

Protective Role of Wheat Germ and Grape Seed Oils in Chlorpyrifos-Induced Oxidative Stress, Biochemical and Histological Alterations in Liver of Rats

Fares K. Khalifa, Fatma A. Khalil, Heba A. Barakat, Marwa M. Hassan

Biochemistry and Nutrition Department Women's College, Ain Shams University, Cairo, Egypt.

Abstract: The purpose of the present study was to assess the antioxidant role of wheat germ oil (WGO) and grape seed oil (GSO) in chlorpyrifos-induced oxidative stress, biochemical and histological changes in liver in male albino rats. Chlorpyrifos (CPF) was added to the different experimental tested diets at two levels of low and high doses (25 and 50 mg/kg diet respectively). WGO and GSO were added to the experimental diets at a level of 200 mg/kg diet. The results showed that the enzyme activities such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (γ GT) were significantly increased in rats administrated only by chlorpyrifos, while total proteins, albumin, and globulin showed a significant decrease at high and low doses of CPF groups. In addition, CPF caused a significant decrease in the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione-s-transferase (GST). Wheat germ oil and grape seed oil supplementation caused significant improvement in different biochemical parameters of all rat groups.

Key words: Chlorpyrifos, Wheat germ oil, Grape seed oil, Biochemical changes, Liver, Oxidative stress.

INTRODUCTION

Some pesticides such as organophosphorus compounds commonly used in agriculture for achieving better quality products, also have brought tremendous benefits to mankind by increasing food production and controlling the vectors of man and animal diseases (Demir *et al.*, 2011). At the same use of these pollutants has posed potential health hazards to the life are toxic substances and lead to generation of reactive oxygen species (ROS) which have harmful effects on human health. (Tuzmen *et al.*, 2008). Chlorpyrifos (CPF) is a broad-spectrum organophosphorus insecticide utilized extensively in agriculture (Saulsbury *et al.*, 2009) and elicits a number of additional effects, including hepatic dysfunction, hematological and immunological abnormalities, embryotoxicity, genotoxicity, and neurobehavioral changes (Mehta *et al.*, 2009). Pesticides are known to increase the production of reactive oxygen species (ROS), which in turn generate oxidative stress in different tissues (Rai and Sharma, 2007). Chlorpyrifos also induces oxidative stress and the accumulation of lipid peroxidation products in different organs (Verma *et al.*, 2007; Mansour and Mossa, 2009). ROS may interact with cellular proteins, lipids and DNA, causing alterations in cell function. Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipids bilayers (Ogutcu *et al.*, 2008), and they may damage membranes by inducing lipid peroxidation (LPO) (Kalender *et al.*, 2010; and Celik and Suzek, 2009).

Cells have several ways to alleviate the effects of oxidative stress. They can either repair the damage or directly reduce the pro-oxidative state via enzymatic and non-enzymatic antioxidants. Non-enzymatic (vitamins E and C, flavonoids, etc.) and enzymatic (superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)) antioxidants have been shown to scavenge free radicals and ROS (Durak *et al.*, 2010). Human diets also contain phytochemicals, such as flavonoids, that are metabolized by the same pathway as pesticides and other environmental pollutants (Panemangalore and Bebe, 2009).

Grape (*Vitis vinifera*) is one of the world's largest fruit crops and grape seed extract is a complex matrix containing approximately 40% fiber, 16% oil, 11% proteins, and 7% complex phenols including tannins, in addition to sugars and mineral salts (Shi *et al.*, 2003). The grape seed oil (GSO) contains 75% linoleic acid, 15% oleic acid, 6% palmitic acid, 3% stearic acid, and 1% linolenic acid (Natella *et al.*, 2002). It has recently become clear that grape seed oil (GSO) has shown various beneficial pharmacological effects such as its chemoprotective properties against reactive oxygen species and oxidative stress as well as being anti-

Corresponding Author: Fares, K., Khalifa, Biochemistry and Nutrition Department Women's College, Ain Shams University, Cairo, Egypt.

inflammatory, anti-bacterial, and anti-cancer (Teresa *et al.* (2010). Moreover, epicatechin is able to scavenge hydroxyl radicals, peroxy radicals, superoxide radicals. Procyanidins are reported to have potent antioxidant activity both in vitro and in vivo (Suwannaphet *et al.*, 2010). Wheat germ oil, which makes up only 7-12% of the seed, is an excellent source of natural vitamin E and tocopherols, the richest known source in nature (Zhu *et al.*, 2011). Wheat germ oil is also rich in unsaturated fatty acids, mainly oleic, linoleic and α -linoleic acids (Sjovall *et al.*, 2000) and in functional phytochemicals, mainly flavonoids, sterols, octacosanols and glutathione (Zhu *et al.*, 2006).

Animal studies show that intake of wheat germ oil results in a rapid increase in the content of vitamin E in the brain, liver, heart, lungs, kidneys, and spleen and gives powerful antioxidant protection to these organs and tissues (Mehranjani *et al.*, 2007; Field *et al.*, 2008). Wheat germ oil has been attributed to improved physical endurance, delayed aging, and compensated the imbalance of the serum biochemical factors in the rats to make it as the control level (Megahed, 2011). The purpose of the present study was to assess the antioxidant role of wheat germ oil (WGO) and grape seed oil (GSO) in chlorpyrifos-induced oxidative stress, biochemical and histological changes in liver in male albino rats.

MATERIALS AND METHODS

Materials:

Chlorpyrifos (CPF) was obtained from Kafr EL-Zayat Pesticides & Chemicals Company, and used as a toxic organophosphorus pesticide. It was added to the experimental tested diets at two levels of low and high (25 and 50 mg/kg diet respectively). Wheat germ oil (WGO) and grape seed oil (GSO) were obtained from Egyptian Indian company for Natural products. Extracted natural oils were added to the experimental diet at a level of 200 mg/kg diet.

Experimental animals:

The health experimental animals used throughout the present work were 70 adult male albino Sprague-Dawley strains, mean weight varied between 98g to 117g. They were obtained from El-Salam-Farm, Giza, Egypt. The animals were allotted to 7 homogenous groups and housed individually in plastic cages fitted with a wire mesh bottoms and fronts in a room maintained at 25-30 °C with about 50% relative humidity. The room was lighted on a daily photo period of 12hr light and dark. Then, they were allocated to the various experimental diets for 30 days.

