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Antioxidant Potential and Hepatoprotective Activity of *Origanum majorana* Leaves Extract against Oxidative Damage and Hepatotoxicity Induced by Pirimiphos-Methyl in Male Mice

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Abstract: The present experiment was designed to evaluate the oxidative damage and hepatotoxicity resulting from Pirimiphos-Methyl (PM) exposure as well as the hepatoprotective potential of *Origanum majorana* L. leaves extract in male mice. The results revealed that Majorana leaves extract exhibited antioxidant capacity manifested by inhibitory effects on DPPH, ABTS, hydroxyl radical and reducing power *in vitro*. Male mice were divided into six groups of six mice each: Control group (I) extract groups (II and III) received an extract at doses of 150 and 300 mg kg^{-1} b.wt.; PM group (IV) received PM (12.0 mg kg^{-1} b.wt., 1/10 LD_{50}) in corn oil; groups (V and VI) received PM along with the two doses of extracts. All the applications were administered via oral route for 28 consecutive days. Exposure of mice to PM caused significant changes in body and relative liver weights as well as significant increases in the activity of serum enzymes alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Also, PM induced significant decreases in the activities of hepatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and in the content of glutathione reduced (GSH), however, induced a significant increase in the level of hepatic lipid peroxidation (LPO) accompanied by histopathological alterations in liver of male mice. Co-administration of *O. majorana* extract to PM ameliorated the above-mentioned parameters. The ultimate effect was achieved by the highest dose of the extract. It could be concluded that PM induced oxidative damage and liver injury in male mice. However, co-administration of *O. majorana* leaves extract attenuated the harmful effects of PM which may be attributed to its antioxidant potential. Results indicated that *O. majorana* leaves could be used for therapeutic option against hepatic injuries resulting from pesticide intoxication.

Key words: Pirimiphos methyl, oxidative stress, antioxidant, liver, *O. majorana*, mice

INTRODUCTION

The main problem arising from the extensive use of pesticides in agriculture and public health, especially in developing countries, is acute and chronic toxicity in man (Abbassy *et al*., 2014). According to WHO (1986), approximately 3 million cases of acute pesticide poisoning, with 20,000 deaths, have been estimated per annum world-wide, with 99% of cases occurring in developing countries (Jeyaratnam, 1990). Currently, long term exposure to low doses of pesticide through food, water or air is a widespread concern because of their adverse effects on human health. It has been reported that many pesticide residues were detected in water, vegetables, fruits, grains, milk, meat and others agriculture and food products (EFSA, 2011).

Organophosphorus insecticides (OPIs) are widely used against a broad spectrum of insects and have adverse toxic effects on human and animal health. The PM (0, 2-diethylamino-6-methylpirimidin-4-yl O, O-dimethyl phosphorothioate) is a fast-acting broad spectrum organophosphorus insecticide with both contact and fumigant action. It is widely used in the world to protect fruits, vegetables, horticultural plants, stored cereal grain, seeds and peanuts against insects. It inhibits acetylcholinesterase in the brain and erythrocytes and plasma cholinesterase (Rajini *et al*., 1989), causes reproductive toxicity at doses of 62.5 and 125 mg kg^{-1} b.wt. (Ngoula *et al*., 2007), induce histopathological alteration in liver, kidney and testes in rat (Nessiem *et al*., 2003). In previous study, Heikal *et al*. (2014) reported that PM induced hepato-renal

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damage and oxidative stress in male mice subjected to different doses of PM when administered for 45 days and they added that the worst effects were achieved by the highest dose of PM (62.11 mg kg^{-1} body weight, 1/20 LD_{50}).

The liver is considered to be one of the most vital organs in the human body. It plays an essential role in metabolism, detoxification and elimination of pesticides, drugs and other xenobiotics. Previous studies showed that pesticides can induce oxidative stress and lipid peroxidation causing liver damage in experimental animals (Nessiem *et al*., 2003; Mossa *et al*., 2013). So, it has been suggested that one molecular mechanism of OP insecticides induced toxicity is the enhancement of Reactive Oxygen Species (ROS) production which in turn leads to oxidative stress (Mossa *et al*., 2013; Mansour and Mossa, 2009). Under normal conditions, production of ROS is counteracted by enzymatic antioxidant enzymes (SOD, CAT, GPx and GST) and non-enzymatic contents (GSH, vitamin E, C and uric acid). In this respect, the overproduction of ROS that were not counteracted by enzymatic and non-enzymatic antioxidant systems can adversely alter lipids, proteins and DNA contents causing aging and several of human illnesses. Currently, many researchers reported the potential role of antioxidants and their free radical scavenging properties in preventing or controlling many of the human diseases.

Marjoram (*Origanum majorana* L.) is a member of the mint family Lamiaceae and has definite therapeutic properties. The essential oil of O. *majorana* has antioxidant, antimutagenic and hepatoprotective activities (Mossa and Nawwar, 2011). Its main constituents are 4-terpineol (29.97%), γ-terpinene (15.40%), trans-sabinene hydrate (10.93%), γ-terpinene (6.86%) and 3-cycolohexene-1-1 methanal,a,a4-trimethyl-,(S)-(CAS) (6.54%). According to Mossa and Nawwar (2011) Majorana leaves extract exhibited a dose-dependent manner inhibitory effect on DPPH•, hydroxyl radical, hydrogen peroxide, reducing power and lipid peroxidation.

There is very little and unsatisfactory information on lipid peroxidation, oxidative stress and hepatotoxicity arising from exposure to PM in mammals. In addition, the antioxidant activity and the use of leaves extract of *O. majorana* to alleviate liver injury, oxidative damage and lipid peroxidation induced by pesticides have not yet been previously examined. The objective of the study was therefore first to evaluate the adverse effects of exposure to PM on lipid peroxidation, oxidative stress and liver dysfunction biomarkers in male mice and second to determine the antioxidant and therapeutic efficacy of *O. majorana* leaves extract against exposure to PM in mice.

MATERIALS AND METHODS

Plant material: The leaves of *O. majorana* were collected in March, 2013, from the local market of Cairo, Egypt. The plant was identified according to the taxonomic characters of Tackholm (1974).

