ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Antioxidant role of olive "Olea europea$_{var}$ picual"leaves extract on profenofos induced oxidative stress in male rats

Samia Mahmoud Khalil, Khaled MaamounTaha, Hoda El-Sayed Farid , Ahmed Fouad Atia*

Biochemistry Dept., Faculty of Agriculture, Menofia University, Egypt

Manuscript Info

Manuscript History:

Received: 12 May 2014
Final Accepted: 23 June 2014
Published Online: July 2014

Key words:
Oxidative stress, Organophosphorus pesticides, olive, Profenofos

Corresponding Author

Ahmed Fouad Atia

Abstract

Evaluation of the antioxidant role of olive leaves extract (OLE)against oxidative stress induced by profenofos (organophosphorus pesticide) in male albino rats was investigated.Olive leaves were extracted with four different solvents (ethanol,hexane,acetone and water) and it was found that the acetonic extract has both the highest content of phenolic compounds and reducing power.Gc/MS analysis of this extract showed presence of seventeen phenolic compounds.The compound present in highest amount was oleuropien. Sixty six adult male albino rats were used for the toxicological experiment (16 for determination of the oral median lethal dose LD_{50}) and the other 50 were divided into 5 groups. They were orally treated as follow: control, olive 400 mg/kg b.wt, profenofos 62.75 mg/kg b.wt (1/8 LD_{50}) , olive 200 mg/kg b.wt + profenofos 62.75 mg/kg b.wt, olive 400 mg/kg b.wt + profenofos 62.75 mg/kg b.wt. Treatments were expanded for 28 days. Blood samples and brain tissue were taken after 14 and 28 days of treatment for biochemical analysis. Also liver tissue was obtained after 28 days of treatment for histopathological examinations.The results revealed that, the oral median leathal dose (LD_{50}) value to male rats was found to be 502 mg/kg b.wt. Brain acetylcholinesterase (AChE) activity was inhibited by profenofos treatment. However, an elevation in oxidative stress biomarker malondialdehyde (MDA) level was recorded with reduction in total reduced glutathione (GSH) concentration and increase in the activities of glutathione peroxidase (GPx) and catalase (CAT) in profenofos treated group. Elevation in the activities of liver function biomarkers alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT),alkaline phosphatase (ALP) and reduction in albumin level were recorded in profenofos treated group. As for lipid profile, there was a reduction in total cholesterol, total lipids and triglycerides concentrations in profenofos treated group. Histopathological examination revealed sinusoidal leukocytosis in liver of profenofos group.Supplementation with olive leaves extract improved the detrimental effects of profenofos in rats and olive leaves extract at a dose of 400 mg/kg was highly effective than 200 mg/kg body weight.

Copy Right, IJAR, 2014,. All rights reserved.

1.Introduction

Organophosphorus pesticides are widely used on vegetables and fruits for the control of insect pests because of their fast action and prolonged protection. These chemicals are generally short persistence and do not accumulate in the animal tissue and environment. The rapid development agriculture in Egypt in the last few years required increased in uses of agriculture chemical and pesticides.

Organophosphorus insecticide namely profenofos is widely used in Egypt to control economic insects especially on vegetables **(Ministry of Agriculture, 2001).**

Profenofos is an organophosphorus insecticide, shows its effect directly by inhibiting the acetylcholinesterase enzyme in blood and nervous system, resulting in the accumulation of acetylcholine (a

major transmitter) and activation of muscarinic and nicotinic receptors, which may lead to death. **(Savolainen,2001 ; Sharma *et al.,* 2005 and Aygun *et al*.,2007).**

Organophosphate insecticides OPIs are known to cause an inhibition to acetylcholinesterase (AChE) activity in the target tissues causing an accumulates ions in acetylcholine and so preventing the smooth transmission of nerve functions (**Jeyaratnam and Maroni 1994).** It is reported that OPIs, besides their inhibitory effect on AChE, also induce changes characteristic of oxidative stress (**Malkovics 1995).** When the free radical production overwhelms the endogenous antioxidant levels, they cause considerable cell damage/death. All the major biomolecules like lipids, proteins, and nucleic acids may be attacked by free radicals, among them lipids are probably the most susceptible one(**Cheeseman and Slater 1992).**The oxidative destruction of lipids (lipid peroxidation) is a destructive, self-perpetuating chain reaction, releasing malonyl aldehyde (MDA) as the end product (**Cheeseman 1993).**

The olive tree (Olea europaea L. [Family: Oleaceae]) has been cultivated in the Mediterranean for more than a thousand years. Not only the olive oil, but also the leaves have been used for medical purposes **(Cherif *et al.,* 1996).** Olive leaf contains the active iridoid constituent oleuropein(chief constituent). Other secoiridoids include11-dimethyloleuropein, 7,11-dimethyl ester of oleoside, ligustroside, oleuroside, and un-conjugated secoiridoid aldehydes. Triterpenes and flavonoids, including luteolin, apigenin, rutin, and diosmetin, are also present. Oleasterol, leine, and glycoside oleoside have been also found in the leaves **(Briante *et al.,* 2002).**

It is well known that oleuropein and its derivatives such as hydroxytyrosol and tyrosol are the main phenolic constituents of olive leaves, which is thought to be responsible for their pharmacological effects. Furthermore, olive leaves contain caffeic acid, p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside **(Bianco and Uccella, 2000; El and Karakaya, 2009).** Like many natural herbs, olive leaves are also known to be an antioxidant and contain some of the most powerful reducing compounds known as antioxidants **(Benavente-Garcia *et al.,* 2002; Lee *et al.,* 2009; Lee and Lee, 2010).**

Since the leaves of olive have been recommended in the literature as a big source of antioxidant agents, we decided to investigate the antioxidant role of olive leaves extract on profenofos induced oxidative stress in male rats.

2. Materials and methods

2.1. Tested material

Profenofos 0-(4-bromo-2-chlorophenyl O-ethyl S-propyl Phosphorthioate) was purchased from Kaffer Elzayat Co. for Insecticide Ind. Kaffr Elzayat, Egypt.

Olive leaves **(*Olea europaea$_{var}$ picual.)*** were collected from El Shaikh Zewaid, Arish, North Sinai Gov., Egypt in September 2012. Leaves were dried and powdered for the extraction.

2.2. Extraction of plant material

Olive leaves were dried and powdered using grinder, then, olive leaves were extracted with water , hexane , acetone or ethanol at ambient temperature (5 gm /50 ml from each solvent). The powder for each extract was extracted three times on each occasion with the mentioned solvents. The extracts were analyzed for its free phenolic compounds and reducing power.

2.3. Determination of free phenolic compounds

The concentration of free phenolic compounds in each extract was determined colorimetrically by the method of Folin-Ciocalteu's as described by **(Gulcin *et al*., 2002)**.

2.4. Determination of Reducing Power

A spectrophotometric method of **(Oyaizu 1986)** was used for the measurement of reducing power.

2.5. Identification and scanning of phenolic compounds in acetonic extract of olive leaves

2.5.1. Instruments

Gas chromatography-mass spectrometry was carried out on a Fisons GC 8000 gas chromatograph coupled to a Fisons MD 800 mass detector under electron impact ionization (70 eV). The interface temperature 230°C, and the MS scan range was 35-450 atomic mass units (AMU). The chromatographic column for the analysis was fused silica OV1 capillary column (25 m X 0.25 mm i.d.). The carrier gas used was helium at a flow rate of 10 ml/min. Two samples were analyzed with the column held initially at 60 °C for 2 min and then increased to 170 °C with a 2 °C / min heating ramp and then kept at 170 °C for 3 min. Finally, temperature was increased to 250 °C with a 3 °C /min heating ramp and the temperature was kept at 250 °C for 120 min. for both samples. The injection was performed in split mode at 220 °C.