Experimental Diets and Design:

The experimental diet used in the present study was the balanced diet prepared according to AIN-1993 adjusted by Reeves *et al.*, (1993). The animals were divided into seven groups of rats (ten for each group). All rats offered a balanced diet for three days for adaptation period on the environmental conditions before starting the experiment. The experimental groups were fed on diets either the balanced diet as control as well as fed on contaminated diets with chlorpyrifos (CPF) alone at the two levels (25 and 50 mg/kg diet) or supplemented with treatment doses of wheat germ oil (WGO) and grape seed oil (GSO) at a level of 200 mg/kg diet. The composition of the different experimental diets was presented in Table (1).

Methods:

1- Biological Evaluation:

During the conditioning period and through out the experiment, food and water were provided ad-libitum. Animals were weighed weekly, and feed efficiency ratio (FER) was calculated. At the end of the experimental period, the animals were fasted for 12hrs, and then anesthetized under diethyl ether anesthesia and whole blood samples were taken from hepatic portal vein in three centrifuge tubes. The second tube contained heparin then centrifuged for 10 minutes at 4000 rpm and plasma kept in plastic vials at -20 °C till used for the biochemical analysis. The second tube were left for 15 minutes at 37°C then centrifuged at 4000 rpm for 20 minutes for separating serum, and then serum were removed and kept in plastic vials at -20 °C until analysis. Livers were separated, rinsed and washed by saline solution (NaCl 0.9%), then blotted on filter paper, weighed and calculated the relative weights. The livers were stored at -20 °C until microscopical examination.

Table 1: Composition of the different experimental diets (g/ kg diet).

Ingredients	G1	G2 (LCPF)	G3 (HCPF)	G4 (LCPF+WGO)	G5 (HCPF+WGO)	G6 (LCPF+GSO)	G7 (HCPF+ GSO)
Corn starch	620.7	620.675	620.65	620.475	620.45	620.475	620.45
Casein	140.0	140.0	140.0	140.0	140.0	140.0	140.0
Sucrose	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Mineral mix.	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Vitamin mix.	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Corn oil	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Cellulose	50.0	50.0	50.0	50.0	50.0	50.0	50.0
L-Cystine	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Choline chloride	2.50	2.50	2.50	2.50	2.50	2.50	2.50
CPF	-	0.025	0.05	0.025	0.05	0.025	0.05
WGO	-	-	-	0.2	0.2	-	-
GSO	-	-	-	-	-	0.2	0.2

-CPF: Chlorpyrifos; WGO: Wheat germ oil; GSO: Grape seed oil; LCPF: Low chlorpyrifos; HCPF: High chlorpyrifos

2- Biochemical Measurements:

Determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum were performed the colorimetric method as described by Reitman and Frankel (1957). Alkaline phosphatase activity in serum was measured by colorimetric method as described by Belfield and Goldberg (1971). Gamma Glutamyl transferase activity (γ GT) was measured in serum by colorimetric method as described by Szasz (1969). The concentration of total protein in serum was determined by colorimetric method as described by Gornall *et al.*, (1949). The albumin level in serum was determined by colorimetric method according to Doumas *et al.* (1971). Globulins were counted by using the following formula:- $\text{Globulins (g/dl)} = \text{Total proteins (g/dl)} - \text{Albumin (g/dl)}$ Determination of lipid peroxides as malondialdehyde concentration in serum was determined by the colorimetric procedure as described by Ohkawa *et al.*, (1979). The activity of superoxide dismutase (SOD) in erythrocyte was determined by the colorimetric method as described by Nishikimi *et al.* (1972). Determination of catalase activity in plasma by the colorimetric method as described by Aebi (1984). Glutathione-S-transferase activity in plasma was determined by the colorimetric procedure as described by Habig *et al.* (1974).

3-Microscopical Examination:

Liver were dissected out and fixed instantaneously in 10% formal saline for 24 hours. The specimens were washed in tap water, dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax at melting point 55-60 °C. Sections of 6mm thickness were prepared and stained with haematoxylin and eosin (Harris, 1990).

4- Statistical Analysis:

Statistical analysis was done by using SPSS 11.5 statistical software completely randomization design in factorial arrangement (ANOVA; F-test) and one way classification to determine least significant difference (L.S.D).

Results:

The results tabulated in table (2) show the effects of consuming chlorpyrifos (CPF) at low and high doses alone or with two oil (GSO& WGO) treatments on food intake (g); body weight gain (g) feed efficiency ratio (FER), and relative weights of liver. From the results, it is clear that there were significant decrease in the level of food intake, body weight gain and FER in groups that fed on low and high CPF. The results showed a significant increase in food intake by 1.06% and 8.48%, and the percentage of change was 26.31% for body weight gain in G4 (LCPF+WGO) and G6 (LCPF+GSO) respectively when compared with G2 (LCPF). While the increments in G5 (HCPF+WGO) and G7 (HCPF+GSO) were reached 18.96% and 21.21% for food intake and 7.50% and 4.38% for body weight gain while the percentages of change of FER were 16.76% and 3.43% respectively when compared to G3 (HCPF). The results demonstrated that the levels of relative weights of liver decreased significantly in G2 and G3 while relative improvement was noticed in other groups. From results of treated groups we observed a great extent of improvement when compared with untreated groups.

Table 2: Effect of different experimental diets on food intake, body weight gain, feed efficiency and liver relative weight of albino rats.

parameters	Food Intake(g)	Body Wt. Gain(g)	Feed Efficiency Ratio(FER) %	Relative Liver Wt. (g%)
(G1)Control	464.0±57.3	55.5± 19.9	0.14± 0.04	5.6±1.0
(G2)LCPF	417.0± 32.0	21.5± 21.6	0.04± 0.05	5.6±1.6
(G3)HCPF	304.3± 27.9	-0.19± 15.5	-0.0003 ±0.06	4.1±0.6
(G4)LCPF+WGO	421.5± 11.5	27.1± 15.9	0.06± 0.04	5.6±1.2
(G5)HCPF+WGO	362.0± 32.5	14.0± 16.6	0.05± 0.05	5.7±1.1
(G6)LCPF+GSO	452.4± 44.7	12.7± 15.4	0.02± 0.03	6.1±1.7
(G7)HCPF+GSO	368.8± 13.7	8.1± 18.8	0.01± 0.05	5.4±1.0

-Values are expressed as means ± S.D, n=10.

The results in table (3) show the effect of different experimental diets on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (γ GT) activities. The results revealed that CPF intake in untreated groups induced liver injury which is reflected by the significant increase in serum level of ALT, AST, ALP and γ GT activities than control and treated groups.