Preparation of plant extract: The air-dried leaves of *O. majorana* (200 g) were powdered coarsely and soaked in 80% (v/v) methanolic aqueous solution at room temperature for 24 h under dark condition. After centrifugation at 4500 rpm for 10 min, the residue was re-extracted twice with 80% methanol as described above. The supernatants were pooled together, concentrated in a rotary evaporator and the dry extract (5.8 g) stored under the refrigerator. The resulting powder extracts were employed for the current study.

Chemicals and reagents: Ascorbic acid was purchased from Sigma-Aldrich Chemie, Steinheim, Germany; 2,2′-diphenylpicrylhydrazyl ($DPPH^{\cdot}$) from Sigma, St. Louis, Missouri, USA; sodium phosphate dibasic and monobasic from Alliance Bio, Irvine, California, USA. Pirimiphos-methyl (95%) was obtained from Taegeuk Corporation, China. The assay kits used for biochemical measurements of aspartate aminotransferases (EC 2.6.1.1.), alanine aminotransferases (EC 2.6.1.2), alkaline phosphatase (EC 3.1.3.1), lactate dehydrogenase (EC 1.1.1.27), catalase (EC 1.11.1.6), superoxide dismutase (EC 1.15.1.1), glutathione peroxidase (EC 1.11.1.9), glutathione reduced, lipid peroxidation and total protein were purchased from Biodiagnostic Company, Dokki, Giza, Egypt.

In vitro studies

Phytochemical: The methanol extract of *O. majorana* was used for determination of phenolic and flavonoids contents. Total phenolic and flavonoids were determined by Folin-Ciocalteu and aluminum trichloride ($AlCl_3$) according to the method of El Kar *et al.* (2011), respectively. Gallic acid and quercetin were used as standard and results were expressed as g of Garlic Acid Equivalents (GAE) or Quercetin (QE) per kg of dry mass.

Antioxidant activity

DPPH• scavenging activity: The scavenging ability of the natural antioxidants of the leaves extract was measured from the bleaching of the purple colored methanol solution of DPPH• and ascorbic acid was used as standard. This spectrophotometric assay uses the stable radical, 2,2′-diphenylpicrylhydrazyl (DPPH•) as a reagent, according to the method cited by Afoulous *et al.* (2013). One milliliter of different concentrations of the methanol

extract of *O. majorana* (5-20 μg mL^{-1}) were mixed with 1.0 mL of 0.1 mM DPPH• in methanol and final volume adjusted up to 3.0 mL with MeOH. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min and then the absorbance was measured at 517 NM using a spectrophotometer (Shimadzu UV-VIS Recording 2401 PC, Japan). Methanol was used as blank and ascorbic acid (0.5-10 μg mL^{-1}) was used as the reference compound. The absorbance of solvent and DPPH• radical without extract was measured as control. The radical-scavenging activities of samples, expressed as percentage inhibition of DPPH•, were calculated according to the equation:

$$I\ (\%) = [(A_C - A_S)/A_C] \times 100$$

where, A_C and A_S are the absorbance of the control and sample, respectively.

ABTS scavenging activity: The antioxidant effect of the leaf extract and ascorbic acid were studied using the spectrophotometric analysis of $ABTS^{\bullet+}$ (2, 2'azinobis-(3-ethylbenzthiazoline -6-sulphonic acid)) radical scavenging activity according to the method of El Kar *et al.* (2011). The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Because ABTS and potassium persulfate react stoichiometrically at a ratio of 1: 0.5, this will result in incomplete oxidation of the ABTS. Oxidation of the ABTS commenced immediately but the absorbance was not maximal. The radical was stable in this form for more than two days when stored in the dark at room temperature. Previous to use, the $ABTS^{\bullet+}$ solution was diluted with distilled water to get an absorbance of 0.700±0.02 at 734 nm with phosphate buffer (0.1 M, pH 7.4). Then, 1 mL of $ABTS^{\bullet+}$ solution was add to 1 mL of extract solution in distilled water at different concentrations (3-15 μg mL^{-1}). After 30 min, the percentage inhibition at 734 nm was calculated for each concentration relative to a blank absorbance. Ascorbic acid (0.5-10 μg mL^{-1}) was used as the reference compound. All determinations were carried out at least five times. The scavenging capability of $ABTS^{\bullet+}$ radical was calculated using the following equation:

$$ABTS^{\bullet+}\ \text{scavenging effect}\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the antioxidant sample.

Reducing power: Total reducing capacity of *O. majorana* extracts and ascorbic acid were determined according to the method of Oyaizu (1986). The mixture reaction contains 1 mL of extract (6.25-100 μg mL^{-1}), 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K_3 Fe $(CN)_6$]. The reaction mixture was incubated for 20 min at 50°C, after that, 2.5 mL of 10% TCA was added and centrifuged. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) and the absorbance was read at 700 nm. Increase in absorbance of sample with concentrations indicates high reducing potential of the samples.

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging ability of the extract and ascorbic acid were carried out by measuring the competition between deoxyribose (2-deoxyribose) and the extract for hydroxyl radicals which attack deoxyribose leading to the formation of thiobarbituric acid reaction system (TBARS), generated from the Fe^{3+}-ascorbate-EDTA-H_2O_2 system according to the method of Kunchandy and Rao (1990). *Origanum majorana* L. extract was applied in different concentrations (2.5-20 μg mL^{-1}). Each reaction mixture contained the following reagents, in the final concentrations stated: $FeCl_3$ (100 μM), EDTA (100 μM), H_2O_2 (2.2 mM), 2-deoxyribose (2.5 μM), L-ascorbic acid (100 μM). Phosphate buffer (pH 7.4) was added up to a final volume of 4 mL. The mixtures were incubated 1 h at 37°C and then 1 mL of 1% (w/v) thiobarbituric acid (TBA) in 0.05 M NaOH and 1 mL of 2.8% (w/v) trichloroacetic acid (TCA) were added in each mixture and heated 15 min at 100°C. After cooling on ice absorbance was measured at 532 nm using spectrophotometer. Inhibition (I) of deoxyribose degradation in percent was calculated in following equation:

$$I\ (\%) = [(A_c - A_s)/A_c] \times 100$$

where, A_c is the absorbance of the control reaction (containing all reagents except the extract) and A_s is the absorbance of the extract. The IC_{50} value represented the concentration of the extract which caused 50% inhibition.