2.5.2. Identification of compounds

Peaks were identified by computer searches in commercial reference libraries. Good spectral matches for some compounds could be found in the Wiley and National Bureau of Standards (NBS).

2.6. Preparation of acetonic olive leaves extract

Leaves were dried and powdered for the extraction by acetone. The powder was extracted three times, on each occasion with acetone. The collective acetone extract was filtered. After filtration process the crude extract was completely dried under reduced pressure in a rotary evaporator and the resulting extract was freeze-dried.

2.7. Animals

Sixty six male albino rats (150-160g) were supplied by the Egyptian Organization of Biological Products and Vaccine. The animals were housed in plastic cages, fed *ad libitum* and allowed to adjust to the new environment for 2 weeks before starting the experiment. The rats were housed at $23 \pm 2\,^{\circ}$C dark/light cycle. All animals were treated according to the standard procedures laid down by **OECD guidelines (1996)** and **EPA protocol (1998)**.

2.8. Experimental design

Rats were divided into two experiments:

2.8.1. Acute oral toxicity

Acute oral toxicity of profenofos was performed to determine the acute oral median lethal dose (LD_{50}).The sixteen male rats were divided into 4 groups of 4 rats for each group.Each group was intubated orally by gavage using stomach tube with different doses of experimental insecticide.Then the treated male rats were kept under observation for 24 hours and symptoms of toxicity and mortality were recorded.The acute oral LD_{50} value was calculated according to the method of **Weil (1952).**

2.8.2. Protective effect of olive leaves extract against sub acute toxicity of tested compound

Fifty males were divided into five groups each containing10 rats and the groups were treated as follow:

Group 1: Rats were kept without any treatments as control.

Group 2: Rats were treated with olive leaves extract at the dosage levels of 400 mg/kg b.wt.

Group 3: Rats were treated with profenofos at the dosage levels of 62.75 mg/kg b. wt. (1/8 LD_{50}).

Group 4: Rats were treated with olive leaves extract at the dosage levels of 200 mg/kg b.wt and profenofos at the dosage levels of 62.75 mg/kg b.wt simultaneously.

Group 5: Rats were treated with olive leaves extract at the dosage levels of 400 mg/kg b.wt and profenofos at the dosage levels of 62.75 mg/kg b.wt simultaneously.

Rats were treated with profenofos and olive leaves extract orally for 28 days (6days/week) and weighed weekly throughout the experiment.

Blood samples were taken at 14 , 28 days of the experimental period for biochemical analysis. On 14th day of the experimental period, 5 rats from each group were sacrificed and dissected for determination of acetylcholinesterase activity in the brain. At the end of the experiment, rats were decapitated and liver was taken to investigate the histopathological changes, Also brain was obtained for determination of acetylcholinesterase activity.

2.9. Sampling

The blood samples of the male albino rats were collected from orbital sinus veins technique (**Schalm 1986)** into heparinized tubes, centrifuged at3600 rpm for 15 min. Plasma were separated, divided into aliquots and kept in a deep freezer at -20 °C till all assays were carried out.

2.10. Preparation of brain homogenate

Brain tissue was homogenized in ratio (1:10 w/v) in pre-cold sodium phosphate buffer (0.2 M) pH 8.0 using potter-El-vehjem glass homogenizer with a Teflon piston.The homogenate was centrifuged at 5000 g for 10 min. at 4 °C.The supernatant was considered to be the enzyme source.

2.11. Biochemical analysis

2.11.1. Oxidative stress parameters

Lipid peroxidation (LPO)in plasma was monitored by determining the concentration of malondialdehyde (MDA) as described by **Ohkawa *et al.* (1979),** Catalase (CAT) activity was determined in plasma as described by **Aebi (1984),** Kinetic determination of glutathione peroxidase (GPx) activity in blood was done according to the method of **Paglia and Valentine (1967),** while reduced glutathione (GSH) concentration was determined in blood according to **Beutler *et al.* (1963).**

2.11.2. Determination of brain acetylcholinesterase (AChE) activity

Brain acetylcholinesterase (AChE) activity was assayed by the method of **Ellman *et al*. (1961).**

2.11.3. Liver Function tests

Determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in plasma were carried out according to the method adopted by **Reitman and Frankel (1957).** Alkaline phosphatase (ALP) activity was determined in plasma according to **Belfield and Goldberg (1971).** Kinetic determination of gamma glutamyltransferase (GGT) activity in plasma was done according to the method of **Szasz (1969).** Albumin concentration was determined in plasma according to **Doumas et al. (1971).**

2.11.4. Lipid profile biomarkers

Total lipids concentration was determined in plasma by the method adopted by **Zollner and Kirsch (1962).** Total cholesterol concentration was determined in plasma by the method described by **Allain *et al. (1974).*** Triglycerides concentration was determined in plasma by the method performed by **Fossati and Prencipe (1982)**. High density lipoprotein – cholesterol concentration was determined in plasma by the method mentioned by **Lopes – Virella et al. (1977).** Calculation of the low density lipoprotein – cholesterol concentration in plasma was done according to the equation of **Friedewald *et al.* (1972)** where:

$$\text{LDL - cholesterol} = \text{total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL - cholesterol}$$

2.12. Histopathological examination

Tissue specimens from liver was collected from all experimental groups at the end of experiment and fixed in 10% neutral buffered formalin, dehydrated in ascending concentration of ethanol and cleared in xylene. The fixed tissue were embedded in paraffin wax and sectioned into 4-5 µm thick, then stained with hematoxylin and eosin (H&E) method **(Bancroft et al.,1996)** . Then the sections were examined under light microscopy at 400 X magnification (DP72, Olympus).

2.13. Statistical analysis

Statistical analysis was done using analysis of variance (ANOVA), Least Significant Difference (LSD) were obtained to compare the means of treatments, using Costat version 6.311 (Copyright 1998-2005, CoHort software. Duncan's multiple range test **(Duncan 1955)** was used to compare between the treatments means. The mean values within each column followed by the same letters are not significantly different at 5% level of probability.

3. Results

In olive leaves extracts, free phenolic content (gm gallic acid /100 g dry matter), as affected by the extracting solvents, were ranged from high to low as follow: acetone extract > ethanol extract > water extract > hexane extract. (Fig.1)

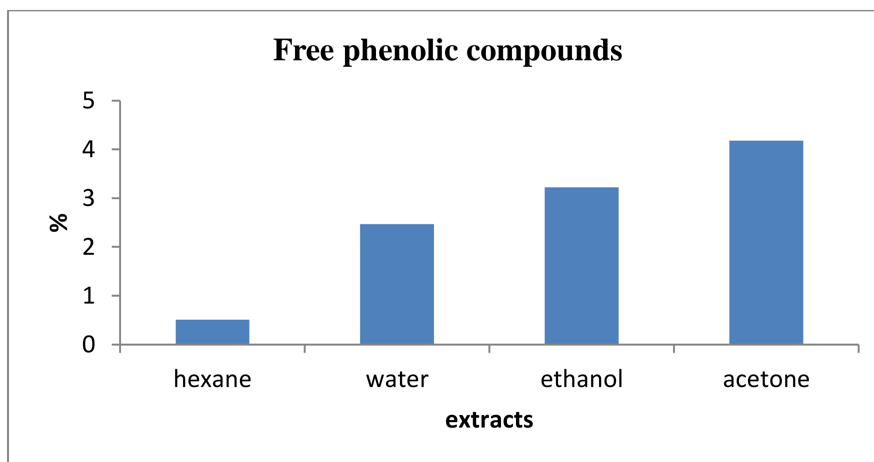


Fig.(1) Concentration of free phenolic compounds as gm gallic acid /100 g dry matter

The results show that the reducing power as affected by the extracting solvents, were ranged from high to low as follow: acetone extract > ethanol extract > water extract > hexane extract against vitamin C.(Fig 2).

