Evaluation of Profenofos Intoxication In White Rats

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Abstract: Profenofos is an organophosphorous insecticide which extensively used in agriculture and household. The present work is under taken to evaluate acute, subchronic and withdrawal effects of profenofos intoxication on some lipid metabolism indices and cytotoxicity enzymes biomarkers as well as on total non-specific esterase in blood of male white rats. Adult male white rats weighing 200±20 g were orally administered with Profenofos at single dose of 47.5mg/kg body weight or repeated dose of 23.75mg/kg body weight. Exposure to single or repeated doses of Profenofos elicited significant increase in TC, TG and LDL-C levels parallel to a decrease in HDL-C level. Also, induction of AcP, ALP, LDH and CK activities were recorded throughout most of the experiments periods as compared to corresponding controls at confidence interval 95% or P value>0.05 respectively. Pointed to the withdrawal effect; all the parameters under investigation restored near the control values except for HDL-C and LDL-C. The present data also explored that acute and subchronic Profenofos intoxication induced significant induction in the total non-specific esterase (NSE) activity and exhibited marked changes in its fractional activity and electrophoretic mobility. Signs of recovery were seen in fractional activity to Profenofos withdrawal. Conclusion: Continuous exposure to Profenofos alters lipid metabolism; increase the activities of cytotoxicity enzymes biomarkers, thereby it may be a causative factor for multiple organs dysfunction as liver, kidney, heart and muscles. Also, the present investigation supports the idea that the estimation of fractional rather than the total activity of NSE is more reliable in reflecting the molecular consequences of acute and chronic oxidative stress induced by single oxidant.

[Nashwah I. Zaki. **Evaluation of Profenofos Intoxication In White Rats. Forests.** *Nat Sci* 2012;10(12):67-77]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 11

Keywords: Acute; subchronic; organophosphorous intoxication; cytotoxicity enzymes; oxidative stress; lipid and protein oxidation

1. Introduction

Organophosphorous compounds (OPs) are arguably one of the most common causes of insecticide poisoning worldwide (Jeyaratnam, 1990). In developing countries, such as Egypt OPs are easily available and cheap, hence a source of both intentional and unintentional poisonings. Pesticide has posed potential health hazards to the life and lead to generation of reactive oxygen species (ROS) which have harmful effects on human health (Tuzmen et al., 2008). Oxidative stress arises when the concentrations of ROS exceed the cellular ability to remove ROS and repair cellular damage and ultimately results in widespread oxidation of biomolecules includes lipids, proteins and nucleic acid. The myriad reactions that ensue can be categorized as peroxidation reactions resulting in the oxidation of polyunsaturated fatty acids that form components of membrane lipids as well as protein oxidation which in turn, results in tertiary structural alterations that promote protein aggregation and amyloid formation (Shinall et al., 2005). Oxidants-other than hydroxyl radical-generally exhibit selectively in the type of biomolecules they react with, then the result is structural alteration of proteins, inhibition of enzymatic activity and interference with the regulatory function and eventual cell death. (Yu, 1994).

Profenofos [(O-4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] is a widely used in Egypt for the control of various caterpillars, white fly and mits on cotton and vegetable crops (Abdel Razik and Shehata, 2007). It is reported to be highly toxic to human and animals (Gotoh et al., 2001; Mustafa et al., 2008; Kavitha and Rao, 2009).The toxicity by Profenofos appeared fatal even at a relatively low plasma concentration as recorded in a case of fatal human poisoning where high concentrations of metabolites were detected suggests Profenofos is rapidly metabolized (Gotoh et al., 2001). Profenofos can induce oxidant stress which may be earlier diagnostic index in profenofos poisoning and can induce serum biochemical alteration even in low concentrations (Lin et al., 2003; Elhalwagy and Hassanin, 2006; Mansour et al., 2009).