From the results we observed that there is a noticeable improvement in all studied enzyme activities according to the following order G6 (LCPF+GSO) and G4 (LCPF+WGO) with respect to G2 (LCPF) and also G7 (HCPF+GSO) and G5 (HCPF+WGO) with respect to G3 (HCPF). It was found that serum activities of (ALT), (AST), (ALP) and (γ GT) was reduced significantly in all treated groups than untreated groups.

Table 3: Effect of different experimental diets on serum ALT, AST, ALP and γ GT activities.

parameters	ALT(U/ml)	AST(U/ml)	ALP(IU/l)	γ GT(U/l)
(G1)Control	24.5± 4.8	36.9± 3.1	93.5± 15.3	2.6± 0.2
(G2)LCPF	50.7± 5.2	63.3± 4.5	211.1± 25.1	4.5± 0.5
(G3)HCPF	63.2± 3.4	76.4± 5.9	242.9± 45.3	7.6±0.3
(G4)LCPF+WGO	41.5± 6.5	41.6± 4.0	133.2± 8.8	2.7±0.5
(G5)HCPF+WGO	48.6± 2.5	54.9± 4.7	38.6± 27.0	3.3±0.1
(G6)LCPF+GSO	35.0± 3.4	43.9± 4.1	123.8± 17.9	3.0±0.4
(G7)HCPF+GSO	44.9± 7.0	60.2± 3.5	132.1± 15.3	3.1±0.6

-Values are expressed as means ± S.D, n=10.

The results presented in table (4) show the other indices of liver function evaluation such as serum total proteins (g/dl), albumin (g/dl), globulins (g/dl) as well as A/G ratio of healthy control as compared with untreated groups of low and high CPF and treated with wheat germ oil (WGO) and grape seed oil (GSO). The results demonstrated that, the presence of CPF either in low or high dose had significantly decreased the level of serum total protein, albumin, and globulin and A/G ratio when compared with control rats.

It is clear from the results that serum total proteins level were elevated in rats fed diets containing WGO and GSO when compared to G2, the percentage of increment reached 22.84% and 17.46% for G4 and G6 respectively. Also, supplementation with WGO and GSO improves and helps to be nearly normal the levels of serum albumin and globulin and A/G ratio when compared with untreated groups subjected to low and high CPF.

The results in table (5) demonstrates the effect of CPF ingestion alone and with treatments of WGO and GSO on serum malondialdehyde (MDA) level, erythrocyte superoxide dismutase (SOD) level, plasma catalase and glutathione-S-transferase (GST) activities. From the results, the level of MDA, SOD, catalase and GST were statistically significant differences in groups that received low and high dose of CPF alone when compared with control.

The findings of the present experimental study demonstrate the improvement effect of WGO and GSO in all enzymes exhibited in treated groups when compared with groups fed on only CPF. Serum MDA level and erythrocyte SOD were improved in treated groups mainly those groups treated with WGO (G4) then GSO (G6) when compared to G2, also G7 (HCPF+GSO) and G5 (HCPF+WGO) represent the same improvement when compared to G3.

Table 4: Effect of different experimental diets on serum total proteins, Albumin, globulins levels and A/G ratio.

parameters	Total Proteins(g/dl)	Albumin(g/dl)	Globulins(g/dl)	A/G ratio
(G1) Control	7.47±0.28	4.95±0.26	2.52±0.16	1.98±0.19
(G2) LCPF	5.21± 0.38	2.88± 0.24	2.33± 0.20	1.25±0.11
(G3) HCPF	4.14±0.27	1.96±0.22	2.18±0.30	0.92±0.18
(G4) LCPF+WGO	6.40± 0.42	3.94±0.35	2.46± 0.26	1.62±0.22
(G5) HCPF+WGO	5.72±0.55	3.40±0.31	2.32±0.42	1.51±0.28
(G6) LCPF+GSO	6.12±0.17	3.72±0.20	2.40±0.19	1.56±0.19
(G7) HCPF+GSO	5.57±0.48	3.09±0.21	2.48±0.40	1.26±0.19

-Values are expressed as means ± S.D, n=10.

Table 5: Effect of different experimental diets on some serum antioxidants enzymatic activities of albino rats.

parameters	MDA (nmole/ml)	SOD (U/g Hb)	Catalase (U/ml)	GST (U/l)
(G1) Control	1.33± 0.3	249.69±16.1	53.20±4.0	13.37±1.5
(G2) LCPF	2.68±0.3	151.46±33.6	35.44±4.1	9.63±0.9
(G3) HCPF	3.48±0.5	147.31±16.8	24.91±3.5	6.27± 0.8
(G4) LCPF+WGO	1.57±0.2	367.14±68.1	42.14±3.6	12.38±0.9
(G5) HCPF+WGO	1.89±0.2	88.41±50.5	38.78±7.0	10.61±1.0
(G6) LCPF+GSO	1.62±0.3	241.97±46.4	47.73±3.8	11.69±1.0
(G7) HCPF+GSO	2.11±0.2	373.18±61.9	40.04±5.8	10.73±1.2

-Values are expressed as means ± S.D, n=10.

The Microscopical Examination:

Examined liver of control rats (G1) showed the normal histological structure (Fig. 1).

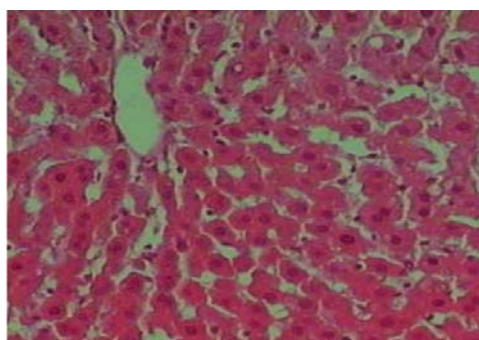


Fig. 1: photomicrograph of liver from control group (G1) showing the normal histological structure (H & E stain-X 150).

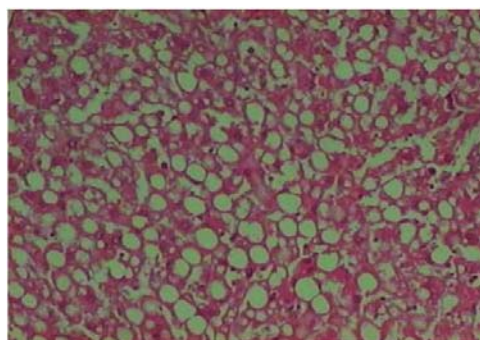


Fig. 2: photomicrograph of liver of rats of (G2) showing fatty change, small and large vacuoles in the hepatocytes with displacement of the nuclei (H& EX 150).