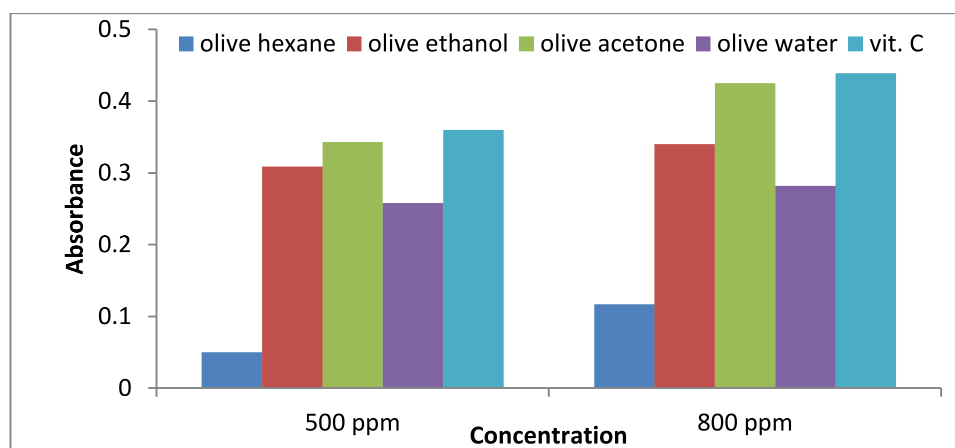


Fig . (2) Reducing power of olive leaf extracts (500 and 800 ppm) against Vit. C.

The GC/MS analysis of olive leaf acetonic extract allowed the identification of seventeen phenolic compounds Table (1): oleuropein, hydroxytyrosol, Quercetin 3-D-galactoside, 4-Ethylcatechol, Tyrosol, 4-Methylcatechol, 4-Methoxymethylphenol, 3,4-Dihydroxyphenylacetic acid, 2-methoxy-3-methyl-phenol, 2-methyl-5-nitro-phenol, oleuropien aglycon isomer1, Genistein, demethyloleuropien, p-Creosol and 5-Sec-butylpyrogallol.

Table (1) The content of phenolic compounds of acetonic extract of olive leaves

Compound	Retention time (min.)	%
oleuropien	4.76	27.99
Hydroxytyrosol	4.83	15.02
Quercetin 3-D-galactoside	5.6	14.00
4-Ethylcatechol	5.94	0.05
Tyrosol	7.61	1.40
4-Methylcatechol	9.36	1.28
4-Methoxymethylphenol	10.91	12.33
3,4-Dihydroxyphenylacetic acid	12.7	0.04
2-methoxy-3-methyl- Phenol	13.05	1.06
unknown	13.53	0.03
2-methyl-5-nitro- Phenol	14.4	9.84
Genistein	15.07	14.07
demethyloleuropien	15.17	0.19
p-Creosol	15.46	1.87
oleuropien aglycon isomer1	15.83	0.18
5-Sec-butylpyrogallol	16.31	0.28
unknown	17.93	0.38

The oral median lethal dose (LD_{50}) value of profenofos to male rats was calculated with **weil (1952)** and found to be 502 mg/kg b.wt.

Concerning activity of brain acetylcholinesterase enzyme, an inhibition in AChE activity in brain was observed in profenofos treated group compared to control after 14 and 28 days of treatment while supplementation with olive leaves extract at a dose of 400 mg/kg b.wt to profenofos group resulted a significant increase in the brain AChE activity compared to profenofos treated group as shown in Table (2).

Table (2) Effect of olive leaves extract supplementation on acetylcholinesterase activity (U/g.) in brain of rats intoxicated with profenofos

Group \ Period	14 days	28 days
C	187.67[a]±7.1	170.95[a]±6.1
O 2	188.98[a]±6.7	159.59[a]±6.5
P	58.92[c]±0.2	58.65 [c]±0.2
O 1 + P	66.75[c]±5.7	58.59[c]±0.2
O 2 + P	105.01[b]±7.5	117.3[b]±0.3

C = control , P = profenofos 1/8 LD_{50} , O 1 = olive leaves extract 200 mg/kg b.wt , O 2 = olive leaves extract 400 mg/kg b.wt
Values represent means ± S.E obtained from 5 animals.
Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$).

The results shown in Table (3) revealed that, the levels of plasma MDA and the activities of plasma CAT and GPx in blood increased significantly while the concentration of GSH in blood significantly decreased in the profenofos treated group after 14 and 28 days of treatment as compared with control. An improvement was recorded when olive leaves extracts (200 and 400 mg/kg b.wt) supplemented to the profenofos group.

Regarding, the liver function biomarkers, No significant changes were noted in the activities of plasma AST, ALT, ALP and GGT and the concentration of plasma albumin in all cases of treatments in comparing with the control group after 14 days of treatment. But, after 28 days of treatment with profenofos caused a significant increase in the activities of the mentioned enzymes, Also a significant decrease in plasma albumin concentration was recorded compared with control. However, supplementation with olive leaves extract at a dose of 400 mg/kg b.wt to profenofos group attenuated the toxic effect of profenofos as shown in Table (4).

Results pointed out in Table (5) show that, all treatments did not alter significantly plasma HDL-cholesterol, LDL-cholesterol,total cholesterol,triglycerides and total lipids concentrations after 14 days of treatment compared to the untreated group. As well as, there was no significant changes in plasma HDL-cholesterol and LDL-cholesterol in any treatment comparing with control after 28 days of treatment.Meanwhile, the concentration of plasma total cholesterol,total lipids and triglycerides were significantly decreased at profenofos treatment compared to the control group after 28 days of treatment.

Administration of olive leaves extract at a dose of 400 mg/kg b.wt to profenofos treated group significantly restored the concentrations of plasma total cholesterol, total lipids and triglycerides near to control.

Histological examination of rats' liver treated with profenofos and olive leaves extract which presented in Fig.(3) revealed that rats treated with olive leaves extract at a dose of 400 mg/kg b.wt showed vacuolar degeneration of some hepatocytes, rats treated with profenofos 1/8 LD_{50} showed sinusoidal leukocytosis, rats treated with olive leaves extract at a dose of 200 mg/kg b.wt and profenofos 1/8 LD_{50} showed kupffer cells activation, while rats treated with olive leaves extract at a dose of 400 mg/kg b.wt and profenofos 1/8 LD_{50} showed no histopathological changes in comparison with control.

