	30.30^{a} ±0.57	29.18^{a} ±0.97	29.24^{a} ±1.21	29.82^{a} ±0.58	28.56^{a} ±0.54	27.78^{a} ±1.02	27.26^{a} ±0.89	27.62^{a} ±0.84	30.56^{a} ±0.32	29.90^{a} ±0.70	30.08^{a} ±0.98	29.62^{a} ±0.36

C: Control, **O**:. olive oil (3.3 ml/kg bw), **D**: Diazinon (36.5 mg/kg bw) and their combination
Values are expressed as means (5 rats) ± standard deviation (SD).
Values in row with different letters are significantly different at ($p \leq 0.05$).

3.3. Biochemical studies

Liver is the major site of organophosphorus metabolism so accumulating a great quantity of its metabolites could be possible **(Giray *et al.,* 2001).** Diazinon (DZ) can be highly toxic for animals and human kind **(Sarabia *et al.,* 2009).** The main mechanism of action of DZ is acetyl-cholinesterase enzyme inhibition **(Kamanyire and Karalliedde, 2004).**The results in Table (5) showed significant ($p < 0.05$) decrease of cholinesterase activity in serum of rats treated with DZ compared to control group, while co-administration of olive oil increased significantly ($p < 0.05$) its activity compared to control.

Oxidative stress is a consequence of imbalance between the body antioxidant system and pro-oxidant state generated by pesticide toxicity. However, DZ may induce imbalance in the free radicals production/elimination processes with consequent induction of cellular damage **(Roegge *et al.,* 2008; Cakici and Akat, 2013)**. Endogenous enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and non-enzymatic antioxidants such as glutathione content (GSH) are essential for the conversion of reactive oxygen species to harmless metabolites as well as to protect and restore normal cellular metabolism and functions **(Bebe and Panemangalore, 2003).** GSH is a peptide containing thiol, one of main cellular antioxidant which is synthesized in the liver cytoplasm and then distributed to the other tissues through blood. GSH plays an important role in scavengering ROS and detoxification of drugs and chemicals **(Meister and**

Anderson 1983)and essential for conversion of dehydroascorbic into ascorbate which acts as antioxidant to neutralize superoxide **(Winkler et al., 1994).**The present study showed significant ($p \leq 0.05$) decreases in the GSH content (89%) of liver tissues under the (DZ) toxication condition. Co-administration of olive oil with DZ modulated significantly ($p \leq 0.05$) the level of GSH. The decreased GSH may be due to increased utilization of GSH for conjugation and/or participation of GSH as an antioxidant in neutralizing free radicals **(John *et al.,* 2001).** It was also a reflection of hepatic reduction of GSH synthesis. The decrease in the levels of GSH in these tissues results in the accumulation of free radicals leading to increased rate of lipid peroxidation which damage cell membranes and developed tissue injury **(Sener *et al.,* 2005).**

These results were in line with **(Buyukokuroglu *et al.,* 2008; Anbarkeh *et al.,* 2014 and Refaie *et al.,* 2014)**. MDA is a stable metabolite of the free radical-mediated lipid peroxidation cascade and known as a marker of oxidative stress. The present data showed a statistically significant ($p < 0.05$) increase in MDA levels (59%) in DZ treated group compared with the control group, while alleviated effect (11.5%) of oil in the DZ plus oil group compared with the DZ group was noticed. The same findings were reported by **(Akturk *et al.,* 2006; Boroushaki *et al.,* 2013 and Mossa *et al.,* 2014).**

Table 5. Biochemical Parameters of Male Rats After Two Weeks of Last Treatment Administered Orally With Diazinon (36.5 mg/kg bw), olive oil (3.3 ml/kg bw) and Their Combination.

Treatments	ChE (µ moles of product/ml/min	GSH (mg/g tissue)	MDA (n moles/ml)	SOD (U/mg tissue)	CAT (U/L)
Control	1.56 ± 0.078^{ab}	30.4 ± 0.76^{c}	2.62 ± 0.13^{b}	216.6 ± 4.2^{c}	2112 ± 10.36^{c}
olive oil	1.62 ± 0.068^{b}	34.52 ± 1.20^{d}	2.26 ± 0.20^{a}	221 ± 2.9^{c}	2122 ± 5.94^{c}
DZ	1.43 ± 0.10^{a}	3.3 ± 0.40^{a}	4.17 ± 0.18^{d}	96.9 ± 4.6^{a}	1252 ± 18.36^{a}
DZ + olive oil	1.68 ± 0.13^{b}	19.6 ± 1.37^{b}	2.90 ± 0.17^{c}	207 ± 5.7^{b}	1881 ± 11.7^{b}

Values are expressed as means (5 rats) ± standard deviation (SD). Values in column with different letters are significantly different at ($p < 0.05$).