Examination of liver sections of rats feeding diet containing low dose of chlorpyrifos (LCPF) G2 showing fatty change in the form of small and large vacuoles in the hepatocytes with displacement of the nuclei (Fig.2).

Histopathological investigation of sections of liver of rats feeding diet containing high dose of chlorpyrifos (HCPF) G3 showing fatty change. The large vacuoles in the hepatocytes with displacement of the nuclei (Fig.3) and congestion of the portal area and inflammatory infiltration (Fig.4) were noticed.

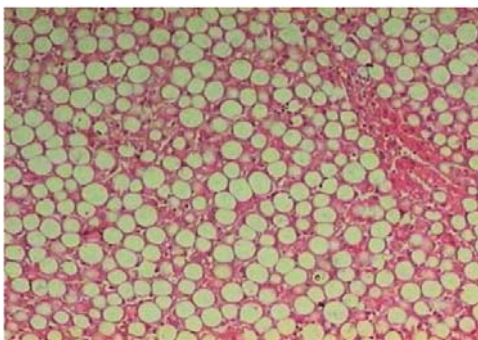


Fig. 3: photomicrograph of liver of rats of (G3) showing fatty change, the large vacuoles in the hepatocytes with displacement of the nuclei (H & E X 150).

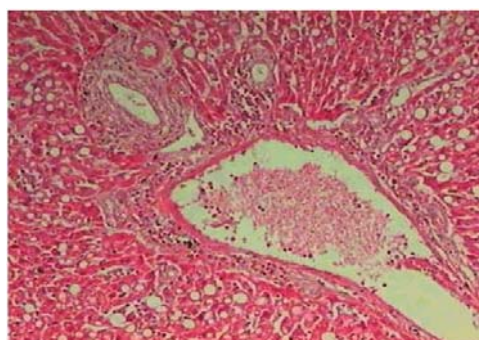


Fig. 4: photomicrograph of liver of rats of (G3) showing congestion of the portal area and inflammatory infiltration (H & E X 150).

Microscopic examination of sections of liver of rats feeding diet containing low dose of chlorpyrifos (LCPF) and wheat germ oil (WGO) (G4) showed few vacuoles in the hepatocytes with displacement of the nuclei and the most of the hepatocytes appear more or less like control (Fig.5).

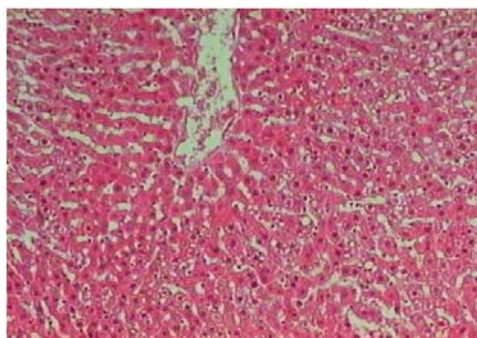


Fig. 5: photomicrograph of liver of rats of (G4) showing few vacuoles in the hepatocytes with displacement of the nuclei (H & E X 150).

Sections of liver of feeding diet containing high dose of chlorpyrifos (HCPF) and wheat germ oil (WGO) (G5) showed few vacuoles in the hepatocytes with displacement of the nuclei and the most of the hepatocytes appear more or less like control (Fig.6).

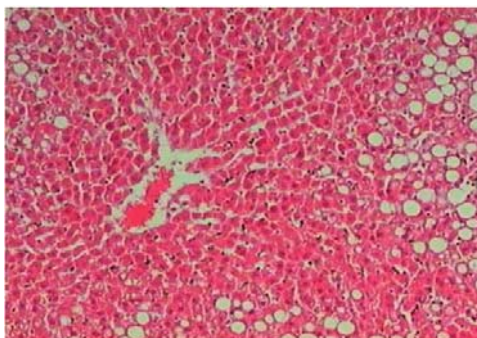


Fig. 6: photomicrograph of liver of rats from (G5) showing few vacuoles in the hepatocytes with displacement of the nuclei (H & E X 150).

In rats feeding diet containing low dose of chlorpyrifos (LCPF) and grape seed oil (GSO) (G6) sections of liver showed fatty change in the hepatocytes and congested portal area that associated with inflammatory infiltration (Fig.7). In another rats, liver showed that the hepatocytes appear more or less like control.

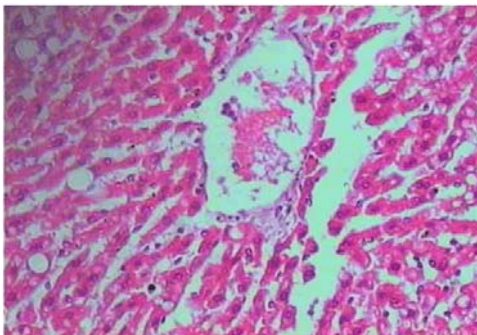


Fig. 7: photomicrograph of liver of rats of (G6) showing the hepatocytes appear more or less like control (H & E X 150).

Sections of liver of rats feeding diet containing high dose of chlorpyrifos (HCPF) and grape seed oil (GSO) (G7) showed few vacuoles in the hepatocytes with displacement of the nuclei and the most of the hepatocytes appear more or less like control (Fig.8).

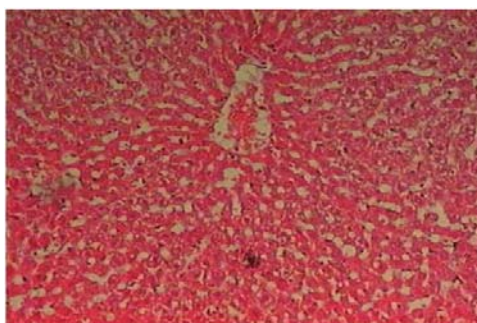


Fig. 8: Photomicrograph of liver of rats of (G7) showing few vacuoles in the hepatocytes (H& EX 150).