***In vivo* studies**

Animals: Healthy male swiss albino mice weighing 29±2 g were obtained from Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt and housed in clean plastic cages. Animals were fed on standard pellet diet and tap water *ad libitum*, under standardized housing conditions (12 h light/dark cycle, the temperature was 20±2°C and a minimum relative humidity of 46%) in the laboratory animal room. Animals

received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals."The Local Ethics Committee at the National Research Centre (NRC), Dokki, Cairo, Egypt, approved the experimental protocols and procedures.

Acute toxicity studies: The acute toxicity studies were performed as per Economic Co-operation and Development (OECD) 423 guidelines (OECD, 2001). Five male albino mice (n = 5) were used in this study. One mouse was fasted overnight with free access to drinking water. It was given *O. majorana* extracts at dose of 3000 mg kg^{-1} and was observed for one day for mortality. The animal was survived after 24 h and then the other four mice were given the same dose of extract (3000 mg kg^{-1} b.wt.). All tested mice were observed for 24 h and daily for 14 days. After 14 day, no mortality or signs of toxicity were observed. Therefore, in the present study we selected 300 mg kg^{-1} (1/10th of 3000 mg kg^{-1}) as maximum safety dose with descending dose levels with 2 fold interval i.e., 150 and 300 mg kg^{-1} b.wt.

Experimental groups and treatments: After one week of acclimatization, mice were divided into six groups of six mice each (n = 6). Group I served as control and received corn oil (0.1 mL kg^{-1} b.wt.). Groups II and III served as extract groups and received aqueous extract of leaves of *O. majorana* at doses of 150 and 300 mg kg^{-1} b.wt., respectively. Group IV administered PM in corn oil at a dose of 12.0 mg kg^{-1} b.wt., 1/10 LD_{50} (Tomlin, 2004). Groups V and VI were administered the same dose of PM (12.0 mg kg^{-1} b.wt.) and the extracts at doses of 150 and 300 mg kg^{-1} b.wt., for V and VI groups, respectively. The extracts were given thirty minutes before the administration of PM. Dosages of PM and *O. majorana* leaves extract were freshly prepared, adjusted weekly for body weight changes and given via oral route for 28 consecutive days. At the end of the administration, the animals were fasted overnight with water *ad libitum* and sacrificed by ether anesthesia with cervical dislocation on 29th day.

Blood collection: Twenty-four hours after the last dose, blood samples were withdrawn from the animals under light ether anaesthesia by puncturing the retero-orbital venous plexus with a fine sterilized glass capillary. The blood samples were then left to clot in clean dry tubes and centrifuged at 3000 rpm (600 g) for 10 min using Heraeus Labofuge 400R, Kendro Laboratory Products GmbH, Germany, to separate serum. The serum was kept in a deep freezer at -20°C until liver biochemical markers (i.e., AST, ALT, ALP and LDH) were analyzed within one week.

Preparation of liver samples: After blood collection, mice were killed by cervical dislocation and livers were dissected out immediately, washed with ice cold saline, cleaned and weighed. Small pieces of liver were cut and transferred into 10% neutral formalin solution for histological studies. Other portions of liver washed with ice cold saline and homogenized in 10% (w/v) ice cold phosphate buffer (0.1M pH 7.4) and centrifugation at 10,000×g for 15 min at 4°C using Heraeus Labofuge 400R, Kendro Laboratory Products GmbH, Germany. The supernatants obtained were kept in deep freezer at -20°C until used for the assays of SOD, CAT, GPx, LPO, GSH and total protein.

Serum liver function enzymes: Serum AST and ALT were determined according to methods of Reitman and Frankel (1957), ALP according to Young *et al.* (1975) and LDH activity as indicator of necrotic cell death was determined according to Vassault (1983). The AST, ALT, ALP and LDH measurements were performed according to the details given in the kit's instructions and expressed as U L^{-1}. All spectrophotometric measurements were performed by using a Shimadzu UV-VIS Recording 2401 PC (Japan).

Oxidative stress biomarkers

Liver lipid peroxidation (LPO): Liver lipid peroxidation (LPO) was determined by a colorimetric method of Satoh (1978) according to the details given in the kit's instructions. A thiobarbituric acid reactive substance (TBARS) was used for the estimation of LPO and expressed in terms of malondialdehyde (MDA) content. For this purpose, 0.5 mL of trichloroacetic acid (10%) solution was added into 0.5 mL sample in test tube. The mixture was centrifuged at 600×g for 10 min and 0.2 mL of the supernatant was transferred into a new test tube containing 1.0 mL of TBA (25 mmol L^{-1}) solutions and boiling for 30 min. The solution was then cooled and a pink color chromogen for samples and standard were read at 534 nm using spectrophotometer. The MDA values were expressed as nmol of MDA/g tissue.

Liver antioxidant enzymes: Determination of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reduced (GSH) were performed according to the details given in Biodiagnostic kit's

instructions and the principals below of different methods are given for each concerned biochemical parameter.

SOD was determined spectrophotometrically at 560 nm according to the method of Nishikimi *et al.* (1972). The method based on the ability of SOD enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Briefly, 0.05 mL sample was mixed with 1.0 mL buffer (pH 8.5), 0.1 mL nitroblue tetrazolium (NBT) and 0.1 mL NADH. The reaction was initiated by adding 0.01 mL phenazine methosulphate (PMs) and then increased in absorbance was read at 560 nm for 5 min. SOD activity was expressed as units/mg protein.

CAT was determined according to the method of Aebi (1984). The method is based on the decomposition of H_2O_2 by catalase. The sample containing catalase is incubated in the presence of a known concentration of H_2O_2. After incubation for exactly one minute, the reaction is quenched with sodium azide. The amount of H_2O_2 remaining in the reaction mixture is then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene, AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) in the presence of H_2O_2 and catalyzed by horseradish peroxidase (HRP). The resulting quinoneimine dye (N-(4-antipyrl)-3-chloro-5-sulfonate-p-benzoquinonemonoimine) is measured at 510 nm. The CAT activity was expressed as μmol mg^{-1} protein.