Furthermore, the toxic potency of OPs in mammals is closely related to their rate of metabolic elimination. Non-specific esterases (NSE) i.e. B-esterases or carboxylesterases) CEs) include a group of hydrolytic enzymes that are important in the metabolism and subsequent detoxification of many xenobiotic including OPs, drugs and endogenous

compounds (Sogorb and Vilanova, 2002; Wheelock et al., 2004; Desai and Desai, 2008). They have an increased affinity over AChE for some OPs and it has been suggested that carboxylesterases act as a "sink" for OPs, thus protecting the organism against OP toxicity (Maxwell, 1992). Previous studies have supported the use of isoenzyme expression and activity rather than total enzyme activity, for better understanding of molecular basis of oxidative stress in male rats (El-Zayate, 2008; Elhalwagy and Zaki, 2009)

The present investigation aimed to assessment acute and subchronic profenofos intoxication induced oxidative stress and its withdrawal changes in lipid metabolism indices; total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) as well as, the activity of some cytotoxicity enzymes as acid phosphatases (AcP), alkaline phosphatases (ALP), lactate dehydrogenase (LDH), creatinine kinase (CK); isoenzyme pattern expression and activity of total non-specific esterase. Also, test the feasibility of using isoenzyme expression and activity beside total enzyme activity as additional markers for better understanding of the molecular basis of acute/chronic oxidative stress in blood of adult male white rats.

2. Materials and methods

2.1. Animals

A total of seventy five sexually mature male white rats weighing 200±20 g (approximately 10 months old) were supplied by National Organization for Drug Control and Research animal house. They were housed in wire cages with natural ventilation and illumination and allowed free water and fed standard diet. The animals were quarantined for ten days before beginning the experiments. All rats were treated according to the standard procedures laid down by OECD (1992) guidelines 407 repeated dose 28 days oral toxicity study in rodents and handled in accordance with the standard guide for the care & use of laboratory animals.

2.2. Chemicals

Pesticide: Coracron (Profenfos 72%; EC) produced by El-Helb pesticides and chemical company, Egypt. The calculated oral LD50 value of Profenofos was (475 mg/kg body weight) according to Weil's method (Weil, 1952). Used dosages equal 1/10 and 1/20 LD50 for the tested pesticide.

2.3. Animal treatment schedule

Animals were divided randomly into two experimental groups which consisted of apparently normal rats. The first experimental group represents acute intoxication group; where fifteen rats exposed to one single dose of Profenofos 47.5 mg per kg body weight. Animals remained alive along the experimental period which extended for 120 hr. Blood samples were collected from retro-orbital plexus just before the treatment start (initial time), 1 hr., 24 hr. ,72 hr. and 120 hr. post treatment. The second experimental group represents subchronic exposure and withdrawal group. Animals included two subgroups control group (C) and treated group (T) of thirty rats each. Control rats were given deionized water only while treated rats exposed to daily oral dose of Profenofos 23.75 mg per kg body weight for 14 days (5 doses/week); then remained without treatment for further 14 days for a withdrawal period. Body weight was monitored twice a week and the dose was adjusted accordingly. Rats were scarified by decapitation at 7, 14 and 28 days post the onset treatment.

2.4. Sampling

Blood samples were collected in two sets of tubes. Heparinized tubes centrifuged at 3600 rpm, then the obtained plasma were separated and kept at -40ºc for estimation of lipid metabolism parameters and non-specific esterase (NSE). Non-heparinized tubes allowed to clot for thirty minutes at room temperature then, centrifuged at 1000 x g and 4ºc per ten minutes. The collected serum samples were stored at -40 ◦c and used for enzymes activities assay.

2.5. Biochemical assays

Total cholesterol level (TC) was assayed according to Flegg, 1973 and Allain et al., 1974. Total triglycerides (TG) was determined by the method of wahlefeld, 1974 using Stanbio reagent Kits; USA. High density lipoprotein (HDL) was measured according to Finley et al., 1978 and Warnick et al., 1983 while low density lipoprotein was calculated using formula of Friedewald et al., 1972.

2.6. Enzyme activities assays

Enzymatic activities of acid and alkaline phosphatase were determined according to Moss, 1984; Bowers and McComb, 1966, respectively; using readymade kits by QCA, Spain. While the activities of lactate dehydrogenase and creatine kinase were measured according to Kachmar and Moss, 1976 using Stanbio reagent Kits; USA.