Discussion:

Oxidative stress caused by various agents (toxins, metals, dioxin and pesticides) is considered as an imminent threat for many organisms since it can lead to death. However, the imbalance between production of oxygen free radicals (OFRs) and antioxidant defenses in the body is called oxidative stress which has

important health implications reported by Ranjbar *et al.*, (2005). If there are too many OFRs or too few antioxidants for protection, a condition of oxidative stress develops, which may cause chronic damage (Abdollahi *et al.*, 2004). It has been indicated that the components associated with such antioxidant defense mechanism are altered under the influence of organophosphorus pesticides and that lipid peroxidation is one of the molecular mechanisms involved in pesticide induced cytotoxicity (Gupta, 2006).

Wheat germ is a rich source of antioxidants that include carotenoids, tocopherols, flavonoids and phenolic acids. (Vaher *et al.*, 2010). Most of the essential amino acids from wheat germ proteins are present at concentrations higher than in the reference egg protein pattern (Ge *et al.*, 2001). Since the rapid increase of the global demand for protein consumption, wheat germ may represent one of the most attractive and alternative source of proteins from cheap vegetable sources (Zhu *et al.*, 2006). Wheat germ is also rich in unsaturated fatty acids, mainly oleic, and α -linoleic acids (Sjovall *et al.*, 2000), and in functional phytochemicals, mainly flavonoids, sterols, octacosanols and glutathione (Zhu *et al.*, 2006).

Xu *et al.*, (2010) reported that grape has been appreciated for their rich content of phenolic compounds such as gallic acid, catechin and resveratrol, and a wide variety of procyanidins. A wide range of biological activities of these phenolic compounds has recently been reported: inhibition oxidation of human low-density lipoproteins, antioxidant properties and radioprotective effects, antihyperglycemic effects, modulation of the expression of antioxidant enzyme systems, anti-inflammatory effects and therapy of cancer. In the present study, the body weight and relative liver weight of animals fed diets containing CPF were markedly less as compared to the control group.

The net body weight gain of the animals intoxicated with CPF was markedly less as compared to the normal controls, suggesting that the poor body weight gain may be due to the overall increased degeneration of lipids and proteins as a result of the direct effects of the CPF.

Also, hepatic toxicity may result in a reduced liver size due to either acute or chronic hepatic injury resulting in cell loss. While the protective effects of wheat germ oil (WGO) and grape seed oil (GSO) in improving the body weight gain of the animals have been emphasized in our study. Our findings are similar to the results of Mansour and Mossa, (2010) who reported that oral administration of CPF at different doses to lactating rats resulted in significant decrease, in body weight as well as reduction of food intake and feed consumption observed in CPF-treated rats may be a result of the combination of increased degradation of lipids and proteins as a result of the direct effects of CPF as an organophosphates.

Our findings are in line with the Goel *et al.* (2005) work, that the net body weight gain of the animals intoxicated with CPF was markedly less as compared to the normal controls. While, Kim *et al.*, (2010) confirmed that food intake, feeding efficiency, after 32 day experimental period were slightly high in the rats fed GSO (4.2 g/day).

Similar results have been reported by Wren *et al.*, (2001) who demonstrated that a significant increase in food consumption was observed in male and female rats provided the grape seed extract diets compared to controls, especially in male rats consuming 2.0% grape seed extract. Grape seed extract appeared to increase the insoluble fraction of the diet. All groups gained weight during the study period.

Liver enzymes activities were used as important biomarkers for detection of hepatotoxic nature of this pesticide. Four serum hepatic marker enzymes (ALT, AST, ALP and γ GT) were evaluated for hepatotoxicity. The liver is the most sensitive organ to preoxidative damage because it is rich in oxidizable substances. The increment of the oxidative stress on the cells of the liver and the consequent decrease in the antioxidant ability of the cells result in the occurrence of aggressive cellular damage to the liver cells with destruction of their membranes and the release of the enzymes into the blood stream. The more severe the liver damage the higher the release of the liver enzymes (El-Khayat *et al.*, 2009).

Increase in serum level of ALT, AST as observed in groups dosed with CPF may reflect damage of liver cells and cellular degeneration or destruction occurs in this organ and the increase in the activities of ALP in plasma might be due to the increased permeability of plasma membrane or cellular necrosis, and this showed the stress condition of the treated animals with CPF. Also, the results of the present study indicate that wheat germ oil (WGO) and grape seed oil (GSO) significantly reduce the toxic effects of CPF by altered the hepatic enzyme activities and thus can be considered a potential hepatoprotective agent in conditions of organophosphate poisoning. Alia *et al.* (2003) explained that the liver is the main detoxifying organ in the body, and as such it possesses a high metabolic rate and it is subjected to many insults potentially causative of oxidative stress. Consequently, a correct status of the hepatic antioxidant defense system is of major importance for the maintenance of health.

The organophosphorus insecticides induce an obvious increase in AST, ALT and ALP, this fact is a conventional indicator of liver injury reported by Rao, (2006). When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream. Elevation of AST and ALT indicates the utilization of amino acids for the oxidation or for gluconeogenesis and is used to determine liver damage (Etim *et al.*, 2006). Also, the elevation in ALP level suggests an increase in lysosomal mobilization and cell necrosis due to pesticide toxicity. (Kalender *et al.*, 2005).

The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and damage or necrosis of hepatocytes (Pari and Arumugam, 2008). The membrane bound enzymes like ALP and γ GT are released unequally into bloodstream depending on the pathological phenomenon. The procyanidins found in grape can inhibit the apoptosis and damage of cells by oxygen free radicals (Li and Zhong, 2004).

Our results go hand in hand with Maheswari and Rao. (2005) who demonstrated that GSO has significantly reduced AST, ALT, and ALP liver enzyme levels further; GSO has increased the level of total proteins, which indicate hepatoprotective activity. Stimulation of protein synthesis accelerates the regeneration process and the production of liver cells (Li and Zhong, 2004).

Yuguang *et al.* (2004) reported that the potential short-term adverse effects of concentrated wheat germs policosanol (WGP) used as a supplement, the results show that WGP caused apparently no adverse effects in the volunteers as indicated by plasma hepatic enzyme activities. For each volunteer, the activities of plasma ALT, AST, and γ GT were within the normal range at the beginning and the end of the trial.

In this study, the CPF treated animals also exhibited significantly lower in total protein and albumin levels than the control animals. Normally, the reduction of albumin level indicates a liver disease. This reduction could be attributed to changes in the protein and free amino acid metabolism and their synthesis in the liver (Ncibi *et al.*, 2008). In the same field, Li *et al.*, (2007) suggested that albumin could be used as a biomarker of CPF toxicity. The same trend was seen with other organophosphate insecticides (Kalender *et al.*, 2005).