The cell has several ways to alleviate the effects of oxidative stress, either by repairing the damage or directly by diminishing the occurrence of oxidative damage by means of enzymatic and non-enzymatic antioxidants. These antioxidants have also been shown to scavenge free radicals and ROS; for instance SOD catalyzes the conversion of superoxide radical to hydrogen peroxide while CAT converts hydrogen peroxide to water. These antioxidant enzymes can, therefore, alleviate the toxic effects of ROS **(Gultekin *et al.,* 2001; Altuntas *et al.,* 2002).** The data showed significant ($p < 0.05$) decrease in the activities of both SOD and CAT, while co-administration of olive oil modulated significantly their activities. There have been several studies of the per-oxidative effects of different OPs on antioxidant enzymes **(Ahmed *et al.,* 2000 and John *et al.,* 2001).** As shown in the present study, MDA level has been significantly increased with lower GSH content. Meanwhile, a significant decrease was recorded in SOD and CAT activities which reflect that impaired oxidant/antioxidant balance which can be partially responsible for the toxic effects of diazinon . These results are correlated with those obtained by **Altuntas *et al.,* 2004; Gokalp *et al*., 2005; Mansour *et al.,* 2009 and Alp *et al.* 2012.** So the need for antioxidants, which are scavengers that detoxify excessive ROS and have an important role in maintaining oxidant/antioxidant balance in the body, was increased **(Agarwal *et al.,* 2012)**. Virgin olive oil appears to be a functional food with various components such as monounsaturated fatty acids that may have nutritional benefits. It is also a good source of phytochemicals, including polyphenolic compounds **(Stark *et al*., 2002; Lavelli, 2002).**

In recent years, scientists have focused on the preventive effects of phenols against degenerative diseases mediated by the ROS. It has been reported that the phenolic compounds are able to interact with the biological systems and as bioactive molecules. The antioxidant activities of olive oil are related to its phenolic compounds content which showed strong antioxidant properties.

3.4. Histopathological studies

The liver is divided into hepatic lobules formed of radially arranged strands of hepatocytes that extend from the central vein to periphery of the lobule. The hepatocytes strands are separated from each other by blood sinusoids that are lined with the endothelial cells and Kupffer cells. Figure 1(A & B) showed histological structure of liver in control and olive oil treated rats with normal architecture. Treating animals with DZ(C) caused several histopathological alterations, such as architecture destruction and fibrous expansion of the portal triad with fibrous septae formation (Fs). The sinusoidal spaces (S) were dilated with increase kupffer cells (D), focal inflammation and infiltration in hepatic parenchyma (E), and vacuolar degeneration (VD) were also noticed (F).

When the oil co-administered with DZ the liver tissue preserved its nearly normal hepatic lobular architecture with central veins and radiating hepatic cords. These results reconfirmed the protective functions of olive oil against DZ induced liver damage (G) and as confirmed

by **Sarhan and Al-Sahhaf, 2011; Abd El-Fattah and Brakat, 2013.**

Conclusion

The results of the present study showed that olive oil has protective effects against oxidative stress and liver damage induced by diazinon and prevents excessive lipid peroxidation and maintains enzymatic and non-enzymatic antioxidant near the normal levels.