Nonspecific esterase (NSE) i.e carboxylesterase activity (CE) was determined according to the method of Gomori (1953) as adapted by Van Asperen (1962). The assay was conducted in a single cuvette, at 25 ºc, using 0.5 ml of 0.05 M phosphate buffer, pH 7.4, 10 ml of diluted plasma in buffer (or 10 ml buffer for the blank) and 5 ml of α-naphthyl acetate (NA) (in 95% ethanol) at a reaction concentration of 0.485 mM as substrate. The reaction was initiated by addition of the substrate and stopped after 10 min by addition of 2.5

ml of a solution of 0-dianiside, tetrazotized zinc chloride complex (fast blue salt BN) (1 mg/ml) freshly prepared in an aqueous solution of sodium dodecyl sulfate (1%). This reagent gives a blue-colored product in the presence of α- naphthol produced by the hydrolysis of α-NA. Its absorbance was measured at 600 nm after 15 min. of storage in darkness.

Electrophoresis of esterases isozymes were carried out for each seven pooling of samples of each treated group. The polyacrylamide gels (12%) for vertical electrophoresis were prepared with 0.37 M Tris-HCl, and pH 8.8 as buffer (Ceron, 1988). The stack gel was prepared with 3.0 mL of acrylamide 10% and bis-acrylamide 0.5% dissolved in 3.0 mL of 0.24 M Tris-HCl, pH 6.8, 30 µL twice-distilled water, 250 µL ammonium persulfate 2% and 30 µL TEMED. Gels were run during 3 h 30 min, at 25 °C, and constant 200 V. The running buffer was 0.1 M Tris-glycine, pH 8.3. Staining technique according to the method described by Ceron, (1988), were used for esterase identification. The gels were soaked for 30 min in 50 mL 0.1 M sodium phosphate, pH 6.2, at room temperature. Esterase activity was visualized by placing the gels for 1hr in a staining solution prepared with 50 mL of sodium phosphate solution, 15 mg of β-naphthyl acetate, 20 mg of α-naphthyl acetate, 60 mg of Fast Blue RR salt, and 5 mL of N-propanol.

2.7.Statstical analysis

Data of the present study are represented in tables as mean±standard error (Mean±SE) and figures. By using the SPSS-PC computer software package version17; Paired t-test at 95% confidence was carried out on the data of acute exposure group. While, the significance of the difference between the groups was calculated by one-way analysis of variance (ANOVA) for subchronic exposure group. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Biochemical parameters

Data of biochemical parameters after Profenofos acute, subchronic exposure and its withdrawal are shown in Tables 1and 2. Exposure to 47.5 mg/kg of Profenofos elicited moderate significant increase in TG and TC levels reaching maximum at 24 hr. post intoxication (p.i) with14.49% and 20.27% from the initial values respectively; at 95% confidence interval (Table1). TG and TC levels were restored near their initial values by 72 and 120 hour p.i, respectively. Concerns low density lipoprptein, LDL-C level showed marked significant induction throughout the experimental period. This elevation showed variable magnitudes reaching maximum at 24hr p.i corresponding to197% form the initial value. In contrast, significant decline in HDL-C level have been demonstrated to acute profenofos intoxication after 24 and 72hr as compared to the initial level at 95% confidence interval. (Table1).

Exposure to Profenofos 23.75mg/kg for 14 days showed the same trend of changes encountered in acute exposure. On other words, significant increases were recorded in TG, TC and LHL-C levels throughout the experimental period. Maximum TG level was recorded after 7 days of treatment corresponding to 10.95% of the control value at $P<0.05$, while the levels of TC and LHL-C reaching maximum after 14 days of the onset treatment (59.49% and 307.06%, respectively at $P<0.05$). In contrast a significant decrease in HDL-C level was recorded after 14days onset treatment (-15.05%; $P<0.05$) versus the respective control value (Table 2). Pointed out the withdrawal, the current data showed that TC and TG levels was restored near the control values among Profenofos treated animals at $P<0.05$. LDL-C and HDL-C levels have shown considerable improvement despite the fact that significantly higher levels still be demonstrated versus the control ($P<0.05$; Table 2). This finding can be supported by the fact that a complete recovery in HDL-C and HDL-C could be seen by time.

Table 1. Effect of acute intoxication with Profenofos on some lipid metabolism parameters in plasma of adult male white rats.