Shin and Moon, (2010) suggested from their results that grape skin or seed ingestion protects the hepatocytes from injuries and improves the liver functions of the treated rats. In chronic liver diseases, the serum albumin levels are reduced due to protein synthesis disruption in the liver. The grape skin and seed treatment blocked these dimethylnitrosamine-induced reductions in serum and total protein.

Abd El Dayem and Moawad, (2001) demonstrated that the increases observed in serum total protein and albumin levels may be due to the improvement in protein synthesis in the liver as a result of antioxidant effect which act as a free radical scavengers and could protect against lipid peroxidation. Biological systems have evolved with endogenous defense mechanisms to help protect against free radical induced cell damage. Catalase (CAT) and superoxide dismutase (SOD) are antioxidant enzymes, which metabolize toxic oxidative intermediates. They require micronutrient as cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity and effective antioxidant defense mechanisms (Halliwell, 2001). In our study CPF has been postulated to have multiple effects on the target cells including generation of reactive oxygen species and induction of intracellular oxidative stress thereby disrupting normal cellular development and differentiation. It is interesting to find that the rats of WGO and GSO supplementation had GST and SOD activities higher as well as of control group, which may indicate that the treated nutrients helped glutathione synthesis, in addition to its role as an essential component of SOD that is vital to cellular antioxidant defense. It has also been reported to interact with cell membranes to stabilize them against various damaging effects, including those due to oxidative injuries (Dulundu *et al.*, 2007).

Superoxide dismutase (SOD) catalyzes the conversion of superoxide radicals to hydrogen peroxide, while CAT converts hydrogen peroxide into water. These antioxidant enzymes can, therefore, alleviate the toxic effects of ROS. In this study, SOD and CAT activities significantly decreased in the erythrocyte of rats respectively that were treated with CPF. Antioxidants have been shown to inhibit free radical formation (Durak *et al.*, 2010). Glutathione is one of the essential compounds for regulation of variety of cell functions. It has a direct antioxidant function by reacting with superoxide radicals followed by the formation of oxidized glutathione (GSSG) and other disulfides. Glutathione S-transferase (GST) catalyses the conjugation of reduced glutathione via the sulfhydryl group, to electrophilic centers on a wide variety of substrates. This activity is useful in the detoxification of endogenous compounds such as peroxidised lipids (Valavanidis *et al.*, 2006). GST activity was decreased in rats by chlorpyrifos exposure (Khan and Kour, 2007).

Goel *et al.* (2005) reported that highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce malondialdehyde, a lipid peroxidation product. This is agreement with our results since CPF have been reported to induce oxidative stress, as shown by enhanced MDA production.

Ahn *et al.* (2002) reported that grape seeds are rich in antioxidant compounds, including phenolic compound (predominantly tannins), and it has been demonstrated that these compounds reduce the risk of chronic disease by protecting against free radical mediated damage. Tannins have been described to have antimutagenic, anticarcinogenic and antioxidant activities. Lipid peroxidation has been and remains one of the most widely used indicators of free radical formation. Thiobarbituric acid reactive substances primarily reflect production of lipid peroxides, which are broken down during the assay to yield malondialdehyde. Li *et al.*, (2001) reported that treatment with grape seed extract reduced the increase in thiobarbituric acid reactive substances. Similar results showing grape seed extract reduced formation of thiobarbituric acid reactive substances.

With respect to the hepatic histoarchitecture of the CPF treated animals there was an increased vacuolization of hepatocytes and focal necrosis in comparison to untreated normal controls. The congestion of the portal area, inflammatory infiltration increased in these animals. These observations indicated marked changes in the overall histoarchitecture of liver in response to CPF, which could be due to its toxic effects. Primarily by the generation of reactive oxygen species causing damage to the various membranous components of the cell. The necrotic conditions observed in liver of CPF treated animals are in corroboration with the observed biochemical changes, wherein an increased level of lipid peroxidation was noticed. The supplementation of grape seed oil (GSO) and wheat germ oil (GSO) is recommended as a concomitant supplement to the routine therapy for the protection against severe tissue damage induced by the organophosphorus pesticides.

Our results are in agreement with the results obtained by Goel *et al.*, (2005) who demonstrated that CPF intoxication alters serum and hepatic activities of liver marker enzymes including AST and ALT and also few hepatocytes were vacuolated, disrupted pattern of hepatic cords around the central vein and there were significant increase in the cells undergoing necrosis in these animals intoxicated with CPF at dose 13.5 mg/kg/ bw.

The histopathological lesions observed in the liver of CPF treated animals are in corroboration with the observed biochemical changes. Our results are in agreement with the results of Maheswari *et al.* (2005), they showed that CPF caused fatty degeneration and necrosis of the liver tissue. Pretreatment with GSO exhibited protection, which confirmed the results of biochemical studies. The results of the present study indicated that simultaneous treatment with GSO protects the liver against chlorpyrifos induced hepatotoxicity. The GSO offers vast possibilities in the treatment of various liver disorders. This may be due to the high level of antioxidant vitamin E, which was claimed to be the mechanism of hepatoprotection.

Tripathi and Srivastav, (2010) showed that the histopathological changes were caused in liver of rats by chlorpyrifos administration. The changes noticed were mainly hepatocytic vacuolation and degeneration of hepatocytes. Focal necrosis of liver as observed in this study following chlorpyrifos intoxication has been reported earlier by Jee *et al.*, (2005) opined that vascular formation is a cellular defense mechanism against injurious substances to cells; these substances were segregated in vacuoles and thus were prevented from interfering with cellular metabolism. It has also been suggested that cytoplasmic vacuolation is mainly a consequence of disturbances in lipid inclusions and fat metabolism occurring during pathological disturbances.

The lymphocytic infiltration observed in this study following chlorpyrifos treatment indicates signs of irritability, inflammation and hypersensitivity to the toxicant used. In the present study Kupffer cells increased in number. This can be explained by the fact that these cells are supposed to be hepatic macrophages which act as phagocytic cells and remove residual materials. Kupffer cells increase in number to engulf the necrotic tissues caused by chlorpyrifos. Similar observations were also reported by Khogali *et al.*, (2005).

ACKNOWLEDGMENTS

The authors are grateful to Dr. Abd El-Razik Hussein, Lecturer of Pathology. National Research Center for reading the histopathological sections.