The GPx was determined spectrophotometrically according to the method of Paglia and Valentine (1967). The estimation of GPx activity based on the oxidation of GSH and NADPH using Glutathione Reductase (GR) and measuring the decrease in absorbance at 340 nm and expressed in units/mg protein.

Liver GSH level was assessed spectrophotometrically 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 The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm. The GSH content was expressed in μmol/mg protein.

Protein determination: Protein was assayed by the method of Lowry *et al.* (1951), with serum bovine albumin as standard.

Histological study: After draining the blood, mice were killed, liver samples were excised, washed with normal saline, fixed in 10% neutral formalin, dehydrated in ascending grades of alcohol and imbedded in paraffin wax. Paraffin sections were taken at 5 μm thick and were stained with haematoxylin and eosin (H and E). The sections were examined for histopathological changes (×160) under light microscope. The liver fields were scored according to Michael (2008) as follows: Normal appearance (-), mild cellular disruption of 1-30% of field area (+), moderate cellular disruption of 31-60% of field area (++), severe cell disruption of 61-90% of field area (+++) and very severe cellular disruption of 91-100% of field area (++++).

Statistical analysis: All results were presented as Means±SE. All data was analysed with the Statistical Package for Social Sciences (SPSS 17.0 for windows). The results were analyzed using one way analysis of variance (ANOVA) followed by Duncan's test for comparison between different treatment groups. Statistical significance was set at $p<0.05$.

RESULTS

Total phenol and flavonoids contents: The total phenolic and flavonoids contents of *O. majorana* leaves extract were calculated and expressed of g Gallic Acid Equivalents (GAE) or Quercetin Equivalents (QE)/kg of dry mass. Total phenolic was found of 62.89±1.37 mg GAE/g of *O. majorana* leaves extract and total flavonoids content was found 45.58±1.18 mg QE/g of *O. majorana* leaves extract.

In vitro antioxidant activity

DPPH and ABTS radical scavenging activity: As shown in Fig. 1a and b, the methanolic extract of *O. majorana* leaves had scavenging activity on DPPH and ABTS. The scavenging activity of the extract was proportional to the increase in extract concentrations. The IC_{50} value of the extract was 12.9 μg mL^{-1} while that of the standard ascorbic acid was 4.70 μg mL^{-1} of DPPH scavenging activity and for ABTS around 9.35 μg mL^{-1} of the extract and 3.98 μg mL^{-1} of ascorbic acid, respectively. The results showed that leaves extract of *O. majorana* possessed high radical scavenging activity.

Reducing power: The reducing power of methanolic extract of *O. majorana* leaves was increased in a dose-dependent manner (Fig. 1c). The IC_{50} value of extract was higher than that of ascorbic acid and recorded 21.16 and 5.26 μg mL^{-1} for the extract and ascorbic acid, respectively. These results demonstrated that the reduction capacity of methanolic extract of *O. majorana* leaves was proportional to the increase in extract concentrations.

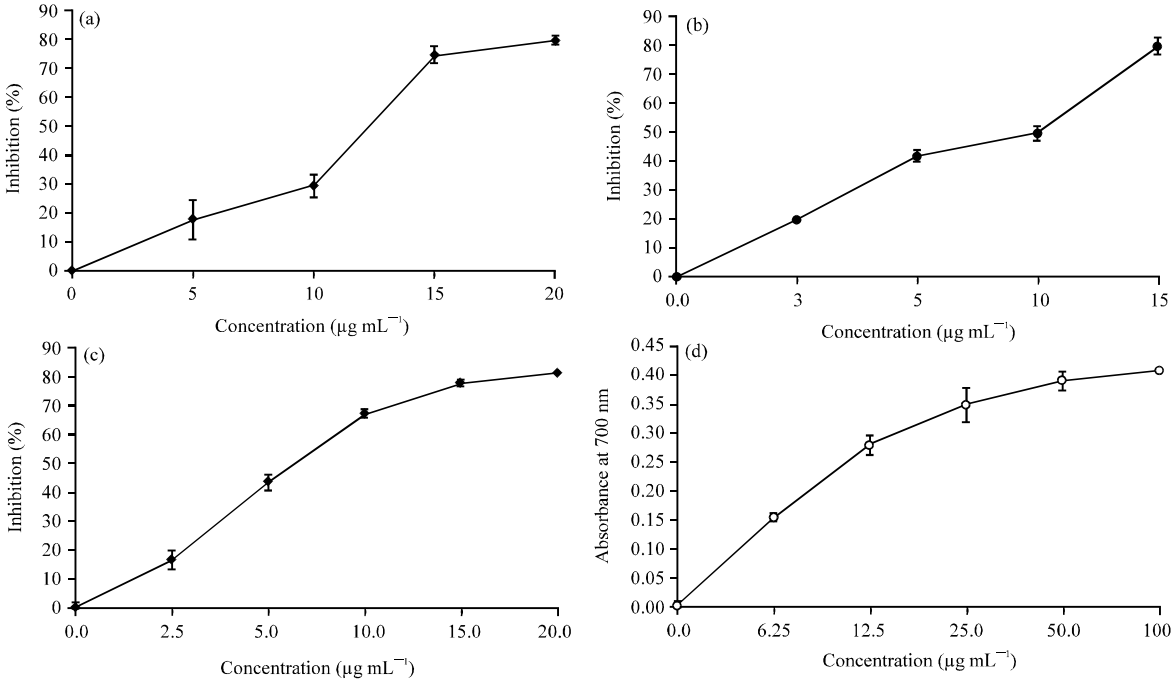

Fig. 1(a-d): Scavenging activity on, (a) DPPH, (b) ABTS, (c) Hydroxyl radicals and (d) Reducing power of methanolic extract of *O. majorana* leaves. Data is presented as Means±SE