Parameters		**TG**	**TC**	**HDL**	**LDL**
Groups	**Duration**				
Untreated	**Initial**	**96.85±0.39**	**101.37±0.82**	**61.05±0.62**	**20.64±0.33**
Treated	1hr	101.88*±0.33	115.54*±0.43	60.14±0.95	34.04*±0.30
	24hr	110.09*±0.37	121.92*±0.72	40.40*±0.77	61.44*±0.26
	72hr	94.56±0.56	115.56*±0.49	51.65*±0.40	44.35*±0.28
	120hr	95.73±0.46	106.21±0.22	56.89±0.63	31.23*±0.27

-Results were expressed as Mean ±SE for each 10 rats.
-*significant at confidence interval 95%.
-Units:(mg/dl).

Table 2. Effect of subchronic intoxication with Profenofos and its withdrawal on some lipid metabolism parameters in plasma of adult male white rats.

Parameters		TG	TC	HDL	LDL
Groups	**Duration**				
Control	7days	**93.31±0.97**	**101.54±1.05**	**58.68±0.69**	**23.06±0.86**
Treated		103.53*±1.62	131.25*±0.99	57.08±1.34	53.47*±2.20
Control	14days	**92.52±0.88**	**100.50±1.71**	**58.01±0.46**	**22.8±0.50**
treated		102.22*±0.88	160.29*±1.47	49.28*±1.18	92.81*±0.94
Control	Withdrawal	**93.86±1.43**	**99.67±1.25**	**57.5±0.89**	**22.54±0.56**
treated		91.03±1.15	106.29±0.70	51.92*±0.47	33.92*±1.13

-Results were expressed as Mean ±SE for each 10 rats.
-*Significance difference versus control group at P<0.05; -Units: (mg/dl).

3.2. Enzymatic activities

The activities of serum enzymes reflecting cytotoxicity measured at different levels of Profenofos exposure as well as its withdrawal are presented in Tables 3 and 4. As seen in Table 3 Profenofos intoxication with single acute dose induced significant increases in AcP, ALP, CK and LDH activities throughout most of the experimental period when compared to their initial values at 95% confidence interval. In other words, the recorded induction in Acp and LDH activities reached its maximal level 24 hr (P.i) corresponding to 71.03% and 92.41% of their initial values, respectively and extended to the end of the experimental period. Meanwhile the maximum increase in CK and ALP were recorded 1hr p.i corresponding to 19% approximately. Deviation in ALP and Ck activities have been correlated quickly and returned near their initial values before the end of the experiment period at 95% confidence interval.

Table 3. Effect of acute intoxication with Profenofos on some cytotoxicity enzymes biomarkers for muscles, heart in serum of adult male white rats.

Parameters		LDH	CK	ALP	AcP
Groups	**Duration**				
Untreated	**Initial**	**742.69±5.24**	**147.11±0.69**	**88.29±0.81**	**11.39±0.17**
Treated	1hr	806.43*±3.06	174.72*±0.77	105.05*±0.50	15.30*±0.13
	24hr	1429.32*±4.10	164.47*±0.84	95.49*±0.54	19.48*±0.38
	72hr	952.99*±3.83	163.56*±0.79	87.74±0.94	15.40*±0.44
	120hr	860.07*±1.73	144.72±0.93	87.27±0.46	12.97*±0.27

- Results were expressed as Mean ±SE for each 10 rats.
-*significant at confidence interval 95%.
-Units: (U/L).

Table 4. Effect of subchronic intoxication with Profenofos and its withdrawal on some cytotoxicity enzymes biomarkers for muscles, heart in serum of adult male white rats.

Parameters		LDH	CK	ALP	AcP
Groups	**Duration**				
Control	7days	**730.19±5.68**	**153.19±0.91**	**82.98±0.97**	**11.8±0.08**
Treated		1095.54*±36.50	253.71*±8.23	92.60*±0.75	17.35*±0.52
Control	14days	**735.5±15.91**	**158.55±1.68**	**83.30±0.90**	**11.72±0.09**
Treated		923.18*±13.04	234.87*±3.31	89.45*±0.61	15.84*±0.55
Control	Withdrawal	**737.49±11.41**	**157.37±0.75**	**82.95±1.04**	**11.14±0.28**
Treated		738.65±6.20	160.10±1.16	84.04±0.59	11.72±0.04

-Results were expressed as Mean ±SE for each 10 rats.
*-Significance difference versus control group at P<0.05; -Units: (U/L).