REFERENCES

- Abd El-Dayem, S.M. and K.M. Moawad, 2001. Toxicity of CCL₄ in rat liver and the effects of antioxidant treatments. *J. Egypt. Soc. Zool.*, 36: 415-442.
- Abdollahi, M., A. Ranjbar, S. Nikfar and A. Rezaie, 2004. Pesticides and oxidative stress a review. *Med. Sci. Monit.*, 10: 141-147.
- Aebi, H., 1984. Catalase in vitro. *Methods Enzymol.*, 105: 121-126.

- Ahn, H. S., J. Tae, Y. L. Joo, G. H. Seong and P. Dong, 2002. Antioxidative activity of persimmon and grape seed extract in vitro and in vivo. *Nutr. Res.*, 22: 1265-1273.
- Alia, M., H. Cesar, B. Laura and G. Luis, 2003. Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. *Nutr. Res.*, 23: 1251-1267.
- Belfield, A. and D.M. Goldberg, 1971. Colorimetric determination of alkaline phosphatase activity. *Enzyme.*, 12: 561.
- Celik, I. and H. Suzek, 2009. Effects of subacute exposure of dichlorvos at sublethal dosages on erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. *Ecotoxicol. Environ. Saf.*, 72: 905-908.
- Demir, F., G.U. Fatma, D. Durak and K. Yusuf, 2011. Subacute chlorpyrifos-induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin. *Pesticide Biochem. and Phy.*, 99: 77-81.
- Doumas, B.T., W. A. Watson and H. G. Biggs, 1971. Biuret method for quantitative estimation of total protein in serum or plasma. *Clin. Chem. Acta.*, 31: 87-91.
- Dulundu, E., Y. Ozel and U. Topaloglu, 2007. Grape seed extract reduces oxidative stress and fibrosis in experimental biliary obstruction. *J. Gastroenterol Hepatol*, 22: 885-892.
- Durak, D., S. Kalender, F. Demir and Y. Kalender, 2010. Mercury chloride induced oxidative stress and the protective effect of vitamins C and E in human erythrocytes in vitro. *Afr. J. Biotechnol.*, 9: 488-495.
- El-Khayat, Z., R.E. Ahmed, S.A. Mahmoud, I.R. Wafaa and R.E. Tahany, 2009. Potential effects of bee honey and propolis against the toxicity of ochratoxin A in rats. *Maced. J. Med. Sci.*, 2(4): 311-318.
- Etim, O.E., E.O. Farombi, I.F. Ushoh and E.J. Akpan, 2006. The protective effect of aloe vera juice on lindane induced hepatotoxicity and genotoxicity. *J. Pharmaceut. Sci.*, 19: 337-340.
- Field, R., M. Verghese, L.T. Walker and J. Boateng, 2008. Feeding wheat germ meal and wheat germ oil reduced azoxymethane-induced aberrant crypt foci in Fisher 344 male rats. *Int. J. Cancer Res.*, 4: 127-136.
- Ge, Y., A. Sun, Y. Ni and T. Cai, 2001. Study and development of a defatted wheat germ nutritive noodle. *European Food Res. and Techn.*, 212: 344-348.
- 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.
- Gornall, A.G., C. J. Bardawill and M. M. David, 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.*, 177: 752.
- Gupta, R.C., 2006. *Toxicology of Organophosphates and Carbamate Compounds*. Elsevier Academic Press, USA, London, UK.
- Habig, W.H., M.J. Pabst and W. B. Jakoby, 1974. Glutathione S-transferase, a novel kinetic mechanism in which the major reaction pathway depends on substrate concentration. *J. Biol. Chem.*, 249: 7130-7139.
- Halliwell, B., 2001. Role of free radicals in the neurodegenerative diseases, therapeutic implications for antioxidant treatment. *Drugs and Aging.*, 18(9): 685-716.
- Harris, J.R., 1990. Rheumatoid arthritis. Pathophysiology and implications for therapy. *New England J. Med.*, 322: 1277-1289.
- Jee, J.H., F. Masroor and K.J. Chan, 2005. Responses of cypermethrin - induced stress in haematological parameters of Korean rockfish, *Sebastes schlegeli* (Hilgendorf). *Aqu. Res.*, 36: 898-905.
- Kalender, S., A. Ogutcu, F. Acikgoz and Y. Kalender, 2005. Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. *Toxicol.*, 211: 197-206.
- Kalender, S., F.G. Uzun, F. Demir and Y. Kalender, 2010. Malathion-induced hepatotoxicity in rats, the effects of vitamins C and E. *Food Chem. Toxicol.*, 48: 633-638.
- Khan, S.M. and G. Kour, 2007. Subacute oral toxicity of chlorpyrifos and protective effect of green tea extract. *Pestic. Biochem. Phys.*, 89: 118-123.
- Khogali, F.A., J.B. Sheikh, A.A. Rahim and M.H. Dagestani, 2005. Histopathological and hematological effects of dimethoate 4EC on some organs of albino mice. *J. King Saud Univ.*, 18: 73-87.
- Kim, D.J., G.J. Jeohye, S.K. Sung and L. Junsoo, 2010. Effect of Grape Seed Oil Supplementation on Plasma Lipid Profiles in Rats. *Food Sci. Biotechnol.*, 19(1): 249-252.
- Li, B., M.S. Lawrence, B. Masson and O. Lockridge, 2007. Matrix-assisted laser desorption/ionization time of flight mass spectrometry assay for organophosphorus toxicants bound to human albumin. *Analyt. Biochem.*, 361: 263-272.
- Li, L. and J. Zhong, 2004. Effect of grape procyanidins on the apoptosis and mitochondrial transmembrane potential of thymus cells. *J. of Hygiene Res.*, 33: 191-194.