Table (3) Effect of olive leaves extract supplementation on oxidative stress parameters in blood and plasma of rats intoxicated with profenofos

Period (day)	14				28			
Parameters / Treatments	MDA (nmol/ml)	CAT (U/ml)	GSH (mg/dl)	GPx (mU/ml)	MDA (nmol/ml)	CAT (U/ml)	GSH (mg/dl)	GPx (mU/ml)
C	21.63[d] ±0.47	0.27[c] ±0.009	22.27[a]±1.3	5645.28[c]±37.1	18.85[c] ±0.49	0.28[c] ±0.004	22.5[a]±1.0	5563.65[d]±47.6
O 2	35.12[a] ±0.7	0.48[a] ±0.015	18.39[b]±1.0	6084.84[ab]±100.7	27.94[a] ±1.12	0.44[a] ±0.012	17.42[b]±0.8	6030.54[ab]±106.5
P	31.77[b] ±1.13	0.45 [a]±0.009	12.5[c]±0.7	6359.06[a]±104.6	29.35[a] ±0.39	0.44[a] ±0.016	11.83[c]±0.4	6302.89[a]±131.9 3
O 1 + P	27.05[c] ±1.08	0.45[a] ±0.014	13.78[c]±0.7	6023.98[b]±106.1	24.18[b] ±2.21	0.35[b] ±0.011	13.18[c]±0.6	5952.72[bc]±99.19
O 2 + P	26.5[c] ±0.6	0.35[b] ±0.017	18.55[b]±0.9	5678.5[c]±104.2	19.15[c] ±0.79	0.34[b] ±0.015	18.29[b]±1.2	5680.38[cd]±72.78

C = control , P = profenofos 1/8 LD_{50} , O 1 = olive leaves extract 200 mg/kg b.wt , O 2 = olive leaves extract 400 mg/kg b.wt
Values represent means ± S.E obtained from 5 animals.
Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$).

Table (4) Effect of olive leaves extract supplementation on parameters of liver functions in plasma of rats intoxicated with profenofos

Period (day)	14					28				
Parameters / Treatments	AST (U/ml)	ALT (U/ml)	ALP (IU/L)	ALBUMIN (g/dL)	GGT (U/L)	AST (U/ml)	ALT (U/ml)	ALP (IU/L)	ALBUMIN (g/dL)	GGT (U/L)
C	98.5^{a} ± 1.13	50.46^{a} ± 1.66	72.45^{a} ± 1.03	2.04^{a} ± 0.11	6.18^{a} ± 0.36	94.34^{c} ± 1.43	48.5^{c} ± 1.45	84.17^{c} ± 1.07	2.19^{a} ± 0.09	4.45^{b} ± 0.39
O 2	100.63^{a} ± 0.94	51.93^{a} ± 0.95	76.15^{a} ± 1.66	1.96^{a} ± 0.09	6.96^{a} ± 0.39	99.11^{b}± 1.26	52.71^{ab} ± 0.91	100.31^{a} ± 1.26	1.87^{bc} ± 0.06	5.28^{ab} ± 0.41
P	100.93^{a} ±0.83	54.57^{a} ± 1.61	76.17^{a} ± 1.14	1.86^{a} ±0.08	7.11^{a} ±0.42	103.64^{a}± 1.31	54.64^{a} ± 1.04	103.18^{a} ± 1.21	1.69^{c} ±0.13	5.93^{a} ± 0.53^{a}
O 1 + P	99.78^{a} ±1.01	51.56^{a} ± 1.70	74.91^{a} ± 1.28	1.95^{a} ± 0.03	6.92^{a} ±0.44	98.8^{b} ± 0.59	52.62^{ab} ± 1.78	99.58^{a} ± 1.10	1.85^{c} ±0.14	4.87^{ab} ±0.32
O 2 + P	99.04^{a} ±0.67	51.4^{a} ± 1.40	74.42^{a} ± 1.57	1.97^{a} ± 0.03	6.49^{a} ±0.27	96.79^{bc} ±0.56	50.81^{bc} ± 1.13	88.87^{b} ± 0.70	2.17^{ab} ±0.08	4.74^{b} ±0.30

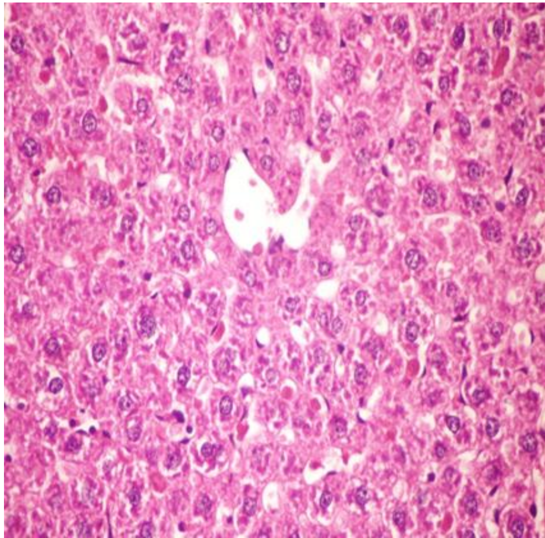
C = control , P = profenofos 1/8 LD_{50} , O 1 = olive leaves extract 200 mg/kg b.wt , O 2 = olive leaves extract 400 mg/kg b.wt
Values represent means ± S.E obtained from 5 animals.
Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$).

Table (5) Effect of olive leaves extract supplementation on parameters of lipid profile in plasma of rats intoxicated with profenofos

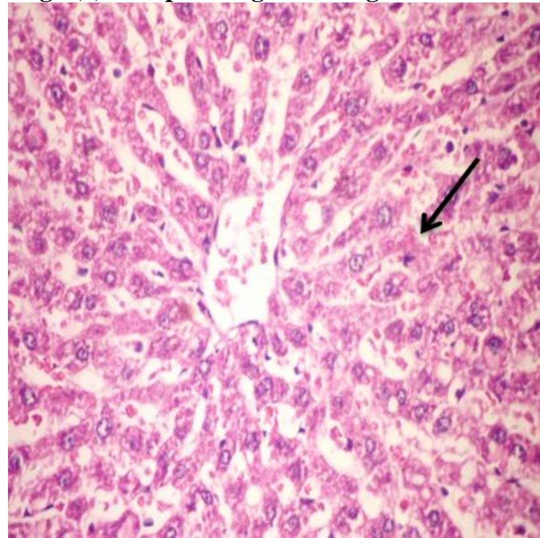
Period (day)	14					28				
Parameters / Treatments	HDL-cholestrol mg/dl	LDL-cholestrol mg/dl	Total Cholesterol mg/dl	Total lipids mg/dl	Triglycerides mg/dl	HDL-cholestrol mg/dl	LDL-cholestrol mg/dl	Total Cholesterol mg/dl	Total lipids mg/dl	Triglycerides mg/dl
C	33.95 [a] ± 1.15	32.59 [a] ± 1.26	82.7 [a] ± o.72	424.66 [a] ± 3.99	80.72 [a] ± 0.30	33.92 [a] ± 0.85	22.77 [a] ± 3.04	75.73[a] ± 1.00	411.08 [a]± 0.92	95.14 [a]± 0.65
O 2	32.01 [a] ± 1.08	32.53 [a] ± 1.48	80.34 [a] ± 0.98	422.61 [a] ± 5.42	79 [a] ± 0.79	31.8 [a] ± 1.16	22.32 [a] ± 1.37	70.31 [c] ± 0.76	405.79[bc] ± 1.81	80.9 [bc] ± 0.49
P	30.98 [a] ± 1.51	33.1 [a] ± 1.58	79.89 [a] ± 0.55	420.55 [a] ± 2.58	79.02 [a] ± 0.78	31.39 [a] ± 1.36	23.43 [a] ± 3.1	69.07[c] ± 1.02	398.85[d] ± 1.12	78.03 [c]± 0.47
O 1 + P	31.97 [a] ± 0.88	32.08 [a] ± 0.90	79.95 [a] ± 0.43	422.04 [a] ± 1.53	79.45 [a] ± 1.22	32.28 [a] ± 0.72	23.26 [a] ± 2.59	70.83[bc] ± 0.91	404.15 [c] ± 1.40	81.98 [bc] ± 0.56
O 2 + P	33.78 [a] ± 0.57	31.97 [a] ± 0.69	81.76 [a] ± 0.91	423.74 [a] ± 2.3	79.98 [a] ± 0.41	33.84 [a] ± 0.89	21 [a] ± 2.75	73.92[ab] ± 1.53	408.89[ab] ± 0.67	89.34 [a]± 0.47

C = control , P = profenofos 1/8 LD_{50} , O 1 = olive leaves extract 200 mg/kg b.wt , O 2 = olive leaves extract 400 mg/kg b.wt
Values represent means ± S.E obtained from 5 animals.
Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$).