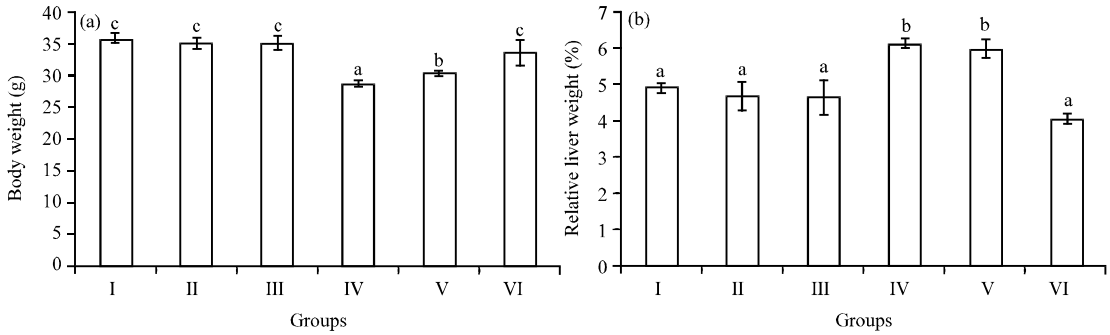

Fig. 2(a-b): Effect of exposure to pirimiphos-methyl on body and relative liver weights of male mice and the protective role of *O. majorana* leaves extract. Bars represent the group Means±S.E., ^{a,b,c,d}values are not sharing superscripts letters (a, b, c, d) differ significantly at $p \leq 0.05$. I: Control group, II and III: Extract groups (150 and 300 mg kg^{-1} b.wt., respectively), IV: Pirimiphos methyl group, V and VI: Extract (150 and 300 mg kg^{-1} b.wt.) along with pirimiphos methyl groups

Hydroxyl radical scavenging activity: As shown in Fig. 1d, hydroxyl radical scavenging activity of methanolic extract of *O. majorana* leaves was increased in response to extract concentrations. The IC_{50} values of methanolic extract of *O. majorana* leaves and ascorbic acid recorded 9.0 and 5.68 µg mL^{-1}, respectively.

In vivo studies

Acute oral toxicity: Methanolic extract of *O. majorana* leaves was studied for acute toxicity according to OECD (NO. 423) guidelines. No mortality or sings of toxicity were recorded in mice received 3000 mg kg^{-1} body weight extract, therefore the extract was found to be safe up to the dose level of 3000 mg kg^{-1} body weight. We select 300 mg kg^{-1} as maximum safety dose in this study.

Body and relative liver weights: Results of final body weight and relative liver weight of mice subjected to different treatments are shown in Fig. 2. It was observed that PM-treated mice achieved a significant decrease

in body weights compared to control (28.78 vs. 35.79 g). Co-administration of *O. majorana* extract at a dose of 300 mg kg^{-1} b.wt., to PM-treated mice showed no significant differences in body weights compared to control group (33.46 vs. 35.79 g) while body weight of mice administered a dose of 150 mg kg^{-1} b.wt., were still statistically different ($p \leq 0.05$) than the normal control values (30.24 vs. 35.79 g). Relative liver weights of mice in PM and PM + *O. majorana* extract at a dose of 150 mg kg^{-1} b.wt., recorded significant decrease in relative liver weight compared to control values. Co-administration of *O. majorana* extract at 300 mg kg^{-1} b.wt., to PM-treated mice restored relative weight of liver to normal value.

Serum liver enzymes: As shown in Table 1, mice treated with PM has significant increased in liver serum enzymes including AST, ALT, ALP and LDH compared to control group. These changes were around 88.48 vs. 40.29 U L^{-1}, 80.96 vs. 28.98 U L^{-1}, 101.56 vs. 41.98 U L^{-1} and 171.97 vs. 94.33 U L^{-1} of AST, ALT, ALP and LDH of PM and control groups, respectively. Co-administration of *O. majorana* extract at dose 150 and 300 mg kg^{-1} b.wt., along with PM treated mice decreased levels of serum AST, ALT, ALP and LDH in dose dependent manner. Also *O. majorana* extract at dose 300 mg kg^{-1} b.wt., restored these enzymes to normal levels and around 43.98 vs. 40.29 U L^{-1}, 33.81 vs. 28.98 U L^{-1}, 43.89 vs. 41.98 U L^{-1} and 104.77 vs. 94.33 U L^{-1} of AST, ALT, ALP and LDH, respectively.

Hepatic antioxidant markers: The change in levels of hepatic antioxidant markers (SOD, CAT, GPx, GSH and LPO) in liver tissue of control and experimental mice were depicted in Table 2. Compared to untreated mice, there were significant decreases in the activity of SOD (5.47 vs. 6.83 μ mg^{-1} protein), CAT (12.60 vs. 17.31 μmol mg^{-1} protein), GPX (4.33 vs. 9.86 μ mg^{-1} protein, level of GSH (0.066 vs. 0.102 μmol mg^{-1} protein) and elevation in LPO (122.35 vs. 75.95 nmol g^{-1} protein) of PM-treated group compared to control. Co-administration of *O. majorana* extract at a dose of 150 mg kg^{-1} b.wt., along with PM improved hepatic antioxidant markers while dose of 300 mg kg^{-1} b.wt., restored the activity of SOD, CAT and level of GSH to within normal levels. The levels of lipid peroxidation marker were restored to normal level after administration of *O. majorana* extract at dose of 150 and 300 mg kg^{-1} b.wt. to PM-treated group.

Histopathological studies: As shown in Fig. 3a-d, histopathological investigation of the liver shows clear difference between the control and PM-treated mice. The PM treatment caused pathological changes in liver including sever congestion, inflammatory cells infiltration and degenerative change of hepatic parenchyma. The liver was almost normal in mice administered *O. majorana* extract at 150 mg kg^{-1} b.wt., along with PM (group V) with mild congestion in the central and portal veins while normal appearance of liver was recorded in mice administered *O. majorana* extract at 300 mg kg^{-1} b.wt.,

Table 1: Effect of exposure to pirimiphos-methyl on serum liver enzymes of male mice and the protective role of *O. majorana* leaves extract