- Li, W.G., X.Y. Zhang and Y.J. Wu, 2001. Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds. *Acta. Pharmacol Sin.*, 22: 1117-1120.
- Maheswari, U.M. and P.G. Rao, 2005. Antihepatotoxic effect of grape seed oil in rat. *Indian J. Pharmacol.*, 37: 179-182.
- Mansour, S.A. and A.H. Mossa, 2009. Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. *Pestic. Biochem. Phys.*, 93: 34-39.
- Mansour, S.A. and A.H. Mossa, 2010. Adverse effects of exposure to low doses of chlorpyrifos in lactating rats. *Toxicol Ind Health.*, 27(3): 213-224.
- Megahed, M.G., 2011. Study on stability of wheat germ oil and lipase activity of wheat germ during periodical storage. *Agriculture and biology J. of North America.*, (1): 163-168.
- Mehranjani, M.S., M.H. Abnosi and M. Mahmodi, 2007. Preventing effects of wheat germ oil on sex hormones, liver enzymes, lipids and proteins in rat serum following treatment with p-nonylphenol. *J. Biol. Sci.*, 7(8): 1408-1411.
- Mehta, A., R.S. Verma and N. Srivastava, 2009. Chlorpyrifos induced alterations in the levels of hydrogen peroxide, nitrate and nitrite in rat brain and liver. *Pestic. Biochem. Phys.*, 94: 55-59.
- Natella, F., F. Belleli, V. Gentili, F. Ursini and C. Scaccini, 2002. Grape seed proanthocyanidins prevent plasma post prandial oxidative stress in humans. *J. Agri. Food Chem.*, 26: 7720-7725.
- Ncibi, S., M.B. Othman, A. Akacha, M.N. Krifi and L. Zourgui, 2008. *Opuntia ficus indica* extract protects against chlorpyrifos-induced damage on mice liver. *Food Chem. Toxicol.*, 46: 797-802.
- Nishikimi, M., N.A. Rao and K. Yagi, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochem. Biophys. Res. Commun.*, 46: 849-853.
- Ogutcu, A., Z. Suludere and Y. Kalender, 2008. Dichlorvos-induced hepatotoxicity in rats and the protective effects of vitamins C and E. *Environ. Toxicol. Pharmacol.*, 26:355-361.
- Ohkawa, H., W. Ohishi and K. Yagi, 1979. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. *Biochem.*, 95: 351-358.
- Panemangalore, M. and F.N. Bebe, 2009. Short- and long-term exposure to low levels of pesticide and flavonoid mixtures modify endogenous antioxidants in tissues of rats. *J. Environ. Sci. Health*, 44: 357-364.
- Pari, L. and S. Arumugam, 2008. Effect of grape (*Vitis vinifera* L.) leaf extract on alcohol induced oxidative stress in rats. *Food and Chemical Toxicol.*, 46: 1627-1634.
- Rai, D.K. and B. Sharma, 2007. Carbofuran-induced oxidative stress in mammalian brain. *Mol. Biotechnol.*, 37: 66-71.
- Ranjbar, A.H., F.J. Solhi, A. Mashayekhi and M. Abdollahi, 2005. Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study. *Environ. Toxicol. Pharmacol.*, 20: 88-91.
- Rao, J.V., 2006. Biochemical alterations in euryhaline fish, *Oreochromis mossambicus* exposed to sub-lethal concentrations of an organophosphorus insecticide, monocrotophos. *Chemosphere*, 65: 1814-1820.
- Reeves, G., F.H. Nielsen and J.G. Fahey, 1993. AIN-1993 reformation of the AIN- 76: Purified final report of the American Institute of Nutrition Purified AdHoc Writing Committee on the Reformation of the AIN-76 Arodent diet. *American Institute J. Nutr.*, 26: 1939-1951.
- Reitman, A. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Amer. J. Clin. Path.*, 28: 56-63.
- Saulsbury, M.D., S.O. Heyliger, K. Wang, and D.J. Johnson, 2009. Chlorpyrifos induces oxidative stress in oligodendrocyte progenitor cells. *Toxicol.*, 259: 1-9.
- Shi, J., Y. Jianmel, E.P. Joseph and K. Yukio, 2003. Polyphenolics in grape seeds biochemistry and functionality. *J. Med. Food*, 6 (4): 291-299.
- Shin, M.O. and J.O. Moon, 2010. Effect of dietary supplementation of grape skin and seeds on liver fibrosis induced by dimethylnitrosamine in rats. *Nutr. Res. Pract.*, 4(5): 369-374.
- Sjovall, O., T. Virtalaine, A. Lapvetelainen and H. Kallio, 2000. Development of rancidity in wheat germ analyzed by headspace gas chromatography and sensory analysis. *J. Agr. Food Chem.*, 48: 3522-3527.
- Suwannaphet, W., A. Meeprom and S. Adisakwattana, 2010. Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats. *Food and Chem. Toxicol.*, 48: 1853-1857.
- Szasz, G., 1969. A kinetic photometric method for serum gamma-glutamyl transpeptidase. *Clin Chem.*, 15(2): 124-136.
- Teresa, S.P., D.A. Moreno and C.G. Viguera, 2010. Flavanols and anthocyanins in cardiovascular Health: A review of current evidence. *Int. J. of Molecular Sci.*, 11: 1679-1703.

Tripathi, S. and A.K. Srivastav, 2010. Liver profile of rats after long-term ingestion of different doses of chlorpyrifos. *Pestic. Biochem. Physiol.* 97: 60-65.

Tuzmen, N., N. Candan, E. Kaya and N. Demiryas, 2008. Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidative defense mechanisms and lipid peroxidation in rat liver cell. *Cell Biochem. Funct.*, 26: 119-124.

Vaher, M., K. Matso, T. Levandi and M. Kaljurand, 2010. Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. *Procedia Chem.*, 2: 76-82.

Valavanidis, A., T. Vlahogiannia and M. Scoullas, 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicol. Environ. Saf.*, 64: 178-189.

Verma, R.S., A. Mehta and N. Srivastava, 2007. In vivo chlorpyrifos oxidative stress attenuation by antioxidant vitamins. *Pestic. Biochem. Phys.*, 88: 191-196.

Wren, A.F., C. Michael, F. Christopher and N. Leslie, 2001. 90-Day oral toxicity study of a grape seed extract in rats. *J. Agric. Food Chem.*, 50(7): 2180-2192.

Xu, C., Y. Zhang, L. Cao and J. Lu, 2010. Phenolic compounds and antioxidant properties of different grape cultivars grown in China. *Food Chem.*, 119: 1557-1565.

Yuguang, L., R. Mike, A. Trautwein and W. Gert, 2004. Wheat Germ Policosanol failed to lower plasma cholesterol in subjects with normal to mildly elevated cholesterol concentrations. *Metabolism.*, 53: 1309-1314.

Zhu, K., C. Lian, X. Guo, W. Peng and H. Zhou, 2011. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. *Food Chem.*, 126: 1122-1126.

Zhu, K., H.M. Zhou and H.F. Qian, 2006. Proteins extracted from defatted wheat germ, nutritional and structural properties. *Cereal Chem.*, 83: 69-75.