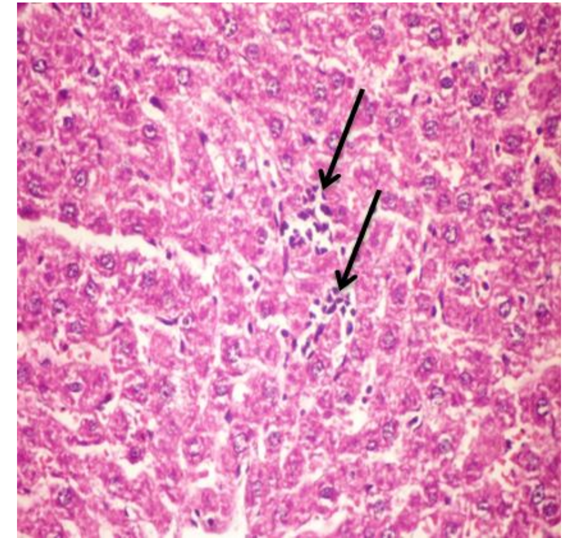
Fig . (3)Histopathological changes of rats' liver



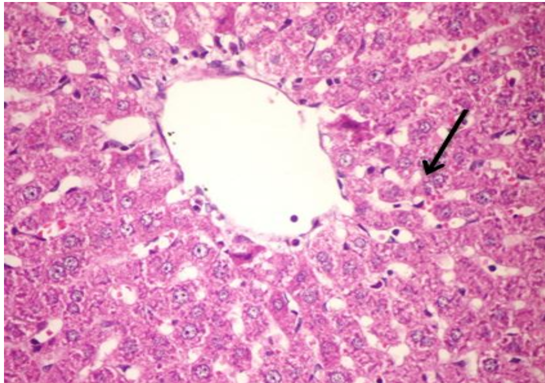
Liver of control, untreated rat showing the normal histological structure of hepatic lobule.



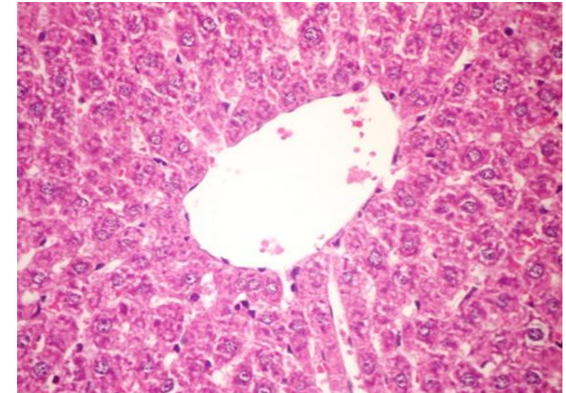
Liver of rat treated with olive leaves extract at a dose of 400 mg /kg b.wt showing vacuolar degeneration of some hepatocytes.



Liver of rat treated with profenofos 1/8 LD50 showing sinusoidal leukocytosis.



Liver of rat treated with olive leaves extract at a dose of 200 mg /kg b.wt + profenofos 1/8 LD50 showing kupffer cells activation



Liver rat treated with olive leaves extract at a dose of 400 mg /kg b.wt + profenofos 1/8 LD_{50} showing no histopathological changes

4. Discussion

The acetone extract of olive leaves showed the highest phenolic content among all solvents studied, these results may be due to that acetone being the most efficient and reproducible extraction solvent for anthocyanins according to **Garcia-Viguera *et al.,*(1998)** and cause the highest yield of most classes of polyphenols according to **Kahkonen *et al.,*(2001)** these results are in line with **Aaby *et al.,*(2007) , Yang *et al.,*(2007) and Seal (2011).**

In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. (**Oyaizu 1986**). Increasing absorbance indicates an increase in reductive ability. The reductive ability of the different olive leaves extracts was proportional to their free phenolic compounds content as previously mentioned in this investigation.

Phenolic compounds in acetonic extract of olive leaves were previously reported to occur in olive leaves (**Benavente-Garcia *et al*., (2002) and Meirinhos *et al*., (2005)**).

Results presented in the present investigation indicate that exposure to profenofos induced increases the lipid peroxidation (LPO) level,catalase (CAT)activity,glutathione peroxidase(GPx)activity and decrease in reduced glutathione(GSH) level.This result agree with **Ahmed *et al.,* (2000), Hazarika *et al.,* (2003) and Amara *et al.,* (2011).** The increase in MDA level might be modulated by profenofos itself inducing LPO or by a possible increase in ROS induced by profenofos. ROS are a part of normal oxidative metabolism, but when they are produced in excess, they cause tissue injury including lipid peroxidation and enzyme inactivation **(Halliwell and Gutterdge 1990 and Dal-Pizzol *et al.,* 2001).**

Glutathione reduced (GSH) content displays remarkable metabolic. Significant decrease in GSH content in blood after profenofos exposure indicated pro-oxidant condition in blood, which may result in oxidative damage. The reduced levels of GSH in profenofos-treated rats may be due to the increased utilization of GSH for conjugation and/or participation of GSH as an antioxidant in terminating free radical production due to profenofos-induced toxicity. It has been reported that GSH play an important role in protecting cells from xenobiotics which induced tissue injury **(Reed and Fariss 1984; and Wu et al., 2004).** The decrease in GSH content in profenofos-treated rats may probably due to an increase in the activity of glutathione-S-transeferase (GST), that may increase the conjugation of –SH groups thus protecting the body from profenofos-induced toxicity.

The corresponding elevation in the activities of GPx and CAT, which are vital in intracellular antioxidant defense, was also not sufficient to neutralize the oxidative stress in terms of an increase in lipid peroxidation and a decrease in GSH content in rats treated with profenofos **(Kaur and Sandhu (2008).**

The reduced lipid peroxidation observed in the olive leaves extract-treated animals may be attributed to the important role of oleuropein, oleuropein aglycone and hydroxytyrosol as antioxidants. This power may be attributed to their ability to decompose free radicals by quenching reactive oxygen species and by trapping radicals before reaching their cellular targets **(Srinivasan *et al.* 2007).**

The observed enhancement in the level of GSH in olive leaves extract with profenofos group could be attributed to the sparing effect of oleuropein in competing with free radicals that burden the antioxidative function of these antioxidants **(Edgecombe *et al.* 2000).**

Recent studies suggest that olive leaves extract suppress inflammation and reduce oxidative stress injury **(Mohagheghi *et al*., 2011).** It is well known that oleuropein, hydroxytyrosol, tyrosol and caffeic acid are the main constituents of olive leaves, which are thought to be responsible for their pharmacological effects **(Bianco and Uccella, 2000; Ryan *et al.,* 2002).** Intervention of oleuropein with already present free radicals may come about through providing hydroxyl group to neutralize and quench free radicals **(Visioli *et al*., 2002).**

Inhibition of acetylcholinesterase activity due to organophosphorus (OP) exposure is the indicator of OP poisoning. OP compounds generally elicit their effects by inhibition of acetylcholinesterase, which leads to an accumulation of the neurotransmitter acetylcholine in synapses; in the neuromuscular junction, overstimulation of postsynaptic cholinergic receptors leads to muscle fasciculation and eventual paralysis **Mileson *et al*., (1988).** Our results agreed with **Binukumar *et al.,* (2010) and Selmi *et al.,* (2010)**.However, supplementation of olive leaves extract at a dose of 400 mg/kg b.wt along with profenofos resulted in a significant increase in the AChE activity.