On the other side, repeated dose of Profenofos (23.75 mg/body weight) for 14 days induced marked significant increases in LDH, CK, ALP and AcP activities versus the respective control values at P<0.05. These induction were maximal after 7days of treatment recorded 50.03%, 65.62%, 11.59% and 47.03% respectively, versus the control groups at P<0.05. However, signs of recovery were seen after Profenofos withdrawal towards all tested enzymes as their levels restored near that of the control ones as demonstrated in Table 4.

3.3. Non-specific esterase

The present observations shown in Tables 5,6 and figures 1,2 explored that acute and subchronic Profenofos intoxication induced pronounced significant induction in total non-specific esterase activity versus the control ones. Similarly, the effect of Profenofos withdrawal on total esterase was pronounced as a significant increase in its activity. It was among the objectives of the present study to check the rate of gene expression as manifested by isozyme pattern of plasma esterase in acute and subchronic treatment as well as withdrawal effects. Measuring the fractional activities of different isozymes was a more practical approach, because in some cases the effect appears on the fractional level not on the total level.

Table 5. Effect of acute intoxication with Profenofos on total and fractional isozyme activities of non-specific esterase in plasma of male white rats.

Parameters	Initial	1hr	24hr	72hr	120hr
Total esterases	**149.56±0.48**	179.30*±0.40	187.76*±0.55	184.34*±0.41	165.84*±0.83
Fractional activity (%)					
E1	0.13(16.0%)	0.10(16.1%)	0.09(11.9%)	0.081(12.4%)	0.081(11.3%)
E2	0.29(18.6.%)	0.26(15.6%)	0.21(11.9%)	0.23(20.1%)	0.38(18.1%)
E3	0.40(15.8%)	0.46(14.8%)	0.46(18.5%)	0.61(26.8%)	0.44(20.4%)
E4	0.78(34.4%)	0.71(18.5%)	0.71(28.6%)	0.74(18.6%)	0.77(23.6%)
E5	0.88(13.8%)	0.87(34.9%)	0.86(29.2%)	0.86(22.1%)	0.87(26.6%)
Total	**100%**	**100%**	**100%**	**100%**	**100%**

Results were expressed as Mean ±SE for each 7 rats.
-*Significant at confidence interval 95%.
-Units: (U/l).

Table 6. Effect of subchronic intoxication with Profenofos and its withdrawal on total and fractional isozyme activities of non-specific esterase in plasma of male white rats.

Parameters	7days		14 days		Withdrawal	
	control	treated	control	treated	control	treated
Total esterases	145.13±0.88	184.99*±0.79	146.86±0.85	175.23*±0.60	146.52±0.72	171.87*±0.67
Fractional activity (%)						
E1	0.036(16.5%)	0.07(24.0%)	0.036(14.9%)	0.035(14.1%)	0.064(17.6%)	0.052(19.1%)
E2	0.38(15.2%)	0.37(20.9%)	0.30(19.9%)	0.32(21.4%)	0.21(22.1%)	0.55(20.7%)
E3	0.44(20.1%)	0.44(19.4%)	0.54(17.6%)	0.68(24.8%)	0. 63 (19.8%)	0.61(20.6%)
E4	0.78(21.8%)	0.78(8.64%)	0.80(15.8%)	0.82(22.3%)	0.78(18.1%)	0.78(18.2%)
E5	0.88(11.9%)	0.88(11.6%)	0.87(15.4%)	---	---	---
E6	0.97(14.5%)	0.96(15.4%)	0.95(16.5%)	0.96(22.3%)	0.96(22.4%)	0.96(21.3%)
Total	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**

Results were expressed as Mean ±SE for each 7 rats.
*-Significance difference versus control group at P<0.05. -Units: (U/l)

Concerns NSE isoenzyme pattern, the present data revealed five distinct phenotypes (E1: E5) to acute Profenofos intoxication and exhibited pronounced changes in the electrophoretic mobility of E2, E3 and E4 as compared to the initial profile (Figure 1). Diffusions were appeared in E5 band throughout the experimental periods as well as in E3 band 72hr post treatment as compared to the initial profile. Intoxication with repeated dose of Profenofos for 7 & 14 days showed six distinct phenotypes (E1:E6) on the gel with slight changes in electrophoretic mobility of treated groups to control ones. In addition, diffusion was seen in E1and E2 bands 7 days of onset treatment while appeared at E3, E4 and E6 bands 14 days of

treatment associated with the missing of E5 band as compared to the respective control profile (Fig. 2). However, good signs of recovery effect were seen post withdrawal period where minor deviations in the % fractional activity have been encountered versus the control group (Figure. 2).