	AST	ALT	ALP	LDH
Treatments	(U L^{-1})			
I	40.29±1.001[a]	28.98±1.801[a]	41.98±1.37[a]	94.33±2.17[a]
II	40.93±1.074[a]	29.94±1.86[a]	43.45±1.19[a]	97.09±1.46[a]
III	41.33±1.128[a]	30.42±1.89[a]	43.04±1.13[a]	96.76±3.22[a]
IV	88.48±1.88[d]	80.96±2.84[c]	101.56±2.68[c]	171.97±6.41[d]
V	59.09±0.933[c]	47.72±2.84[b]	62.06±0.88[b]	154.72±2.92[c]
VI	43.98±1.78[a]	33.81±2.10[a]	43.89±0.71[a]	104.77±2.32[b]

Each value is a mean of 6 Mice±SE, [a,b,c,d]Values are not sharing letters differ significantly at $p<0.05$, AST: Aspartate aminotransferases, ALT: Alanine aminotransferases, ALP: Alkaline phosphatase and LDH: Lactate dehydrogenase. I: Control group, II and III: Extract groups (150 and 300 mg kg^{-1} b.wt., respectively), IV: Pirimiphos methyl group, V and VI: Extract (150 and 300 mg kg^{-1} b.wt.) along with pirimiphos methyl groups

Table 2: Effect of exposure to pirimiphos-methyl on oxidative stress biomarkers in liver of male mice and the protective role of *O. majorana* leaves extract

	Oxidative stress biomarker				
Treatments	SOD (U mg^{-1} protein)	CAT (μmoL mg^{-1} protein)	GPx (U mg^{-1} protein)	GSH (μmoL mg^{-1} protein)	LPO (nmoL g^{-1} protein)
I	6.83±0.08[b]	17.31±0.31[b]	9.86±0.42[d]	0.102±0.004[b]	75.95±1.14[a]
II	6.82±0.14[b]	18.37±1.09[b]	9.06±0.63[cd]	0.098±0.002[b]	75.55±1.36[a]
III	6.86±0.23[b]	17.998±1.71[b]	9.90±0.65[d]	0.10±0.007[b]	76.34±1.02[a]
IV	5.47±0.07[a]	12.60±0.52[a]	4.33±0.55[a]	0.066±0.001[a]	122.35±2.24[b]
V	5.71±0.40[a]	15.64±0.49[b]	6.11±0.39[b]	0.074±0.001[a]	81.18±2.37[a]
VI	6.80±0.17[b]	16.48±0.34[b]	8.15±0.42[c]	0.096±0.001[b]	78.17±3.23[a]

Each value is a mean of 6 mice±SE, [a,b,c]Values are not sharing superscripts letters differ significantly at $p<0.05$, SOD: Superoxide dismutase, CAT: Catalase, Gpx: G lutathione peroxidase, GSH: Glutathione reduced, LPO: Lipid peroxidation. I: Control group, II and III: Extract groups (150 and 300 mg kg^{-1} b.wt., respectively), IV: Pirimiphos methyl group, V and VI: Extract (150 and 300 mg kg^{-1} b.wt.) along with pirimiphos methyl groups

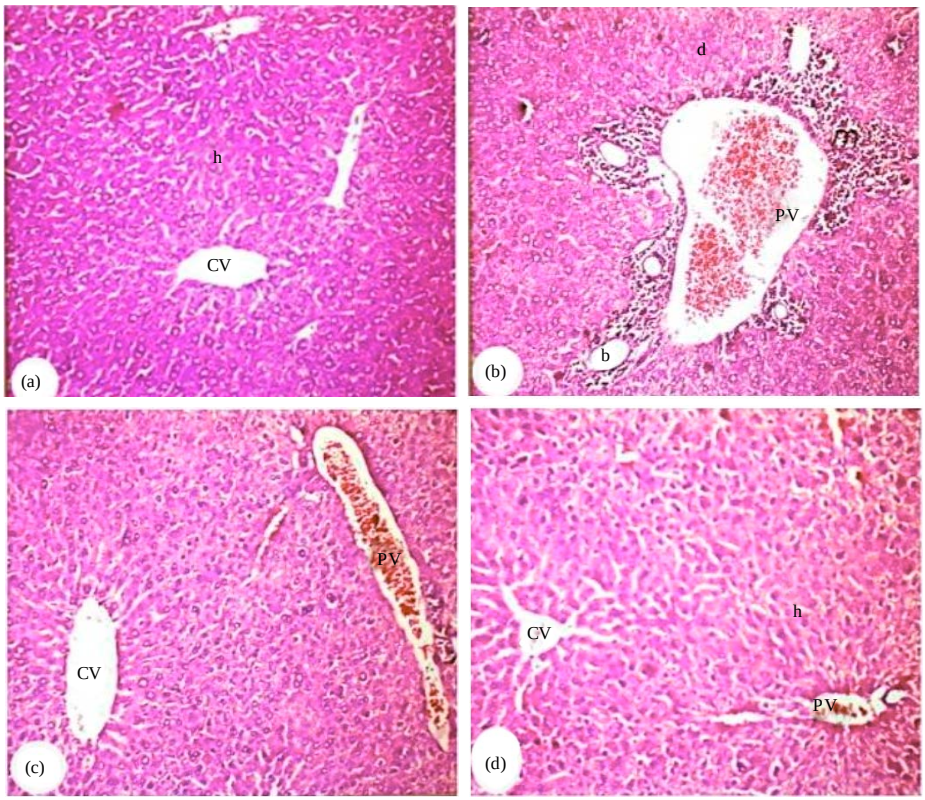

Fig. 3(a-d): Photomicrograph of liver sections stained by haematoxylin and eosin (H and E) for histopathological changes. Group 1 showing (a) Normal histological structure of the central vein and surrounding hepatocytes were recorded (x40). Mice administrated *O. majorana* extract at 150-300 mg kg^{-1} b.wt. showed the normal appearance of liver (group II and III) without any histological alterations as like control rats (group I). Group IV (pirimiphos methyl at 1/10 LD_{50}) showing (b) Sever congestion in the portal vein with multiple number of newly formed bile ducts as well as periductal inflammatory cells infiltration and degenerative change of hepatic parenchyma (x40). Group V (pirimiphos methyl along with extract at 150 mg kg^{-1} b.wt.) showing (c) Congestion in the central and portal veins (x40). Group VI (pirimiphos methyl along with extract at 150 mg kg^{-1} b.wt.) showing (d) No histopathological alteration (x40)

Table 3: Severity of the reaction in liver tissue of different groups according to the histopathological alterations

Histopathological alterations	Treatments					
	I	II	III	IV	V	VI
Portal inflammatory	-	-	-	+++	-	-
Congestion	-	-	-	++	+	-
Degeneration change in hepatocytes	-	-	-	+++	-	-

+++Sever, ++Moderate, +Mild, -Nil. I, control group, II and III, extract groups (150 and 300 mg kg^{-1} b.wt., respectively), IV, pirimiphos methyl group, V and VI, extract (150 and 300 mg kg^{-1} b.wt.) along with pirimiphos methyl groups

along with PM (group VI). Mice treated with *O. majorana* extract at 150 or 300 mg kg^{-1} b.wt., showed the normal appearance of liver like control mice (Table 3).