Antioxidants can protect biological systems against the potentially harmful effects of processes or reactions that can cause excessive oxidation **(Arnao *et al.,* 2001).**

Because of the phytochemical diversity and aqueous and lipid phases in foods, it is reasonable to suggest the existence of various substances that interact for common purposes. Thus, a substance responsible for protecting plants can act as an antioxidant and also as an acetylcholinesterase inhibitor (**Omena *et al.,* 2012).** It was shown that polyphenolic compounds can be effective antioxidants towards neuronal cells in

different brain parts **(Ishge *et al*., 2001).** Polyphenols can be considered as possible active neuroprotectants **(Szwajgier 2013)**.

Liver plays a central role in the detoxification process and along with kidney faces the threat of maximum exposure to xenobiotics and their metabolic by-products. The susceptibility of liver tissues to this stress due to exposure to pesticides is a function of the overall balance between the degree of oxidative stress and the antioxidant capability (**Khan *et al* 2005**). Results of the present study revealed that profenofos treatment caused an increase in the activities of AST, ALT, ALP and GGT in plasma of male rats. Our results agree with **(Mansour and Mossa, 2010, Al-Attar 2010,Ambali *et al*.,2011)** The increase in these enzymes may be due to liver dysfunction with alteration in the permeability of liver membrane takes place **(Meyer and Kulkarni, 2001; Khan *et al*., 2005).**Also, elevation in these enzymes concentration was associated with generalized hepatotoxicity**(Cornish 1971).**

. **Awad et al. 1998** found that cell damage exhibited good correlation with the enzyme leakage.

The hypoalbuminemia may be due to a decrease in serum albumin synthesis as a result of hepatic impairment and/ or increase urinary excretion due to impaired renal function arising from renal injury **(Ambali et al., 2011)** our results are agree with **Ambali *et al.,*(2011).**

Besides, the role of albumin as an antioxidant **(Roche et al., 2008)** it has been found that it is used up in the process of combating oxidative stress evoked by profenofos, thereby contributing to the low albumin concentration in the profenofos group. Furthermore, the ability of albumin to scavenge OP and bind to it, therefore reducing the measurable free albumin available in the system resulting decline in its concentration **(Ambali et al., 2011)**.

Olive leaves extract at a dose of 400 mg/kg b.wt to profenofos group restored the plasma enzymes near to control, This may be due to the ability of the antioxidant to protect against oxidative damage to the liver by profenofos **(Ambali *et al*., 2007). Abaza et al., 2007** indicated that flavonoids and phenolic compounds obtained from olive leaves are known to have diverse biological activities and may also be responsible for the pharmacological actions of olive leaves or, at least synergistically reinforcing those actions.

Oleuropein prevents formation of free radicals by its ability to chelate metals such as copper and iron, which catalyze free radical generation reactions such as lipid oxidation. As a protective action oleuropein may also directly neutralize radicals by providing hydroxyl groups **(Galli and Visioli, 1999).**

The mitigation of the hypoalbuminemia by olive leaves extract at a dose of 400 mg/kg b.wt may be due to protection of the liver from oxidative damage provoked by exposure to profenofos, apparently due to its antioxidant effect.

Similar hypocholesterolaemina was previously recorded in serum of experimental animals that were treated with various insecticides, including acephate, dichlorovos and cypermethrin **(Choudhari and Chakraharti, 1984; Ryhanen *et al*., 1984; Shakoori *et al*., 1988; respectively).** It has been suggested that OPs may phosphorylate and inhibit hydroxylmethyl glutaryl CoA reductase, the key enzyme in cholesterol production **(Ryhanen *et al*., 1984).** Also, a decrease of total cholestrol may be due to the OP-induced stimulation of LDL receptors which promote the clearance of cholesterol from circulation **(Brown *et al*., 1981).**

The present study has also demonstrated a relatively lower triglycerides and total lipids levels in the profenofos group and this observation agreed with those obtained in rats by **Barna-Lloyd *et al*. (1990).** This decrease in the profenofos group may have resulted from liver damage **(Kaneko *et al*., 2008; Stojevic *et al*., 2008).**

Pretreatment with the olive leaves extract caused apparent normalization in the total cholesterol, triglycerides and total lipids levels. This may be due to the ability of the antioxidant to protect against oxidative damage to the liver by profenofos **(Ambali *et al*., 2007),** which brings about an improvement in the entire processes that are associated with lipid metabolism.

Supplementation of olive leaves extract to rats intoxicated with profenofos may ameliorate the toxic effects of profenofos. The protective effects of natural antioxidants in olive leaves extract against profenofos-induced histopathological and biochemical alterations are the most important evidences for involvement of oxidative stress in profenofos-induced toxicity.

References

Aaby, K.; Ekeberg, D. and Skrede, G. (2007): Characterization of Phenolic Compounds in Strawberry (*Fragaria ananassa*) Fruits by Different HPLC Detectors and Contribution of Individual Compounds to Total Antioxidant Capacity. J. Agric. Food Chem., Vol. 55, pages 4395-4406.

Abaza, L.; Talorete, T.P.N. ; Yamada, P. ; Kurita, Y. ; Zarrouk, M. and Isoda, H. (2007): Induction of Growth Inhibition and Differentiation of Human Leukemia HL-60 Cells by a Tunisian Gerboui Olive Leaf Extract. Biosci. Biotechnol. and Biochem. 71, 1306-1312.

Aebi,H.(1984): Methods in Enzymatic Analysis, vol. 3, Academic Press, New York, pp. 276–286.

Ahmed, R.S. ;Vandana, S.; Pasha, S.T. and Banerjee, B.D. (2000): Influence of dietary ginger *(Zingiber officinales Rosc)* on oxidative stress induced by malathion in rats. Food and Chemical Toxicology, Vol. 38, Issue 5, Pages 443-450.

Al-Attar, A.M. (2010): Physiological and Histopathological Investigations on the Effects of α-Lipoic Acid in Rats Exposed to Malathion, J Biomed Biotechnol.; Published online 2010 May 5. doi: 10.1155/2010/203503.

Allain, C.C.; Poon, L.S.; Chan, C.S.G.; Richmond, W. and Fu, P.C. (1974): Enzymatic determination of total serum cholesterol. Clin. Chem., 20(4), 470-475.

Amara ,I.B. ; Soudani ,N. ; Hakim ,A. ; Troudi,A. ; Zeghal ,K.M. ; Boudawara ,T. and Zeghal,N.(2011): Selenium and vitamin E, natural antioxidants, protect rat cerebral cortex against dimethoate-induced neurotoxicity. Pesticide Biochemistry and Physiology.Vol. 101 .pages: 165–174.