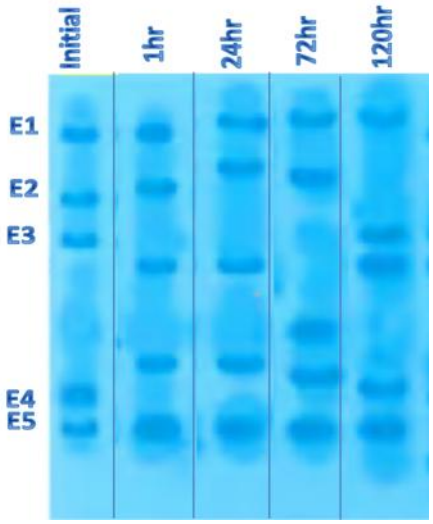

Figure. 1. Impacts of acute profenofos toxicity on plasma total esterases isozyme of male white rats. Lane 1 (initial) just pretreatment, lane 2 (1hr) 1hour post profenofos intoxication (47.5mg/kg body weight), lane 3 (24hr) 24hour post profenofos intoxication, lane 4 (72hr) 72hour post profenofos intoxication, lane 5 (120hr) 120 hour post profenofos intoxication.

4. Discussion

OPs are primarily recognized for their ability to induce toxicity in mammals through inhibition of acetylcholinesterase (AChE) and subsequent activation of cholinergic receptors (Costa, 2006). Both acute and chronic OPs poisoning have been identified to have potential to generate free radical in biological system (Altuntas *et al*., 2002; Tuzmen *et al*., 2008). All the major biomolecules like lipids, protein and nucleic acid may be attacked by reactive oxygen species (ROS), but lipids are probably the most susceptible (Cheesman and Slater, 1992). The present data explored that, acute and subchronic Profenofos intoxication significantly increased the levels of serum total triglyceride(TG), total cholesterol (TC) and low density lipoprotein (LDL-C) concurrent with significant decreased in the level of high density lipoprotein (HDL-C). The present results are in agreement with Attia and Nasr, 2009; Mansour *et al*., 2009 and particularly with Kalender *et al*., 2005; Yousef *et al*., 2006; Elhalwagy and Zaki, 2009. Disturbances of lipids level in tissues and serum are usually associated with many abnormalities, including gallstone formation, atherosclerosis, and coronary artery disease (Moss *et al*., 1987).

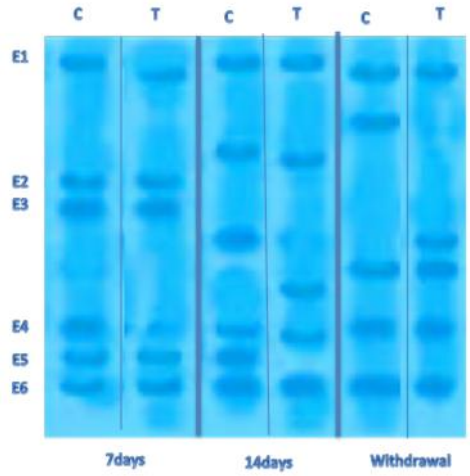

Figure 2. Impacts of subchronic profenofos toxicity and its withdrawal on plasma total esterases isozyme of male white rats. Lane 1 (C) control of 7days post profenofos intoxication (23.75mg/kg body weight), lane 2 (T) 7days post profenofos intoxication, lane 3 (C) control of 14days post profenofos intoxication, lane 4 (T) 14days post profenofos intoxication, lane 5 (C) control post 14day of profenofos withdrawal, lane 6 (T) post 14day of profenofos withdrawal.