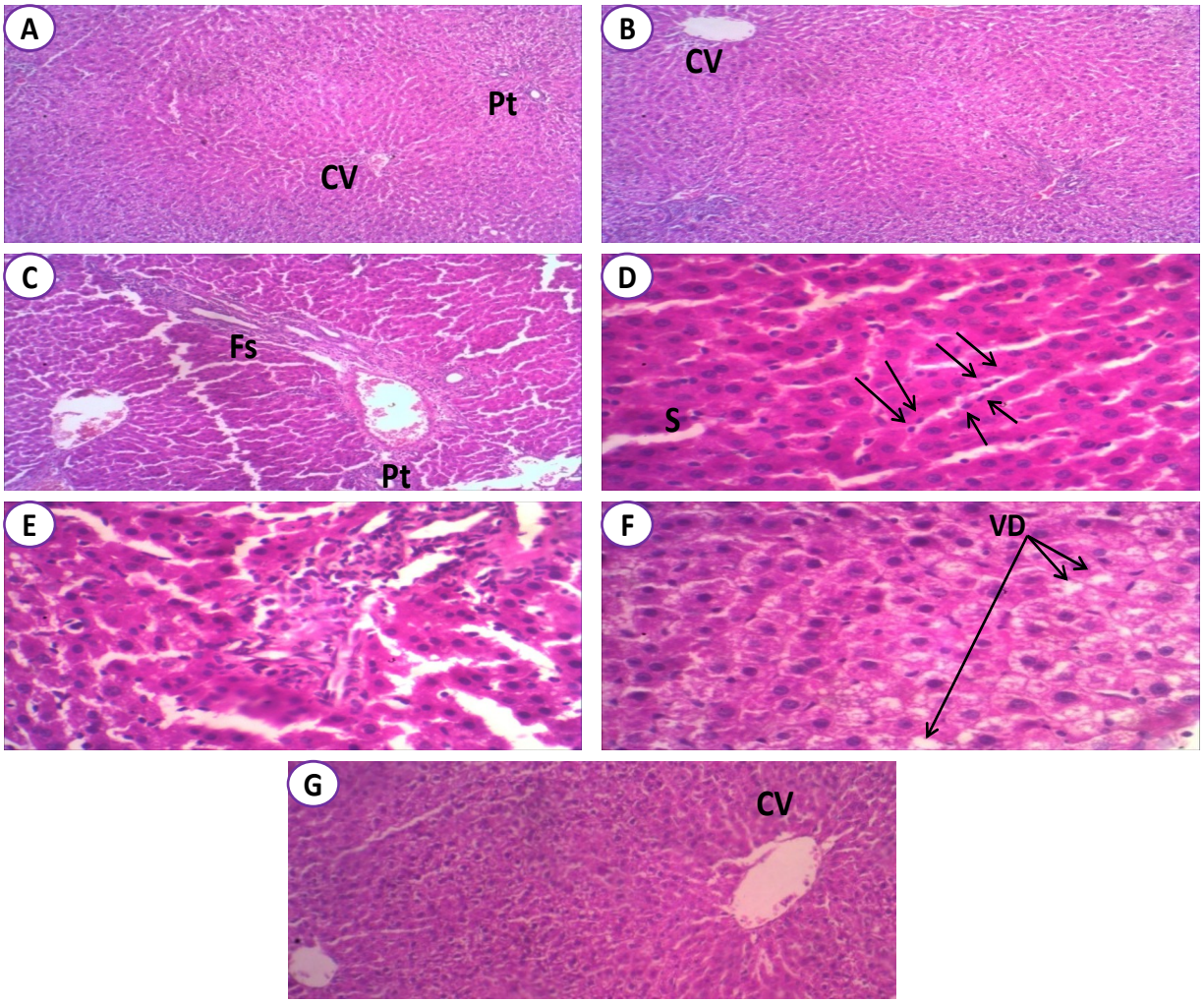

Figure 1. (A): Liver section of control showed central vein (CV) and portal triad (Pt) with normal architecture (H&E stain X100).

(B): Liver section of virgin olive oil - treated rats showed pathological free hepatic central vein with normal architecture (H&E stain x 100).

(C): Liver section of rat treated with 36.5 mg diazinon / Kg bw showed architecture destruction and fibrous expansion of the portal triad with fibrous septae formation (Fs) (H&E stain x 100).

(D): Liver section of rat treated with 36.5 mg diazinon / Kg bw showed sinusoids dilation (s) with increase kupffer cells (arrows) (H&E stain x 100).

(E): Liver section of rat treated with 36.5 mg diazinon / Kg bw showed Focal inflammation and infiltration in hepatic parenchyma (H&E stain x 400)

(F): Liver section of rat treated with 36.5 mg diazinon / Kg bw showed vacuolar degeneration (VD) (H&E stain x 400).