Ambali, S.; Akanbi, D. ; Igbokwe, N.; Shittu, M. and x Kawu, M. *(*2007): Evaluation of subchronic chlorpyrifos poisoning on hematological and serum biochemical changes in mice and protective effect of vitamin C. J. Toxicol. Sci., Vol.32 pages: 111-120.

Ambali, S.F.; Angani, M.; Adole, A.O.; Kawu, M.U.; Shittu, M.; Akande, M.G. and Oladipo, O.O. (2011): Protective effect of vitamin C on biochemical alterations induced by subchronic co-administration of chlorpyrifos and lead in Wistar rats. Environmental & Analytical Toxicology. 1: 3.

Arnao, M. B.; Cano, A. and Acosta, M. (2001): The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry.Vol.73 pages: 239–244.

Awad, M.E. ; Abdel-Rahman, M.S. and Hassan, S.A. (1998): Acrylamide toxicity in isolated rat hepatocytes, Toxicol. In Vitro 12 (1998) 699–704.

Aygun, D.; Erenler, A.K. ; Karataz, A.D. and Baydin, A.(2007): Intermediate syndrome following acute organophosphate poisoning: correlation with initial serum levels of muscle enzymes, Basic Clin. Pharmacol. Toxicol. 100, pp 201‑204.

Bancroft, J.D.; Stevens, A. and Turner, D.R. (1996) : Theory and practice of histological techniques. - Edinburgh, London, Melbourne: Churchill Livingstone. - 4th ed.

Barna-Lloyd, T.; Szabo, J.R. and Davis, N.L. (1990): Chlorpyrifos-methyl (Reldan R) rat subchronic dietary toxicity and recovery study. Unpublished Report from Dow Chemical Co., TX, USA, submitted to WHO by Dow AgroSciences, Midland, MI, USA.

Belfield, A.and Goldberg, D.M.(1971): Revised assay for serum phenyl phosphatase activity using 4-aminoantipyrine. *Enzyme* 12, Pages :561-573.

Benavente-Garcia, O. ; Castillo, J., ; Lorente, J. ; Ortuno, A. and Del Rio, J.A. (2002): Antioxidant activity of phenolics extracted from Olea europea L. leaves. Food Chemistry 68, 457–462.

Beutler,E.;Duron,O,andKelly,M.B.(1963): Improved method for the determination of blood glutathione, J. Lab. Clin. Med. 61 Pages: 882–888.

Bianco, A. and Uccella, N. (2000): Biophenolic components of olives. Food Research International33, 475–485.

Binukumar, B.K.;Bal,A. ; Kandimalla,R.J.L. and Gill,K.D.(2010): Nigrostriatal neuronal death following chronic dichlorvos exposure: crosstalk between mitochondrial impairments, α synuclein aggregation, oxidative damage and behavioral changes. Mol Brain. Pages 3-35.

Briante, R.; Patumi, M.; Terenziani, S. ;Bismuto, E. ;Febbraio, F. and Nucci, R. (2002): Olea europaea L. leaf extract and derivatives: antioxidant properties. Journal of Agricultural and Food Chemistry 50Pages: 4934–4940.

Brown, M.S. ; Kovanen, P.T. and Goidstein, J.L., (1981): Regulation of plasma cholesterol by lipoprotein receptors. Science 212pages 628 –635.

Cheeseman, K.H. (1993): Lipid peroxidation in biological system. In:DNA and Free radicals. Pages 201-11.

Cheeseman, K.H. and Slater JF. (1992): An introduction to free radical biochemistry. In: Free radicals in medicine.. Churchill Livingstone.Pages: 481-93.

Cherif, S. ;Rahal, N. ; Haouala, M. ; Hizaoui, B.; Dargouth, F. and Gueddiche, M. (1996): A clinical trial of a titrated Olea extract in the treatment of essential arterial hypertension. Journal de Pharmacie de Belgique 51Pages: 69–71.

Choudhari, P.D. and Chakraharti, C.H., (1984): Effect of acephate (orthene), an organophosphorus insecticide, on lipid metabolism in albino rats. Ind. J. Exp. Biol.Vol. 22 pages 45 – 49.

Cornish, H.H. (1971). Problems posed by observations of serum enzyme changes in toxicology. CRC Crit. Rev. Toxicol., Vol.1.pages 1-32.

Dal-Pizzol, F.; Klamt, F.; Dalmolin, R.J.S.; Bernard, E.A. and Moreira, J.C.F. (2001): Mitogenic signaling mediated by oxidants in retinal treated Sertoli cells, Free Radic. Res., Vol. 35, pages 749–755.

Doumas, B.T.; Watson, W.A. and Biggs H.C. (1971): Albumin standards and Management of serum albumin with bromocresol green. *Chin. Chim. Acta*. 31Pages:87-96.

Duncan, D. B. (1955): Multiple range and Multiple F. Tests. Biometrics, 11: 1-42.

Edgecombe, S.C.; Stretch, G.L.and Hayball, P.J. (2000). Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. Journal of Nutrition .pages: 2996–3002.

El, S.N. and Karakaya, S. (2009): Olive tree (Olea europaea) leaves: potential beneficial effects on human health. Nutrition Review 67, 632–638.

Ellman, G.C.; Courtney, K.O.; Andres, V. and Featherstone, R.M., (1961).A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, Pages: 88–95.

EPA (Environmental Protection Agency) (1998): Reproduction and fertility.

Fossati, P. and Prencipe, L. (1982): Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28(10), 2077-2080.

Friedewald, W.T.; Levy, R.S. and Fredrickson, D.S. (1972): Estimation of the conc. of LDL-cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.

Galli, C.and Visioli, F. (1999): Antioxidant and Other Activities of Phenolics in Olives/Olive Oil, Typical Components of the Mediterranean Diet. Lipids., 34: 23-26.

Garcia-Viguera, C.; Zafrilla, P. and Toma´s-Barbera´n, F. A. (. 1998): The use of acetone as an extraction solvent for anthocyanins from strawberry fruit. Phytochem. Anal., Vol. 9, pages 274-277.

Gulcin I., Oktay M., Kufrevioglu I. and Aslan A. (2002): Determination of antioxidant activity of lichen *Cetraria islandica* (L.) Ach. *J. Ethnopharmacol*. 79: 325-29.

Halliwell, B. and Gutterdge, J.M.C. (1990): The antioxidants of human extracellular fluids, Arch. Biochem. Biophys., Vol. 280, pages 1–8.

Hazarika, A.; Sarkar, S.N. ; Hajare,S.; Kataria, M. and Malik, J.K.(2003): Influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. Toxicology Vol.185 (1-2) pages : 1 – 8.

Ishge K.; Schubert D.and Sagara Y.(2001): Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free Rad. Bio. Med., Vol. 30 pages: 433–446.

Jeyaratnam, J. and Maroni, M. (1994): Organophosphorus compounds, Toxicology 91Pages: 15–27.

Kahkonen, M. P.; Hopia, A. I. and Heinonen, M. (2001): Berry phenolics and their antioxidant activity. J. Agric. Food Chem., Vol. 49, pages 4076-4082.

Kaneko, J.J.; Harvey, J.W. and Bruss, M.(2008): Clinical Biochemistry of Domestic Animals. 6th Edn., Academic Press, Amsterdam, Boston, pp: 916.

Kaur and Sandhu (2008): In *vivo* changes in antioxidant system and protection role of selenium in chlorpyrifos-induced subchronic toxicity in *bubalus bubalis*.Environmental Toxicology and Pharmacology.Vol.26,pages 45-48.