DISCUSSION

In the present study, the total phenolic and flavonoid contents of *O. majorana* leaves extract recorded 62.89±1.37 mg GAE/g and 45.58±1.18 mg QE/g, respectively. It has been reported that phyto-compounds e.g., phenolic and flavonoid commonly found in plants providing bioactive properties of the plant including antioxidant activity (Kalaivani and Mathew, 2010). Moreover, the antioxidant activities of phenolic compounds are playing an essential role in neutralizing free radical, quenching singlet and triplet oxygen, decomposing peroxides, stabilizing lipid peroxidation and protecting the cells against oxidative damage

(Zheng and Wang, 2001; Mossa *et al*., 2013). In addition to phytochemical properties, the antioxidant activity of *O. majorana* leaves extract was determined by using DPPH, ABTS, hydroxyl radical and reducing power assays. In the present study, the results showed a dose-dependent manner scavenging capacity of DPPH, ABTS, hydroxyl radical and reducing power which may be attributable to the hydrogen-donating ability of *O. majorana* leaves extract. Also, it has been reported that the antioxidant activity of the compounds were related to various antioxidant mechanisms including breakdown of peroxides, prevention of continued hydrogen abstraction, reduction in the capacity of chain initiation, binding with transition metal ion catalysts and ability of radical scavenging property (Liu *et al*., 2007). So, the reducing power of the extract may serve as a significant indicator of its potential antioxidant activity (Liu *et al*., 2013). Also, hydroxide radical can attack bio-macromolecule in cells and cause damage to cells by stimulating lipid peroxidation which occurs when hydroxyl radical is generated close to membranes and attacks the fatty acid side chains of the membrane phospholipids (Valko *et al*., 2007). The *in vitro* antioxidant assays performed on *O. majorana* extract revealed significant antioxidant potential compared with the standard (ascorbic acid).

Long term exposure to pesticides residues in water, vegetables, fruits, grains, milk, meat and others agriculture food products are widespread concern because it causes adverse health effect to human health. In toxicological studies, organ and relative organ weights are important criteria for evaluating organ toxicity (Crissman *et al*., 2004). In the present study, the body weight of PM treated mice was markedly less while relative liver weight was increased as compared to the control group. The reduced body weights may be due to the overall increased degradation of lipids and proteins as a result of the direct effects of PM as an organophosphate compound (Goel *et al*., 2005). However, the increase in liver weight in PM intoxicated mice may be due to the increment of biotransformation enzymes (Singh *et al*., 2005). Liver is playing a vital role in metabolism and detoxification of pesticides and other xenobiotics, therefore it is the first target organ against toxicity. Consequently, any changes of hepatic function biomarkers (AST, ALT, ALP and LDH) considered an indicative of tissue damage and hepatic dysfunction. The results in the current study suggested that the administration of PM increased the activity of liver function enzymes (AST, ALT, ALP and LDH) in serum of mice which are indicatives of cellular leakage and hepatotoxicity (Drotman and Lawhorn, 1978). However, co-administration of *O. majorana* at a dose of 300 mg kg^{-1} b.wt. shifted the activities of these enzymes to normal values.

Our present study demonstrated significant increases in hepatic LPO level and significant decreases in hepatic antioxidant enzyme activities (SOD, CAT and GPx) and the level of GSH in PM-treated mice. As well documented, SOD, CAT and GPx worked together to counteract the overproduction of ROS in the body. So, the decrease in theses enzymes in PM-intoxicated mice reflected the imbalance of the equilibrium normally held between the production and elimination of ROS. In addition, the antioxidant enzymes limited the harmful effects of oxidant molecules in hepatic tissues by means of their free radical scavenger's property (Abdollahi *et al*., 2004; Mansour and Mossa, 2009; Mossa *et al*., 2013). In our previous study, Heikal *et al*. (2014) concluded that PM increased the oxidative stress in liver and kidney of male mice as evidenced by enhanced levels of LPO accompanied by decreases in antioxidant enzymes activities. Other previous studies supported the present findings regarding the alterations in the enzymatic and non-enzymatic hepatic antioxidant systems as well as the liver dysfunction enzymes as a result of ODIs intoxication (Akhgari *et al*., 2003; Mansour and Mossa, 2009; Mossa *et al*., 2013). Form the other side, our study revealed the potential of *O. majorana* extract to attenuate the hepatic histopathological findings and the biochemical perturbations induced by PM in male mice.

CONCLUSION

It could be concluded that PM induced oxidative damage and hepatotoxicity in male mice. The mechanism of PM to induce liver toxicity mainly may be due to promotion of free radical. However, co-administration of *O. majorana* leaves extract attenuated the harmful effects of PM. The hepatoprotective effect of *O. majorana* may be attributed to the antioxidant potential of the extract where it reduced lipid peroxidation and improved antioxidant defense system in hepatocytes. The results indicated that *O. majorana* leaves could be used as therapeutic option against hepatic injuries resulting from pesticide intoxication.

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REFERENCES

Abbassy, M.A., A.E.S.M. Marei, M.A.M. Al-Ashkar and A.T.H. Mossa, 2014. Adverse biochemical effects of various pesticides on sprayers of cotton fields in El-Behira Governorate, Egypt. Biomed. Aging Pathol., 4: 251-256.