(G): Liver section of rats treated with diazinon + virgin olive oil showed no histopathological changes denoting recovery (H&E stain x 100).

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الإجهاد التأكسدي الناجم عن الديازينون والتأثير التخفيفى لزيت الزيتون البكر في ذكور الجرذان البيضاء

نادية على حامد و رضا خميس عبدالرازق

قسم بحوث سمية المبيدات للثديات- المعمل المركزى للمبيدات – مركز البحوث الزراعية - الاسكندرية

الملخص العربي:

تستخدم المبيدات على نطاق واسع من أجل حماية المواد الغذائية من خلال مكافحة الحشرات الضارة وناقلات الأمراض في الزراعة. يتم استخدام ديازينون (DZ)، وهو مبيد حشرى من مبيدات الفوسفات العضوية ، على نطاق واسع لمكافحة الحشرات وفي الطب البيطري للسيطرة على الطفيليات الخارجية. وقد أجريت هذه الدراسة لبحث تأثير جرعات تحت المميتة المتكررة (٣٦,٥ مجم / كجم من وزن الجسم / يوم لمدة أسبوعين) من DZ على بعض القياسات الدموية، القياسات البيوكيميائية والتغيرات التشريحية المرضية في أنسجة الكبد من ذكور الجرذان البيضاء و تقدير التأثير الوقائي المحتمل من زيت الزيتون ضد التأثير السلبي الذى يسببه DZ. المعاملة عن طريق الفم ٣ جرعات / أسبوع على مدى أسبوعين، ثم أسبوعين دون معامله بمثابة فترة ملاحظه.

وقد قسمت الجرذان الى اربع مجموعات : المجموعة الأولى اعطيت الماء و هى - (المجموعة الضابطة) - المجموعة الثانية: أعطيت الجرذان زيت الزيتون البكر ٣,٣مل/ كجم من وزن الجسم - المجموعة الثالثة: أعطيت الجرذان ٣٦,٥ DZمليجم / كجم من وزن الجسم و هذه الجرعة تمثل ١٠/١ من قيمة الـ LD50 (قيمة الـ LD50= ٣٦٥ مليجم / كجم من وزن الجسم) - المجموعة الرابعة: أعطيت الجرذان DZ ٣٦,٥مليجم / كجم من وزن الجسم بالإضافة الى زيت الزيتون ٣,٣مل / كجم من وزن الجسم. مبيد DZوزيت الزيتون يعامل بهما عن طريق الفم بالسرنجة الموصلة للمريء.

أوضحت نتائج الدراسة أن مبيد الديازينون أحدث التأثيرات التالية-:

- نقص وزن الحيوانات المعاملة و زيادة وزن الكبد النسبى.
- زيادة معنوية فى كل من عدد كرات الدم البيضاء، مستوى الهيماتوكريت .بينما حدث انخفاض فى عدد صفائح الدم فى الاسبوع الاول بعد المعاملة.تبع ذلك انخفاض معنوى فى عدد كرات الدم البيضاء، تركيز الهيموجلوبين، مستوى الهيماتوكريت بينما حدث زيادة فى عدد صفائح الدم وذلك فى الاسبوع الثانى بعد المعاملة.فى نهاية مدة التجربة انخفض عدد كرات الدم البيضاء و مستوى الهيماتوكريت، بينما زاد عدد صفائح الدم لم يحدث تغيير معنوى فى ثوابت الدم .
- انخفاض فى مستوى الجلوتاثيون المختزل وكذلك نشاط انزيمى السوبر اوكسيد ديسميوتيز والكتاليز بينما حدثت زيادة ملحوظة فى مستوى أكسدة الدهون وأيضا تأثيرات معنوية فى نشاط انزيم الأسيتيل كولين استيراز مقارنة بالمجموعة الضابطة
- وتم تأكيد التأثيرات السابقة للمبيد على الحيوانات المعاملة بالتغيرات التشريحية فى الكبد للحيوانات المعاملة بالمبيد.

وايضا أوضحت الدراسة أن زيت الزيتون بالجرعة المستخدمة تمكن من تقليل الضرر الناتج من المبيد على الحيوانات المعاملة مما يعكس الدور الفعال له ضد السمية الكبدية الناجمة عن الديازينون.