Khan, S.M. ; Sobti, R.C. and Kataria, L. (2005): Pesticide-induced alteration in mice hepatooxidative status and protective effects of black tea extract, Clin. Chim. Acta 358 Pages : 131–138.

Lee, O.H. and Lee, B.Y. (2010). Antioxidant and antimicrobial activities of individual and combined phenolics in Olea europaea leaf extract. Bioresource Technology 101Pages :3751–3754.

Lee, O.H.; Lee, B.Y.; Lee, J. ;Lee, H.B.; Son, J.Y. ;Park, C.S.; Shetty, K. and Kim, Y.C. (2009). Assessment of phenolics-enriched extract and fractions of olive leaves and their antioxidant activities. Bioresource Technology 100 Pages: 6107–6113.

Lopes-Virella, M.F.; Stone, P.; Ellis, S. and Colwell, J.A. (1977): Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem., 23(5), 882-884.

Malkovics, B. (1995): Effect of organophosphates on the antioxidant systems of fish tissue. Acta Biol Hung Pages: 9-11.

Mansour, S.A. and Mossa, A.T.H.(2010): Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pesticide Biochemistry and Physiology .pages: 14–23.

Meirinhos, J. ; Silva, B.M. ; Valentao, P. ; Seabra, R.M. ; Pereira, J.A. ; Dias, A. ; Andrade,P.B. and Ferreres, F. (2005): Analysis and quantification of flavonoidic compounds from Portuguese olive (Olea europaea L.) leaf cultivars. Natural Product Research 19, 189-195.

Meyer, S.A. and Kulkarni, **A.P. (2001):** Hepatotoxicity in Introduction to Biochemical Toxicology, Hodgson, E. and R.C. Smart, John (Eds.).3rd Edn, Wiley and Sons New York, pp: 487 -490.

Mileson B.E.; J.E. Chambers, W.L. Chen, W. Dettbran and Ehrich. M. (1988): Common mechanism of toxicity: a case study of organophosphorus pesticides, Toxicol. Sci., Vol. 41, pages 8–20.

Ministry of Agriculture, A.R.E. (2001): Pest Control Program (in Arabic).

Mohagheghi, F. ; Bigdeli, M.R. ; Rasoulian, B. ; Hashemi, P. and Pour, M.R.(2011) : The neuroprotective effect of olive leaf extract is related to improved blood-brain barrier permeability and brain edema in rat with experimental focal cerebral ischemia. *Phytomedicine*. 15;18(2-3) Pages:170-5.

OECD (Organization for Economic cooperation and development) (1996) : Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test, No. 422.

Ohkawa,H.;Ohishi,W and Yagi,K.(1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 Pages : 351–358.

Omena,C.M.B. ; Valentim ,I.B.; Guedes,G.D.S ; Rabelo,L.A.; Mano,C.M. ; Bechara,E.J.H. ; Sawaya, A.C.H.F. ; Trevisan, M.T.S. ; Costa,J.G. ; Ferreira,R.C.S. ; Sant'Ana, A.E.G. and Goulart,M.O.F.(2012): Antioxidant, anti-acetylcholinesterase and cytotoxic activities of ethanol extracts of peel, pulp and seeds of exotic Brazilian fruits Antioxidant, anti-acetylcholinesterase and cytotoxic activities in fruits. Food Research International.Vol.49 pages: 334–344.

Oyaizu, M. (1986): Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition. pages : 307–315.

Paglia,D.E. and Valentine,W.N.(1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med. 70, Pages : 158–169.

Reed, D.J. and Fariss, M.W. (1984): Glutathione depletion and susceptibility, Pharmacol. Rev., Vol. 36, pages 25S–33S.

Reitman, S.and Frankel, S.(1957): Colorimetric estimation of AST and ALT activities, J. Lab. Clin. Med. 28 (1957) 56–63.

Roche,M.; Rondeau,P.; Singh,N.R.; Tarnus,E. and Bourdon,E.(2008): The antioxidant properties of serum albumin. FEBS Letters, Volume 582, Issue 13,Pages 1783-1787.

Ryan, D. ; Antolovich, M. ; Prenzler, P. ; Robards, K. and Lavee, S. (2002): Biotransformations of phenolic compounds in *Olea europaea* L. *Scientia Horticulturae* 92 Pages : 147–176.

Ryhanen, R.; Herranen, J.; Korhonen, K.; Penttila, I.; Polvilampi, M. and Puhakainen, E. (1984): Relationship between serum lipids, lipoproteins and pseudocholinesterase during organophospate poisoning in rabbits, int. J. Biochem., Vol. 16(6), pages 687- 690.

Savolainen,K.(2001): Understanding the toxic action of organophosphates, Handbook of Pesticide Toxicology, II Ed, Academic Press, USA, pp. 1013–1043.

Schalm, O.W. (1986): Veterinary Haemotology.4th Ed., Lea and Febiger,Philadelphia, pages :21-86.

Seal, T. (2011): Antioxidant Activity of Some Wild Edible Fruits of Meghalaya State in India. Advances in Biological Research, Vol. 5 (3), pages 155-160.

Selmi, S., El-Fazaa, S. and Gharbi, N. (2010): Oxidative stress and cholinesterase inhibition in plasma, erythrocyte and brain of rats pups following lactational exposure to malathion. Environ Toxicol Pharmacol., Vol. 34(3) pages:753-60.

Shakoori, A.R.; Ali, S.S. and Saleem, M.A., (1988): Effects of six months feeding of cypermethrin on the blood and liver of albino rats. J. Biochem. Toxicol. Vol.3 pages 59 – 72.

Sharma,Y.; Bashir,S.; Irshad, M.; Gupta, S.D. and Dogra, T.D. (2005): Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats, Toxicology 206 ,pp 49–54.

Srinivasan, P. ; Sabitha, K.E. and Shyamaladevi, C.S.(2007): Attenuation of 4- nitroquinoline 1-oxide induced *in vitro* lipid peroxidation by green tea polyphenols, Life Sci. pages: 1080–1086.

Stojevic, Z.; Filipovic, N.; Bozic, P.; Tucek, Z. and David, J.(2008): The metabolic profile of Simmental service bulls. Vet. Arhiv., Vol.78 pages: 123-130.

Szasz, G.(1969): A kinetic photometric method for serum γ -glutamyltransferase J. Clin Chem 15, Pages:124-136.

Szwajgier, D. (2013): Anticholinesterase Activities of Selected Polyphenols – a Short Report. Pol. J. Food Nutr. Sci., Vol. 64, No. 1, pp. 59-64.

Visioli, F.; Poli, A. and Galli, C. (2002): Antioxidant and other biological activities of phenols from olives and olive oil. Medicinal Research Reviews, Vol. 22, pages 65–75.

Weil, C.S. (1952): Tables for convenient calculation of medium effective dose(LD_{50} or ED_{50}) and instruction in their use. Biometrics, Vol. 8, pages 249-263.

Wu, G.; Fang, Y.Z.; Yang, S.; Lupton, J.R. and Turner, N.D. (2004): Glutathione metabolism and its implications for health, J. Nutr., Vol. 134, pages 489–492.

Yang, D.; Wang, Q.; Ke, L.; Jiang, J. and Ying, T. (2007): Antioxidant activities of various extracts of lotus (*Nelumbo nuficera Gaertn*) rhizome. Asia Pac J Clin Nutr., Vol. 16 (Suppl 1), pages 158-163.

Zollner, N. and Kirsch, K.Z. (1962): A colorimeteric method to determine total lipid. Ges. Exp. Med., 135: 545.