Abdollahi, M., A. Ranjbar, S. Shadnia, S. Nikfar and A. Rezaie, 2004. Pesticides and oxidative stress: A review. Med. Sci. Monit., 10: RA141-RA147.

Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.

Afoulous, S., H. Ferhout, E.G. Raoelison, A. Valentin, B. Moukarzel, F. Couderc and J. Bouajila, 2013. Chemical composition and anticancer, antiinflammatory, antioxidant and antimalarial activities of leaves essential oil of *Cedrelopsis grevei*. Food Chem. Toxicol., 56: 352-362.

Akhgari, M., M. Abdollahi, A. Kebryaeezadeh, R. Hosseini and O. Sabzevari, 2003. Biochemical evidence for free radicalinduced lipid peroxidation as a mechanism for Subchronic toxicity of malathion in blood and liver of rats. Hum. Exp. Toxicol., 22: 205-211.

Beutler, E., O. Duron and B.M. Kelly, 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Crissman, J.W., D.G. Goodman, P.K. Hildebrandt, R.R. Maronpot and D.A. Prater *et al*., 2004. Best practices guideline: Toxicologic histopathology. Toxicol. Pathol., 32: 126-131.

Drotman, R.B. and G.T. Lawhorn, 1978. Serum enzymes as indicators of chemically induced liver damage. Drug Chem. Toxicol., 1: 163-171.

EFSA, 2011. Risk assessment for pirimiphos-methyl residues resulting from cross-contamination. EFSA J., 9: 2436-2484.

El Kar, C., A. Ferchichi, F. Attia and J. Bouajila, 2011. Pomegranate (*Punica granatum*) juices: Chemical composition, micronutrient cations and antioxidant capacity. J. Food Sci., 76: C795-C800.

Goel, A., V. Dani and D.K. Dhawan, 2005. Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. Chem. Biol. Interact., 156: 131-140.

Heikal, T.M., A.T.H. Mossa, A.W. Ibrahim and H.F. Abdel-Hamid, 2014. Hepato-renal damage and oxidative stress associated with pirimiphos-methyl exposure in male mice. Oxid. Antioxid. Med. Sci., 3: 109-117.

Jeyaratnam, J., 1990. Acute pesticide poisoning: A major global health problem. World Health Stat. Q., 43: 139-144.

Kalaivani, T. and L. Mathew, 2010. Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Wild. *ex* Delile, an Indian medicinal tree. Food Chem. Toxicol., 48: 298-305.

Kunchandy, E. and M.N.A. Rao, 1990. Oxygen radical scavenging activity of curcumin. Int. J. Pharm., 58: 237-240.

Liu, C.H., C.H. Wang, Z.L. Xu and Y. Wang, 2007. Isolation, chemical characterization and antioxidant activities of two polysaccharides from the gel and the skin of *Aloe barbadensis* Miller irrigated with sea water. Process Biochem., 42: 961-970.

Liu, J., L. Jia, J. Kan and C.H. Jin, 2013. *In vitro* and *in vivo* antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). Food Chem. Toxicol., 51: 310-316.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mansour, S.A. and A.T.H. Mossa, 2009. Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. Pestic. Biochem. Physiol., 93: 34-39.

Michael, J.D., 2008. The Toxicologist's Pocket Handbook. 2nd Edn., CRC Press, New York, ISBN-13: 978-1420051384, Pages: 392.

Mossa, A.T. and G.A. Nawwar, 2011. Free radical scavenging and antiacetylcholinesterase activities of *Origanum majorana* L. essential oil. Hum. Exp. Toxicol., 30: 1501-1513.

Mossa, A.T.H., A.A. Refaie, A. Ramadan and J. Bouajila, 2013. Antimutagenic effect of *Origanum majorana* L. essential oil against prallethrin-induced genotoxic damage in rat bone marrow cells. J. Med. Food, 16: 1101-1107.

Nessiem, A.L., N.S. Bassily and S.A. Metwally, 2003. Comparative histopathological evaluation of permethrin, pirimiphos methyl and bendiocarb toxicities in testes, liver and kidney of rat. Egypt. J. Hosp. Med., 11: 58-73.

Ngoula, F., P. Watcho, M.C. Dongmo, A. Kenfack, P. Kamtchouing and J. Tchoumboue, 2007. Effects of pirimiphos-methyl (an organophosphate insecticide) on the fertility of adult male rats. Afr. Health Sci., 7: 3-9.

Nishikimi, M., N.A. Rao and K. Yagi, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun., 46: 849-854.

OECD, 2001. OECD/OCDE guidelines for acute toxicity of chemicals. OECD No. 423, Organization for Economic Co-operation and Development (OECD), Paris, France.

Oyaizu, M., 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr., 44: 307-315.

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

Rajini, P.S., Muralidhara and M.K. Krishnakumari, 1989. Inhibitory pattern of tissue esterases in rats fed dietary pirimiphos-methyl. J. Environ. Sci. Health Part B, 24: 509-524.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.

Satoh, K., 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta, 90: 37-43.

Singh, V.K., P. Dixit and P.N. Saxena, 2005. Cybil induced hepatobiochemical changes in Wistar rats. J. Environ. Biol., 26: 725-727.

Tackholm, V., 1974. Studies Flora of Egypt. 2nd Edn., Beirut Press, Lebanon.

Tomlin, C.D.S., 2004. The e-Pesticide Manual: A World Compendium. Version 3.1, The British Crop Protection Council, London, UK.

Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39: 44-84.

Vassault, A., 1983. Lactate Dehydrogenase: UV-Method with Pyruvate and NADH. In: Methods of Enzymatic Analysis, Bergmeyer, H.U., J. Bergmeyer and M. Grabl (Eds.). Verlag-Chemie, USA., ISBN-13: 9783527260430, pp: 119-126.

WHO, 1986. Informal consultation on planning strategy for the prevention of pesticide poisoning, Geneva, November 25-29, 1985. WHO/VBC/86.926, World Health Organization, Geneva, Switzerland, pp: 1-28.

Young, D.S., L.C. Pestaner and V. Gibberman, 1975. Effects of drugs on clinical laboratory tests. Clin. Chem., 21: 1D-432D.

Zheng, W. and S.Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem., 49: 5165-5170.