A positive association between occupational exposure to pesticides and an increase in lipid profile was found with Op (Nakagawa *et al*., 1982). OP insecticides generally cause increase of total cholesterol and total lipids levels (Makhija and Pawer, 1977; Hassan *et al*., 1988; Azza *et al*.,1994). The current increase in serum cholesterol can be attributed to the effect of pesticide on the permeability of liver cell membrane (Adham *et al*., 1997) as well as to the blockage of liver bile ducts causing reduction or cessation of its secretion to the duodenum (Zaahkouk *et al*., 2000). Regarding triglycerides, the current results are consistent with the ones recently found by Elhalwagy and Zaki, 2009; Mansour *et al*., 2009 who reported an elevation in TG of rats treated with the Diazinon and Profenofos, respectively. This elevation might be attributed to an inhibition of lipase enzyme activity of both the hepatic TG and plasma lipoproteins (Goldberg *et al*., 1982). Also, the current alteration of lipoprotein fractions might be related to the protective role of paraoxonase1 (PON) to secure against free radicals. It is known that organophosphate inhibits PON1 activity (Ellenhorn *et al*., 1997) and therefore it might decrease the ability of (PON) to metabolizes toxic oxidized lipids associated with both low density lipoprotein and high density lipoprotein (Mackness *et al*., 2000; Costa *et al*., 2005). The current study explored gradual increase in LDL-C, TC levels with time duration to subchronic Profenofos intoxication which might be declared that Profenofos reacts in an

apparent dose-time dependent manner. Also, delayed improvement in HDL-C and LDL-C level to profenofos intoxication might be evidenced that profenofos exposure has been associated with an increased risk of coronary artery disease and or other organs complication.

Regarding the enzymatic activities induction in their activity were recorded to both acute and subchronic intoxication. OP insecticides like Profenofos may cause increase in activities of AcP, ALP, CK and LDH (Azza *et al*.,1994; Jalili *et al*., 2007; Ahmed and Zaki, 2009 ; Al-Attar, 2009). The present findings might reflect the necrotic effect of oxidative stress induced by profenofos toxicity on different body cells. Previous experimental studies have shown that single and repeated administration of OP compounds including Profenofos (Azza *et al*., 1994; Rahman *et al*., 2000; Lukaszewiez and Moniuszko, 2005; Ahmed and Zaki, 2009; Attia and Nasr, 2009) and carbamates (Rahman *et al*., 1990) produced significant increases in AcP activity in rat and chicken sera. Additionally, clinically significant amounts of AcP have been reported in many diseases included metastasized prostate cancer, diseases of blood cells, lysosomal storage diseases, systemic infection, anemia, kidney disease, liver disease, heart attack and oxidative stress conditions (Baron *et al*., 1985; Bakheet, 2010). The current induction in AcP activity could be interpreted as a result of increased permeability of plasma membrane or cellular necrosis, indicating a stress condition in the exposed animals or might suggest an increase in the synthesis of this enzyme, which may be an adaptive mechanism to the toxicant stress. It is worthy here to point out that the impact of acute profenofos intoxication on AcP activity was more pronounced than that encountered to subchronic one. This conclusion can be supported by a recent study (Hernández *et al*., 2004) who hypothesized increase of AcP activity along with the decrease of butyrylcholinesterase (BChE) activity. Concerns alkaline phosphatase activity, recently ALP could be used as marker of cardiometabolic risk (Webber *et al*., 2010) besides its usual use as marker for skeletal and hepatobiliary disorders; it's significantly associated with a higher frequency of cardiovascular disease, hypertension, hypercholesterolemia and diabetes. Hence, The present disruption of phosphatase from the normal values may be denote biochemical impairment and lesions of tissues and cellular functions because they are involved in the detoxification process, metabolism and biosynthesis of energetic macromolecules for different essential functions (Azza *et al*., 1994).

Likewise, elevation in serum LDH and CK activities have been reported to different pesticides intoxication including organophosphate (Sauviat and Pages, 2002; Kalender *et al*., 2004; Manna *et al*., 2004; Ogutcu *et al*., 2006; Jalili *et al*., 2007; Omurtag *et al*., 2008; Attar, 2009; Eraslan *et al*., 2009). It is known that LDH is present in almost all cells of the body and particularly in higher level in liver, heart, erythrocytes, skeletal muscles and kidneys (Lott and Nemensanszky, 1987). Also, it recognized as a potential marker for assessing the toxicity of a chemical (Abdul Naveed *et al*., 2011). Consequently, elevations in its activity have been recorded to be associated with diseases affecting these organs as myocardial infarction, renal infarction and haemolysis. The present increase in LDH activity to Profenofos intoxication indicated damage to any of the organs producing this enzymes. Additionally, CK is one of the most valid and reliable methods for assessing muscular damage including heart muscle (Cardinet, 1989; Moss and Henderson, 1994). Regarding data on humans, Friedman *et al.* (2003) and khan *et al.* (2008) reported similar elevations in creatine kinase after acute and chronic exposure to anticholinesterases. In clinical application, creatine kinase (CK) can provide information about the severity and the stage of damage to the heart muscle. Ck or CK-MB is first heart enzyme to appear in the blood few hours after onset heart attack symptoms and then returns to its baseline.

The last interesting finding of our study is the association of profenofos exposure with plasma total non-specific esterase, although the fact that it is an inverse association makes it difficult to interpret. However, previous studies explored different patterns of changes to esterase; Elhalwagy and Zaki, 2009 reported significant decline in NSE level in male rats exposed to repeated dose of Diazinon for 14 days, whereas El-Zayat, 2008 recoded non-significant changes against acute Paraquat exposure in female albino rats. Preliminary study by Zemaitis *et al*.(1976) coincided with our results and reported increased levels of plasma and liver esterase to a single oral dose of dieldrin, or diet containing dieldrin for up to 8 weeks to male and female rats. Nevertheless, Satoh and Hosokawa, 1998 stated that exposure to environmental pollutants could result in induction of carboxylesterase activity. Also, changes in the activity of some detoxifying enzymes, including esterases, were detected in organisms residing in contaminated sites which might help in interpreting such vague increase. Therefore, these enzymes have been suggested as biomarkers of physiological stress provoked by the presence of toxicants in the environment (Wilczek *et al*., 1997; Stone *et al*., 2002).

The oxidation of proteins plays an essential role in the pathogensis of an important number of degenerative diseases which is now widely recognized (Berlett and Stadtman, 1997; Stadtman and Berlett, 1998). Many different types of protein oxidative

modification can be induced directly by Reactive Oxygen Species (ROS) or indirectly by the reactions of secondary by- products of oxidative stress (Berlett and Stadtman, 1997). The toxic effects of oxidative stress on cells depend upon the intensity of the stress and or the cell type. Low ROS concentrations have been shown to increase the antioxidant enzyme activity and to promote cell proliferation. Conversely, high ROS levels induced apoptosis or necrosis (Dong-Yun *et al*., 2003; Kang and Zheng, 2004). In the current study, the fractional activities of plasma isoenzyme exhibit different interesting trends under acute and subchronic Profenofos toxicity. A marked rise in E3 and E5, reduction in E1 and E4 and changeable levels in E2 have been detected to acute Profenofos intoxication but fluctuated alternative levels in % fractional activities has demonstrated to subchronic intoxication. This might be explained in the current study as oxidative damaging effect of profenofos intoxication on DNA and protein. Regarding the *in vitro* studies Das *et al* (2006 a; b) explored that subchronic toxicity profenofos induced apoptosis and necrosis as well as single strand break in DNA using lymphocyte from peripheral blood samples of healthy human donors via DNA diffusion and comet assay under *in vitro* condition. The present study has also revealed major changes in electrophoric mobility of some speared isoenzymes among both acute and subchronic profenofos intoxication groups. These changes might reflect a considerable degree of oxidation to protein molecules which might in turn resulting in tertiary structural alteration that promote protein aggregation and amyloid formation as support by the study of Shinall *et al*., 2005.the mechanism involved in the oxidation of protein by ROS has been described by the work of Stadtman, 2004. Up to date, few studied have been recorded about the effect of acute and or chronic intoxication induced oxidative stress on the hereby measured isoenzyme expression (El-Zayate, 2008; Elhalwagy and Zaki, 2009). The above findings are of special importance and could prove the possibility to use the isoenzyme expression as an additional marker to differentiate between the acute and subchronic molecular effect of single and or different oxidants in male rats. As conclusion the current data suggested that Profenofos intoxication creates a condition of physiological stress possible by altering lipid metabolism and induction enzymes activities as AcP, ALP, CK, LDH and total non-specific esterase which may be an adaptive mechanism to the toxicant stress. Also, the present investigation suggested that Profenofos intoxication may be contributed for multi organs dysfunctions as liver, heart, erythrocytes, skeletal muscles and kidneys.

Acknowledgment

Deepest gratitude and many thanks to Prof. Dr. Hany Hassan, prof. of immunology (Animal Reproduction Research Institute (ARRI), for his help in carrying out the part of non-specific esterase.

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5/10/2012